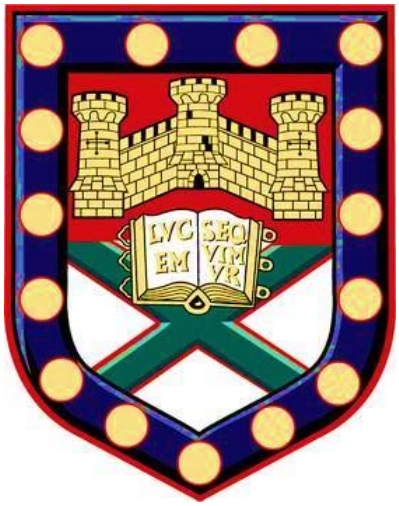
The effect of acute consumption of non-specific COX-inhibitors on neuromuscular fatigue and exercise performance

PAUL MORGAN



Submitted by Paul Morgan to the University of Exeter as a thesis for the degree of Doctor of Philosophy in Sport and Health Sciences

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

P.T.Morgan

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ABSTRACT

Exercise-induced fatigue is a complex, multifactorial process, the physiological bases of which are still widely debated. Recent research has implicated pain sensation in the aetiology of exercise-induced neuromuscular fatigue. Indeed, acute consumption of acetaminophen (ACT, or paracetamol), a non-specific inhibitor of the cyclooxygenase (COX) enzymes which synthesise prostaglandins and sensitise group III/IV muscle afferents, has been shown to improve exercise performance concomitant with a lower pain and effort sensation. However, the neurophysiological bases for the potential ergogenic effect of acute ACT ingestion, and how this influences the power/torque-duration relationship, have yet to be determined. Therefore, the purpose of this thesis was to assess the effect of acute consumption of the recommended therapeutic dose (1 g of ACT and 400 mg of IBP) of common pain-relieving, COX-inhibiting medicines on performance and the mechanisms of neuromuscular fatigue development in a variety of different exercise tests. **Study 1:** Mean torque (61 ± 11 vs. 58 ± 14% pre-exercise MVC) and end-test torque, reflective of critical torque, (44 ± 13 vs. 40 ± 15% pre-exercise MVC) were greater in the ACT trial compared to placebo (PL) when completing 60 maximum voluntary contractions (MVCs) of the knee extensors (both $P<0.05$). Voluntary activation (VA; a marker of central fatigue) and potentiated twitch (pTw; a marker of peripheral fatigue) declined at a similar rate in both conditions (both $P>0.05$). However, the decline in electromyography (EMG) amplitude, a marker of muscle activation, was attenuated in the ACT trial with EMG being greater compared to PL from 210 s onwards ($P<0.05$). **Study 2**: Compared to PL, ACT ingestion increased end-test power, reflective of critical power (ACT: 297 ± 32 vs. PL: 288 ± 31 W, $P<0.001$), and total work done (ACT: 66.4 ± 6.5 vs. PL: 65.4 ± 6.4 kJ, $P<0.05$) without impacting W' (ACT: 13.1 ± 2.9 vs. PL: 13.6 ± 2.4 kJ, $P>0.05$) or the M-wave amplitude ($P>0.05$) during a 3-min all-out cycling test. EMG declined throughout the 3-min protocol in both the PL and ACT conditions; however, the decline in EMG was attenuated in the ACT condition, with the EMG amplitude being greater compared to PL over the last 60 s of the test ($P<0.05$). **Study 3:** Time-to-exhaustion (T_{lim}) during severe-intensity knee extensor exercise in the right leg was 19% shorter after completing prior severe-intensity knee extensor exercise to exhaustion in the left leg following placebo ingestion (Leg_{2PL}) compared to no prior fatigue in the left leg (Leg_{2CON}) (Leg_{2CON}: 385 ± 104 vs. Leg_{2PL}: 311 ± 92 s; $P<0.05$). ACT ingestion did not improve

T_{lim} in the left leg without prior contralateral fatigue (Leg_{1ACT}) compared to the placebo condition (Leg_{1PL}) (Leg_{1CON}: 396 ± 105 vs. Leg_{1PL}: 390 ± 106 vs. Leg_{1ACT}: 402 ± 101 s; P<0.05). Moreover, ACT ingestion did not improve T_{lim} in the right leg following prior contralateral fatigue in the left leg (Leg_{2ACT}) compared to the placebo condition (Leg_{2PL}) (Leg_{2ACT}: 324 ± 85 vs. Leg_{2PL}: 311 ± 92 s; P>0.05). There were no changes in intramuscular phosphorous substrates and metabolites, ratings of perceived exertion or EMG amplitude after ACT ingestion compared to PL ingestion in the severe-intensity single-leg knee extensor exercise tests completed with or without prior contralateral fatigue. **Study 4:** Mean torque (IBP: 60 ± 12 vs. PL: 58 ± 14% of pre-exercise MVC) and end-test torque (IBP: 41 ± 16 vs. PL: 40 ± 15% of pre-exercise MVC) were not different between the IBP and PL conditions when completing 60 MVCs of the knee extensors (P>0.05). Similarly, end-test power output (IBP: 292 ± 28 W vs. PL: 288 ± 31 W) and work done (IBP: 65.9 ± 5.9 kJ vs. PL: 65.4 ± 6.4 kJ) during the 3-min all-out cycling tests were not different between the IBP and PL conditions (all P>0.05). Neuromuscular fatigue markers developed at a similar rate in both exercise tests in the PL and IBP conditions (all P>0.05).

In conclusion, acute consumption of ACT, but not IBP, increased muscle activation and critical torque (and power), and attenuated fatigue development during maximal-intensity knee extensor and cycle ergometry exercise. However, acute ACT ingestion did not influence T_{lim} during continuous severe-intensity knee-extension exercise completed with or without prior fatigue of the contralateral limb. The original findings from this work suggest that acute ACT ingestion, but not IBP ingestion, might have implications for improving performance during maximal-intensity, but possibly not severe-intensity constant work rate exercise with these ergogenic effects linked to an increase in muscle activation. These findings offer insights into the potential for non-specific COX inhibitors to influence performance and neuromuscular fatigue development during different forms of exercise.

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LIST OF ABBREVIATIONS

^{31}P-MRS	^{31}Phospherous-magnetic resonance spectroscopy
ACT	Acetaminophen
aEMG	Average rectified EMG
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
Ca^{2+}	Calcium
CMEP	Cervicomedullary motor evoked potential
CMD	Central motor drive (i.e. neural drive)
CMO	Central motor output
CNS	Central nervous system
COX	Cyclooxygenase
CP	Critical power
CSP	Cortical silent period
CT	Critical torque
CV	Coefficient of variation
CWR	Constant work rate
EMG	Electromyography
EMG_{RMS}	Electromyography amplitude (Root mean square)
GET	Gas exchange threshold
H^+	Hydrogen ion
IBP	Ibuprofen
K^+	Potassium
Leg_{1CON}	Left leg knee extensor exercise completed without capsule ingestion
Leg_{1ACT}	Left leg knee extensor exercise completed following acetaminophen ingestion
Leg_{1PL}	Left leg knee extensor exercise completed following placebo ingestion
Leg_{2CON}	Right leg knee extensor exercise completed without capsule ingestion

$Leg_{2ACT\text{-}CONTRA}$	Right leg knee extensor exercise completed following exhaustive left leg knee extensor exercise following acetaminophen ingestion
$Leg_{2PL\text{-}CONTRA}$	Right leg knee extensor exercise completed following exhaustive left leg knee extensor exercise following placebo ingestion
M1	Primary motor cortex
MEP	Motor evoked potential
M_{max}	Maximal M-wave amplitude
MVC	Maximum voluntary contraction
MRI	Magnetic resonance imagery
MRS	Magnetic resonance spectroscopy
***m.*VL**	Vastus lateralis
***m.*VM**	Vasus medialis
Na^+	Sodium
NLMF	Non-local muscle fatigue
NSAID	Non-steroidal anti-inflammatory drug
PCr	Phosphocreatine
PG	Prostaglandin
Pi	Inorganic phosphate
PL	Placebo
pTw	Potentiated twitch
RER	Respiratory exchange ratio
$RT_{0.5}$	One half relation time
RPE	Ratings of perceived exertion (via BORG 6-20 scale)
SR	Sarcoplasmic reticulum
sTw	Superimposed twitch
T_{lim}	Time to the limit of exercise tolerance
TMS	Transcranial magnetic stimulation
TT	Time-trial
TTE	Time to exhaustion
VA	Voluntary activation
$\dot{V}CO_2$	Volume of carbon dioxide production
$\dot{V}E$	Minute ventilation

$\dot{V}O_2$	Volume of oxygen uptake
$\dot{V}O_{2peak}$	Peak oxygen uptake
W'	Curvature constant of the hyperbolic power-duration (or Torque-T_{lim}) relationship
WADA	World anti-doping association
WR_{peak}	Peak work rate

DECLARATIONS, PUBLICATIONS AND COMMUNICATIONS

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

REFEREED JOURNAL ARTICLES FROM THE THESIS

Morgan, P. T., Bowtell, J. L., Vanhatalo, A., Jones, A. M., & Bailey, S. J. (2018). Acute acetaminophen ingestion improves performance and muscle activation during maximal intermittent knee extensor exercise. *Eur J Appl Physiol*, *118*(3), 595–605. http://doi.org/10.1007/s00421-017-3794-7.

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CURRENTLY UNDER REVIEW

Morgan, P.T., Bailey, S.J., Banks, A.R., Fulford, J., Vanhatalo, A., & Jones, A.M. Contralateral fatigue during severe-intensity single-leg exercise: influence of acute acetaminophen ingestion. *Original investigation*. *J Appl Physiol.*

CONFERENCE ACTIVITY

Morgan PT, Black MI, Bailey SJ, Jones AM and Vanhatalo A. Conventional critical power test predicts 16.1-km road time trial performance. Oral presentation: presented at ECSS annual congress 2015 and Physiological Society conference 2015.

Morgan PT, Bowtell JL, Vanhatalo A, Jones AM and Bailey SJ. Acute acetaminophen ingestion improves performance and muscle activation during maximal intermittent knee extensor exercise. Oral presentation: presented at BASES conference 2016 and ECSS annual congress 2016

Morgan PT, Banks AR, Fulford J, Vanhatalo A, Bailey SJ and Jones AM. Neuromuscular and muscle metabolic basis of contralateral lower limb fatigue. ECSS annual congress 2018.

CHAPTER 1
INTRODUCTION

The development of fatigue during exercise (i.e., exercise-induced fatigue) is a complex phenomenon which arises from impairments in multiple physiological systems and compromises exercise performance. Exercise-induced fatigue can be defined as a condition in which there is a loss in the capacity for developing force and/or velocity in response to a load, regardless of whether or not the task can be sustained and which is reversible by rest (Enoka & Duchateau, 2008; Gandevia, 2001; Macklem, 1990). The complexity of resolving the mechanisms that underpin exercise-induced fatigue is exemplified by the fact that fatigue development is influenced by various factors including, but not limited to, age, gender, exercise duration, intensity and mode of exercise as well as environmental conditions (Enoka & Duchateau, 2008). Moreover, research into the development and underpinning mechanisms of fatigue spans several disciplines, which often adopt different definitions of fatigue (Abbiss & Laursen, 2005 *for review*). Although this multidisciplinary approach has been valuable in helping to generate multi-organ, integrative models of the putative mechanisms of exercise-induced fatigue (e.g., Amann & Calbet, 2008; Lambert et al. 2005; Noakes, 2005; St. Clair Gibson et al. 2018), there is currently no consensus on the causes of exercise-induced fatigue and no universal agreement on one model that fully explains its development.

Controversy surrounding the mechanisms that underpin exercise-induced fatigue can also be attributed to variations in the testing procedures employed and the nomenclature adopted when assessing fatigue development. Indeed, the terms ‘fatigue’, ‘exhaustion’ and ‘task failure’ are often used interchangeably, despite describing different concepts and perhaps being underpinned by different mechanisms. Accordingly, research conducted within the field of exercise-induced fatigue should be interpreted with consideration of such inconsistencies. Moreover, the task-, intensity- and condition-dependence of exercise-induced fatigue further complicates the advancement of understanding. Therefore, for clarity and precision, it is important to define fatigue, exhaustion and task failure, as well as performance, at the outset of this

document so that the implications of, and mechanistic insights from, this work can be placed in the correct context. It is also pertinent to note that 'exercise-induced fatigue' and 'neuromuscular fatigue' will be used interchangeably throughout this thesis.

Exhaustion (or task failure) refers to the point where skeletal muscle force (power or velocity) production drops below the force required to continue the exercise task. Time to exhaustion or task failure (T_{lim}) occurs due to a failure of the central nervous system (CNS) to activate the skeletal muscle and/or of the skeletal muscle to generate the required force to continue the exercise task despite sufficient central motor output (CMO). Moreover, exhaustion/task failure might occur due to task disengagement, where the participant voluntarily ceases the exercise task despite having the *potential* to continue. To place these different concepts into context, if an individual was instructed to run at a set velocity until they were no longer able to run at this velocity and if regular measurements of the maximum capability of the lower limb muscles to produce force were performed, a decline in maximum force would be manifest due to a combination of central and peripheral factors (e.g., fatigue, *discussed below*) before exhaustion/task failure or task disengagement occurred (e.g., T_{lim}, figure 1.1). Accordingly, exercise-induced fatigue, exhaustion/task failure and task disengagement, and the likely underpinning mechanisms, can be dissociated. Performance, on the other hand, refers to the accomplishment of a given task measured, typically, against pre-set known standards of time or distance. As such, tests to assess performance are often characterised by 'closed-loop' tests. Closed-loop tests are defined by the presence of a known endpoint (e.g., time or distance) such as a time-trial (TT) whereby individuals are able to regulate exercise intensity (e.g., self-pace). By direct contrast, open-loop tests are defined by the *absence* of a known endpoint such as a time-to-exhaustion test whereby individuals are required to maintain the required intensity for as long as possible before exhaustion/task failure or task disengagement occurs. Ultimately, the mechanisms that govern task failure (i.e., during open-loop tests) and work rare regulation (i.e., during self-paced closed-loop exercise) should be considered as different constructs. Whilst providing some use and benefit to improving understanding of exercise-induced fatigue, the difficulty with

assessing constant work rate (or open-loop) tests in the context of performance is that the mechanisms responsible for the final cessation of effort will be distinctly different from those responsible for the manipulation of work output during 'self-paced' exercise.

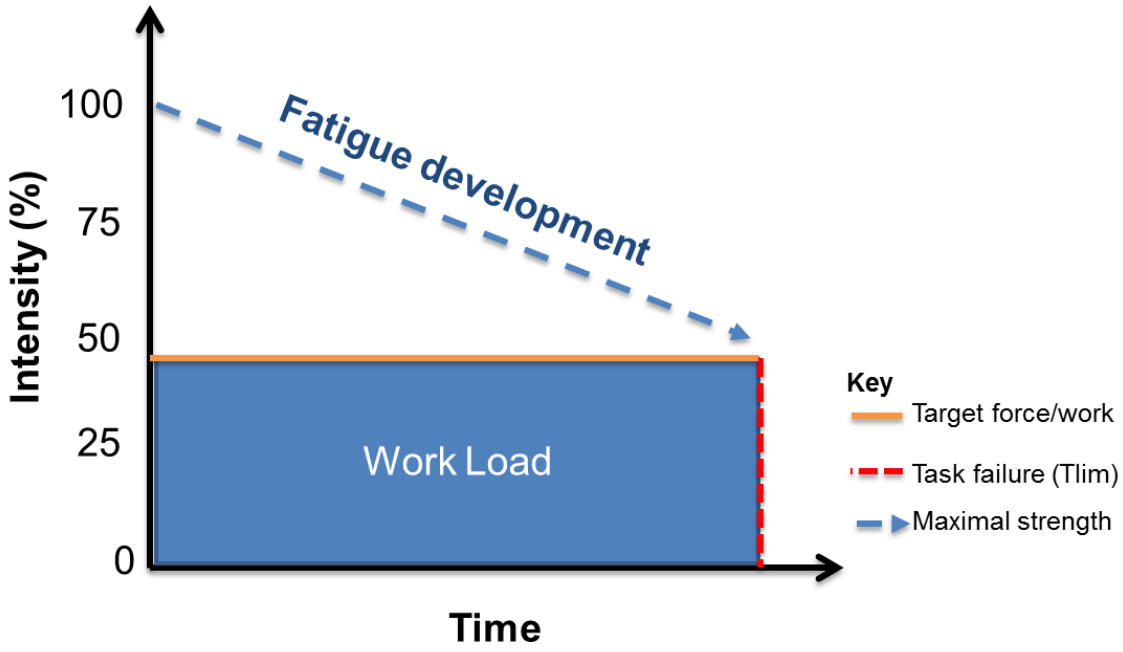

Figure 1.1. *Schematic illustrating the differences between exercise-induced fatigue and exhaustion. The y-axis represents a given intensity of exercise (% of maximum voluntary contractile force). Adapted from Millet (2016).*

Exercise-induced fatigue has been broadly categorised into two forms: peripheral fatigue, which occurs within the skeletal muscle, and central fatigue which is linked to the CNS. Historically, attainment of T_{lim} and the development of exercise-induced fatigue has often been attributed to predominantly one contributing mechanism such as perceptual, neurophysiological, cardiovascular or intra-myocyte bioenergetics and contractility. However, more recent evidence suggests that it is likely the sum and/or interaction of changes taking place throughout the body's physiological and psychological systems which underpins the cessation of effort and exercise-induced fatigue (i.e., Barry & Enoka, 2007; Enoka & Duchateau, 2008; Lambert et al. 2005; St Clair Gibson & Noakes, 2004; St. Clair Gibson et al. 2018).

Chapter 1: Introduction

For many years, work conducted by the pioneering exercise physiologist, A.V. Hill, was accepted to account for the mechanisms that underpinned exercise-induced fatigue (Hill & Lupton, 1923). The series of experiments conducted by Hill reasoned that fatigue develops due to the oxygen requirements of the contracting muscles exceeding the capacity of the heart to supply oxygen (figure 1.2). Subsequently, the development of an 'an anaerobic state' within the working muscles and the resulting accumulation of lactic acid was argued to provoke peripheral failure and thus task cessation. From this seminal work, it was evident that exercise-induced fatigue had both central cardiovascular and peripheral components and that both contributed, to an extent, to fatigue development. Although this work laid the foundations for research to pursue the underling mechanisms of exercise-induced fatigue, recent research challenges some of the ideas championed in this model. In the years that followed, and on the basis of Hill's postulate (Hill & Lupton, 1923) that metabolic changes within the contracting skeletal muscle were integral to exercise-induced fatigue, the majority of research investigating exercise-induced fatigue focused on peripheral components, without considering the potential contribution of central nervous system (CNS) limitations.

Many disparate models of exercise-induced fatigue have since been developed and championed to explain how the integration of central and peripheral components conflate to dictate muscle fatigue development during exercise. For example, large parts of A.V Hill's model have since been dismissed in the 'the central governor model' (Noakes et al. 2001; 2005, Noakes, 2000; 2007; 2012; St Clair Gibson et al. 2001; Ulmer, 1996) which proposes that efferent discharge from the central nervous system in response to metabolically triggered afferent feedback in the periphery, regulates exercise intensity in an anticipatory manner to prevent '*catastrophic'* failure to homeostasis. Numerous other models have since been proposed including, but not limited to, the 'psychobiological' model of fatigue (Marcora et al. 2009), the 'Critical Peripheral Fatigue Threshold' and/or a 'sensory tolerance limit' model (Amann & Calbet, 2008; Hureau et al. 2018) and, more recently, the 'Integrative Governor theory' (St Clair Gibson et al. 2018). To further complicate the matter, such models are not consistently *labelled* within the literature. A number of comprehensive

reviews on fatigue and its candidate mechanisms, however, have been written (e.g., Allen et al. 2008; Amann, 2011; Ament & Verkerke, 2009; Bigland-Ritchie, 1984; Boone et al. 2013; Enoka & Duchateau, 2008; Enoka & Stuart, 1992; Enoka et al. 2011; Ferreira & Reid, 2008; Fitts, 2008; Gandevia, 2001; Gibson & Edwards, 1985; Kent-Braun et al. 2012; Kirkendall, 1990; Morales-Alamo et al. 2015; Sahlin, 1992; Taylor et al. 2016; Thomas et al. 2018; Van Cutsem et al. 2017; Weir et al. 2006) and an overview of some of the literature harboured in support of these models of exercise-induced fatigue is provided in the review of literature (Chapter 2). Given that muscle contraction starts at the brain it is, perhaps, unsurprising that more recent attempts to explain fatigue have incorporated the brain in their model as oppose to previous 'brainless' models to explain endurance performance, for example (i.e., Joyner & Coyle, 2008). Indeed, it is generally accepted that peripheral muscle requires the CNS to function and the CNS requires afferent information from peripheral muscle to regulate work output. Ultimately, one (i.e., peripheral machinery) needs the other (i.e., CNS) to 'function' and to 'function' is to 'fatigue' appropriately, in order to conserve cellular integrity (see Chapter 2: Literature review).

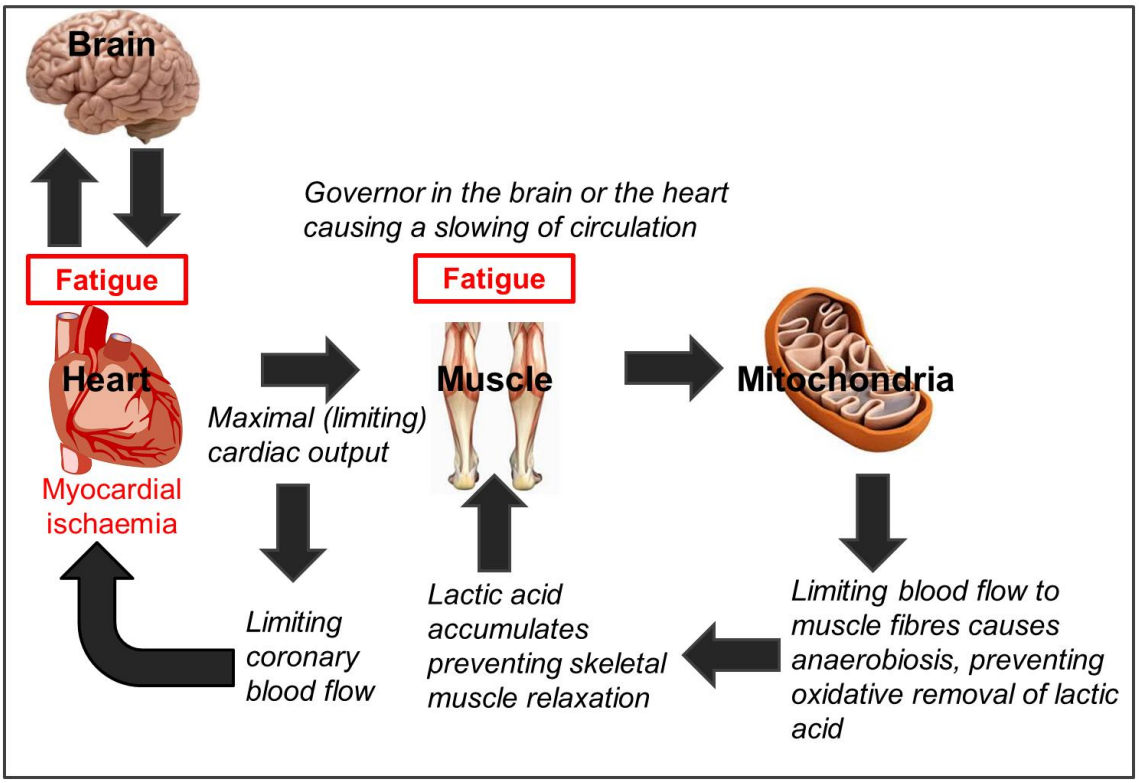

Figure 1.2. *Schematic of A.V Hill's theoretical model of exercise performance (A.V Hill, 1923). Schematic adapted from Noakes (2012).*

Whilst it is well established that factors such as maximal oxygen uptake ($\dot{V}O_{2peak}$) and the lactate threshold are important determinants of endurance performance (Joyner & Coyle, 2008), work rate regulation is ultimately *controlled* by the brain (Ulmer, 1996). Consequently, measures of sensation during exercise have been suggested to play an important role (i.e., Marcora et al. 2009; Mauger, 2014; St Clair Gibson et al. 2003; Tucker, 2009). Specifically, another factor that might contribute to exercise-induced fatigue development but has received comparatively limited research attention in existing models, is pain sensation (Foster et al. 2014; Mauger et al. 2010). This is despite emerging evidence linking pain sensation (and the manipulation of pain) to fatigue and/or performance (e.g., Astokorki & Mauger, 2017; Foster et al. 2014; Mauger et al. 2010). However, further research is needed to assess whether exercise-induced pain can be included in models of fatigue and in which exercise contexts pain sensation has a greater (or a lesser) effect on fatigue development. Over-the-counter analgesics, such as acetaminophen (ACT) and the non-steroidal anti-inflammatory drug (NSAID), ibuprofen (IBP), are widely consumed by athletes to reduce pain sensation and inflammation. Both ACT

and IBP act primarily by inhibiting cyclooxygenase (COX) activity and thus prevent the synthesis of prostaglandins (PGs) following the release of arachidonic acid. PGs are known to sensitise group III/IV muscle afferents and thus are implicated in exercise-induced pain sensation (Hayes et al. 2006; Rueff et al. 1993; Schaible et al. 2011). There is emerging evidence to support the acute use of ACT in enhancing endurance performance, possibly by reducing pain sensation associated with intense exercise (Foster et al. 2014; Mauger et al. 2010; Mauger et al. 2014). In addition, ACT may favourably alter neuromuscular function to optimise performance by attenuating components of central fatigue development (Mauger, 2013; Mauger & Hopker, 2013). However, despite the prevalence of consumption of ACT and IBP amongst athletes, observations of an ergogenic effect of these drugs is limited. Therefore, the purpose of this thesis is to assess the efficacy of ACT and IBP to mitigate exercise-induced fatigue development and the mechanisms by which this potential ergogenic effect might be elicited in a range of exercise tasks.

CHAPTER 2
LITERATURE REVIEW

Exercise-induced fatigue is fundamentally important to the fields of sport and exercise physiology and exercise prescription and rehabilitation. Therefore, understanding the mechanisms of exercise-induced fatigue development has potential application in improving exercise performance in athletes and improving adherence to exercise training/rehabilitation programmes to improve health and wellbeing in the general public and patient populations. Moreover, from a basic science standpoint, resolving the mechanistic bases of fatigue is central to advancing our understanding of physiological control processes.

The pioneering work of A.V Hill (Hill & Lupton, 1923) laid the foundations for understanding some of the mechanisms of fatigue, and suggested that fatigue develops due to the oxygen requirements of the exercising muscles exceeding the capacity of the heart to supply oxygen. Subsequently, seminal work including that of Bergstrom and colleagues (1967) demonstrated that, during prolonged heavy-intensity, constant-load exercise, fatigue occurred concomitantly with muscle glycogen depletion. This provided a novel and important advancement in understanding the mechanisms of exercise-induced fatigue. Since then, a plethora of other intra- and extra-muscular factors have been implicated in the development of exercise-induced fatigue (see Finsterer, 2012 *for brief review of the biomarkers of peripheral muscle fatigue during exercise*). Some of the components linked to peripheral fatigue include increased intramuscular metabolite accumulation, such as adenosine diphosphate (ADP) (e.g., McLester, 1997), inorganic phosphate (Pi) (e.g., Allen & Trajanovska, 2012; McLester, 1997; Westerblad et al. 2002), hydrogen ions (H^+) (e.g., Sahlin, 1986; Westerblad et al. 2002; Wilkie, 1986) and thiol oxidation and reactive oxygen species (e.g., Juel, 2006; Reid, 2016); a reduction in the intramuscular substrates, phosphocreatine (e.g., Allen & Westerblad, 2001; Hirvonen et al. 1992) and glycogen (e.g., Ørtenblad et al. 2013); impairments in muscle calcium (Ca^{2+}) regulation (e.g., Allen & Westerblad, 2001; Allen et al. 2002; 2007; 2008; Debold et al. 2016; Fitts, 2008), cross-bridge cycling (e.g., Fitts, 2007) and sodium-potassium pump function (e.g., Lindinger & Sjøgaard, 1991; McKenna, 1992; McKenna et al. 2007; 2008); as well as metabolic acidification (e.g., Fitts, 2016)

and alterations in thermoregulation (e.g., González-Alonso et al. 1999; Tucker, 2008; Westerblad et al. 1997), cardiovascular limitations, oxygen (O_2) availability and respiratory function (e.g., Amann & Calbet, 2007; Romer & Polkey, 2007), endocrine function and hormonal regulation (e.g., Clausen et al. 1996; Cordeiro et al. 2017; Meeusen & De Meirleir, 1995; Meeusen et al. 2003). Additionally, regulation of excitability of the sarcolemma via interplay between muscle specific CLC-1 chloride channel regulation and extracellular potassium (K^+) accumulation have been implicated in regulating muscle function (e.g., Bækgaard Nielsen et al. 2017).

In addition, more recently, centrally-mediated mechanisms have also been linked to the fatigue process including impaired motor unit firing (e.g., Gandevia, 2001; Woods et al. 1987), decreased motoneuron excitability (e.g., Johnson et al. 2004; Taylor & Gandevia, 2008), reduced motor cortical drive and excitability (e.g., Klass et al. 2008; Macefield et al. 2001; Sogaard et al. 2006; Taylor & Gandevia, 2008), cerebral blood flow (e.g., Secher et al. 2007) as well as a number of other supraspinal (cortical) and spinal mechanisms which are influenced by intrinsic changes within the CNS, including, for example, increases in the magnitude of inhibitory feedback from muscle afferents (Taylor et al. 2016 *for review*). This series of complex, multi-organ processes have recently been suggested to operate in an integrated and coordinated manner to regulate exercise-induced fatigue development (e.g., Amann & Calbet, 2008; Lambert et al. 2005; Noakes, 2005). However, a criticism of models such as the central governor (CGM) is its lack of ability to consider the complex interplay between inhibitory and facilitatory physiological signals with inhibitory and facilitatory psychological drives to regulate work output (St. Clair Gibson et al. 2018). Indeed, exercise-induced fatigue should be considered to have both sensory/emotional and physiological (central and peripheral) components and not simply exclusively one or the other. In addition, the complex and individually-dependent nature of exercise-induced fatigue does not lend itself well to be explained by a simplistic model across a number of different scenarios. Figure 2.1 provides a brief summary of the complex chain of events involved in voluntary force production and thus the complexities inherent in investigating exercise-induced fatigue.

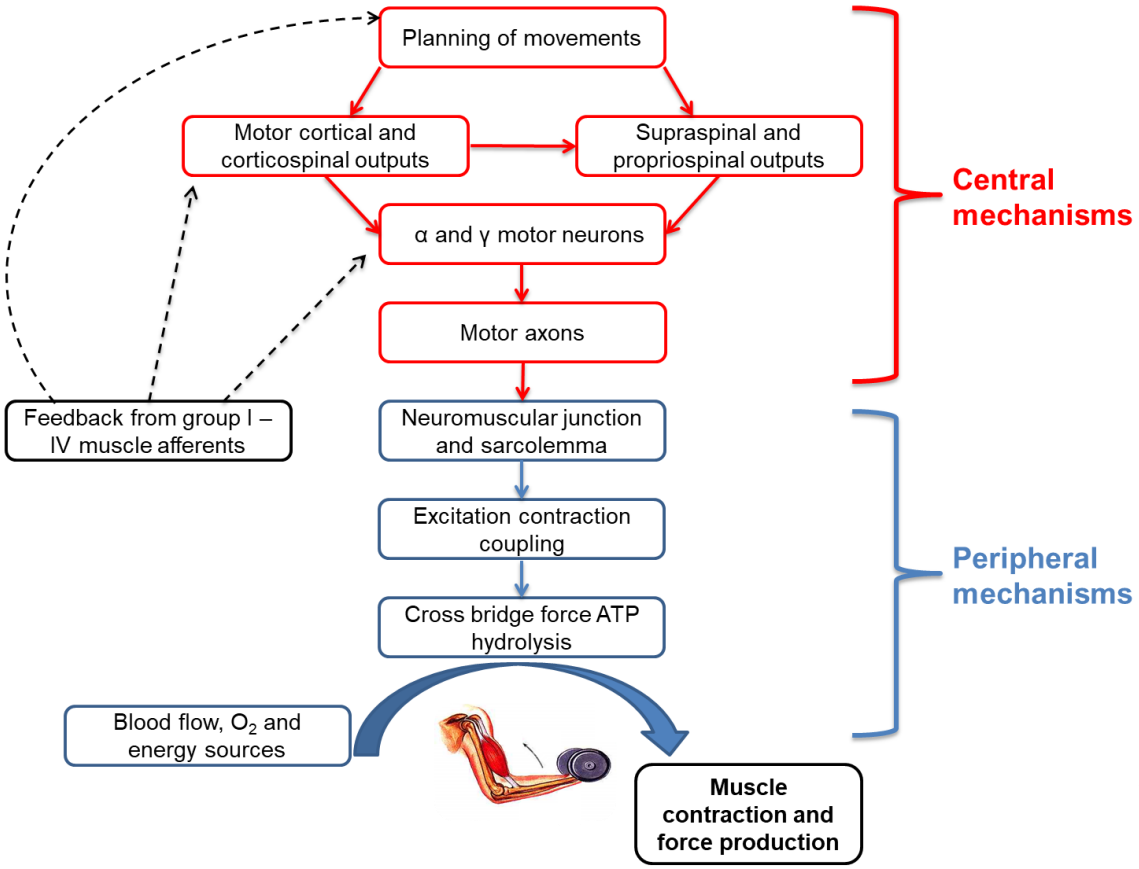

Figure 2.1. *The complex chain of events involved in voluntary force production. Adapted from Gandevia (2001).*

This chapter will: 1) outline the methods used to assess exercise-induced fatigue development and its underpinning mechanisms; 2) review the mechanisms and task-dependence of exercise-induced fatigue; and 3) provide a summary of recent research linking exercise-induced pain sensation in the aetiology of exercise-induced fatigue and areas for further research to enhance understanding in this emerging topic within the field of exercise-induced fatigue. Finally, a brief review of some of the literature surrounding the effect of non-specific COX-inhibitors on attenuating fatigue development is provided as an evidenced-based rationale for the experimental chapters that comprise this PhD thesis.

2.1 Measuring exercise-induced fatigue

To improve understanding of exercise-induced fatigue, a number of tools and methodologies have been used to isolate specific sites along the motor pathway

that contribute to the reduction in force production that accompanies exercise of a sufficient intensity and/or duration (Cairns et al. 2005; Vollestad, 1997 *for review*). Figure 2.2 provides a brief summary of the methodological apparatus that can be utilised at different anatomical locations throughout the motor pathway to investigate central and peripheral mechanisms of exercise-induced fatigue.

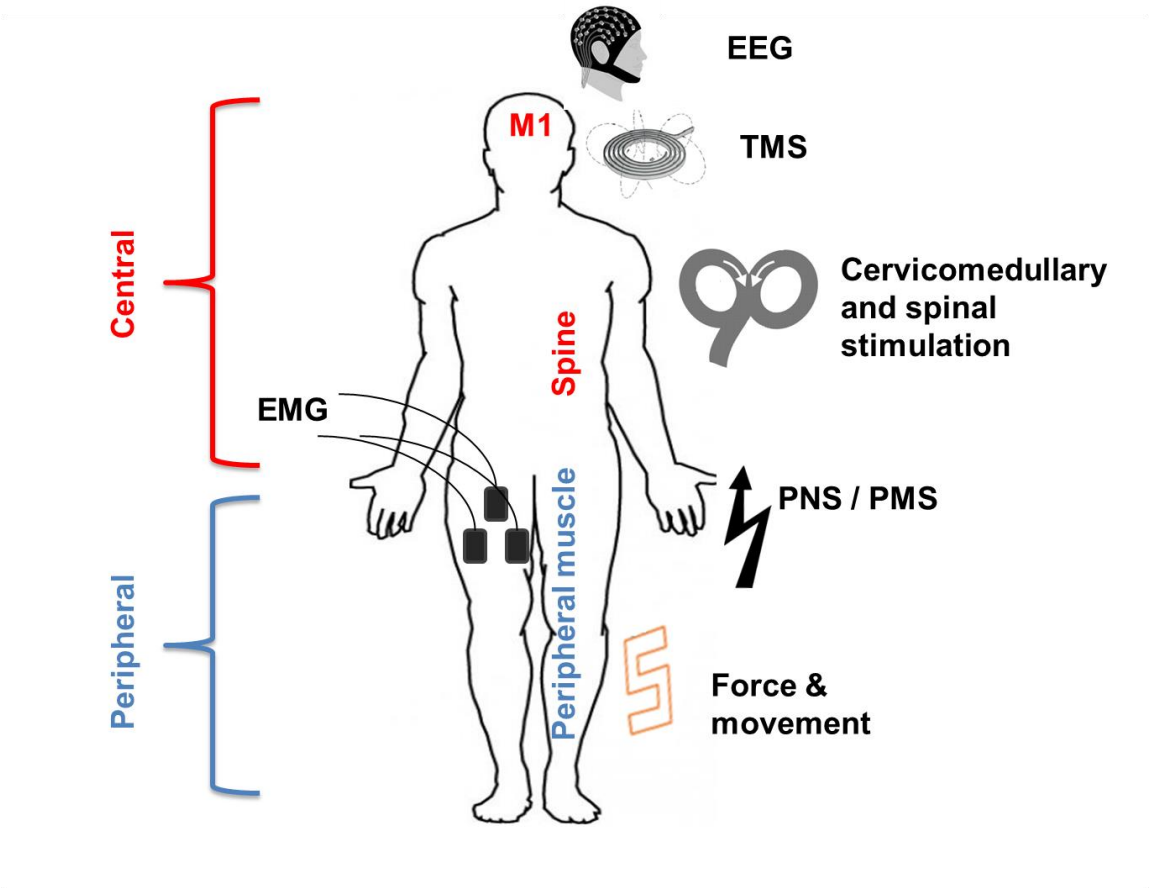

Figure 2.2. *The development of exercise-induced fatigue involves numerous complex mechanisms. Assessment of muscle fatigue can be segregated into peripheral and central components by motor nerve and muscle stimulation techniques. Supraspinal fatigue, a subset of central fatigue, can be assessed by motor cortex stimulation. Briefly, peripheral fatigue is assessed via changes to twitch force, excitability (i.e., M-wave) and contractility characteristics following supramaximal peripheral stimulation of a nerve or muscle. Whilst central fatigue can be assessed indirectly using similar techniques, stimulation of the spinal tract and/or the motor cortex allows for a more direct approach to assess changes in cortical voluntary activation (i.e., neural drive) and spinal and supraspinal excitability. Electromyography (EMG) is a technique used to record myoelectric signals formed by physiological variations in the muscle fibre membranes and*

can be used to infer changes to peripheral and central fatigue. More specifically, EMG can be used to assess changes in muscle activation as well as motor unit recruitment and firing frequency. EEG; electroencephalogram, TMS; transcranial magnetic stimulation, PNS; peripheral nerve stimulation, PMS; peripheral muscle stimulation, M1; primary motor cortex. Adapted from Goodall et al. (2010).

2.1.1 Methods to assess neuromuscular fatigue

The most common method of assessing the neuromuscular bases of exercise-induced fatigue is via supramaximal stimulation of a peripheral nerve innervating the contracting musculature (or the muscle directly) by means of either electrical or magnetic methods to monitor changes in force production (e.g., potentiated; pTw and superimposed twitches; sTw *described below*), excitability parameters (e.g., M-wave response) and contractility characteristics (e.g., rates of force development and relaxation). Electromyography (EMG) can be used in combination with evoked stimulation techniques to assess muscle excitability and without evoked stimulation techniques to assess muscle activation as well as motor unit recruitment and firing frequency. These 'pulses' of stimulation can be administered as a single pulse (e.g., singlet), paired pulse (e.g., doublet) or continuous pulse (e.g., tetanus) and can be administered at different locations throughout the motor pathway to assess relative contributions of central (spinal and/or supraspinal) and peripheral fatigue mechanisms. In addition, these pulses can be manipulated (e.g., output/current and duration) to investigate a range of different fatigue mechanisms (i.e., high and low-frequency fatigue).

The name given to the electrical signal evoked in the muscle fibres as a result of peripheral nerve stimulation is a compound muscle action potential or 'M-wave', whereas, a motor evoked potential or 'MEP' refers to the signal evoked from cortical stimulation. A 'CMEP' refers to a cervicomedullary motor-evoked potential following stimulation at the cervicomedullary junction (Gruet et al. 2013; Inghilleri et al. 1993; Taylor, 2004; 2006). The amplitude and/or area of the waveform are measured to characterise the M-wave (e.g., peripheral membrane excitability, figure 2.3), CMEP (e.g., spinal excitability) and/or MEP (e.g., supraspinal excitability). Therefore, decrements in these excitability parameters provide insight into peripheral and central fatigue development. Whilst changes to M-wave amplitude have been reported during fatiguing contractions (e.g.,

Lepers et al. 2002), the maintenance of M-wave amplitude suggests no or little change to membrane/motoneuron excitability. In this instance, the site of failure likely originates proximal to the sarcolemma. On the other hand, MEPs can be used to examine the ability of the motor cortex to activate skeletal muscles e.g., changes in corticospinal excitability (Taylor & Gandevia, 2001).

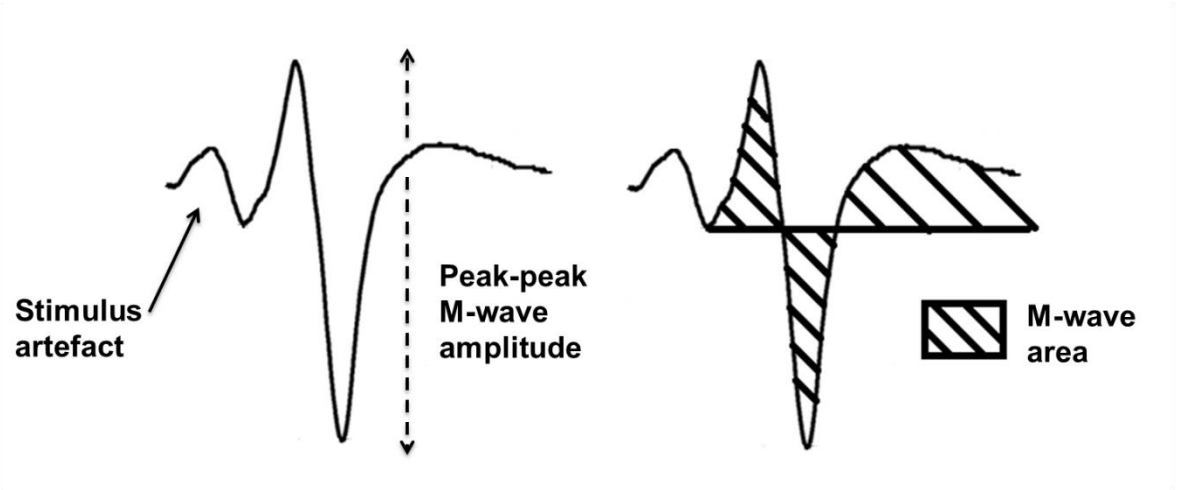

Figure 2.3. *Compound muscle action potential (e.g., M-wave) evident within an EMG trace following supramaximal peripheral nerve stimulation. The M-wave amplitude is calculated via the peak-to-peak trace of the electrical potential (dotted arrow), whereas the M-wave area is calculated as the total integral of the curve (both positive and negative deflections).*

As well as monitoring changes in the M-wave response, peripheral fatigue can also be inferred via a reduction in the resting muscle twitch (e.g., pTw) following supramaximal peripheral nerve stimulation (Alway et al. 1987; Kufel et al. 2002). Alternatively, central fatigue can be identified via a decrease in voluntary activation (VA) using, for example, the interpolated twitch method (Merton, 1954) which is revealed as an increase in the twitch superimposed (e.g., sTw) onto a maximal voluntary contraction (MVC). The sTw typically increases during intense prolonged exercise, reflecting impaired VA (e.g., the participants' inability to recruit sufficient muscle fibres, see below, figure 2.4). However, this method has received some criticism including providing a lack of sensitivity in the measurement of motoneuronal excitation at near-maximal forces (e.g., Herbert & Gandevia, 1999). A more direct approach of assessing the contribution from spinal and supraspinal structures is with the use of transcranial (TMS) and cervicomedullary magnetic stimulation. These techniques have been used to

further investigate the independent contributions of supraspinal and spinal fatigue (e.g., Gruet et al. 2013; Inghilleri et al. 1993; Taylor, 2004; 2006).

For these methods, the subject is required to perform a maximal voluntary contraction (e.g., MVC). When the force produced by the subject reaches its peak, the investigator superimposes a supramaximal stimulation of the peripheral muscle (*either by stimulating the muscle directly or the main nerve innervating the contracting musculature*) and quantifies the change in force (figure 2.4). When a sTw is observed, this provides evidence that motor drive (conscious and/or sub-conscious) was submaximal as the electrical or magnetic pulse was able to evoke additional force beyond that voluntarily produced (Merton, 1954). A 'twitch' is then produced again after the MVC with the same pulse to provide the investigator with a 'reference value' (e.g., pTw), which is used to infer peripheral fatigue development and peripheral muscle function (figure 2.4). However, contrary to peripheral stimulation methods, following a TMS evoked contraction of the target muscle group and production of a MEP, there is a silent period evident in the EMG trace. This period of EMG 'inactivity' is also referred to as the cortical silent period (CSP) and has been suggested to represent increased cortical inhibition (Inghilleri et al. 1993; Taylor et al. 1996; Taylor & Gandevia, 2001).

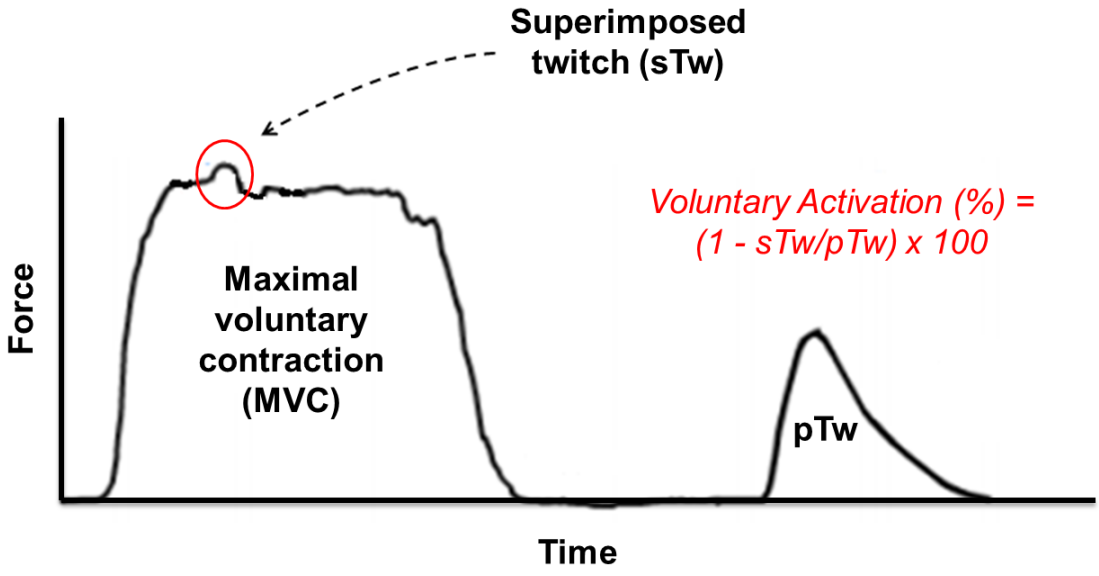

Figure 2.4. *Illustration of superimposed (sTw) and potentiated (resting, pTw) twitches, produced during and following a maximal voluntary contraction (MVC) for the determination of voluntary activation and peripheral function (edited from Merton, 1954).*

2.1.2 Contraction characteristics

For each pTw, contraction time, maximum rate of force development and one-half relaxation time ($RT_{0.5}$) can be obtained through analysis of the force trace. Changes to such characteristics are thought to represent alterations to Ca^{2+} release and uptake (Eleveld et al. 2004; Frank, 1982; Klitgaard & Clausen, 1989). In addition, a reduced rate of force development (or 'MRFD') is considered to reflect a decrease in the rate of cross-bridge formation (Fitts, 2008; Maffiuletti et al. 2016; Stein & Parmiggiani, 1981). Reductions in maximal relaxation rate and a prolonged $RT_{0.5}$, however, are thought to reflect decreases in the maximal rate of weak-to-strong cross-bridge formation and decreases in the maximal rate of cross-bridge detachment, respectively (Westerblad, Bruton, & Lannergren, 1997; Westerblad, Lannergren, & Allen, 1997).

2.1.3 Electromyography (EMG)

EMG is a technique used in neuromuscular physiology which records myoelectric signals formed by physiological variations in the muscle fibre membranes and can be measured via surface electrodes placed on the skin superior to the muscle of interest, and more directly, via needles inserted into the muscle of interest (Enoka & Duchateau, 2008). For the purpose of this thesis, EMG amplitudes were rectified, since electrical activity appears positive as depolarization approaches the electrode and negative as it passes the recording electrode (which would cancel each other out). Reduced EMG amplitude during maximal contractions suggests reduced muscle activation, reduced motor unit recruitment and firing frequency and/or a change in muscle fibre-type recruitment (Sogaard et al. 2006). However, excitability of the sarcolemma can also be altered with fatigue (e.g., Lepers et al. 2002) and thus, the reduced EMG amplitude during maximal contractions may also be explained by changes in the maximal M-wave amplitude (Millet & Lepers, 2004). Thus, EMG can be normalised to the M-wave amplitude in order to exclude any changes to the trace due to changes in local membrane excitability. Although EMG has its limitations such as its inherent variability and a 'complex relationship with fatigue (see below) (Amann & Calbet, 2008; Enoka & Stuart, 1992; Martinez-Valdes et al. 2018; Taylor & Gandevia, 2008), it can also be used as a valid measure to estimate the development of both central and peripheral fatigue during exercise such that, for example, central fatigue from impaired alpha motor cord neuron firing will manifest as a reduced mean spectral

frequency (Bigland-Ritchie et al. 1978; 1981; 1983; 1986; Edwards, 1981; Enoka & Stuart, 1992; Gandevia, 2001; Stephens & Taylor, 1972). Reduced amplitude of EMG signals at task failure indicates either loss of recruitment or synergistic activation of multiple muscles (Gandevia et al. 1995; Taylor & Gandevia, 2008). However, surface EMG has recently been criticised in its use to indirectly infer changes to neural drive (Martinez-Valdes et al. 2018).

The electromyographic response to exercise shares a highly complex relationship with fatigue. For example, during sustained maximal exercise, EMG declines as a function of time concomitant with decrements in force (e.g., Kent-Braun, 1999). However, during sustained submaximal exercise, EMG typically increases until exercise is terminated as, at the onset of exercise, sub-maximal muscle activity does not induce maximal motor unit recruitment (Dimitrova & Dimitrov, 2003; Enoka & Stuart, 1992; Taylor & Gandevia, 2008). This is markedly different compared to maximal contractions where EMG activity (due to high firing rates) is at its highest at the start of exercise and continues to decline as a function of time (e.g., Burnley, 2009; St Clair Gibson et al. 2001). However, during repeated submaximal contractions there is also a large inter-individual variability in the EMG response. As the relationship between EMG and fatigue can change dependent on the type of task being completed (i.e., an increase or decrease in the EMG trace doesn't necessarily infer fatigue development), this does not necessarily mean that EMG is always a robust indicator of muscle fatigue (Enoka & Stuart, 1992; Taylor & Gandevia, 2008). In addition, the use of EMG during locomotion, particularly within field-based research, adds further complexity. For example, during cycling exercise, the terrain can have a direct impact on muscle activation at a given speed (i.e., Wang et al. 2013). In this instance, during self-paced exercise and independent of fatigue development, an individual may consciously decide to reduce muscle activation despite the lack of observation of significant central, peripheral and/or mental fatigue development. As a consequence, care should be taken when interpreting changes in the EMG signal during an exercise task.

2.1.4 Voluntary activation (VA)

VA is defined as '*the level of neural drive to a muscle during exercise*' (Merton, 1954) and reflects the ability to fully activate the target muscle group or the ability

of the CNS to maximally drive a muscle during voluntary contraction. In a fatigued state, a larger twitch is evoked during an MVC (e.g., sTw) and VA is incomplete; that is, some motor units are not recruited or not firing fast enough to generate fused contractions (Gandevia et al. 1998; Todd et al. 2003). For the purpose of the investigations that comprise this thesis, VA was determined using the following equation:

$$VA\ (\%) = (1 - sTw/pTw) \times 100$$

Where pTw relates to the potentiated twitch produced by the quadriceps (i.e., for knee extension exercise). The presence of a sTw produced by TMS during the peak force production of an MVC suggests that the drive from the motor cortex is sub-optimal. Importantly, this conclusion cannot be made specific to the motor cortex from peripheral methods as the impairment may be located anywhere 'upstream' from the peripheral nerve (Gandevia et al. 1998; Todd et al. 2003).

Most healthy individuals can activate quadriceps muscle to near maximum (~95%; Kent-Braun, 1999). As the voluntary drive varies at different muscle groups, the degree of central fatigue will depend on the muscle tested. For example, maximal VA of the diaphragm is less than the biceps brachii as twitch force during maximum contraction has been shown to be relatively greater with the diaphragm than with the biceps (Gandevia et al. 1995). VA of a single muscle is also reduced when multiple muscles are simultaneously contracted (Howard & Enoka, 1991). VA is also reduced with pain, likely due to feedback inhibition to spinal alpha motor neurons via spinal oligosynaptic pathways (Gandevia, 2001; Martin et al. 2006; Taylor et al. 2000; Woods et al. 1987; Amann et al. 2008; Amann & Dempsey, 2008). In addition, whilst physical training has been shown to increase VA, immobilization is known to reduce it (Duchateau & Hainaut, 1987).

2.2 Multi-faceted, task-dependent nature of fatigue

The aetiology of exercise-induced fatigue is recognised as being multi-factorial such that it can be caused by a number of different mechanisms that result in an acute reduction in the ability to perform an exercise task. However, exercise-induced fatigue also appears to depend on the task characteristics, with variations in contraction intensity (or duration), speed of movement, or type of

muscle contraction influencing the relative contributions of central (spinal and supraspinal) and peripheral fatigue development (Enoka, 1995 for review). Specifically, the muscle mass engaged (single-limb vs. locomotor exercise; Amann et al. 2011; 2013, single vs. double limb exercise; Rossman et al. 2012; 2014), nature of the task (e.g., intermittent vs sustained; Bigland-Ritchie et al. 1986; Duchateau et al. 2002), duration and intensity (Thomas et al. 2014; 2016) of the task being performed (e.g., short vs prolonged; Bigland-Ritchie et al. 1986; Ross et al. 2010; Schillings et al. 2003; Smith et al. 2007; Sogaard et al. 2006) and even the target muscle group (e.g., elbow extensors vs knee extensors; Kawabata et al. 2000) or action (e.g., eccentric vs isometric; flexors vs extensors; (Smith & Newham, 2007) influence the bases of exercise-induced fatigue. This task-dependence of fatigue development makes resolving the mechanisms of exercise-induced fatigue highly complex. Therefore, caution should be applied when extrapolating findings on the mechanisms of exercise-induced fatigue to other exercise tasks. Moreover, the task-dependent manner of exercise-induced fatigue complicates the pursuit of a unifying model of exercise fatigue.

2.2.1 Peripheral and central fatigue mechanisms

Peripheral fatigue is associated with an attenuation of muscle force production caused by processes distal to the neuromuscular junction (Ament & Verkerke, 2009; Gandevia, 2001). As such, peripheral fatigue develops *outside* of the CNS consequent to changes in energy supply and/or metabolite accumulation and is manifest as impaired muscle membrane excitability, contractility and/or impaired neurotransmission. In peripheral skeletal muscle fatigue there are a number of sites where repeated contractions may cause impairment, either at the neuromuscular junction, muscle membrane, sarcoplasmic reticulum or within the sarcomeres (the proteins actin and myosin) of the working muscle. A brief summary of some of the main proposed mechanisms of peripheral fatigue is presented below:

- **Excitation-contraction uncoupling:** Impaired Ca^{2+} release and active reuptake from sarcoplasmic reticulum, SR (e.g., excitation contraction uncoupling), as well as impaired interactions between myosin and actin during cross-bridge cycling and calcium sensitivity (e.g., lower force for a given intramyocyte [Ca^{2+}]).

- **Sodium (Na^+) & potassium (K^+) pump activity**: Loss of electrical conduction from muscle membrane to t-tubule system.
- **Metabolite accumulation:** accumulation of intramuscular lactic acid and H^+ causes a reduction in the pH of the cell, while the accumulation of inorganic phosphate (Pi) and adenosine diphosphate (ADP) have also been implicated in peripheral fatigue development.
- Impaired **adenosine triphosphate (ATP)** turnover via **glycogen** and/or **phosphocreatine (PCr)** depletion**.**

Perhaps the most frequently contested discussion surrounding peripheral fatigue development and its contribution to T_{lim} and work output regulation, is the lack of observation of absolute depleted levels of substrates (i.e., whole muscle PCr, glycogen). In addition, when methods to manipulate central fatigue development are introduced (i.e., fentanyl administration, *discussed below*), further development of peripheral fatigue is observed, suggesting that peripheral fatigue (or more specifically, metabolite accumulation and/or substrate depletion) may be regulated by the CNS in order to prevent complete failure of the peripheral machinery. However, it is important to consider that localisation of [PCr] (i.e., Cannon et al. 2013; Sahlin et al. 2017) and glycogen (i.e., Nielsen et al. 2011; 2012; 2014) depletion have been observed which can have profound effects on force production. In addition, it is pertinent to note that in the example of reduced glycogen levels, the rate of fat oxidation cannot meet the required ATP demand alone that is associated with high-intensity exercise and thus work output must reduce to allow exercise to continue (Baker et al. 2010). Although beyond the scope of this thesis, it is also worthy to note that factors of central (i.e., neural drive) and mental fatigue (i.e., effort perception, *both discussed below*), are also rarely observed at their 'true' zero/maximal, providing further support that a number of mechanisms likely coordinate to bring about fatigue development. For example, voluntary activation (a measure of neural drive) and RPE using the 6-20 Borg scale (a measure of perceived effort) in healthy individuals are rarely observed <60% and at 20, respectively, during exhaustive exercise (i.e., Ross et al. 2010). A VA of >0% and RPE of <20 would, therefore, suggest a 'reserve capacity' located proximal to the neuromuscular junction within the CNS and/or that factors associated with mental fatigue (i.e., motivation) are sub-maximal at task-end, respectively.

In contrast, central fatigue encompasses mechanisms that originate *within* the CNS and manifests as a reduction in VA or neural drive to the target muscle due to, for example, decreases in the number of recruited motor units and/or their discharge rate from the primary motor cortex, M1 (Gandevia, 2001; Verin et al. 2004). Such changes are likely due to alterations in cerebral metabolism and neurotransmitter responses (Gandevia, 2001; Verin et al. 2004 *for review*). As a consequence of central fatigue development, impairments upstream of the neuromuscular junction are observed such as alterations to spinal propagation, and corticospinal, intracortical and supracortical excitability changes (Gandevia, 2001). In contrast to research on peripheral fatigue mechanisms, limited research has been conducted on the role of the CNS in human fatigue and thus less in known of the central regulation of human performance. This is likely because most research historically supported the theory of peripheral fatigue and that, comparatively, there were many limitations (and still are) in the ability to measure components of central fatigue. This is often because of a lack of objective measurement tools available to measure central fatigue. However, the relatively recent introduction of TMS in underpinning a failure of central mechanisms during exercise now affords the researcher with the opportunity to explore exercise-induced central fatigue development with relative ease (e.g., Taylor et al. 2006; Smith et al. 2007; Goodall et al. 2009; Sidhu et al. 2009; Goodall et al. 2012). The main proposed mechanisms of central fatigue include:

- Loss of recruitment of high threshold motor units
- Reduced central drive from increased inhibitory interneuron input to M1
- Central conduction block from demyelination or motor neuron dropout
- Increased inhibitory feedback from group III/IV muscle afferents
- Loss of facilitatory feedback from muscle spindle group I sensory afferents
- Deficit in brain serotonin

The majority of research has demonstrated that peripheral fatigue mechanisms are the predominant contributors to fatigue development during the early stages of exercise (e.g., Bigland-Ritchie et al. 1983; 1986). Peripheral fatigue mechanisms also seem to dominate during higher-intensity exercise (e.g., Bigland-Ritchie et al. 1983; 1986; Thomas et al. 2014) as evidenced by a decreased pTw, decreased M-wave amplitude and changes to contraction characteristics such as an increase in relaxation rate (e.g., Bigland-Ritchie et al.

1986; Gandevia, 2001; Schillings et al. 2003; Smith et al. 2007; Sogaard et al. 2006). Centrally mediated mechanisms have also been identified in a range of exercise tasks via significant reductions in VA, decreased MEP amplitude and extended CSP length (Taylor et al. 1996) indicative of a reduction or a cease in firing rates of some motoneurons (Bigland-Ritchie et al. 1983; 1986). It appears that central fatigue mechanisms become increasingly important during extreme conditions (i.e., heat, hypoxia) and with increasing exercise duration where there is a concomitant decrease in exercise intensity (Froyd et al. 2016; Thomas et al. 2014; Taylor et al. 2016 *for review*). The relative proportion of central fatigue development is also known to increase under extreme environmental conditions (e.g., hypoxia, extreme heat/cold) such that spinal and supraspinal contributions accelerate and interact with peripheral muscle to restrict exercise capacity (e.g., Amann et al. 2006; Goodall et al. 2010; Meeusen & Roelands, 2010; Nybo, 2010).

Central fatigue can be further categorised into spinal and supraspinal fatigue. Supraspinal fatigue is defined as an exercise-induced decline in force caused by suboptimal output from the motor cortex demonstrated through a decreased descending drive or increased perceived exertion (or effort, RPE), whereas spinal fatigue relates to impairments within the spinal cord (Gandevia, 2001). From a technical perspective, central fatigue refers to any exercise-induced reduction in MVC force which is not accompanied by the same reduction in maximal evocable force (evoked by supramaximal stimulation of the target muscle group or nerve innervating the muscle group). For example, a decrease in VA of up to 10% has been observed after prolonged cycling (Lepers et al. 2002). After prolonged fatiguing muscle contractions, the amplitude of MEP in response to TMS has also been shown to be reduced, indicating a reduction in cortical excitability (Ross et al. 2007; Schillings et al. 2004). In addition, central fatigue has been identified during exercise tasks via impairments in the motor cortex (e.g., Gandevia et al. 1996; Sidhu et al. 2009; 2013), impaired reflex responses at the spinal cord (e.g., Garland & McComas, 1990; Klass et al. 2008) and alterations to intrinsic properties of motoneurons (e.g., McNeil et al. 2011a; 2011b). Central fatigue development has also been attributed to a reduced drive from motor neurons via reflex effects from muscle afferents. Specifically, stimulation of group III/IV afferent nerves (chemo- and nociceptive afferents) induces a decrease in motor neuron firing frequency and an inhibition of the motor cortical output (e.g., Amann

et al. 2013). Greater discussion on the role of group III/IV afferents in the development of central fatigue is provided in *section 2.3.* The M1 behaviour has been found to be altered in a complex manner during exercise as the excitability of cells within the cerebral cortex is known to increase as a result of physical activity (e.g., Wrightson et al. 2016). However, as fatigues ensues as a function of increased duration and intensity of exercise, the excitability of cortical cells decreases, leading to impaired exercise performance (e.g., Sharples et al. 2016).

Mental fatigue, which has been defined as a psychobiological state caused by prolonged periods of demanding cognitive activity and is characterised by subjective feelings of "tiredness" and "lack of energy" (Boksem & Tops, 2008), was once considered a strand of central fatigue. However, most research now describes mental fatigue as a 'sensation' and its contribution to central fatigue development is unclear. Specifically, whilst some studies have demonstrated a decrease in endurance performance and earlier attainment of task disengagement (i.e., T_{lim}) (e.g., Bray et al. 2008; Marcora et al. 2009; Pageaux et al. 2013), others have shown no effect (e.g., Martin et al. 2015; Pageaux et al. 2015), following a mentally fatiguing task. Nevertheless, it would seem that perceived exertion (i.e., Bayne et al. 2008; Crewe et al. 2008; Pires et al. 2011; Romer et al. 2007) and exercise-induced pain (discussed below) may have the potential to influence exercise performance irrespective of their underpinning mechanisms. For example, studies attempting to attenuate the negative effects of mental fatigue have shown improvements in T_{lim} and exercise performance by way of providing an external motivation to participants (i.e., self-talk, Blanchfield et al. 2014; monetary reward, Zedelius et al. 2014; competition, Corbett et al. 2012). In addition, some studies have also observed beneficial effects on performance using methods of deception (Mauger et al. 2009; Stone et al. 2012; Williamson et al. 2011). However, it is possible that such effects of mental fatigue and interventions aimed at reducing the perceived demands of the task may be limited in trained subjects (i.e., Corbett et al. 2012; Hulleman et al. 2007; Skorski et al. 2017). It is also possible that the effect of mental fatigue is specific to the task and the type of 'mentally fatiguing' intervention (Van Cutsem et al. 2017 *for review*). In addition, during exercise it is likely that mechanisms of mental fatigue and the psychological demands of exercise interact with multiple physiological systems to bring about the final cessation of effort and exercise-induced fatigue

development (Inzlicht, & Marcora, 2016; McCormick et al. 2015; Van Cutsem et al. 2017 *for review*).

There is also ongoing debate as to whether sensory input (i.e., group III/IV muscle afferents) contribute to the perception of effort (de Morree et al. 2012; Khan et al. 2011; Kjaer et al. 1989) and/or whether perception of effort can be disassociated from perceived exertion (Peñailillo et al. 2018) and the perception of exercise-induced pain (i.e., Pageaux, 2016). Instead, group III/IV muscle afferent feedback may simply act as a means to inform pain and numerous other sensations during exercise and thus not provide sensory signals for generating perception of effort (de Morree et al. 2012; Khan et al. 2011; Kjaer et al. 1989; Marcora, 2008). However, it is pertinent to note that the generation and interpretation of effort is likely multi-factorial (i.e., sensory and emotional) and that central command does not always increase linearly with the perception of effort during exercise (Abbiss et al. 2015; Nybo & Nielsen, 2001; Pageaux, 2016).

It has also been suggested that RPE may be set at the beginning of the exercise task in 'teleoanticipation' as part of a feedforward control mechanism (i.e., O_2 levels of ambient air, skin temperature, glycogen levels), comparing a 'template' of previous experiences as well as knowledge of distance/duration, the level of motivation and competition, to the instantaneous actual power (or velocity) and pacing profile to prevent a threat to homeostasis (i.e., Central Governor Model, Noakes & St. Clair Gibson, 2004; Crew et al. 2008; Tucker, 2009). In this case, the RPE will rise to reach maximum levels at exercise completion, irrespective of the exercise task (i.e., time to exhaustion vs. time-trial) (Noakes & St. Clair Gibson, 2004 *for review*; Tucker et al. 2004). In support of this notion, Swart et al. (2008) found that RPE was strongly correlated with the duration of exercise when participants had prior knowledge of the 'end-point'. Indeed, this model of fatigue is of interest for self-paced exercise as work output is typically reduced much before any whole muscle 'limiting environment' is attained (Noakes & St. Clair Gibson, 2004 *for review*; Tucker et al. 2004). However, originally thought to be solely a sub-conscious mechanism, it is now generally accepted that work output during self-paced exercise is likely regulated by a combination of conscious and sub-conscious mechanisms and that different regions of the brain as an 'integrative controller' acts as a complex system to regulate output (i.e.,

Crewe et al. 2008; St Clair Gibson et al. 2018). Interestingly, Crewe et al. (2008) found that the rate of rise in RPE accurately predicted T_{lim} almost from the onset of exercise. Ultimately, whilst beyond the scope of this thesis, there is evidence to suggest that many factors contribute to the sensation of effort (i.e., Marcora, 2008; Pageaux, 2016; Smirmaul, 2012; St Clair Gibson et al. 2003). Furthermore, given that corollary discharge represents the efferent output to increase activation of skeletal muscle (and work output), it is perhaps unsurprising of its association with RPE (Marcora, 2008). However, it is important to note that the CNS must receive information from periphery muscle in order to determine corollary discharge to inform the exercising human of when and to what extent to increase and/or decrease effort to drive skeletal muscle (i.e., Smirmaul, 2012). However, an extensive review on the role of mental fatigue and perceived effort (and/or exertion) in determining T_{lim} and performance during a range of tasks is beyond the scope of this thesis.

2.2.2 Sustained maximal exercise

During sustained maximal exercise, force declines as fatigue ensues. During the initial stages of sustained maximal exercise, this decline has been attributed almost exclusively to peripheral factors (e.g., Bigland-Ritchie et al. 1978; Schillings et al. 2003) and manifests as a reduction in twitch force and slowed twitch dynamics (e.g., rate of force development, $RT_{0.5}$), with no change in M-wave parameters, suggesting that the contractile machinery, and not impulse propagation, is impaired (Bigland-Ritchie et al. 1978). By contrast, central fatigue manifests as a reduction in voluntary force that is faster than the force decrement elicited via peripheral nerve or muscle stimulation and its relative contribution increases with duration (e.g., Kent-Braun, 1999; Froyd et al. 2016; Thomas et al. 1989; Schillings et al. 2003). The development of supraspinal fatigue during an MVC is accompanied by an increase in the sTw evoked by TMS, the development of which increases as a function of time (Todd et al. 2005). In addition to changes in the sTw twitch, MEP size decreases during sustained MVCs with time, revealing rapid reductions in motoneuron excitability that are compensated for by sufficient on-going voluntary drive (McNeil et al. 2009; Taylor et al. 1996). During sustained maximal exercise tasks, EMG is at its highest on initiation of the task and continues to decline until task cessation reflecting a gradual decrease in input

from the motor cortex, development of peripheral fatigue and thus reduced muscle activation.

In a recent study, neuromuscular fatigue development was assessed during the completion of a 60 MVC protocol over a period of 5 min (60 repetitions of 3 s MVCs, and 2 s passive recovery) (Burnley, 2009). During the MVCs, force declined to approximately 30% of baseline values, which was shown to be not different from the critical torque (CT), the asymptote of the torque-duration relationship (figure 2.5). VA declined from ~91% to 67% and pTw declined to ~48% of resting levels, indicating the development of central and peripheral fatigue, respectively. During prolonged maximal exercise, alongside the development of central and peripheral fatigue, distinct changes to the muscle metabolic milieu (e.g., Pi, PCr and H^+ accumulation) and muscle activation (EMG) are also evident (e.g., Burnley, 2009; Fulford et al. 2013). At the onset of exercise, there is a rapid decline in muscle [PCr] and pH coupled with a rapid rise in [Pi], [ADP] and [AMP] before reaching a peak that coincides with task cessation, the achievement of CT and a plateau in the decline of muscle activation to approximately 70% of maximal baseline levels (as measured via EMG) (Burnley, 2009; Fulford et al. 2013).

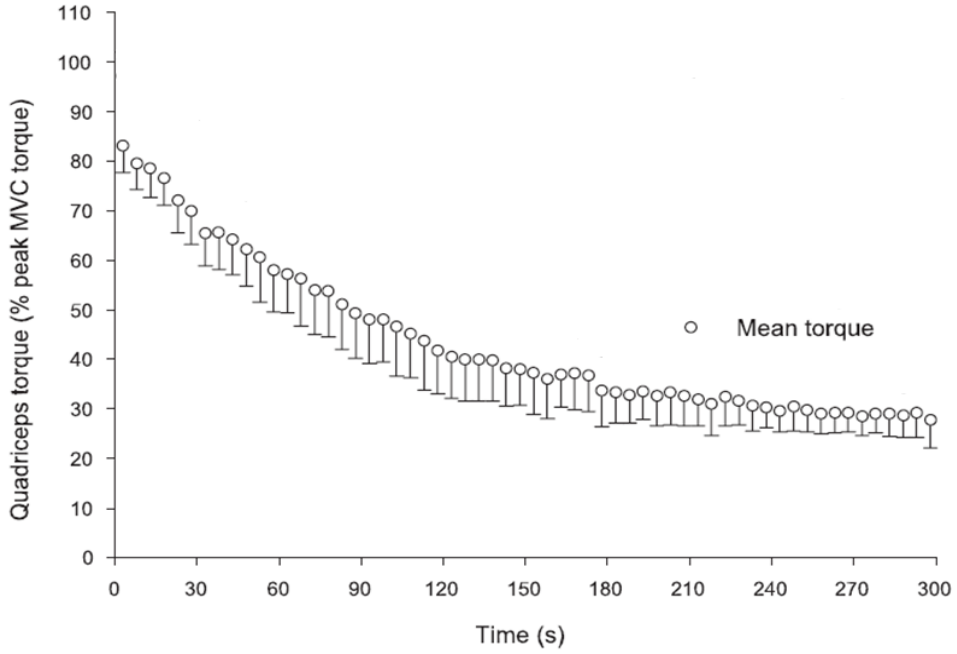

Figure 2.5. *Mean torque ± SD during the 60 maximal contractions that comprised the 5-min all-out test. Edited from Burnley (2009).*

2.2.3 Exercise intensity domains

Submaximal constant work rate exercise can be categorised into three distinct intensity domains based on kinetics of oxygen consumption ($\dot{V}O_2$) and the blood [lactate] response: moderate, heavy and severe (Black et al. 2017; Whipp & Ward, 1992). The upper limit of the moderate-intensity exercise domain is indicated by the lactate (LT) and/or gas exchange threshold (GET), which comprises work rates for which there is no change (or only a transient increase) in blood [lactate] and a steady state in O_2 uptake is attained (Burnley & Jones, 2007; Wilkerson et al. 2004). The boundary between the heavy and severe exercise intensity domains is demarcated by critical power (CP, or critical torque, CT, as an exercise modality equivalent), which reflects the asymptote of the power (or torque)-duration relationship. In addition, the CP represents a threshold for neuromuscular fatigue development (Burnley, 2009; Burnley et al. 2012; Moritani et al. 1993). During heavy-intensity exercise, a $\dot{V}O_2$ slow component is evident, causing a delayed and elevated steady state in $\dot{V}O_2$ and blood [lactate] (Poole et al. 1988). Whereas, the severe-intensity domain encompasses work rates above CP in which maximal oxygen uptake ($\dot{V}O_{2max}$) can be elicited (~2-15 min in duration) and is associated with a progressive increase in muscle metabolic perturbation (Jones et al. 2008; Poole et al. 1988, figure 2.6).

2.2.4 Submaximal exercise

During submaximal exercise, the extent of neuromuscular fatigue and thus the relative contributions of central and peripheral mechanisms are highly dependent on the intensity of exercise or contractions. For example, during sustained lower intensities (e.g., <30% MVC) the contribution of central fatigue is substantially higher than that observed during sustained higher intensity submaximal contractions (e.g., >30% MVC) where peripheral fatigue dominates and central fatigue is modest or even absent (Bigland-Ritchie et al. 1986; Eichelberger & Bilodeau, 2007; Yoon et al. 2007). Indeed, the CT has been identified as a distinct point at which the rate of peripheral fatigue development precipitously accelerates, whereas, below CT, central fatigue gradually increases (Burnley et al. 2012). At intensities below CT, exercise tolerance is typically >20 min, whereas, peripheral fatigue continues to increase above the CT until exhaustion ensues within ~20 min. The EMG response to submaximal exercise is also distinctly different to maximal exercise tasks. Unlike maximal activity, submaximal muscle activity does not induce maximal motor unit recruitment from

exercise/contraction onset. Instead, a progressive rise in EMG, reflecting an increase in motor unit recruitment and/or motor unit firing rate, is evident (Dimitrova & Dimitrov, 2003). The size of the MEP, CSP length and sTw evoked by TMS has also been shown to increase during submaximal exercise as a function of time (particularly during lower intensity contractions), reflecting a decrease in corticospinal excitability and increases in cortical inhibition and supraspinal fatigue (Sogaard et al. 2006; Smith et al. 2007; Taylor et al. 1996). However, when volitional drive is reduced and/or absent, MEP and CMEP size are reduced and thus excitability is impaired (McNeil et al. 2011).

Similar to sustained maximal exercise, during submaximal exercise within the severe-intensity domain, distinct changes in muscle metabolism are evident (e.g., Black et al. 2017; Chidnok, et al. 2013a; 2013b; Jones et al. 2008). During exercise in the severe-intensity domain, the termination of exercise is believed to coincide with the depletion of the finite anaerobic reserves and an accumulation of metabolites (e.g., H^+, Pi, ADP), which have been linked to exercise-induced fatigue (Jones et al. 2008; Vanhatalo et al. 2010). Recent research has implicated a critical threshold of accumulation of these by-products in the fatigue process, suggesting that the development of metabolic perturbation and peripheral fatigue development may be restricted by the attainment of a 'sensory tolerance limit' which limits CMD and expedites central fatigue development (Amann & Calbet, 2008; Gandevia, 2001 *for review*). The sensory tolerance limit is a hypothetical construct thought to relate to a specific, individually determined tolerable level of peripheral fatigue development to protect homeostasis (Amann & Calbet, 2008; Gandevia, 2001; Hureau et al. 2018). Section 2.3 and 2.4 below discusses in further detail how metabolites activate group III/IV afferent firing in contributing to the fatigue process due the attainment of the sensory tolerance limit.

During exercise below CP, PCr, Pi and muscle pH are able to achieve steady state values within minutes of initiation of an exercise task (Black et al. 2017; Jones et al. 2008; Newham & Cady, 1990; Vanderthommen et al. 2003). However, during severe-intensity exercise there is a progressive loss of muscle homeostasis (Black et al. 2017; Burnley & Jones, 2010; Burnley, et al. 2010; Hogan et al. 1999; Jones et al. 2008; Sogaard et al. 2006). A rapid decline in pH coupled with an increase in Pi and a steady fall in PCr are evident, with

exhaustion soon occurring perhaps to an individually determined 'critical level' of muscle metabolic perturbation (Black et al. 2017; Chidnok et al. 2013a; 2013b; Jones et al. 2008; Vanhatalo et al. 2010). However, whilst there is evidence to suggest that inhibiting group III/IV muscle afferent feedback during severe-intensity exercise, via lumbar intrathecal administration of fentanyl, lowers central fatigue development and facilitates increased skeletal muscle metabolic perturbation via greater increases in muscle [Pi], other markers of muscle metabolic perturbation (pH, [PCr] and [ADP]) are not different (i.e., Amann et al. 2009, 2011; Blain et al. 2016; Broxterman et al. 2017a, 2017b; 2018; Sidhu et al. 2014, 2017, 2018). Further research, therefore, is needed to provide clarity on the direct role of group III/IV afferents on muscle metabolic perturbation in light of these discrepancies.

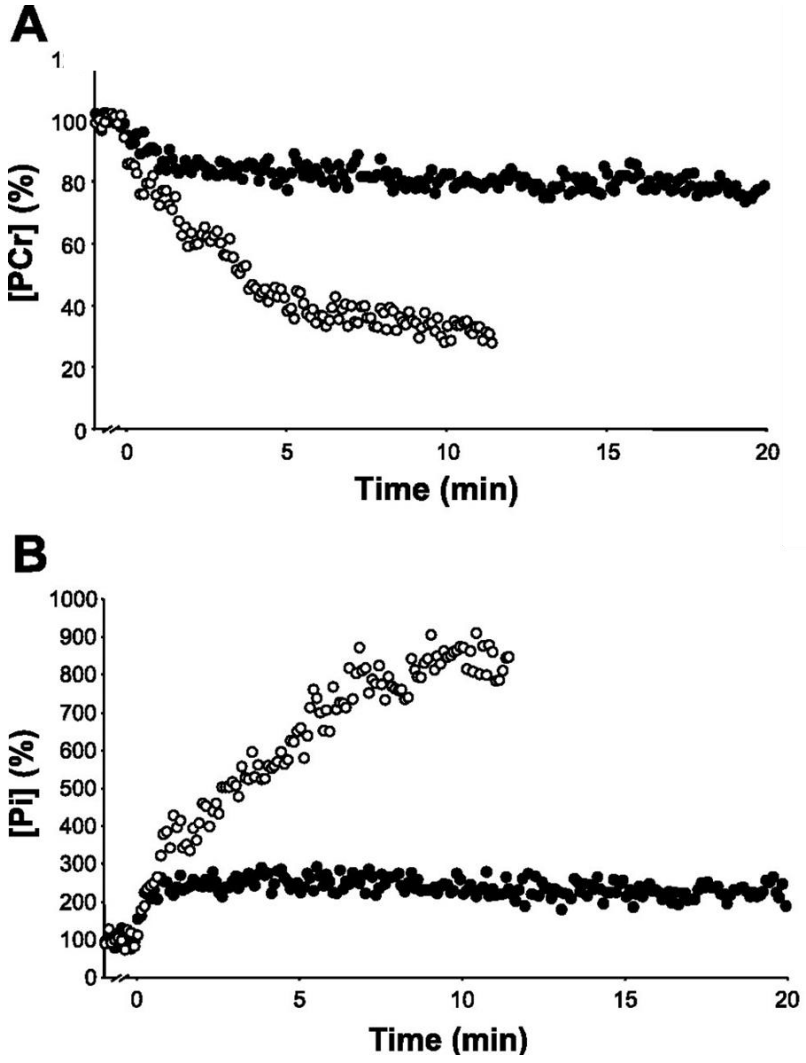

Figure 2.6. *Muscle metabolic response during constant work rate exercise just above and just below critical torque. A – Phosphocreatine [PCr] B – Inorganic*

phosphate [Pi]. A steady fall in PCr (B) and a rapid increase in Pi (B) are evident when exercising above (open circles), compared to below (filled circles), the critical torque (From Jones et al. 2008).

In addition to the muscle metabolic responses to severe-intensity exercise, Moritani and colleagues (1993) showed that the EMG response could be stabilised at a given work rate, but continued to gradually increase once CP was exceeded. Furthermore, Burnley et al. (2012) found that the rate at which fatigue developed, and the site of neuromuscular fatigue (e.g., central or peripheral fatigue) differed above and below CT. The rate of change in the MVC torque and the pTw torque were disproportionately larger during exercise above, compared to below the CT (figure 2.7). At exercise intensities just above CT, exercise duration was ~18 min and was accompanied by a 24% drop in VA. Conversely, at the highest exercise intensity, (~3 min), the drop in VA was only 6%. The drop in pTw, on the other hand, was not different between trials, providing evidence for the existence of a threshold for peripheral fatigue during exercise to exhaustion above CT (Burnley et al. 2012). It was concluded that exercise above the CT is limited by the development of peripheral failure, whereas central fatigue likely plays a greater role during exercise below the CT.

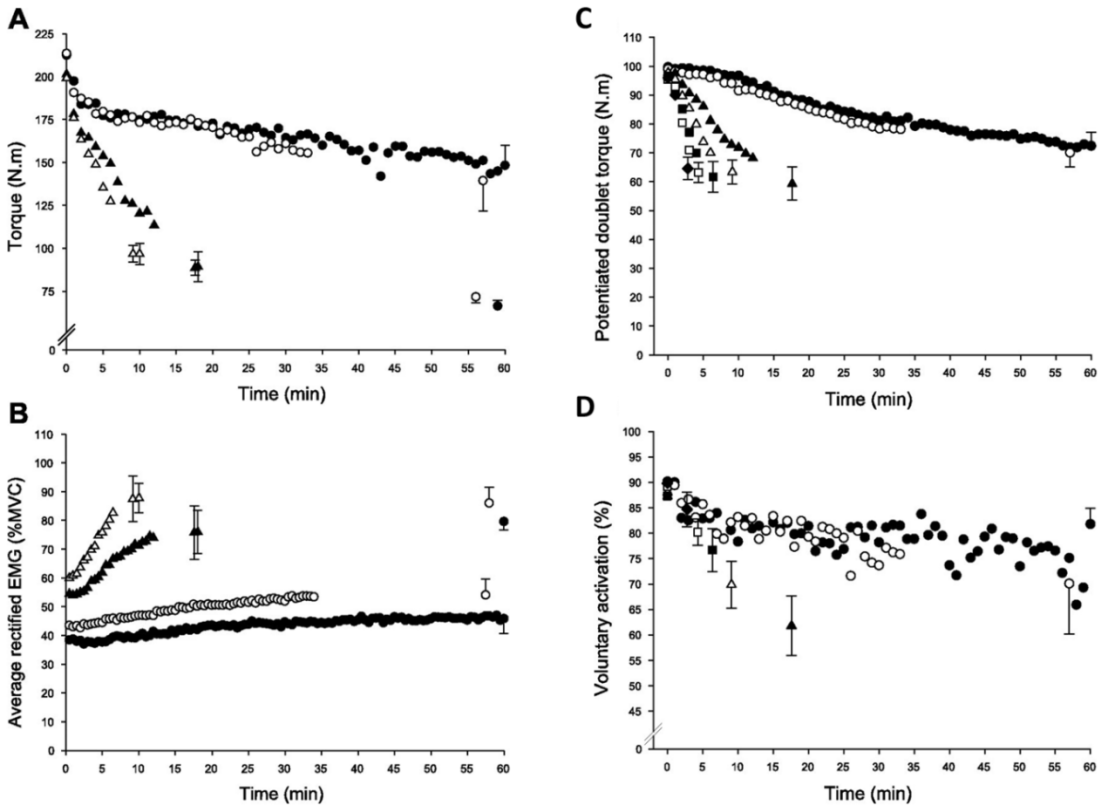

Figure 2.7. *Maximal voluntary contraction torque [A], electromyograhy (EMG) [B], potentiated twitch torque [C] and voluntary activation (VA) [D] responses to contractions performed 20% below (filled circles), 10% below (open circles) and five works rates of increasing intensity above critical torque (CT) (filled triangles, open triangles, filled squares, open squares and filled diamonds, respectively) (Burnley et al. 2012). During exercise below CT, there is an initial decrease in torque, EMG, twitch torque and VA before plateauing. Note increases in the relative contribution from centrally-mediated mechanisms as a function of time (i.e. VA). During exercise above CT, there is a rapid decline in torque, twitch torque and VA and continued rise in EMG before task cessation. Note peripheral mechanisms dominate during severe-intensity exercise (i.e. twitch torque) perhaps to individually specific levels of peripheral fatigue development. Taken from Burnley et al. (2012).*

2.2.5 Locomotor exercise

The cause of fatigue during locomotion is highly sensitive to the type, intensity and duration of exercise, even more so during smaller, isolated exercise tasks (Enoka & Stuart, 1992; Barry & Enoka, 2007). Many studies that have attempted to characterise the neuromuscular fatigue response during locomotor exercise

have been constrained by methodological issues and the fact that fatigue assessment is also highly sensitive to the type of task. Much of the available literature has been based on the assessment of neuromuscular fatigue immediately post-exercise on a separate apparatus, which is confounded by a significant recovery of neuromuscular function when transferring between the different experimental set ups (Carroll et al. 2017). However, recent studies have employed novel approaches to assess neuromuscular fatigue during ergometry (e.g., Black et al. 2017; Doyle-Baker et al. 2017; Sidhu et al. 2012). As with maximal contractions, short duration high-intensity locomotor exercise appears to be primarily limited by mechanisms of peripheral fatigue (as evidenced by reduced pTw and impaired contraction characteristics), whilst central fatigue becomes increasingly prevalent as exercise duration is prolonged (as evidenced by decreases in maximal VA, e.g., Decorte et al. 2012; Lepers et al. 2000; 2002; Millet et al. 2003; Place et al. 2004; Ross et al. 2010; Sidhu et al. 2009; Thomas et al. 2014; 2015). There is less clear evidence of neurotransmission failure contributing to fatigue, with some authors reporting reductions (e.g., Millet et al. 2003) and others observing no change (e.g., Ross et al. 2010) in M-wave responses. A limited number of studies have adopted the use of TMS during locomotion and demonstrated the presence of supraspinal (Goodall et al. 2012; Ross et al. 2007; Sidhu et al. 2009; 2012; 2013) and corticospinal fatigue (Sidhu et al. 2012; Sidhu et al. 2013). However, more research is required to reveal a more comprehensive understanding of the central regulation of fatigue during locomotion.

2.3 Group III/IV muscle afferents and fatigue

Muscle afferents have been implicated in the development of exercise-induced fatigue. The nervous system has different types of neurons that can be sub-categorised into afferent and efferent neurons. Afferent neurons receive information from sensory organs and communicate this to the CNS via ascending tracts, whereas, efferent neurons send impulses from the CNS to peripheral limbs and organs via descending tracts (figure 2.8). In the peripheral nervous system, an afferent nerve fibre is the axon of an afferent sensory neuron. There are many types of afferent and efferent fibres, each of which have different functions. In particular, group III (myelinated) and IV (unmyelinated) afferent fibres monitor changes in contraction-induced mechanical and chemical stimuli within skeletal

muscle and have respective conduction velocities of 4-20 and 0.2-3 m/sec (Boyd & Davey, 1968).

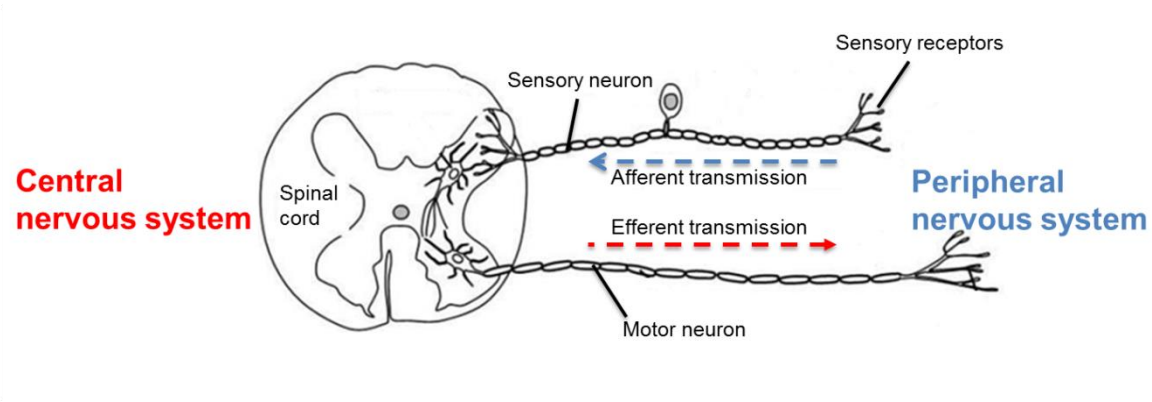

Figure 2.8. *Afferent neurons receive information from sensory organs and communicate this back to the CNS via ascending tracts, whereas, efferent neurons send impulses from the CNS to peripheral limbs and organs via descending tracts (adapted from https://www.studyblue.com/).*

At the onset of exercise, changes in contraction-induced mechanical stimuli and noxious chemicals activate molecular receptors which are located on the terminal end of both group III/IV muscle nerve fibres. The exercise-induced activation of these receptors increases the discharge of the thin fibre muscle afferents that project to various sites within the CNS at both spinal and supraspinal levels, many of which are currently unknown, via the lumbar dorsal horn of the spinal cord (Adreani et al. 1997; Brooks et al. 2005; Katayama et al. 2007; Light et al. 2008; Wilson et al. 2002; Wilson & Hand, 1997). These noxious chemicals either sensitise (e.g., reduce the threshold of these nociceptors to fire) or cause the discharge of nociceptors with the resulting response subsequently perceived as pain (*along with many other sensations*) by the exercising human (Dubin & Patapoutian, 2010; Mense, 2008; Miles & Clarkson, 1994; O'Connor & Cook 1999; McCord & Kaufmann 2010; Pollak et al. 2014). During exercise, group III/IV muscle afferents play a major role in regulating the cardiovascular and ventilatory responses (Amann et al. 2010; 2011; 2015 *for review*), CMD (the output of spinal motoneurons) (Amann et al. 2009; 2011; 2012; Blain et al. 2016; Laurin et al. 2015; Hilty et al. 2011; Sidhu et al. 2017) and efficiency of skeletal muscle contractile function (Broxterman et al. 2017a; 2017b; 2018). Specifically, continuous afferent feedback from working locomotor muscle is essential for

evoking appropriate ventilatory and circulatory responses during exercise in order to prevent premature peripheral fatigue by allowing sufficient blood flow and thus oxygen delivery to the working muscle (Amann & Calbet, 2008 *for review*). However, group III/IV muscle afferents are also known to provide a regulatory role by limiting CMD and the development of peripheral fatigue to a 'critical threshold', likely to protect the organism from excessive exhaustion and potentially harm (Amann et al. 2009; 2010; 2015 *for review*, figure 2.9).

As such, group III/IV muscle afferent feedback has been suggested to contribute to the attainment of the 'sensory tolerance limit' (Gandevia, 2001; Hureau et al. 2016), a hypothetical construct relating to a specific critical level of peripheral fatigue prior to the withdrawal of CMD. Indeed, with intact feedback from muscle afferents, numerous studies have revealed that the inability to continue high-intensity, constant-load endurance exercise coincides with the attainment of a specific critical level of peripheral fatigue and/or metabolic perturbation (e.g., Amann & Dempsey, 2008; 2016; Amann, et al. 2006; 2007; Black et al. 2017; Chidnok et al. 2013a; 2013b; Duffield et al. 2010; Gagnon et al. 2009; Romer et al. 2007; Saey et al. 2005; Vanhatalo et al. 2010; 2016). However, other studies have also refuted such claims (e.g., Froyd et al. 2016; Thomas et al. 2018 *for review*) suggesting that performance and fatigue are not regulated to a peripheral critical threshold and that, instead, fatigue is determined by the mode and intensity of the task which dictate the active muscle mass and demand on other physiological systems. Together, these systems interact to determine fatigue development and exercise tolerance (Thomas et al. 2018 *for review*). Nevertheless, such studies support the notion that the absolute magnitude (or *ensemble*) of muscle afferent feedback from intense exercise regulates performance.

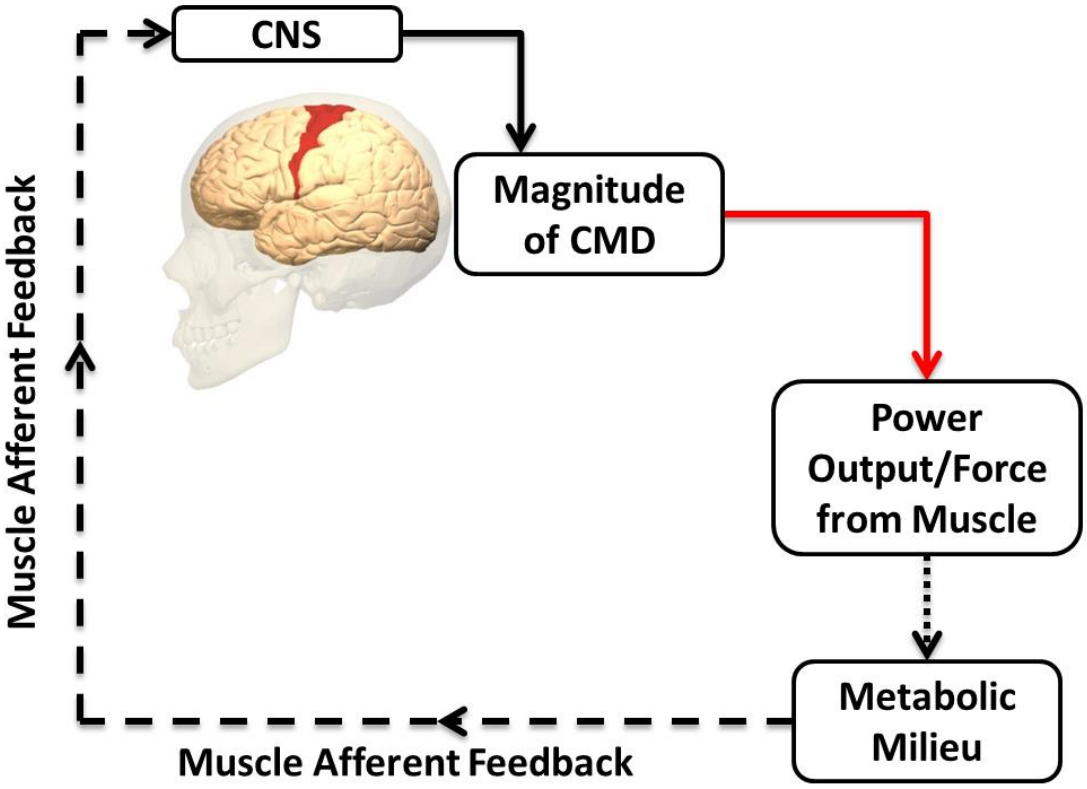

Figure 2.9. *Schematic illustration of the afferent-feedback model of fatigue proposed by Amann & Dempsey (2008). Briefly, afferent neurons, and the associated inhibitory feedback that projects towards cortical regions of the central nervous system, have been suggested to regulate the magnitude of central motor drive which, in turn, determines the sustainable intensity of the peripheral muscles. Adapted from Amann & Dempsey (2008).*

Whilst muscle afferent feedback appears to be of critical importance during whole-body exercise (i.e., Amann & Calbet, 2008; Amann & Dempsey, 2008; Amann et al. 2008; 2009; 2010; 2011; Blain et al. 2016; Gandevia, 1996; Rossman et al. 2014; Sidhu et al. 2014; Taylor et al. 1996; Weavil et al. 2015; 2016), it should be acknowledged that muscle afferent feedback is only one of several potential factors that may account for the inability to continue high-intensity, constant load endurance exercise and a reduction of CMD (see figure 2.1). Moreover, two different subtypes of chemosensitive group III/IV muscle afferents have been characterised. The so-called 'metaboreceptors', only respond to levels of intramuscular metabolites (e.g., lactate, ATP, protons) associated with 'normal' (e.g., freely perfused) exercise (Amann et al. 2015;

Bangsbo et al. 1993; Hellsten et al. 1998). In contrast, the other subtype, metabonociceptors, only respond to higher (noxious) levels of metabolites present in muscle during ischaemic contractions or following hypertonic saline infusions, but not to non-noxious metabolite concentrations associated with 'normal' exercise (Jankowski et al. 2013; Light et al. 2008; Pollak et al. 2014). This suggests, perhaps, that methodologies such as the use of occlusion techniques or hypertonic saline infusions may no longer be considered optimal techniques for investigating the role of group III/IV muscle afferents in exercise-induced fatigue. Consequently, further research is required to elucidate the importance of afferent feedback as a candidate mechanism for exercise-induced fatigue.

To assess the importance of afferent feedback as a candidate mechanism for exercise-induced fatigue, a number of recent studies have attempted to experimentally manipulate group III/IV muscle afferent feedback during exercise (e.g., Amann, et al. 2006; 2007; 2008; 2009; 2010; 2011; 2013; Amann & Dempsey, 2008; Blain et al. 2016; Broxtermnn et al. 2017; 2018; Dempsey et al. 2008; 2014; Hilty et al. 2011; Katayama et al. 2007; Romer et al. 2007; Rossman et al. 2012; 2014; Sidhu et al. 2014; 2017; Weavil et al. 2015; 2016). The same level of peripheral fatigue has been observed despite manipulating oxygen availability (e.g., hypoxia, hyperoxia, Amann et al. 2006; 2007; Vanhatalo et al. 2010) and pre-existing muscle fatigue (e.g., Amann & Dempsey, 2008), factors which would be expected to increase type III/IV muscle afferent feedback. However, the administration of fentanyl, a pharmacological agent that abolishes pain sensation and type III/IV afferent feedback in the lower limbs (e.g., Amann et al. 2009, figure 2.10; Amann et al. 2010; 2011; Blain et al. 2016; Broxterman et al. 2018; Sidhu et al. 2017), permitted a greater development of peripheral fatigue. Specifically, Amann et al. (2011) suggested that participants were able to delay the attainment of the sensory tolerance limit which permitted further development of peripheral muscle fatigue beyond their "critical threshold" with intact afferent feedback. In further support of the role of group III/IV afferent feedback regulating central function, abolishing neural input via fentanyl attenuates intracortical inhibition, via a reduced cortical silent period during a single leg exercise task (Hilty et al. 2011). This supports the notion that central

effects of muscle afferents might accelerate intracortical inhibition and thus the development of central fatigue.

Although there is robust experimental evidence to support a role for fentanyl administration to modulate the bases of exercise-induced fatigue, the effects of fentanyl administration on exercise performance and/or T_{lim} is unclear (e.g., Amann et al. 2009; 2010; 2011; Blain et al. 2016; Broxterman et al. 2017b; 2018; Hilty et al. 2011; Sidhu et al. 2017). Therefore, further research is needed to assess the role of afferent feedback in exercise performance/fatigue development. In support of the notion that group III/IV muscle afferent feedback impairs exercise performance by accelerating central fatigue, Sidhu et al. (2014) demonstrated that the decline in VA observed post-exercise could be abolished with impaired feedback (via fentanyl blockade) during severe-intensity cycling. Indeed, the use of fentanyl has also been shown to reduce the CSP, thought to reflect attenuation in inhibitory intercortical interneuron activity that is a likely contributor to central fatigue as well as enhance spinal excitability (Sidhu et al. 2017). However, during non-fatiguing contractions, the facilitatory effects of muscle afferents was also abolished, highlighting a complex role of muscle afferents on exercise performance (Sidhu et al. 2017).

Whilst studies administering fentanyl provide evidence for an inhibitory effect of afferent feedback on output from spinal motoneurones and peripheral muscle fatigue, a limitation of this approach is that it has been shown that the blocking of group III/IV muscle afferents impairs the cardiopulmonary responses during exercise, particularly as fatigue ensues (Amann et al. 2009; 2010; 2011; Blain et al. 2016), which can compromise exercise performance in certain experimental conditions (Amann et al. 2009; 2010; 2011; Blain et al. 2016). An alternative approach to investigate the influence of group III/IV muscle afferents is to increase the inhibitory feedback. For example, when peripheral locomotor muscle fatigue has been induced prior to a cycling test (Amann & Dempsey; 2008; Gagnon et al. 2009) (which would be expected to provoke higher afferent feedback) a lower output of spinal motoneurones (estimated via EMG) and an impaired cycling performance during a subsequent trial has been reported (Amann & Dempsey, 2008; Gagnon et al. 2009).

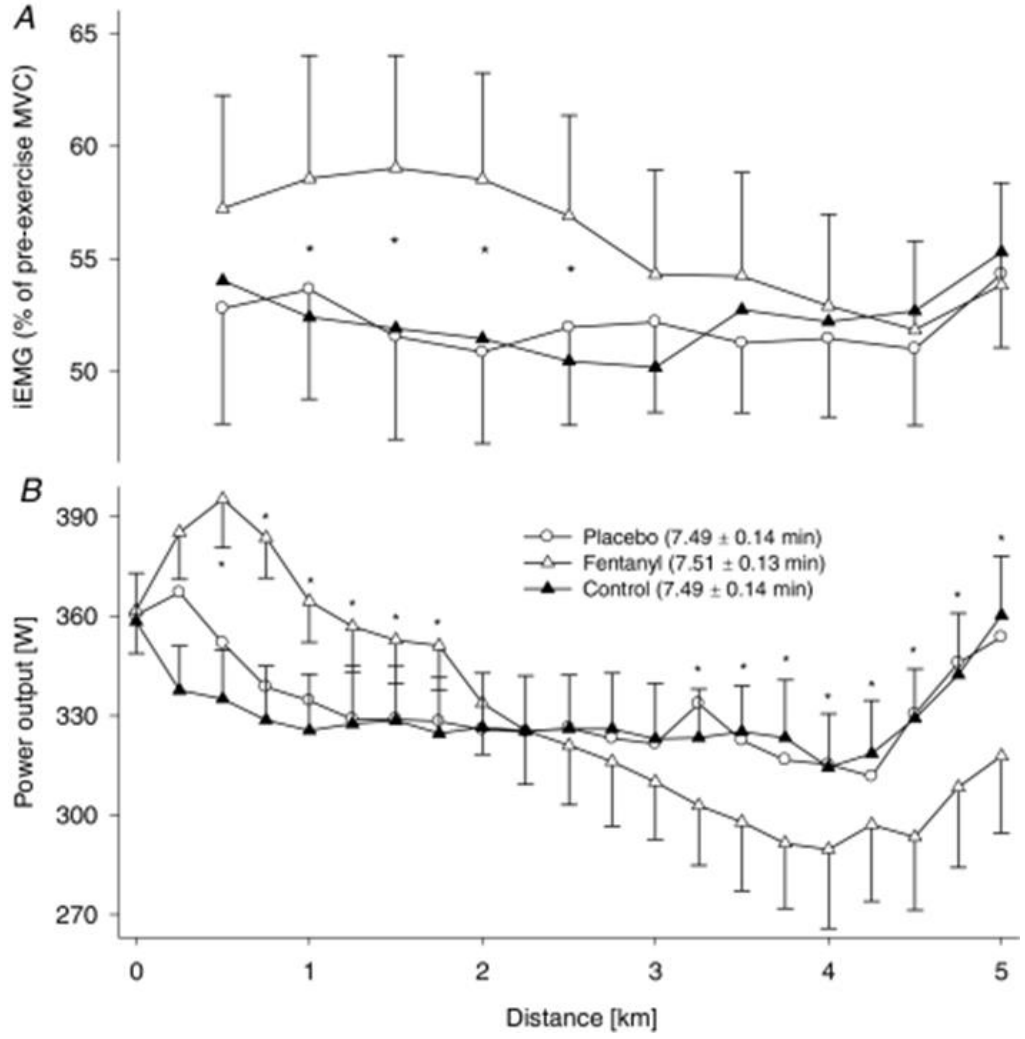

Figure 2.10. *Effect of reduced afferent feedback on central motor drive and power output during a 5-km cycling time trial; (A) Effects of intrathecal fentanyl on EMG and; (B) Power output during the 5 km time trial with and without neural feedback blockade. Mean EMG during the time-trial (TT) was significantly increased following intrathecal fentanyl administration as the CNS permitted a higher degree of central motor drive, enhancing exercise performance. However, the administration of fentanyl allowed a higher degree of peripheral fatigue development which impaired performance in the second half of the trial, with no overall net effect of 5-km TT performance. From Amann et al. (2009).*

Interestingly, as found with impaired feedback, a greater degree of peripheral fatigue development has also been found during small compared to large muscle mass exercise (i.e., single leg vs. whole body cycling; single vs. double leg exercise) (Rossman, et al. 2012; 2014). This has been attributed to confining group III/IV muscle afferent feedback to a small muscle mass, enabling the CNS

to ‘tolerate’ a greater degree of peripheral fatigue. Specifically, trials performed with single-leg knee-extension resulted in significantly greater peripheral quadriceps fatigue than double-leg knee-extension, as evidenced by changes in the EMG signal (147 and 85%), MVC (-25 and -12%), and pTw (-44 and -33%), for single-leg and double-leg knee-extension, respectively (Rossman et al. 2014). It is, therefore, likely that the magnitude of ‘ensemble’ afferent feedback is more important in determining the magnitude of CMD during fatiguing exercise. This is in agreement with recent reviews (i.e., Thomas et al. 2018) criticising the existence of a critical peripheral threshold and that, instead, the critical development of peripheral fatigue is specific to the task characteristics. However, it would have been of interest in this line of studies to normalise iEMG to a presentative M-wave amplitude to provide more conclusive evidence of the effect of abolishing afferent feedback to neural drive.

It would also seem that group III/IV afferent feedback associated with exhaustive exercise has the ability to compromise performance of remote non-exercised muscle groups during locomotion (e.g., Sidhu et al. 2014). Indeed, Sidhu and colleagues (2014), found that following exhaustive leg cycling, maximal voluntary muscle activation (during and after exercise) of a non-local muscle group e.g., elbow flexors (not directly involved in the task) was reduced (Sidhu et al. 2014). Specifically, Sidhu et al. (2014) completed constant-load leg fatiguing (i.e., to exhaustion) and non-fatiguing (30 s of sub-maximal exercise) cycling exercise both under control conditions and with lumbar intrathecal fentanyl to impair feedback from μ-opioid receptor-sensitive lower limb muscle afferents. Interestingly, central fatigue was manifest during control fatiguing conditions in the, rested, elbow flexors (i.e., reduced MVC, VA and MEP). However, when the central projection of afferent feedback was attenuated, these effects were abolished. Interestingly, despite the non-fatiguing bout enhancing corticospinal excitability (increased MEP) fentanyl administration attenuated this blockade, resulting in a 14% lower corticospinal responsiveness during this bout (thus abolishing the facilitatory effects of muscle afferents) and highlighting the complex role of group II/IV muscle afferents during exercise. Taken together, these observations suggest that in the absence of muscle fatigue (i.e., during non-fatiguing contractions), group III/IV muscle afferent facilitate responsiveness of the motor pathway to non-local muscle. However, by direct contrast, in the

presence of locomotor muscle fatigue (i.e., during fatiguing contractions), group III/IV afferents exacerbate supraspinal fatigue and inhibit the responsiveness of corticospinal projections in remote muscle non-exercised muscle groups (Sidhu et al. 2014).

2.4 Contralateral limb fatigue

Cross-over (or non-local muscle fatigue; NLMF) is characterised by performance impairments in remote non-exercised muscles following a fatiguing protocol of a contralateral or ipsilateral muscle group (Halperin et al. 2015, *for review*; e.g., Doix et al. 2013; 2014; Halperin et al. 2014; Halperin et al. 2014; Kennedy et al. 2014; Martin & Rattey, 2007; Sidhu et al. 2014). The mechanisms of NLMF are poorly understood but are thought to be due to a number of complex neurological, biochemical, biomechanical and psychological factors (Halperin et al. 2015, *for review*). The topic of contralateral (or ipsilateral) limb fatigue is of interest as it offers insights into the potential determinants of exercise-induced fatigue. Growing evidence suggests that humans voluntarily terminate high-intensity, constant-load endurance exercise once their 'sensory tolerance limit' is reached (Gandevia, 2001 *for review*) by inhibiting CMD (Gandevia, 1996; Taylor et al. 1996; Todd et al. 2003; 2005). However, whilst many studies have attempted to isolate the inhibitory effect of muscle afferents via pharmacological inhibition (i.e., fentanyl), these studies are confounded or complicated by impeding the cardiopulmonary response to exercise and/or inhibiting different afferent fibres to those emanating from the exercising skeletal muscles (e.g., Amann et al. 2009; 2010; 2011; Blain et al. 2016; Amann & Calbet, 2008 *for review*). Previous studies have also been confounded by issues such as changes in peripheral muscle function (pre-fatigue studies e.g., Amann & Dempsey, 2008) and to the external environment (hypoxia studies e.g., Amann, et al. 2006; 2007).

To bypass these issues, Amann and colleagues (2013) conducted a study that sought to determine whether muscle afferent feedback associated with peripheral muscle fatigue inhibits CMD via a single leg model where subjects were asked to perform single leg knee-extensor exercise to exhaustion in one leg immediately followed by the identical task in the other, rested, leg (e.g., leg_2). Specifically, participants performed constant-load, single-leg knee extensor exercise to exhaustion at 85% of peak work rate with each leg. In addition, the test was

repeated but with the contralateral limb performed immediately at the cessation of the first leg task (Amann et al. 2013). T_{lim} (e.g., exercise tolerance, time-to-exhaustion) of the consecutively exercising contralateral leg was 49% shorter when compared to its single leg control (without prior contralateral fatigue). Additionally, pTw following the contralateral limb protocol was 19% less in leg_2 (33 vs 52%). End-exercise EMG amplitude was also 26% lower and perceived exertion was consistently 28% higher, all providing evidence that peripheral fatigue and associated afferent feedback limits the development of further peripheral fatigue and compromises endurance exercise performance by inhibiting CMD. Therefore, it is possible that, during contralateral limb exercise (via a feedback loop), group III/IV muscle afferents have an inhibitory impact on the CNS, leading to decrements in CMD to both the working and non-exercised muscles (e.g., Amann et al. 2013; Sidhu et al. 2014). It could, therefore, be likely that the termination of exercise during this constant load task was due to participants reaching their 'sensory tolerance limit' (Amann et al. 2013; Gandevia, 2001; Hureau et al. 2018). Importantly, any possible humoral cross-over effect was excluded as neither handgrip MVC nor quadriceps muscle function of the rested contralateral leg were altered by exhaustive single-leg knee-extension (Amann et al. 2013). Figure 2.11 summarises the proposed mechanisms for the existence of contralateral limb fatigue and the interactions between afferent feedback, peripheral fatigue, CMD and endurance exercise performance. Importantly, further research is necessary to understand whether T_{lim} is linked to the sensory tolerance limit (i.e., via an increased magnitude of group III/IV muscle afferent feedback) and/or to direct local effects of changes to muscle metabolic milieu (i.e., high $[H^+]$, low [PCr]) on muscle contraction (i.e., not reliant on group III/IV muscle afferent feedback or changes in CMD).

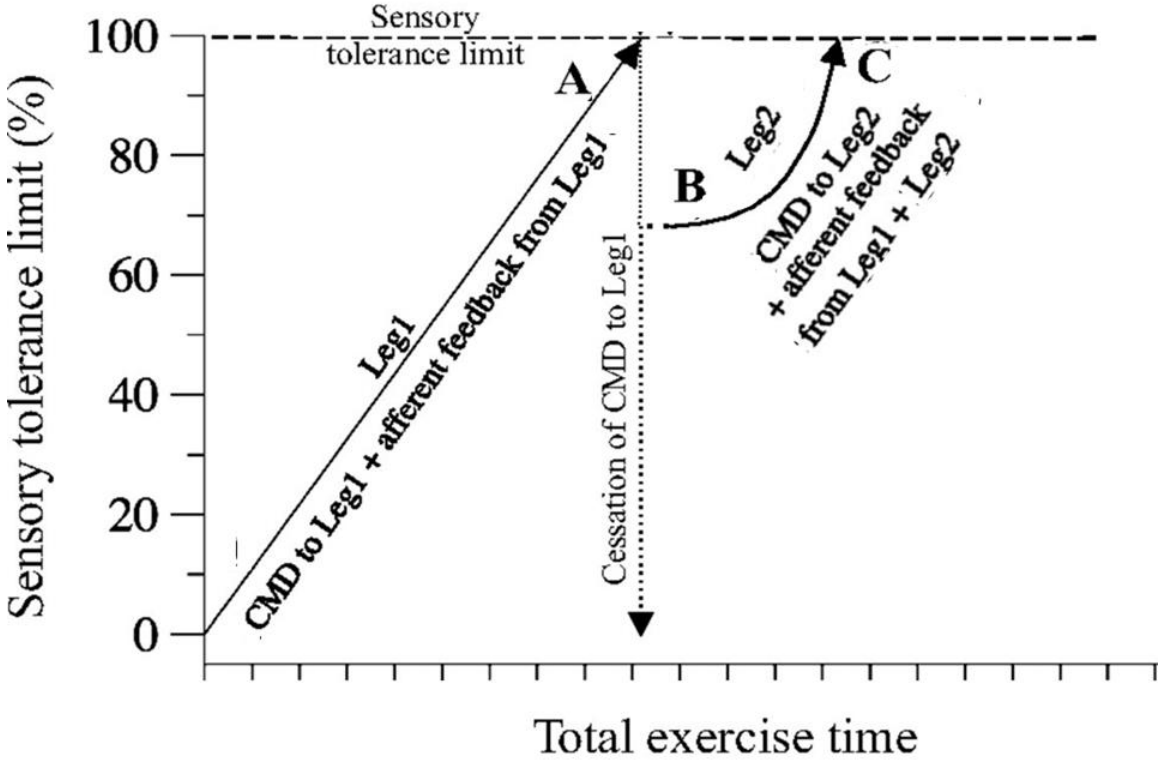

Figure 2.11. *Theory proposed by Amann et al. (2013) for the interactions between afferent feedback, peripheral fatigue, central motor drive (CMD) and endurance exercise performance. Specifically, task disengagement of the prior limb exercise (i.e., leg$_1$) coincides with the attainment of the sensory tolerance limit [A], a hypothetical construct that coincides with a certain level of peripheral fatigue and associated intramuscular metabolic milieu. Upon task cessation of leg$_1$, the contralateral limb (i.e., leg$_2$) completes the identical exercise task. However, whilst there is some recovery from afferent firing, afferent feedback remains elevated from leg$_1$ exercise [B], and the sensory tolerance limit is reached at an earlier time point during leg$_2$ exercise [C], contributing to 49% impairment in single-limb knee-extension exercise performance when compared to the identical exercise task without pre-existing fatigue in the opposing limb.*

Several other studies have also documented a significant reduction in CMD to the contralateral rested muscle (e.g., Rattey et al. 2006; Todd et al. 2003; Zijdewind et al. 1998). However, the effect of contralateral limb fatigue was quite pronounced in the Amann et al. (2013) study, with a 49% impairment in leg$_2$. This observation is consistent with some (e.g., Amann et al. 2013; Doix et al. 2013; Halperin et al. 2014; Kennedy et al. 2013; Rattey et al. 2006; Takahashi et al. 2011), but not all (e.g., Elmer et al. 2013; Grabiner & Owings, 1999; Regueme et

al. 2007; Todd et al. 2003; Zijdewind et al. 1998), previous studies reporting increased fatigue development after prior contralateral or non-local muscle fatigue. Some studies have also reported reductions in the output of spinal motoneurons to the contralateral rested muscle, but with no performance impairment (e.g., Rattey et al. 2006; Todd et al. 2003; Zijdewind et al. 1998). This inter-study discrepancy in the potential ergolytic effect of contralateral fatigue (and NLMF) has been attributed to differences in sex, exercise modality, utilised muscle mass (and thus the total magnitude of group III/IV muscle afferent feedback), and/or the use of flexor vs. extensor muscles (Amann et al. 2013). However, this 'task-dependency' requires further investigation (Halperin et al. 2015 *for review*).

These discrepancies might be also explained, in part, by the possibility that sub-maximal non-local muscle exercise can potentiate contractility of resting muscles (Sidhu et al. 2014). A recent study provides further controversy on contralateral limb fatigue and the significance of the role of afferent feedback during exercise (Kennedy et al. 2015). In this study, participants completed a series of MVCs with the contralateral knee extensors after completing a two minute continuous unilateral MVC of the knee extensors with and without lower limb occlusion to maintain or increase firing frequency of group III/IV muscle afferents. However, no differences in MVC torque was observed with blood flow occlusion. Thus, further investigation is required to confirm the contribution of group III/IV muscle afferents to contralateral limb impairments, particularly in light of recent research that has criticised techniques such as occlusion to alter the magnitude of afferent feedback (Bangsbo et al. 1993; Hellsten et al. 1998; Jankowski et al. 2013; Light et al. 2008; Pollak et al. 2014).

2.5 COX-inhibitors and exercise performance

Emerging evidence shows that blunting afferent feedback (via the use of mild analgesics), but not completely abolishing sensory input (via fentanyl administration), can improve exercise performance (Foster et al. 2014; Mauger et al. 2010; 2014). In addition to their known role in regulating the exercise pressor reflex (Amann et al. 2012; Secher & Amann, 2012 *for review*), the discharge of group III/IV muscle afferents in response to mechanical and metabolic stimuli also contributes to the sensation of muscle pain during exercise

(O'Connor & Cook 1999; McCord & Kaufmann 2010; Pollak et al. 2014). Over-the-counter pharmacological agents, such as anti-inflammatory drugs (NSAIDs) and paracetamol (or acetaminophen, ACT) which act primarily by inhibiting the activity of COX enzymes (isoforms: COX-1, COX-2, COX-3), are known to reduce the sensation of pain. Consumption of an acute dose of ACT may improve exercise performance (Foster et al. 2014; Mauger et al. 2010; 2014) but the potential mechanism(s) of action is/are unclear. Therefore, further studies are required to better understand the physiological mechanisms by which analgesic drugs might enhance exercise performance. The following sections will focus on the literature that has assessed the effects of consuming non-specific COX-inhibitors (e.g., ACT and IBP) on exercise performance and its underpinning mechanisms to help generate the rationale for the current thesis and justify the experimental hypotheses.

2.5.1 ACT mechanism of action and pharmacokinetics

ACT and IBP (a non-steroidal anti-inflammatory drug, NSAID) are competitive inhibitors of the enzyme, COX, which mediates the bioconversion of arachidonic acid to PGs, endogenous algesic exokines (and its derivatives i.e., TXA_2, PGD_2, PGE_2, PGF_2). It is believed that NSAIDs act centrally and peripherally to reduce the sensation of pain and tissue inflammation, respectively (Fridén & Lieber, 1992). ACT is a commonly used non-prescription pain reliever (e.g., an analgesic) and antipyretic (e.g., able to reduce fever) and is considered one of the safest non-opioid analgesics at therapeutic doses (1 g, Toussaint et al. 2010). Following oral ingestion, ACT is rapidly absorbed from the gastrointestinal tract and then metabolized by the liver into toxic and non-toxic compounds (Hodgman & Garrard, 2012) with a resultant bioavailability of 70 to 90% (Forrest et al. 1982). However, whilst gastric emptying can be slowed by the intake of food, the absorption of ACT is not affected (Prescott, 1980). Research has demonstrated peak plasma [ACT] to be attained at ~60 min (e.g., Anderson et al. 2008; Forrest et al. 1982) and up to 120 min (1.5 g dose, Foster et al. 2016) following oral ingestion. The plasma half-life of acute ACT ingestion at the therapeutic doses ranges between 1.5 and 4 h in humans (Forrest et al. 1979; 1982; Prescott et al. 1971; Prescott, 1980). Whilst the mechanisms that underlie the analgesic effect of ACT are not completely understood *in vivo*, the analgesic effect is considered to be predominantly mediated by central factors (Anderson

2008; Graham et al. 2013; Smith 2009; Toussaint et al. 2010). Conventionally, the analgesic effect of ACT has been attributed to the inhibition of COX enzymes, which stimulate nociceptor discharge through the synthesis of PGs (Graham et al. 2013; Jóźwiak-Bębenista & Nowak 2014, figure 2.12). There is also evidence that the analgesic effect of ACT is linked to potentiation of descending serotoninergic pathways (Andersson et al. 2011Pickering et al. 2006; 2008), and modulating opioid and cannabinoid receptors (Graham et al. 2013; Ottani et al. 2006). These mechanisms likely interact to lower pain sensation (as well as inflammation and body temperature) following acute ACT ingestion by increasing the nociceptive stimuli required to evoke a given pain sensation.

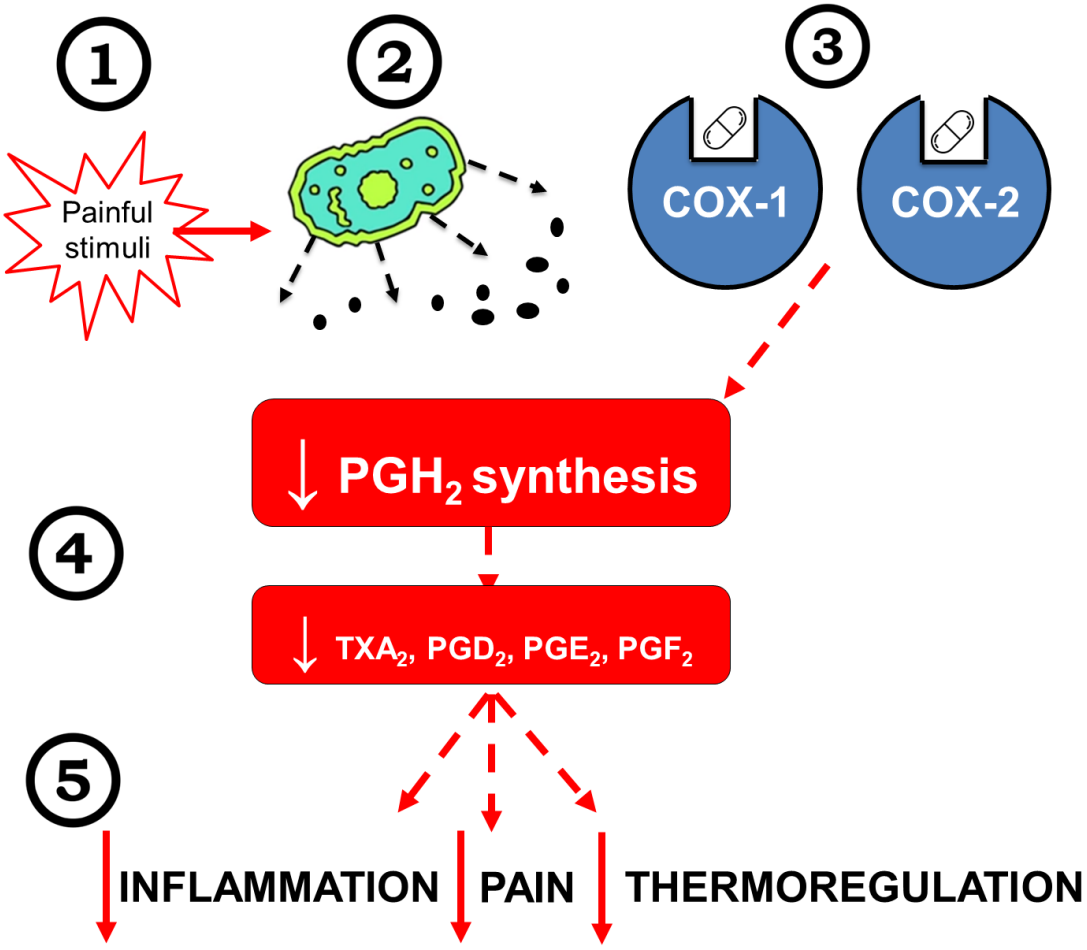

Figure 2.12. *Mechanism of action of non-specific COX-inhibitors (e.g., acetaminophen, ACT, ibuprofen, IBP) on cyclooxygenase (COX) and prostaglandin (PG) synthesis. Pain is considered the body's 'early warning system' as its primary purpose is to protect the body from further harm. Nociceptors, which are specialised nerve cells that stretch from the spinal cord to*

areas including skeletal muscle, skin, bones and internal organs, relay information back to the brain to inform the central nervous system of a painful stimulus. Nociceptors, in contrast to other nerve cells, have a threshold to fire and thus are only stimulated on exceeding this threshold. Following the delivery of a painful stimulus [1] and the subsequent damage to cells, PGs (or PGH_2) are produced following the bioconversion of arachidonic acid (released following painful stimuli [2]) by cyclooxygenase enzymes (e.g., COX-1, COX-2) [3]. PGs sensitise nociceptors, thus lowering the threshold to fire. Following the conversion of arachidonic acid, PGs are then converted to a number of different chemicals (e.g., TXA_2, PGD_2, PGE_2, PGF_2) [4] and subsequently have a number of effects on the body including raising the body temperature, causing inflammation and lowering the pain threshold [5]. Non-specific COX-inhibitors (e.g., ACT and IBP) provide analgesic, antipyretic and inflammatory effects through (non-selectively) binding to the active sites of COX [e.g., 3], blocking the metabolism of arachidonic acid and thus preventing PG synthesis.

2.5.2 ACT and neuromuscular function

PGs are lipid autacoids that are derived from arachidonic acid by the action of COX isoenzymes and contribute to the sensation of pain and to the desensitizing of group III/IV muscle afferent fibres. Indeed, as PGs biosynthesis is blocked by non-specific COX-inhibitors, manipulating PG synthesis in this way may act as a less invasive means to assess the potential contribution of group III/IV muscle afferent feedback to exercise performance in a more applied and ‘real-world’ setting when compared to the invasive nature of spinal fentanyl administration that has previously been discussed. In addition, acute pain has been observed to increase corticospinal inhibition which likely inhibits optimal motor cortex activation during voluntary movement and thus impairs voluntary muscle activation and, ultimately, muscle function (Dubé & Mercier, 2011). In direct contrast, ACT ingestion is known to reduce pain and has been reported to increase resting corticospinal excitability, as inferred from an increased MEP amplitude (Mauger & Hopker 2013). This increased resting corticospinal excitability would be expected to manifest during exercise if inhibitory feedback from group III/IV muscle afferents was attenuated with ACT ingestion and could explain its ergogenic capabilities. In support of these observations, it has previously been demonstrated in mice that ACT increases the activity of TRPA1

at the spinal level and increases the activity of endocannabinoid CB1 receptors within the brain (Andersson et al. 2011; Ottani et al. 2006). Together, these factors might contribute to the enhanced excitability of the corticospinal tract in resting conditions. Indeed, as suggested by Mauger & Hopker (2013), it would perhaps be unsurprising if this effect would translate to improvements in exercise performance and/or the tolerable duration of exercise, as exercise-induced fatigue has been linked with decreased cortical excitability (e.g., Ross et al. 2010). However, despite these observations, the extent to which these neuromuscular alterations at rest relate to improved exercise performance during and following intense exercise is currently untested. In addition, a prior investigation performed by Mauger & Hopker (2013) of a subset of the subjects in the same study revealed that ACT had no effect on compound muscle action potentials at rest (e.g., M-wave) in further support that its effect is likely located distal to the neuromuscular junction. In agreement, there is currently no evidence to support the ingestion of ACT evoking a systemic physiological response during exercise or affecting skeletal muscle peripheral fatigue development. Further research is, therefore, required to investigate the effect of non-specific COX-inhibitors on neuromuscular function as relatively little is known of its potential to favourably enhance neuromuscular parameters during exercise.

2.5.3 ACT effect on performance and exercise capacity

ACT is widely consumed by athletes and recreational sports men and women alike (e.g., Brewer et al. 2014; Feucht & Patel, 2010; Garcin et al. 2005; Tscholl & Dvorak, 2012; Warden, 2010). Indeed, in support of its ingestion, emerging evidence suggests that ACT can improve exercise performance (Mauger et al. 2010; 2014; Mauger & Hopker, 2013; Foster et al. 2014). Specifically, Mauger et al. (2010) identified a 2% improvement in power output across a 10-mile cycling TT following the ingestion of 1.5 g of ACT in trained male cyclists (figure 2.13). The ergogenic potential of acute ACT ingestion (at 20 $mg \cdot kg^{-1}$ lean body mass) has also been reported during cycling in the heat (30°C, 50% relative humidity) by improving exercise tolerance by ~17% in recreationally active, untrained, males (Mauger et al. 2014). This was accompanied by significantly lower core (-0.15°C) and skin (-0.47°C) temperature as well as lower reported ratings of thermal sensation (*subjective perception of thermal comfort*). However, whilst thermoregulatory adjustments following ACT consumption have been linked to

improved performance in the heat (Mauger et al. 2014), the tests we will use in this thesis will be conducted in temperate conditions and will be short in duration and thus not limited by the tolerance of heat.

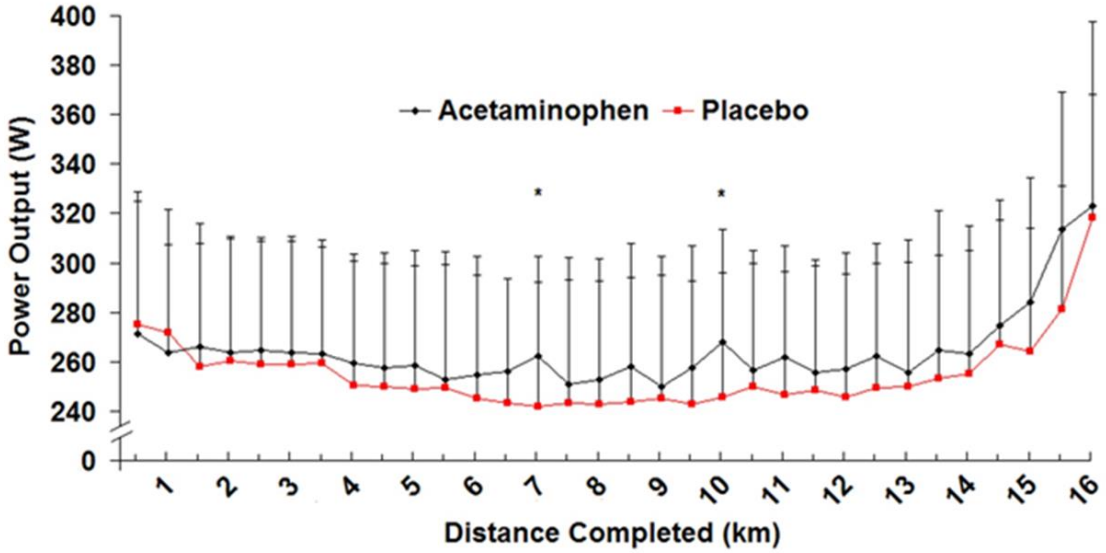

Figure 2.13. *Acetaminophen (black line) reduced the extent to which power output declined towards the middle section of the time-trial, resulting in a 2% improvement on performance time compared to placebo (red line). From Mauger et al. (2010).*

Foster et al. (2014) have also demonstrated a significant increase in mean power output (391 ± 74 vs. 372 ± 90 W) during a repeated sprint cycling protocol (Wingate anaerobic tests: 8 × 30 s sprints, 2 min active rest intervals) following the ingestion of a 1.5 g dose of ACT in nine recreationally active male participants. The observed improvement in repeated sprint ability was the result of an attenuated decline of power output in the latter stages of the protocol (e.g., sprints 6, 7 and 8). In direct contrast, more recently, data has also been presented to suggest ACT does not influence sprint interval treadmill running despite reducing pain sensation (Park et al. 2016),

2.5.4 Pain as a determinant of endurance performance

Pain is a sensory and emotional experience associated with performing intense activity (International Association for the Study of Pain, 2018; Treede, 2018). This suggests that pain is a subjective experience and influenced by previous history of pain. As a consequence, pain is not always proportionally dependent on the

nociceptive signal. Exercise-induced pain is processed by the brain following feedback from group III/IV muscle afferent fibres, which are activated or sensitised by mechanical pressure, heat, cold and algesic substances, relaying information to the CNS regarding tissue damage (O'Connor & Cook, 1999). These chemicals (e.g., metabolites) are released when there is a disturbance to homeostasis. As increases in relative work rate are associated with increased metabolic perturbation (e.g., Vanhatalo et al. 2011) and therefore stimuli for group III/IV muscle afferent feedback, it is unsurprising that exercise-induced pain increases as a function of work rate (e.g., Astokorki & Mauger, 2017a; Cook et al. 1997; Sgherza et al. 2002). However, different types of pain are sensed and processed by the body differently (i.e., thermal pain, chemical pain, pressure pain etc.) and the same 'signal' does not always result in the same level of pain sensation (or 'perception'), complicating understanding of the potential role of exercise-induced pain sensation in the aetiology of exercise-induced fatigue (Astokorki & Mauger, 2017a).

Although previous research has reported no relationship between pain and exercise performance, a limitation of these studies is that they are confounded by pain being induced through interventions (i.e. pain pressure tests) that differ markedly from exercise-induce pain (e.g., Astokorki & Mauger, 2017a), questioning the validity of some of these early studies on exercise-induced pain. This is pertinent to note as different causes of pain (i.e., mechanical, chemical, thermal) activate different pain pathways (Olesen et al. 2012). Therefore, despite other techniques that have been adopted to assess exercise-induced pain (i.e., cold pressor test, algometry) having excellent validity and reliability in studies of clinical pain, the processing and subsequent response to exercise-induced pain is likely fundamentally different as each may arise from different stimuli (i.e., neuropathic, inflammatory, and nociceptive) as well as be perceived differently, and so exert a different '*response*' (Olesen et al., 2012).

More recent evidence using valid measures of exercise-induced pain suggests that the sensation of pain during exercise exhibits an inverse relationship with endurance performance (e.g., Astokorki & Mauger, 2017a). Indeed, Astokorki & Mauger (2017a) found exercise-induced pain, using a pain perception score, predicted performance by 7.5% (P<0.05), stronger than other traditional

endurance parameters such as $\dot{V}O_{2peak}$ and GET. Indeed, it is also attractive to consider that the typical day-to-day variability in exercise performance regularly observed amongst elite performers (i.e., Hickey et al. 1992) may be explained, at least in part, to differences in the sensations associated with intense activity. This is particularly pertinent within homogenous groups of elite athletes with similar physiological qualities. In further support of the significance of pain to exercise performance, athletes generally report higher levels of pain tolerance during exercise when compared to recreationally active individuals (Janal et al. 1994; O'Leary et al. 2017; Tesarz et al. 2013) and, consequently, the willingness to tolerate pain during exercise has been identified as an important predictor of endurance performance (e.g., Astokorki & Mauger, 2017a; 2017b; Mauger, 2014; Mauger et al. 2010).

Whilst studies have been able to demonstrate that exercise-induced pain shares a strong relationship with exercise intensity (i.e., Cook et al., 1997), and that interventions (i.e., paracetamol, caffeine, amphetamines) aimed at reducing pain can enhance exercise performance (i.e., Astorino et al., 2011, 2012; Felippe et al. 2018; Foster et al., 2014; Gonglach et al. 2016; Hudson et al., 2008; Jenkins et al., 2008; Mauger et al., 2010, 2014; Swart et al. 2009), other studies have questioned the role of pain in regulating work output and performance (i.e., aspirin, Cook et al., 1997; ginger, Black & O'Connor, 2008; Black et al., 2010; paracetamol, Park et al. 2016; ibuprofen, Da Silva et al. 2015). However, whilst other factors may contribute to its ergolytic effect, it is important to note than when pain is increased during exercise through muscle damage (i.e., Black & Dobson, 2013) and intramuscular saline injections (i.e., Graven-Nielsen et al., 2002; Khan et al., 2011), exercise performance is impaired. This may suggest that the aforementioned analgesics (i.e., paracetamol, caffeine, amphetamines) may enhance exercise performance by altering pain as well as other aspects that contribute to exercise performance and/or that the effect of analgesics are specific to the task and/or population. For example, caffeine, which binds to adenosine receptors involved in nociceptive signalling, is also known to provide analgesia via a similar mechanism to paracetamol (*discussed below*) via inhibition of COX-enzymes (i.e., Fiebich et al. 2000). However, caffeine may also act to increase fat oxidation and motor unit recruitment as well as alter dopamine and serotonin release (Baratloo et al. 2016; Felippe et al. 2018; Gonglach et al.

2016; Jenkins et al. 2008; Walton et al. 2003). Similar to caffeine, amphetamines (i.e., methylphenidate), whilst also reduce pain sensation, also act as a central stimulant and favourably alter the ratio of dopamine/serotonin, permitting a higher metabolic stress (i.e., Swart et al. 2009).

2.5.5 ACT effect on pain sensation during exercise

Research suggests that interventions, such as ACT, aimed at reducing pain sensation during exercise (may have the potential to enhance performance (Foster et al. 2014; Mauger et al. 2010; 2014). However, whilst acute ACT ingestion may improve exercise performance independent of its effect on pain (and effort and/or exertion) sensation by alleviating the development of central fatigue (Mauger & Hopkins, 2013), ACT may also help to maintain cortical excitability, enhance muscle activation and/or reduce cortical inhibition by modulating pain sensation (e.g., Dubé & Mercier 2011). In addition, ACT may enhance corticospinal excitability in the absence of acute pain (Mauger & Hopker, 2013) which may enhance exercise performance and attenuate exercise-induced fatigue development (e.g., Ross et al. 2010). There is also evidence to suggest that, in athletes, ACT ingestion reduces RPE at the lactate threshold (Garcin et al. 2005) and can reduce exercise-induced pain during the latter stages of sprint interval running (Park et al. 2016). However, pain at the lactate threshold is, typically, very low if present at all (Garcin et al. 2005). In addition, as aforementioned, caffeine, a widely accepted ergogenic aid, also provides mild analgesia which can reduce muscle pain at a given intensity (e.g., Cotl et al. 2003) providing further support for interventions aimed at reducing pain sensation in enhancing exercise performance. However, further research is required to assess the effects of ACT ingestion on exercise performance and the mechanisms underpinning any ergogenic effect, particularly as Park et al. (2016) recently demonstrated an acute dose of ACT to reduce exercise-induced pain during the latter stages of sprint interval treadmill running but without influencing performance. This, possibly, highlights a task specific effect of ACT on performance and/or that the effects of ACT ingestion on performance and pain sensation can be dissociated.

Despite this, Mauger et al. (2010) demonstrated a significant improvement in cycling TT performance via increasing power output at a given sensation of pain

and effort (and thus likely lowering pain and effort sensation for a given power output). Specifically, although Mauger et al. (2010) demonstrated no difference in RPE or perceived pain following acute ACT ingestion, participants cycled at a higher mean power output, with an increased heart rate and blood [lactate] response, for the same RPE and pain rating. These findings, therefore, support the notion that performance can be influenced by pain perception, and that an increased pain tolerance can improve exercise capacity possibly by permitting further development of peripheral fatigue into a 'metabolic reserve' (Mauger, 2014 *for brief review*). This is also supported by data from Mauger et al. (2014) who demonstrated a 17% improvement in time-to-exhaustion during cycling in the heat (30°C) after ingestion 20 mg·kg lean body mass^{-1} ACT and by Foster et al (2014) who found improved performance during a repeated sprint protocol following ACT ingestion to be associated with a reduction of pain for a given work rate. Collectively these findings highlight the potential for pain sensation to mediate the effect of ACT ingestion on enhancing exercise performance (Foster et al. 2014; Mauger et al. 2010; 2014).

Whilst improving pain tolerance or reducing exercise-induced pain sensation can be crucial to athletic performance (e.g., Astokorki & Mauger, 2017a; 2017b; Mauger et al. 2010), the manipulation of pain by modulating type III/IV muscle afferent feedback has marked effects on the exercise pressor reflex (i.e., Amann et al. 2009; 2011; 2012; 2015; Blain et al. 2016; Secher & Amann, 2012 *for review*). Indeed, muscle afferents are not only responsible for communicating the 'pain signal' to the brain, but also regulate the cardiopulmonary response to exercise, which can have implications for exercise performance. This might account for observations of impaired performance in the second half of a 5 km cycling TT when all feedback from group III/IV muscle afferents is removed via intrathecal fentanyl administration despite an elevated CMD and performance in the first half of the 5 km TT (Amann et al. 2009; Blain et al. 2016). ACT administration, on the other hand, which seemingly acts predominantly through central processes to blunt, but not completely abolish, pain sensation and afferent feedback (Anderson, 2008; Graham et al. 2013; Smith, 2009; Toussaint et al. 2010) may act as a 'real-world' and less invasive means to enhance CMD and permit further development of peripheral fatigue without impairing the cardiopulmonary response to whole-body exercise. However, the potential

mechanisms by which analgesics that can modulate type III/IV muscle afferent feedback might improve exercise performance are unclear and will be addressed in this thesis.

2.5.6 Ibuprofen and exercise performance

IBP also contains analgesic properties and is predominantly used to treat pain and reduce inflammation and fever. IBP is considered safe for oral ingestion within healthy populations at the standard therapeutic dose (Albert & Gernaat, 1984; García-Martín et al. 2004). Consequently, utilisation of analgesic and anti-inflammatory medication, such as IBP, has also emerged as a popular pre-competition strategy in elite athletes attempting to enhance athletic performance (Alaranta et al. 2008; Corrigan & Kazlauskas, 2003; Da Silva et al. 2015; Gorski et al. 2011; Huang et al. 2006). It is believed that NSAIDs act centrally and peripherally to respectively reduce pain perception and tissue inflammation (Fridén & Lieber, 1992) by inhibiting COX and the subsequent synthesis of PG (e.g., PGE_2, Albert & Gernaat, 1984; Fridén & Lieber, 1992). However, in contrast to the effects of ACT consumption on exercise performance, there is currently very limited published research on NSAID and NSAID-like compounds during exercise when *muscle damage* is not present during whole body exercise in humans (e.g., Cleak, & Eston. 1992; Da Silva et al. 2015; Nosaka & Clarkson, 1996; Tokmakdis et al. 2003). Despite the prevalence of its use amongst athletic populations, it is unclear whether IBP provides an ergogenic benefit to performance in a 'fresh' state. Nonetheless, while there is some evidence to suggest that the increases in muscle soreness, pain, damage and contractile dysfunction after contraction-induced muscle damage can be attenuated following NSAID administration (Ebbeling & Clarkson, 1989; Hasson et al. 1993; Pizza et al. 1999; Tokmakdis et al. 2003), there is also evidence that NSAID administration does not impact these variables after muscle damage is induced (Da Silva et al. 2015; Donnelly et al. 1990; Grossman et al. 1995; Tokmakdis et al. 2003; VanHeest et al. 2002). Taken together, these observations suggest that the ergogenic potential of administering IBP, a purported analgesic and anti-inflammatory agent (Fridén & Lieber, 1992), is limited without prior induction of muscle damage, and even when muscle damage is induced, and inflammation and pain sensation are correspondingly increased, the effect of IBP ingestion on exercise performance is equivocal. Further research is therefore required to

assess the potential of IBP to enhance performance and the mechanisms that might underpin any ergogenic effect of IBP ingestion.

2.6 Study aims and hypotheses

The overall aim of the thesis was to assess the efficacy of acute oral consumption of non-specific cox-inhibitors (e.g., ACT and IBP) to improve exercise performance and the potential underlying neurophysiological mechanisms for any ergogenic effects of ACT and IBP ingestion.

Study 1: The effect of acute acetaminophen ingestion on performance during maximal intermittent knee extensor exercise.

Aims: Acute consumption of acetaminophen has been shown to improve exercise performance by increasing pain tolerance/lowering pain sensation. However, the neurophysiological bases of this effect and how it impacts the parameters of the torque-time relationship, critical torque and W′, had yet to be established. The purpose of this initial study was to test the efficacy of acute acetaminophen consumption to improve total work, critical torque, muscle activation and the rate of neuromuscular fatigue development, including markers of central and peripheral fatigue development, using a controlled experimental model. Specifically, this study sought to assess the ergogenic potential of 1 g acetaminophen ingestion prior to completion of a 5 min single-leg maximal intermittent knee-extension test as this afforded the opportunity to robustly assess performance, and neuromuscular, peripheral and central fatigue development.

Study 2: The effect of acute acetaminophen ingestion on muscle activation and performance during a 3-min all-out cycling test.

Aims: The subsequent study sought to assess whether the effect of acetaminophen on performance in a small muscle mass model was transferable to a larger muscle mass model. Therefore, a standard therapeutic dose of acetaminophen (1 g) was administered prior to assessing performance in an experimental model with improved translation to whole body exercise performance by conducting cycling exercise. This is important since the exercise modality employed, and volume of skeletal muscle mass engaged, is known to influence the extent, and underpinning mechanisms, of neuromuscular fatigue development.

Study 3: The neuromuscular and muscle metabolic responses to contralateral limb fatigue following acute acetaminophen ingestion.

Aims: The existence of non-local muscle fatigue is thought to be predominantly centrally mediated. Since the mechanisms for the purported ergogenic effect of acetaminophen ingestion are also thought to be predominantly centrally mediated, this study sought to test the potential for acetaminophen ingestion to attenuate contralateral limb fatigue and whether this effect was greater compared to short-duration unilateral severe-intensity exercise in which fatigue development is predominantly peripherally mediated. This study also used ^{31}P-MRS to assess whether acute acetaminophen ingestion (1 g) permitted the attainment of a greater degree of perturbation to intramuscular phosphorous substrates and metabolites during exercise to provide novel insight into the mechanisms by which acute acetaminophen ingestion might be ergogenic.

Study 4: The effect of acute ibuprofen ingestion on performance and fatigue development during maximal intermittent knee extensor exercise and an all-out cycling test.

Aims: Whilst research suggests that acute consumption of pharmacological analgesics can improve exercise performance, the ergogenic potential of ibuprofen administration is poorly understood, particularly when muscle damage is not present or inflammation is not elevated above baseline levels. This is an important research question as ibuprofen is regularly consumed by athletes to enhance performance. In addition, as ibuprofen is known to act both centrally and peripherally to respectively reduce the sensation of pain and tissue inflammation, this study tested the efficacy of oral ibuprofen ingestion to improve performance using the same protocols as administered in studies 1 and 2 and how this compared to the effects observed following acetaminophen ingestion. This may yield important information on the relative ergogenic efficacy of COX inhibitors after acute ingestion of the recommended therapeutic dose (400 mg).

CHAPTER 3
GENERAL METHODS

The general methods that have been applied throughout this thesis will be explained in this chapter. The specific methods in each experimental chapter will be fully described in the methods section of the respective chapters. The experimental studies included in this thesis entailed 524 exercise tests. All exercise tests in the following experimental chapters took place in an air conditioned exercise physiology laboratory or MRI suite at sea level with an ambient temperature of 18-22ºC.

3.1 Pre-test procedures

3.1.1 Ethical approval

Prior to the commencement of data collection, ethical approval for each study (*chapters 4-7*) was obtained from the University of Exeter Research Ethics Committee (Sport and Health Sciences). For each study, participants were provided with an information sheet providing an explanation of the potential risks and benefits of participation, and information regarding data storage safety, publication and anonymity. After reading this information sheet, participants were afforded the opportunity to ask any questions they had about the study and the participation requirements. Subsequently, participants provided their written, informed consent to take part if they were happy to participate. In addition, participants completed a health questionnaire prior to data collection for each study. This health questionnaire incorporated specific questions for each study relating to the safety of the electrical and magnetic stimulation protocols, exercise protocols and, where appropriate, the ingestion of pain relief medication prior to performing intense exercise (appendix 9.1). Participants were made aware that they were free to withdraw from study participation at any point with no disadvantages to themselves.

3.1.2 Health and Safety

All procedures and protocols utilised in the studies that comprise this PhD thesis conformed to the health and safety guidelines and risk assessments of the Department of Sport and Health Sciences at the University of Exeter. Exercise physiology laboratories and other experimental testing areas were

clean, safe and suitable for conducting human exercise physiology experiments. Routine safety checks on all testing areas and equipment used were also conducted by members of the technical staff.

3.1.3 Participants

A range of active male volunteers, aged 18-45 yrs, were recruited across the experimental chapters including participants from the University student and staff population and from the local community. All volunteers were screened prior to recruitment to ensure suitability for participation. Participants were excluded when the prior completion of the health questionnaire revealed any health concerns that might have put them at risk during the experimental conditions. For example, any history of motor or neurological disorders or any complications relating to the ingestion of paracetamol and/or non-steroidal anti-inflammatory medication. In each study, participants were familiarised with the experimental protocols and procedures (i.e., measurements of neuromuscular function) until the coefficient of variation (CV %) between trials was considered acceptable. Subjects were instructed to arrive at the laboratory on each occasion in a rested and fully hydrated state, at least 3 h post-prandial, to avoid strenuous exercise in the 24 h preceding each testing session and to refrain from consuming caffeine and alcohol in the 12 h preceding each visit. All tests were performed at a similar time of day (± 2 h) to account for daily variations in neuromuscular excitability (Küüsmaa et al. 2015; Tamm et al. 2009). Participants were also required to refrain from analgesics and any form of anti-inflammatory medication during the testing period. Following the first experimental visit, participants were instructed to replicate their usual diet and exercise regime as closely as possible for each subsequent trial.

3.1.4 Anthropometry

All experimental testing procedures (excluding tests within MRI scanner) were conducted in the Sport and Health Sciences department at the University of Exeter. Prior to the commencement of data collection, participants' height, body mass, age and, where appropriate, leg dominance were recorded. Height (m) and body mass (kg) were measured using a stadiometer (Seca 220, Seca Limited, Birmingham, UK) and calibrated electronic scales (Seca 220, Seca Limited, Birmingham, UK), respectively, using the standard procedures for

height and weight measurement. Upon returning to the laboratory for each experimental test, participants had their body mass determined before performing exercise tests.

3.1.5 Supplementation

For Chapters 4-6, 1 g of maltodextrin (placebo, PL) or 1 g of ACT were ingested orally, 60 min prior to the exercise bout such that the start of the exercise trial was expected to coincide with attainment of peak plasma [ACT] (Anderson et al. 2008; Forrest et al. 1982). Following oral ingestion, ACT is rapidly absorbed from the gastrointestinal tract and its bioavailability ranges from 70 to 90% (Forrest et al. 1982). Both 0.5 g and 1.0 g doses of ACT have been shown to have an analgesic effect superior to that of placebo from 1 and up to 5 h after administration, with peak analgesia effects achieved between 1 and 2 h following oral ingestion (Nielsen et al. 1992). For chapter 7, 400 mg IBP was ingested prior to the exercise bout (s). Following oral administration, IBP is rapidly absorbed but both peak plasma concentrations and maximal analgesic onset are achieved within 1.5-2 hours of oral administration (Albert & Gernaat, 1984). However, there has been shown to be a significant correlation between plasma IBP levels and the resultant degree of pain relief, particularly ~1 hour after administration (Laska et al., 1986). The absolute bioavailability of IBP indicates a complete absorption when administered (Martin et al. 1990).

3.2 Apparatus, procedures & measurements

To investigate exercise-induced fatigue and its underlying mechanisms, a number of experimental apparatus and a range of methodologies were used. Techniques such as magnetic resonance spectroscopy (MRS), femoral nerve stimulation and EMG were employed to provide insight into the specific site of failure and mechanistic basis for the reduction in force production. The following sections will outline the main procedures used in this thesis. More specific information is provided in each experimental chapter.

Exercise testing procedures

Subjects completed (a) preliminary trial(s) for familiarisation to the measurement techniques and experimental protocol for all chapters. For all experimental chapters, trials started with a standardised warm-up routine and

testing of the optimal EMG electrode, anode, and cathode placement and intensity required for peripheral nerve stimulation.

3.2.1 Cycle ergometry and ramp incremental test

All cycling exercise tests in Chapters 4 and 7 were performed on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands, figure 3.1). During the initial visits to the laboratory, ramp incremental tests to volitional exhaustion were performed in order to acquire individual metrics including peak work rate and $\dot{V}O_{2peak}$ and the gas exchange threshold (GET). A linear factor was used to determine the work rates during a 3-min maximal cycling test (described in chapters 4 and 7). The work rate imposed by the linear function is cadence-dependent and is given by the following equation: Linear factor = Power output ÷ Cadence^2. The ramp incremental tests used in chapters 4 and 7 commenced with the completion of a 3 min period of pedalling at 20 W (baseline period) at a self-selected cadence (80-100 rpm). Upon completion of baseline cycling, the work rate was linearly increased at a rate of 30 W·min^{-1} until the limit of tolerance (T_{lim}), using the cadence-independent power-time function. The test was terminated when the pedal rate fell by >10 rpm below the self-selected cadence despite strong verbal encouragement. The self-selected cadence, as well as the position of the handle bars and saddle, were recorded during the initial visit and repeated for all subsequent visits. During all cycle tests, pulmonary gas exchange and ventilation were measured breath by breath.

Figure 3.1. *Participant performing a 3-min all-out cycling test on an electronically braked cycle ergometer. Note neuromuscular function is also being assessed via peripheral nerve stimulation and electromyography of the quadriceps.*

3.2.2 Isokinetic dynamometry

An isokinetic dynamometer (Biodex System 3 Medical Systems 830-200, Shirley, N.Y. 11967 USA) was used in chapters 4 and 7 for assessment of torque and fatigue of the knee extensors and adjusted so that the axis of rotation of the lever arm was in line with the lateral epicondyle of the right femur. Subjects were seated with the hip and knee joints at relative angles of 155° and 90°, respectively (figure 3.2). The remainder of the chair settings were recorded and replicated in all subsequent trials to ensure an identical body position was assumed throughout the experimental trials. The semi-supine position was employed as it permitted better access to the superficial femoral nerve for peripheral nerve stimulation as verified during pilot testing. Inelastic padded Velcro straps were fastened at the ankle, quadriceps, hip and shoulders to maintain a stable body position.

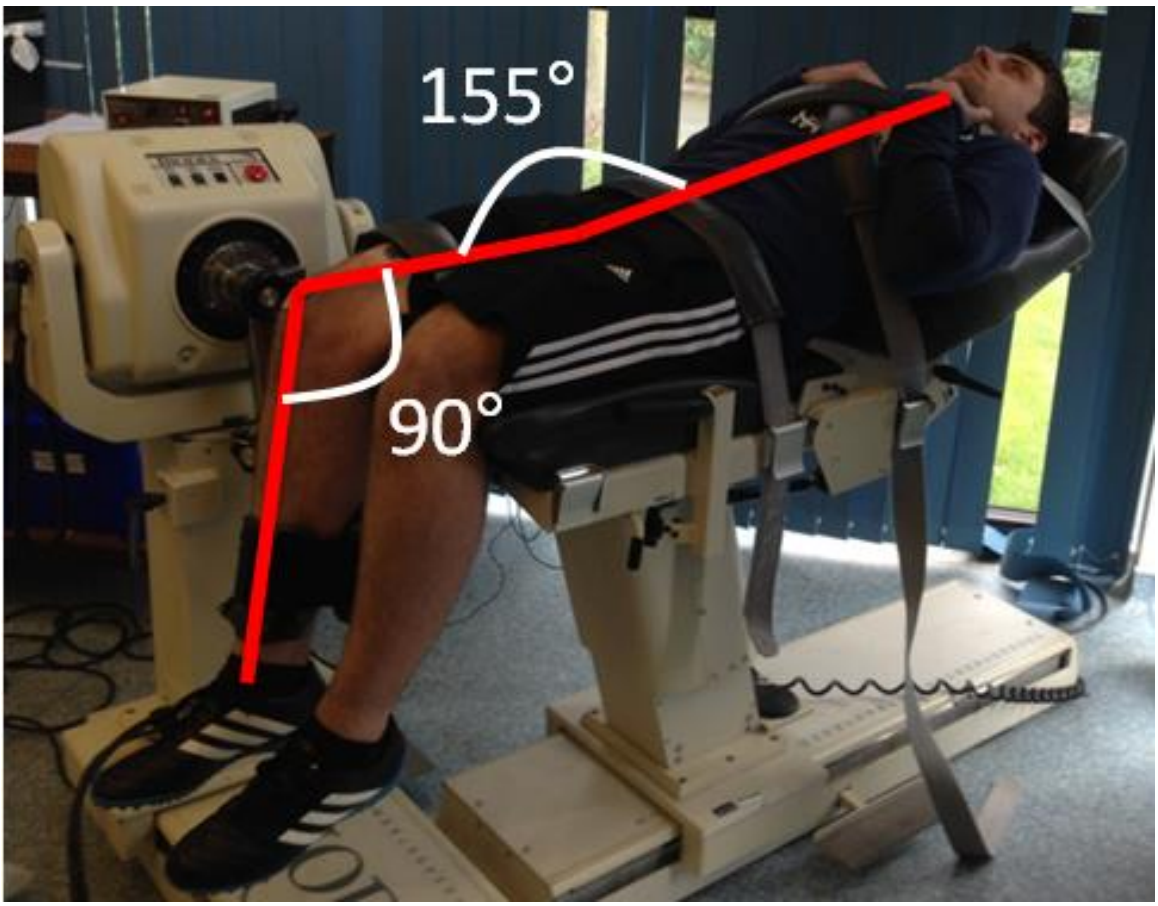

Figure 3.2. *A participant preparing to undertake a maximal voluntary contraction of the quadriceps muscles.*

3.3 Measurements

3.3.1 Reliability

To assess the reliability of the mean maximal voluntary contraction (MVC) performance and EMG amplitude during the 60 MVC test used in chapters 4 and 7, 12 individuals completed two 60 MVC tests on separate days yielding a coefficient of variation of 2.9% and 4.8% for mean MVC torque and EMG amplitude, respectively. The cycling ergometer used in chapters 5 and 7 has been shown to be highly reproducible, making it a reliable method for monitoring cycling performance (CV 1.0-5.0%; Driller, 2012; Earnest et al. 2005). For chapters 5 and 7, participants completed a 3 min all-out cycling test. End power-output has been demonstrated to yield a coefficient of variation within <2% (Vanhatalo et al. 2007, Wright et al. 2017). The coefficient of variation for severe-intensity knee-extension exercise performed within the MRI for chapter 6 for time to task failure in our laboratory is 5–7% (Bailey et al. 2010). To assess the within and between-day reliability of all remaining

neuromuscular measures used throughout this thesis in chapters 4, 5 and 7, a variety of evoked and voluntary contractions were administered and/or performed at rest with 12 individuals yielding respective coefficients of variation of 4.7%, 6.2% and 4.5% for voluntary activation (VA), M-wave amplitude and quadriceps potentiated twitch (pTw), respectively.

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

The GET was determined as: 1) the first disproportionate increase in $\dot{V}CO_2$ versus $\dot{V}O_2$; 2) an increase in minute ventilation ($\dot{V}E$) relative to $\dot{V}O_2$ with no increase in $\dot{V}E/\dot{V}CO_2$ and; 3) the first increase in end-tidal O_2 tension with no fall in end-tidal CO_2 tension. The GET was estimated independently by three experienced assessors using code labelled data files such that the assessors were blind to the subject being assessed and others estimations. $\dot{V}O_{2peak}$ was determined as the highest 30-s mean value attained during ramp incremental tests. Oxygen uptake and other ventilatory and gas exchange responses (e.g., $\dot{V}_E$, $\dot{V}CO_2$ and RER) were measured using a Cortex MetaLyzer (Cortex, Leipzig, Germany). Briefly, subjects wore a mask connected to an impeller turbine transducer assembly. Expired gas volume and concentration signals were continuously sampled at 100 Hz. These analysers were calibrated before each test with gases of known concentration, and the turbine volume transducer was calibrated using a 3-L syringe (Hans Rudolph, KS).

3.3.3 Torque

Knee extensor torque during voluntary and evoked contractions was measured via the use of isokinetic dynamometry (Biodex System 3 Medical Systems 830-200, Shirley, N.Y. 11967 USA). Knee-extensor torque from the Biodex® was sampled at 1,000 Hz and low-pass filtered at 40 Hz, before being displayed on a wide screen monitor using Spike2 (CED, Cambridge, UK). Voluntary torque was expressed as a percentage (%) of pre-exercise MVC.

Assessment of neuromuscular function

Throughout this thesis, neuromuscular function was assessed before, during and after the exercise tests. This included the characteristics of the M-wave response to supra-maximal femoral nerve stimulation, contractility of the muscle (e.g., maximal rate of force development, contraction time and rate), voluntary

activation (VA) and potentiated twitch (pTw). Data were analysed using a custom written script developed in Spike2 software (Cambridge Electronic Design, Cambridge, UK). Responses from peripheral nerve stimulation were recorded as described below in order to assess changes in membrane excitability (via M-wave changes), muscle activation and neural drive drive (via changes in EMG and VA) and peripheral muscle function (via changes in pTw).

3.3.4 Electromyography

Electromyography refers to the technique used in neuromuscular physiology to record myoelectric signals formed by physiological variations in the state of muscle fibre membranes. In this thesis, EMG was measured via surface electrodes placed on the skin superior to the muscle of interest. Electrodes placed on the surface of the skin record the sum of all action potentials propagated along the sarcolemma to the muscle fibres at that point in time. For all experimental chapters, surface EMG activity was recorded from *m.*vastus lateralis, *m.*vastus medialis and *m.*rectus femoris of the quadriceps and lateral head of the *m.*biceps femoris of the hamstring of the right leg using active bipolar bar electrodes single differential configuration (DE2.1, DelSys Inc, Boston, MA, USA, figure 3.3). It was necessary to measure activity from the biceps femoris as inadvertent co-activation of antagonist muscles can affect the measurement of voluntary activation when using twitch interpolation (Allen et al. 1995). Surface EMG activity of both the right and left leg were recorded for chapter 6. The two electrodes, which were housed into one unit, were placed over the respective muscle bellies parallel to the longitudinal axis of each muscle in parallel with the Surface Electromyography for the non-invasive assessment of muscles (SENIAM) guidelines. Final electrode positions were recorded and marked with indelible ink to ensure consistent placement between experimental sessions. The skin area underneath each EMG electrode was shaved, then exfoliated and cleaned with alcohol to minimise the skin impedance. Double-sided adhesive tape and a hypoallergenic medical tape were used to ensure the EMG sensor stability during exercise.

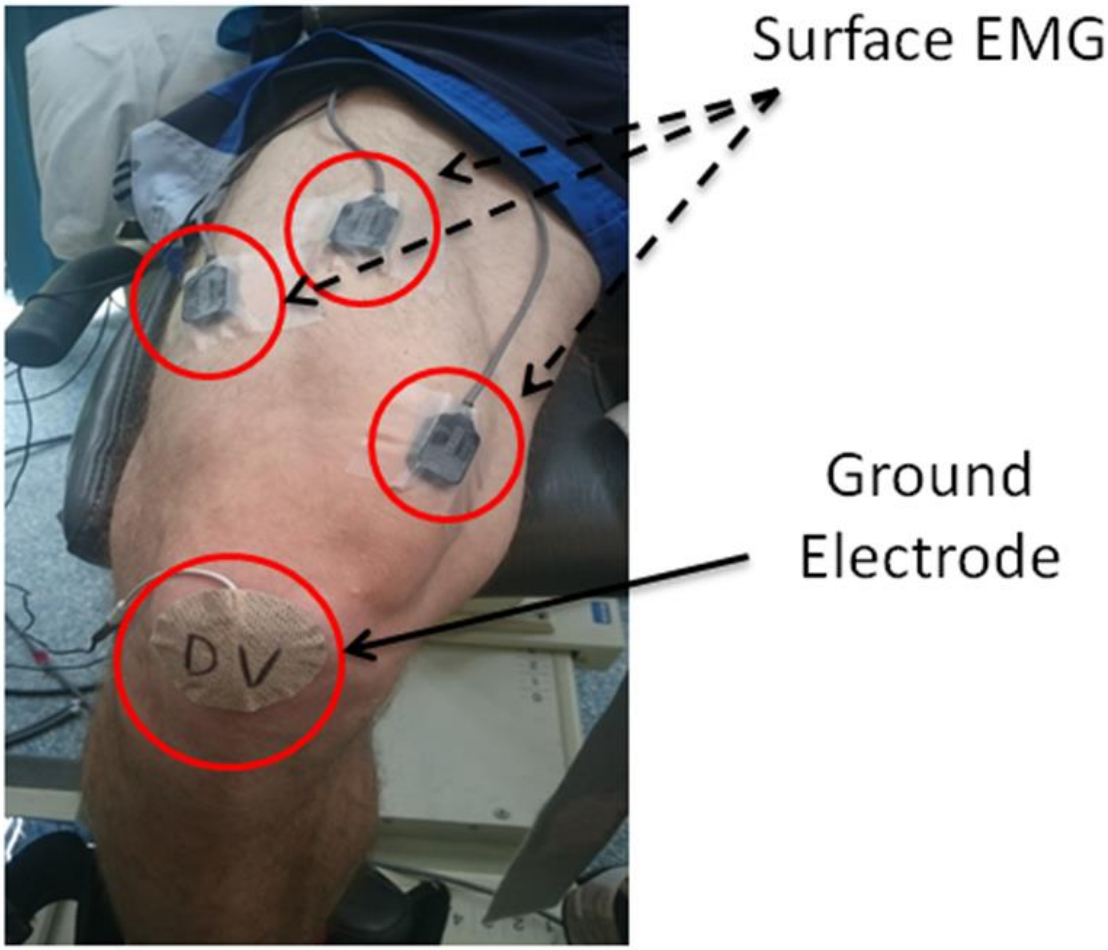

Figure 3.3. *Experimental set up of surface EMG electrodes superficial to the quadriceps muscles. Surface EMG activity was recorded from vastus lateralis, vastus medialis and rectus femoris of the quadriceps and biceps femoris of the hamstring of the right leg using active bipolar bar electrodes single differential configuration. The ground electrode was placed over the patella of the right leg.*

The EMG signal was pre-amplified (1,000 x), band-pass filtered (20–450 Hz, Bagnoli-8, DelSys Inc, Boston, MA, USA), and then transferred to a computer with a sampling frequency of 2 kHz. EMG data were recorded continuously and digitised synchronously with 16 bit resolution via an A/D converter (±5 V range, CED 1401 power, Cambridge, UK). Throughout, a digital smoothing algorithm was used to provide a mean EMG value over time as bursts of raw EMG during voluntary activity are not consistently formed. We used the root mean square method (EMG_{RMS}), which reflects the mean power of the signal and is recommended for measuring muscle activation during contractions of constant force (De Luca, 1997). The RMS is calculated by squaring each data point, summing the squares, dividing the sum by the number of data points and then

taking the square root over a given time frame (Konrad, 2005). EMG_{RMS} was normalised to the pre-exercise maximum (or maximal EMG signal) and the local M-wave amplitude (closest measure of the M-wave to the MVC) to exclude any changes to the EMG trace as a result of changes in local excitability (Millet & Lepers, 2004). The ground electrode was placed over the patella of the right leg so that the electrical potential could be compared to that of a 'reference' electrode located in an environment that is electrically quiet (Basmajian & De Luca, 1985). Throughout this thesis, the signal from the *m.*vastus lateralis was used as the primary measure of EMG activity. It has been shown that, during exercise, EMG activity from the *m.*vastus lateralis can be considered as a surrogate measure of EMG activity of the knee-extensors as a whole (Alkner et al. 2000; Amann et al. 2006; Lepers et al. 2001; Millet et al. 2002; Place et al. 2007; Romer et al. 2006).

3.3.5 Peripheral Nerve Stimulation

Single electrical stimuli were delivered to the femoral nerve applied by a constant current stimulator (Digitimer Stimulator DS7, Digitimer, Welwyn Garden City, Hertfordshire, UK). Specific protocols to assess changes in peripheral and central function are outlined in each experimental chapter. The electrical current is passed to the body tissues by means of two stimulating pads placed on the surface of the skin. Frequency, duration and intensity can be used to describe single pulses delivered by electrical stimulation. The frequency of stimulation refers to how many pulses are delivered per second; this can be from 1 Hz upwards. The duration of each pulse is referred to in microseconds (μs) and the intensity of current is described in milliamps (mA) or voltage (V) (Low & Reed, 1994). The stimulation current was identified by constructing a stimulus-response curve for each individual. Single stimuli (1 Hz, 200 μs duration) were delivered in 20 mA step-wise increments from 100 mA until plateaus were evident in the potentiated twitch force and M_{max}, indicating maximum depolarisation of the femoral nerve. The positive electrode (cathode) was placed over the femoral nerve approximately 3–5 cm below the inguinal ligament in the femoral triangle, and the negative electrode (anode) was placed midway between the iliac crest and the greater trochanter (Sidhu et al. 2009b). The cathode was systematically moved vertically and horizontally and the amplitude of the muscle action potential (M-wave) was monitored to identify the

optimal position of the cathode for attaining maximal peak-to-peak M_{max}. Once the M-wave was elicited, the maximum amplitude (peak-to-peak) of the M-wave was determined (M_{max}) for the *m.*vastus lateralis and *m.*vastus medialis.

To account for activity dependent changes in axonal excitability (Burke, 2002), it was necessary to use a supramaximal level of stimulation. To ensure a supramaximal response, the current was increased by an additional 30% (Goodall et al. 2010; Neyroud et al. 2014; Sidhu et al. 2017). The mean M_{max} was obtained from 3 stimuli, with ~30 s separating each pulse at rest to minimise the effect of potentiation (Hodgson et al. 2005). Maximal rate of force development, maximal relaxation rate, contraction time and contraction rate were analysed for all pTw. For chapters 5 and 7, during cycling exercise, the crank angle at which peripheral nerve stimulation was to be delivered during the trials, was determined for each subject as described by Black et al. (2017) and Sidhu et al. (2012). Briefly, stimulations were delivered at the identified crank angle for all subsequent trials for that participant to align with maximal muscle activation. A custom written sequencer script triggered 3 stimulations, with at least 1 and up to 10 pedal revolutions between each stimulus.

3.3.6 Excitability parameters

Voluntary muscular activity results in a quantifiable EMG response that can be measured on the surface of the skin. The name given to the electrical signal manifest in the muscle fibres leading to the activation and contraction of the muscle fibres is a compound muscle action potential (or M-wave). When responses were evoked by electrical stimulation of the femoral nerve, the M-wave was used to assess changes in membrane excitability. The properties of the waveforms produced following stimulation included the peak-to-peak amplitude and area. The peak-to-peak amplitude was manually identified post-experiment and defined as the absolute difference between the maximum and the minimum points of the waveform (negative and positive deflections). The area was calculated as the integral of the reflected value of the entire waveform (Fowles et al. 2002).

3.3.7 Twitch parameters

In addition to M-waves evoked in skeletal muscle, changes in the resting potentiated muscle twitch and twitches superimposed onto an MVC were measured. A reduction in the potentiated twitch, for example, is indicative of peripheral failure. It has previously been demonstrated that the potentiated twitch is more sensitive to the changes that occur in fatigued muscle when compared to the 'unpotentiated' twitch (Kufel et al. 2002). One proposed mechanism of the potentiated twitch is the phosphorylation of myosin regulatory light chains via myosin light chain kinase, rendering actin-myosin interaction more sensitive to Ca^{2+} released from the sarcoplasmic reticulum (Hodgson et al. 2005). An increased sensitivity of Ca^{2+} increases the rate by which myosin cross-bridges move from a non-force producing state to a force producing state, thus increasing the size of a resting muscle twitch for a given intramyocyte [Ca^{2+}] (Szubski et al. 2007).

Contraction time, maximum rate of force development, maximum relaxation rate and one half relaxation time ($RT_{0.5}$) were analysed by Spike2 for each pTw whereby changes to such characteristics may represent alterations to Ca^{2+} release and uptake (Klitgaard et al. 1989). Contraction time was defined as the time between the stimulus and peak twitch force in response to peripheral nerve stimulation. $RT_{0.5}$ was defined as the time taken for twitch force to decay to half of the peak twitch amplitude. Maximal rate of force development occurs during the initial linear incline of the force response and was calculated as the slope of the tangent drawn to the steepest portion of the curve. Maximal relaxation rate occurs during the initial decline in the force response and was calculated as the slope of the tangent drawn to the steepest portion of the curve. A reduced MRFD is considered to reflect a decrease in the rate of cross-bridge formation (Stein & Parmiggiani, 1981). Reductions in relaxation rate and a prolonged $RT_{0.5}$, are thought to reflect decreases in the maximal rate of weak-to-strong cross-bridge formation and decreases in the maximal rate of cross-bridge detachment, respectively (Westerblad et al. 1997).

3.3.8 'Peripheral' voluntary activation

The sTw can be used to infer changes to VA. VA is defined as the level of neural drive to a muscle during exercise and reflects the ability to fully activate

the target muscle group or the ability of the central nervous system to maximally drive a muscle during voluntary contraction (Merton, 1954) and can be estimated from peripheral, cortical and spinal methods. Briefly, when peak force (approximately 0.5-1-s into a 3-s contraction) was attained during an MVC, a single stimulus was delivered to the femoral nerve to evoke an increment in force. The superimposed twitch torque was calculated as the increment in torque immediately following the pulse during MVCs. The amplitude of the twitch evoked by the peripheral stimulus was compared with the amplitude of the resting potentiated twitch delivered immediately after (1-2 s post) the MVC (figure 2.4) and VA was determined using the following equation:

$$\textit{Voluntary Activation (\%)} = (1 - sTw/pTw) \times 100$$

In a fatigued state, a larger twitch is evoked during an MVC and voluntary activation is incomplete. This indicates that that some motor units are not recruited or not firing fast enough to generate fused contractions e.g., the drive from the motor cortex is sub-optimal. This conclusion cannot be made specific to the motor cortex from peripheral methods as the impairment may be located anywhere upstream from the peripheral nerve.

3.4 Statistical analyses

Statistical analyses in all experimental chapters were performed using the Statistical Package for Social Sciences (SPSS v.23 (Chapter 4 and 7) and SPSS v.24 (Chapters 5-6)). The statistical procedures specific to each chapter are outlined in the Statistical analyses sections of Chapters 4-7. For calculation of effect size, partial eta squared (η^2) was used for omnibus tests. Cohen's d was used to calculate the effect size for paired t-tests and post-hoc comparisons. In all chapters, data are presented as mean ± SD unless otherwise stated and statistical significance was accepted at $P<0.05$.

Acute acetaminophen ingestion improves performance and muscle activation during maximal intermittent knee extensor exercise

Original investigation

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Paul T. Morgan[1], Joanna L. Bowtell[1], Anni Vanhatalo[1], Andrew M. Jones[1] and Stephen J. Bailey[1,2]

[1]Sport and Health Sciences, College of Life and Environmental Sciences, University of Exeter, St. Luke's Campus, Heavitree Road, Exeter, EX1 2LU, UK.

[2]School of Sport, Exercise and Health Sciences, Loughborough University, Ashby Road, Loughborough, Leicestershire LE11 3TU, UK.

Chapter 4: Acute acetaminophen ingestion improves performance and muscle activation during maximal intermittent knee extensor exercise

ABSTRACT

Aim: Acetaminophen is a commonly used medicine for pain relief and emerging evidence suggests that it may improve endurance exercise performance. This study investigated some of the physiological mechanisms by which acute acetaminophen ingestion might blunt muscle fatigue development. **Methods**: Thirteen active males completed 60 × 3 s maximum voluntary contractions (MVC) of the knee extensors with each contraction separated by a 2 s passive recovery period. This protocol was completed 60 min after ingesting 1 g of maltodextrin (placebo) or 1 g of acetaminophen on two separate visits. Peripheral nerve stimulation was administered every 6th contraction for assessment of neuromuscular fatigue development, with the critical torque (CT), which reflects the maximal sustainable rate of oxidative metabolism, taken as the mean torque over the last 12 contractions. Surface electromyography was recorded continuously as a measure of muscle activation. **Results**: Mean torque (61 ± 11 vs. 58 ± 14 % pre-exercise MVC) and CT (44 ± 13 vs. 40 ± 15 % pre-exercise MVC) were greater in the acetaminophen trial compared to placebo (both $P<0.05$) , respectively. Voluntary activation and potentiated twitch declined at a similar rate in both conditions ($P>0.05$). However, the decline in electromyography amplitude was attenuated in the acetaminophen trial, with electromyography amplitude being greater compared to placebo from 210 s onwards ($P<0.05$). **Conclusion**: These findings indicate that acute acetaminophen ingestion might be ergogenic by increasing CT and preserving muscle activation during high-intensity exercise.

Key words: Analgesic; critical torque; electromyography; neuromuscular fatigue; single leg exercise

Chapter 4: Acute acetaminophen ingestion improves performance and muscle activation during maximal intermittent knee extensor exercise

INTRODUCTION

Neuromuscular fatigue is defined as a decrease in skeletal muscle force production capacity (Gandevia, 2001). This neuromuscular fatigue development can arise from physiological perturbations within the central nervous system, termed central fatigue, or within, or distal to, the neuromuscular junction, termed peripheral fatigue (Gandevia, 2001). Exercise-induced fatigue is a complex, multi-factorial process, the physiological bases of which are still widely debated (Enoka & Duchateau, 2008; Gandevia, 2001; Hureau et al. 2016; Place et al. 2010; Taylor et al. 2016). Indeed, recent research suggests that peripheral fatigue and central fatigue develop inter-dependently and are likely to interact in a coordinated manner to determine neuromuscular fatigue development (Hureau et al. 2016).

Following the onset of muscle contractions, group III and IV afferents discharge in response to mechanical and metabolic stimuli, contributing to the sensation of muscle pain during exercise (McCord and Kaufman, 2010; O'Connor & Cook, 1999; Pollak et al. 2014). Group III/IV muscle afferent feedback, and the associated sensation of pain, appears to play a role in neuromuscular fatigue development through modulating both central and peripheral fatigue. Indeed, when the ascending projection of group III and IV muscle afferents is attenuated via intrathecal fentanyl administration, central motor drive is increased (as inferred via electromyography, EMG, and peripheral fatigue development is expedited (Amann et al. 2009, 2011; Blain et al. 2016). Therefore, interventions that can modulate skeletal muscle pain sensation have the potential to impact muscle activation and exercise-induced fatigue development.

Acetaminophen (ACT; or paracetamol) is a commonly used non-prescription pain reliever which is considered one of the safest non-opioid analgesics at therapeutic doses (Toussaint et al. 2010). Acute ACT consumption has been shown to improve exercise performance concomitant with a lower pain sensation (Foster et al. 2014; Mauger et al. 2010). The mechanisms that underlie the analgesic effect of ACT are not completely understood but are considered to be predominantly mediated by central factors (Anderson, 2008; Graham et al. 2013; Smith, 2009; Toussaint et al. 2010). Conventionally, the

analgesic effect of ACT has been attributed to the inhibition of cyclooxygenase enzymes, which stimulate nociceptor discharge through the synthesis of prostaglandins (Graham et al. 2013; Jóźwiak-Bębenista and Nowak, 2014). There is also evidence that the analgesic effect of ACT is linked to potentiation of descending serotoninergic pathways (Andersson et al. 2011; Pickering et al. 2006, 2008), and to modulation of opioid and cannabinoid receptors (Graham et al. 2013; Ottani et al. 2006). These mechanisms likely interact to lower pain sensation after acute ACT ingestion by increasing the nocioceptive stimuli required to evoke a given pain sensation. Although ACT ingestion has been reported to increase resting cortico-spinal excitability, as inferred from an increased motor evoked potential amplitude (Mauger & Hopker, 2013), the neuromuscular bases for blunted exercise-induced fatigue development following ACT ingestion have yet to be investigated.

In addition to altering pain sensation and neuromuscular function, ACT ingestion might attenuate fatigue development and enhance performance by modulating aspects of the power or torque-duration relationship. The asymptote of the hyperbolic torque-duration relationship is termed the critical torque (CT) and can be estimated from the mean torque produced over the last 12 maximum voluntary contractions (MVC) of a 60 MVC knee extension protocol (Burnley, 2009). The CT reflects the maximal sustainable rate of oxidative metabolism (Jones et al. 2008 *for review*) and represents a critical threshold for neuromuscular fatigue development (Burnley et al. 2012). The curvature constant of the torque-duration hyperbola is termed the W′ and represents a fixed amount of torque-impulse (surrogate measure of ‘total work’) that can be completed above CT (Burnley and Jones, 2016; Jones et al. 2008; Poole et al. 1988). Together, CT (or critical power, critical speed for other forms of exercise) and W′ (or its equivalent) can be used to accurately predict exercise performance (Black et al. 2014, Burnley et al. 2012). An intervention that enhances high-intensity exercise tolerance or performance, or attenuates fatigue development, would be expected to enhance CT or W′ (or their exercise-modality-specific equivalents). For example, critical power, but not W′, is increased following endurance training concomitant with improved endurance exercise performance (Vanhatalo et al. 2008). Therefore, the reported

improvement in exercise performance with acute ACT ingestion (Foster et al. 2014; Mauger et al. 2010, 2014) would be expected to be linked to improvements in CT and/or W′, but this has yet to be investigated.

The purpose of this study was to test the hypotheses that, compared to placebo, acute consumption of 1 g ACT would reduce the rate of neuromuscular fatigue development, and increase total impulse (torque-time integral), CT and muscle activation during a 5-min single-leg intermittent MVC test.

METHODS

Participants

Thirteen healthy male volunteers (mean ± SD: age 31 ± 7 years, height 1.76 ± 0.08 m, body mass 75 ± 11 kg) provided written, informed consent to participate in the present study, which was approved by the Ethics Committee of Sport and Health Sciences (University of Exeter). After being informed of the experimental procedures and associated risks, all participants completed a medical health questionnaire to ensure it was safe to consume ACT prior to performing exhaustive exercise. Subjects were not consumers of any 'pain relief' medication (prescription or non-prescription) over the course of the study. None of the subjects had a history of motor or neurological disorders.

Experimental Design

Subjects visited the laboratory on three occasions over a 3- to 4-week period with all tests separated by at least 1 week and conducted at a similar time of day (± 90 min) to limit changes in quadriceps strength and to account for diurnal variations in neuromuscular excitability. The first laboratory visit was used to familiarise subjects to the measurements and experimental protocol described below. During these sessions, the settings and placement of the dynamometer and electromyography (EMG) and peripheral nerve stimulation electrodes were recorded for each subject. Subsequently, subjects performed the fatiguing protocol under two conditions *(see 'Experimental Protocol')*: placebo (PL) and ACT.

Experimental Protocol

Subjects completed a preliminary trial for familiarisation to the measurement techniques and experimental protocol. An isokinetic dynamometer (Biodex System 3, Shirley, NY, USA) was used in all tests and adjusted so that the axis of rotation of the lever arm was in line with the lateral epicondyle of the right femur. Subjects were seated with the hip and knee joints at relative angles of 155° and 90°, respectively. The remainder of the chair settings were recorded and replicated in all subsequent trials to ensure an identical body position was assumed throughout the experimental trials. The semi-supine position was employed as it permitted better access to the superficial femoral nerve for peripheral nerve stimulation as verified during pilot testing. Inelastic padded Velcro straps were fastened at the ankle, quadriceps, hip and shoulders to maintain a stable body position. The procedures adopted during the familiarisation trial were replicated in all experimental trials.

The trials on visits 2 and 3 were completed in a double-blind, randomised fashion using a counter-balanced cross-over experimental design. Prior to each visit, subjects were required to refrain from caffeine (for at least 12 h), strenuous exercise and alcohol (for at least 24 h), analgesics and any form of anti-inflammatory drug (for the duration of the experimental trial) and to arrive in a fully rested, hydrated state. Subjects were instructed to maintain their usual diet and exercise regime during the study. 1 g of maltodextrin (placebo) or 1 g of acetaminophen was ingested orally, 60 mins prior to the exercise bout such that the start of the exercise trial was expected to coincide with attainment of the peak plasma [acetaminophen] (Anderson et al. 2008; Forrest et al. 1982). Following oral ingestion, ACT is rapidly absorbed from the gastrointestinal tract and its bioavailability ranges from 70 to 90% (Forrest et al. 1982). The trials started with a standardised isometric warm-up routine (10 isometric contractions for 3 s at 50% of pre-exercise MVC as determined during familiarisation testing) and testing of the optimal EMG electrode, anode, and cathode placement and stimulation intensity for peripheral nerve stimulation. Neuromuscular function was assessed pre-trial, during and (<10 s) post-trial. Single peripheral nerve stimulation pulses were manually triggered at rest to determine pre-exercise neuromuscular function, namely the characteristics of the M-wave response (M-

wave amplitude; M_{max}) to supra-maximal nerve stimulation, contractility of the muscle (e.g., maximal rate of force development, half relaxation time and contraction time), voluntary activation (VA) and potentiated twitch force (pTw). During maximal voluntary contractions (MVCs), peripheral nerve stimulation pulses were triggered to occur as soon as a peak torque was achieved (typically 1.5 s into a 3 s contraction), each separated by a 45 s rest. The stimuli were also delivered 1-2 s after the cessation of the contraction to provide a resting pTw. Identical measurements were repeated as soon as possible (<10 s) after the fatiguing exercise to determine post-exercise neuromuscular function (see figure 4.1).

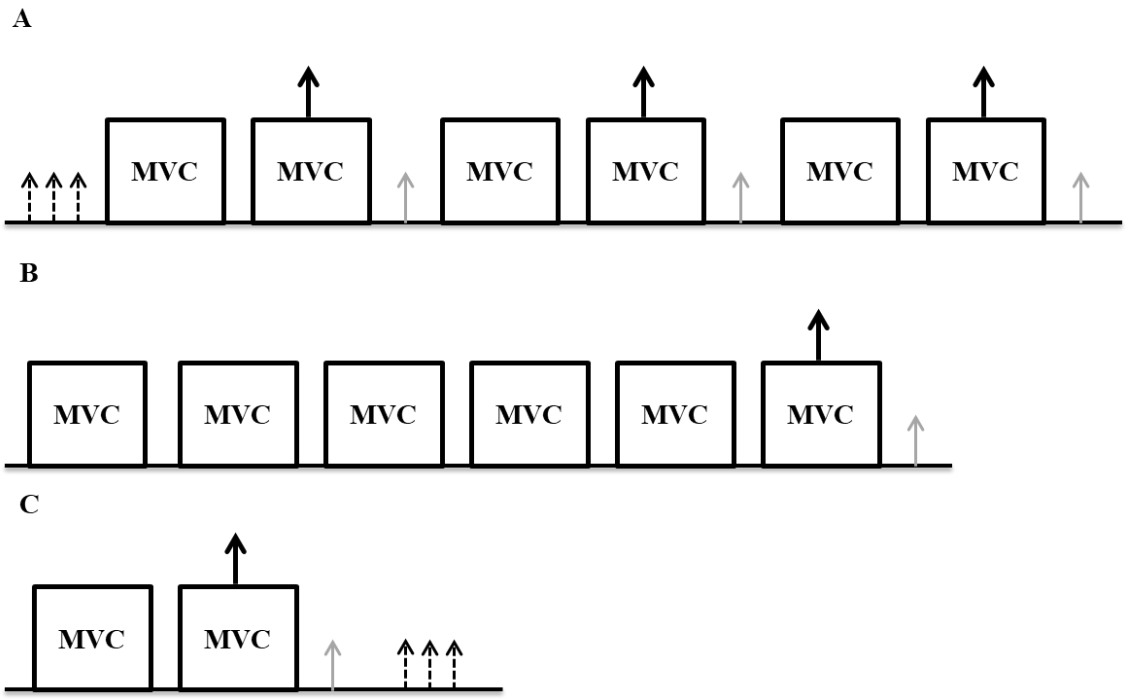

Figure 4.1. *Graphic overview of the procedures used in the current study. A and C: Procedures employed pre and post (<10 s) high-intensity exercise, respectively. 10 s separated each single pulse stimulation administered at rest (small dashed arrows). A 45 s rest period separated maximal efforts (MVCs). Single pulse stimuli were administered during peak force production of MVCs (large solid arrow) and immediately (1-2 s post; small grey arrows). B: 60 MVC protocol of the knee extensors. The figure presents a period of 30 s which is repeated sequentially for 5 min. Each MVC was held for 3 s and interspersed by a 2 s passive recovery period. Every 6th MVC was accompanied by single pulse stimuli administered during peak force production (Large solid arrow) and immediately following (<2 s post; small grey arrows). This cycle was repeated 10 times such that the protocol spanned 5 minutes requiring the completion of 60 MVCs. MVC, maximal voluntary isometric contraction. Surface electromyography (EMG) was measured throughout.*

The intermittent isometric contraction protocol used in this study had a similar design to that presented by Burnley (2009). The protocol consisted of repeated brief MVCs (3 s contraction, 2 s rest), timed via a visual prompt to ‘go’ and ‘relax’ (separate to the torque output screen), accompanied by the same verbal instructions from the experimenter. The protocol was terminated when the subjects completed the 60th MVC. Every 6th contraction was accompanied by

peripheral nerve stimulation during and post MVC (as described for pre- and post-trial measurements). Subjects were not given a visual representation of the torque produced during each MVC or made aware of the number of MVCs elapsed during the protocol. Subjects were instructed to continue to perform maximal contractions throughout. The coefficient of variation for MVC performance (mean torque through 60 MVCs) and electromyography root mean square (EMG_{RMS}) amplitude using this experimental set up was 2.9% and 4.8%, respectively as calculated during pilot testing.

Measurements

Torque

Knee-extensor torque from the Biodex dynamometer was sampled at 1,000 Hz and low-pass filtered at 40 Hz, before being displayed on a wide screen monitor using Spike2 (CED, Cambridge, UK). Torque was expressed throughout as a percentage (%) of pre-exercise MVC.

Electromyography

Surface EMG activity was recorded from vastus lateralis, vastus medialis and rectus femoris of the quadriceps and biceps femoris of the hamstring of the right leg using active bipolar bar electrodes single differential configuration (DE2.1, DelSys Inc, Boston, MA, USA). The electrodes were placed over the respective muscle bellies (SENIAM guidelines). Double-sided adhesive tape and a hypoallergenic medical tape were used to ensure the EMG sensor stability for recording electrodes. The skin area underneath each EMG electrode was shaved, then exfoliated and cleaned with alcohol to minimise the skin impedance. The EMG and torque signals were pre-amplified (1,000 x), band-pass filtered (20–450 Hz, Bagnoli-8, DelSys Inc, Boston, MA, USA), and then transferred to a computer with a sampling frequency of 2 kHz. EMG and torque data were recorded continuously and digitised synchronously with 16 bit resolution via an A/D converter (±5 V range, CED 1401 power, Cambridge, UK). The electrodes were used to record: 1) evoked muscle action potentials (peak-to-peak amplitude of the M-wave); and 2) EMG to estimate muscle activity and the output of spinal motoneurons (motor unit recruitment and firing frequency). EMG was average rectified using the root mean square method (EMG_{RMS}).

EMG_{RMS} was then normalised to the pre-exercise maximum (or maximal EMG signal) and the local M-wave amplitude (closest measure of the M-wave to the MVC) in order to exclude any changes to the EMG trace to changes in local membrane excitability. The ground electrode was placed over the patella of the right leg.

Peripheral Nerve Stimulation

Electrical stimulation was applied by a constant current stimulator (Digitimer Stimulator DS7, Digitimer, UK). M-waves were elicited by supramaximal percutaneous electrical stimulation of the femoral nerve (200 μs duration). The cathode was placed over the femoral nerve in the inguinal fossa, approximately 3–5 cm below the inguinal ligament in the femoral triangle. Once the M-wave was elicited, the maximum amplitude (peak-to-peak) of the M-wave was determined (M_{max}) for the vastus lateralis and vastus medialis. To determine the stimulation intensity (current), single stimuli were delivered in 20 mA step-wise increments from 100 mA until a plateau in quadriceps pTw and M-wave were observed. To ensure a supramaximal response, the current was increased by an additional 30% (mean ± SD current = 194 ± 81 mA; Burke, 2002; Goodall et al. 2010; Neyroud et al. 2014). The average M_{max} was obtained from 3 stimuli, with ~8-10 s separating each pulse at rest. Peak torque, maximal rate of force development, half relaxation time and contraction time were analysed for all pTw.

Data Analyses

Data were analysed using a custom written script developed in Spike2 software (CED, Cambridge, UK). Mean torque for each 3 s contraction was determined for all tests as the mean value over a 1 s period which approximated the plateau level of the highest torque (e.g., 500 ms before and after the peak torque). The pTw was calculated as the peak torque achieved following the single pulse delivered 1 s post-MVC. The twitch torque superimposed onto the peak force production of the MVC (sTw) was calculated as the increment in torque immediately following the pulse during MVCs. The torque impulse was calculated as the area under the torque-time curve by accumulating the time integral of each MVC (3 s) by the difference in torque between MVCs. The end-

test torque (e.g., CT) during the 60 MVC test was defined as the mean of the last 12 contractions (e.g., the last 60 s; Burnley, 2009). The W' was calculated as the area above the CT from the torque-time curve (e.g., impulse above CT). Central fatigue was assessed as the maximal voluntary activation of the motoneuron pool (VA, %), calculated using the interpolated twitch method from peripheral nerve stimulation (Merton, 1954). Specifically, the increment in torque evoked during the MVCs was expressed as a fraction of the amplitude of the potentiated twitch produced with the same stimulus in the relaxed muscle post MVC. The level of voluntary drive was then quantified as a percentage: [1 − evoked torque (superimposed on voluntary torque, sTw)/ (mean control evoked response, pTw) × 100] (e.g., Allen at al. 1998).

The changes in maximal voluntary torque, pTw, VA and EMG_{RMS}, were used to quantify peripheral fatigue and central fatigue. The maximal EMG was taken from the first MVC during the 60 MVC task and compared to the last MVC at task end. The neuromuscular parameters extracted from the three sets of maximal contractions completed post-exercise were tested for statistical differences between sets of contractions and then compared to the first set of MVCs completed pre-exercise (Froyd et al. 2013; Pageaux et al. 2015; Doyle-Baker et al. 2017). Neuromuscular function was also measured for each of the stimulated contractions during the exercise and normalised to the corresponding pre-exercise values at 100% MVC. All neuromuscular parameters and torque were averaged across the protocol using 10 (30 s) bin averages.

Statistics

Paired-samples *t*-tests were used to compare the mean torque, total impulse, CT and W' (e.g., impulse above CT) between ACT and PL conditions. In addition, paired samples *t*-tests were used to assess parameters of neuromuscular function at task end between trials including pTw, maximal rate of force development, half relaxation time and contraction time. The profiles of torque, VA, pTw and m-wave amplitude were analysed using two-way ANOVAs with repeated measures (using 12 contraction averages; e.g., 6 time points). Normalised EMG_{RMS} were analysed using two-way ANOVAs with repeated

measures using 30 s mean values (e.g., 10 time points). Where the ANOVA revealed a significant interaction effect, post-hoc tests were completed using a Bonferroni correction. For calculation of effect size, partial eta squared (η^2) was used for omnibus tests. Cohen's *d* was used to calculate the effect size for paired t-tests and post-hoc comparisons. A t-test was also conducted on the differences in EMG and force production (torque) from task end (e.g., 300 s) to 150 s (mid-point) to assess the rate of change as the protocol progressed. All statistical tests were performed both on % change and raw data. Where sphericity was violated, a greenhouse-geisser correction factor was used. For all tests, results were considered statistically significant when $P<0.05$. Data are presented as means ± SD unless otherwise indicated. All statistical analyses were conducted using IBM SPSS Statistics version 23.

RESULTS

The mean MVC torque achieved prior to the 60 MVC protocol was 232 ± 47 and 228 ± 48 N·m for PL and ACT, respectively. Voluntary activation of the knee extensors achieved during the preliminary MVCs was 88 ± 7% and 87 ± 5 for PL and ACT, respectively. Baseline MVC and VA were not different between conditions ($P>0.05$).

60 MVC Performance

The profile for mean torque across all subjects and a representative individual plot during each contraction for the 60 MVC protocol is shown in figure 4.2. During the PL trial, torque declined from a peak of 99 ± 3% MVC (relative to pre-exercise MVC) during the first contraction to 40 ± 15% MVC during the last 12 contractions ($P<0.0001$, $\eta^2=0.908$; table 4.1, figure 4.2). The mean torque (relative to pre-exercise MVC) achieved across the 60 MVCs was greater with ACT (61 ± 11%, 97.1 ± 23.2 N·m) compared to PL (58 ± 14%, 83.8 ± 22.7 N·m; $P=0.030$, $d=0.656$) and there was a significant interaction effect (time × condition; $P=0.036$, $\eta^2=0.260$). Post-hoc tests revealed significant differences in torque between conditions at task end ($P=0.044$, $d=0.656$) but at no other time-points (all $P>0.084$ and $d<0.548$). CT was higher with ACT compared to placebo (ACT: 44 ± 13 % vs. PL: 40 ± 15%, $P=0.011$, $d=0.691$), with no between-condition differences in W' (PL: 6.97 ± 2.43, ACT: 6.91 ± 2.54 N·m·s;

P=0.879, *d*=0.026). Total impulse in the 60 MVC protocol was higher with ACT (24386 ± 3793 N·m·s) compared to PL (22055 ± 3885 N·m·s; *P*=0.006, *d*=0.973). As time progressed, the difference in force production between conditions increased as evidenced by a significant difference in rates of change between conditions (e.g., difference between 300 and 150 s time points; *P*=0.035, *d*=0.696). The individual responses following ACT supplementation to the torque-impulse can be seen in figure 4.3. Table 4.2 illustrates the parameters of the 60 MVC test for PL and ACT for individual subjects.

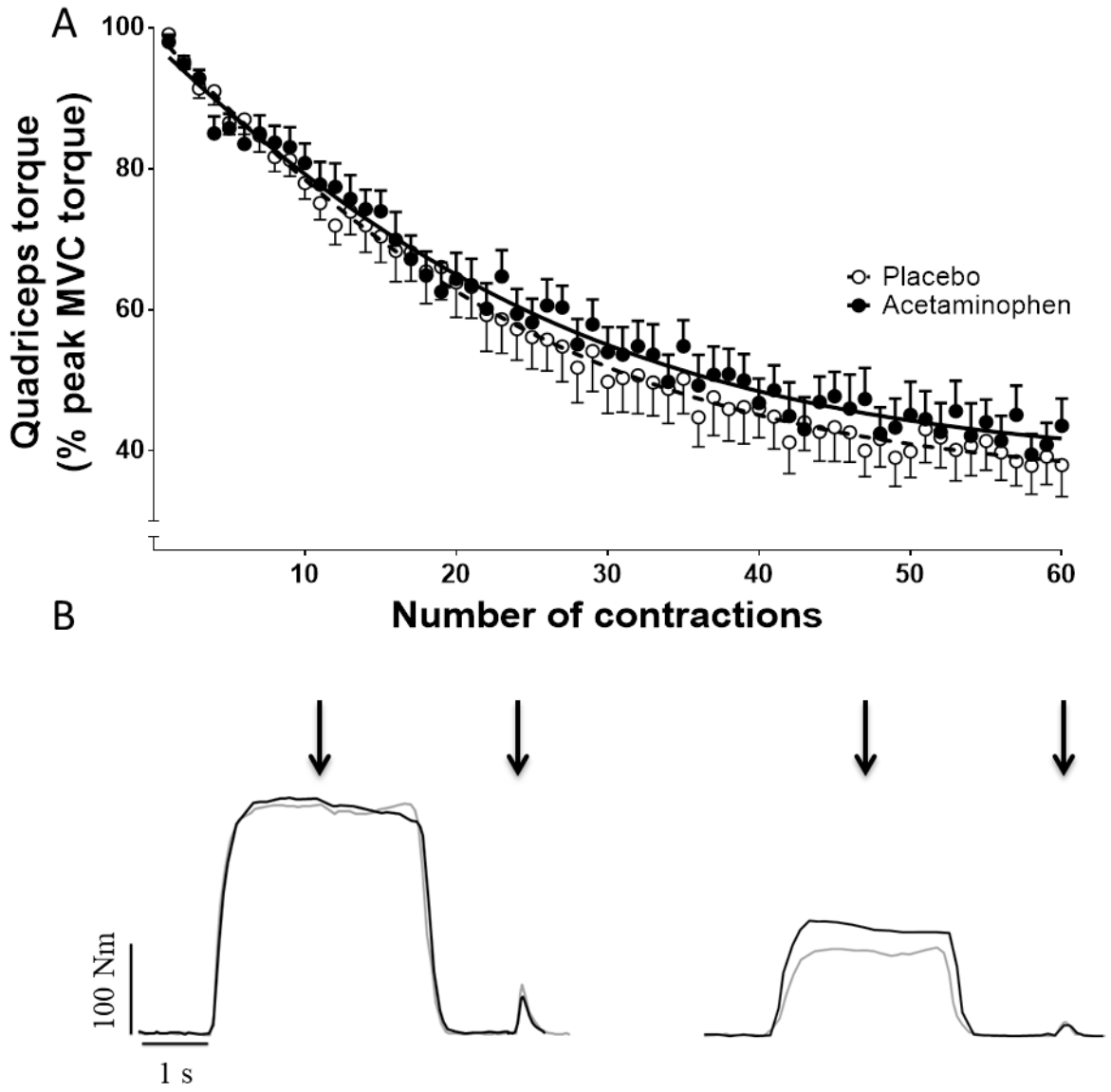

Figure 4.2. *Torque profile during the 60 maximal contractions for placebo (clear circles) and acetaminophen (filled circles) trials. All contractions were normalized to a control maximal voluntary contraction (MVC) performed before the test commenced. Mean ± SE torque responses are presented in panel A with the torque response from a representative individual presented in panel B for the 6th and 54th contractions, respectively, for PL (grey line) and ACT (black line). Note that torque falls over the first ~150 s before reaching stable values between 240 and 300 s (the end-test torque; last 12 MVCs). For panel B, black arrows indicate point of peripheral nerve stimulation.*

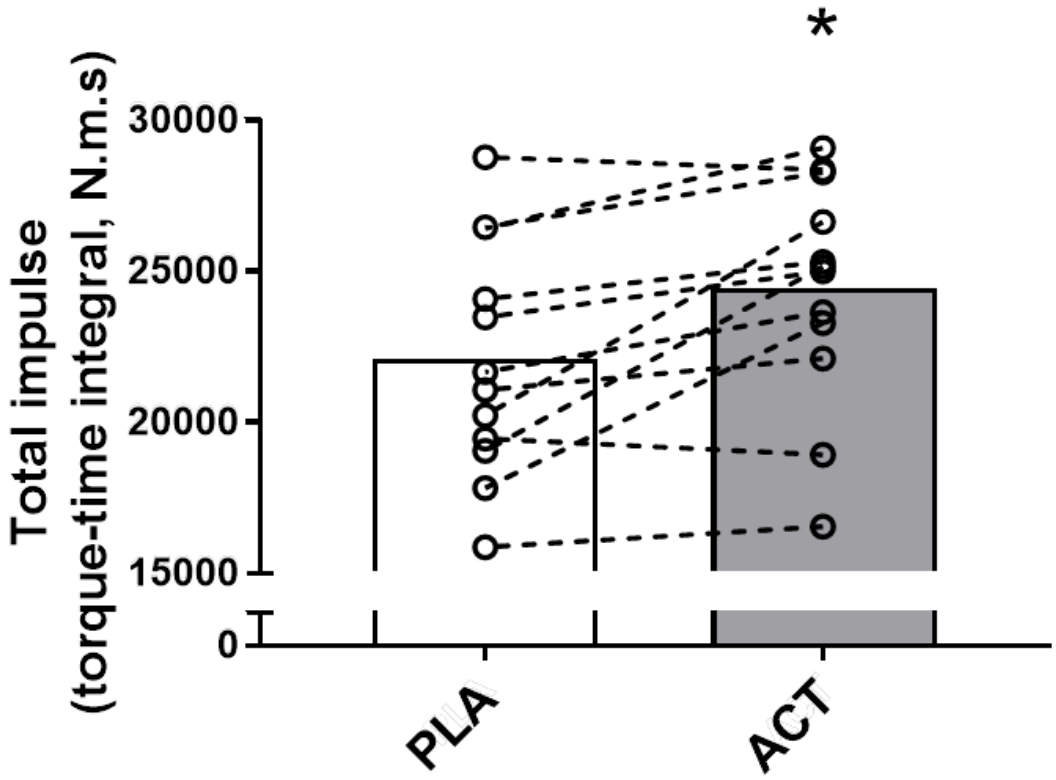

Figure 4.3 *Individual responses to ACT supplementation on 60 MVC performance (torque-impulse). *Significantly different from placebo (P<0.05).*

Table 4.1: *Performance and neuromuscular function parameters of the 60 MVC test following placebo and acetaminophen ingestion*

	Placebo	**Acetaminophen**
Performance		
Peak MVC (N·m)	221 ± 49	230 ± 38
Mean torque (% MVC)	58 ± 14	61 ± 11*
Total torque-impulse (N·m·s)	22,055 ± 3,885	24,386 ± 3,792*
CT (% MVC)	40 ± 15	44 ± 12*
W' (N·m·s)	6,971 ± 2,432	6,906 ± 2,537
Neuromuscular function		
End-exercise pTw (N·m)	30 ± 20	31 ± 23
End-exercise:		
Contraction time (ms)	83.2 ± 10.9	81.3 ± 14.0
Half relaxation time (ms)	72.5 ± 17.2	75.5 ± 17.5
MRFD (N·m·s)	1330 ± 471	1230 ± 433
End-exercise VA (%)	59 ± 19	62 ± 16
End-exercise M-wave amplitude (%)	96 ± 14	95 ± 18
End-exercise M-wave amplitude (mV)	5.64 ± 2.59	5.44 ± 2.72
End-exercise EMG amplitude (%)	59 ± 17	87 ± 28*

MVC, maximal voluntary contraction; CT, critical torque measured in the last 6 contractions; mean torque measured as to average torque across the 60 MVC test; pTw, potentiated twitch force; MRFD, maximal rate of force development; VA, voluntary activation measured using the interpolated twitch method technique; EMG, electromyography; N·m, newton metres; N·m·s, newton metres per second; ms, milliseconds. *Significantly different from placebo (P<0.05).

Neuromuscular Function

There was a main effect for time on pTw (figure 4a, $P<0.001$, $\eta^2=0.754$), voluntary activation (figure 4b, $P<0.001$, $\eta^2=0.647$) and M-wave amplitude figure (4c, $P=0.005$, $\eta^2=0.281$), which all declined as the protocol progressed. VA declined from 89 ± 8% to 59 ± 19% and from 88 ± 5% to 62 ± 16% in the PL and ACT conditions ($P<0.0001$, figure 4.3), respectively. However, there were no main condition effect on M-wave amplitude ($P=0.733$, $\eta^2=0.012$), pTw ($P=0.783$, $\eta^2=0.032$) or VA ($P=0.841$, $\eta^2=0.004$) between the PL and ACT conditions (figure 4.4c). Likewise, there was no significant interaction effect (e.g., time × condition) on M-wave amplitude ($P=0.993$, $\eta^2=0.009$) or VA ($P=0.387$, $\eta^2=0.097$). In addition, pTw declined from 68 ± 8 to 30 ± 20 N·m and from 71 ± 12 to 31 ± 23 N·m (both $P<0.0001$) for PL and ACT, respectively, but there was no significant difference in end-exercise pTw between conditions ($P=0.763$, $d=0.047$). There was also no significant difference in end-exercise maximal rate of force development ($P=0.181$, $d=0.383$), half relaxation time ($P=0.234$, $d=0.341$) or contraction time ($P=0.595$, $d=0.232$) between conditions.

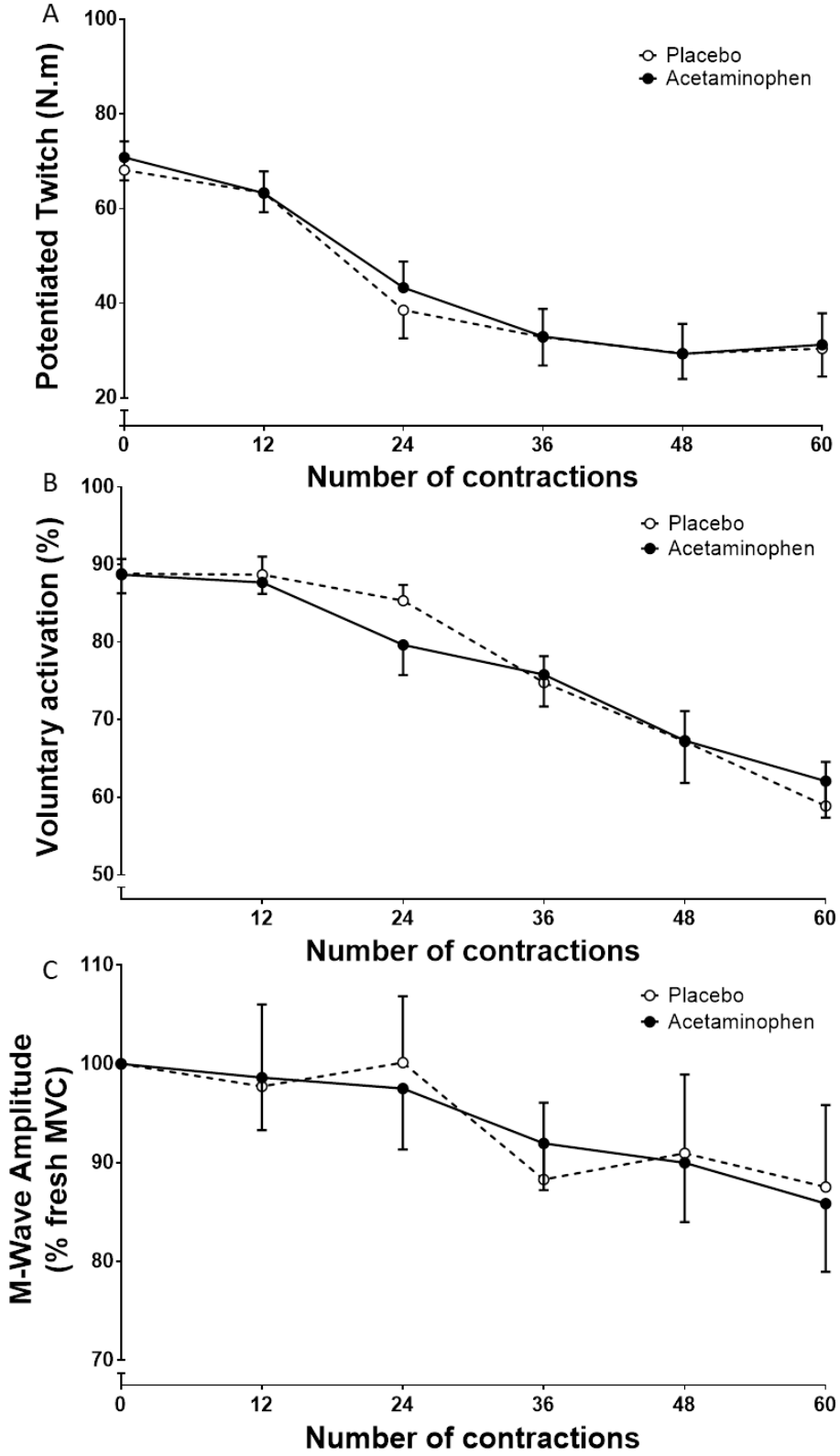

Figure 4.4. *Mean ± SE potentiated twitch (A), voluntary activation (B), and M-wave amplitude (C) responses during the 60 maximal voluntary contraction (MVC) test for placebo (clear circles) and acetaminophen (filled circles) trials.*

Using 6 contraction (30-s) bin means, EMG_{RMS} decreased from 99 ± 4% to 59 ± 17% in the PL trial (from first 6 to last 6 contractions; $P<0.0001$, $\eta^2=0.502$; figure 4.5). This decline in EMG_{RMS} was attenuated following ACT ingestion (100 ± 5 to 87 ± 28%), demonstrating a significant main effect of condition ($P=0.033$, $\eta^2=0.381$) and an interaction effect ($P=0.043$, $\eta^2=0.229$). The EMG_{RMS} was elevated at 60 s ($P=0.036$, $d=0.730$), 90 s ($P=0.020$, $d=0.835$), 210 s ($P=0.070$), 240 s ($P=0.015$, $d=0.886$), 270 s ($P=0.019$, $d=0.844$) and 300 s ($P=0.001$, $d=1.306$) in ACT compared to PL (figure 4.5). As time progressed, the difference in EMG amplitude between conditions increased as evidenced by a significant difference in rates of change between conditions (e.g., difference between 300 and 150 s time points; $P=0.024$, $d=0.804$).

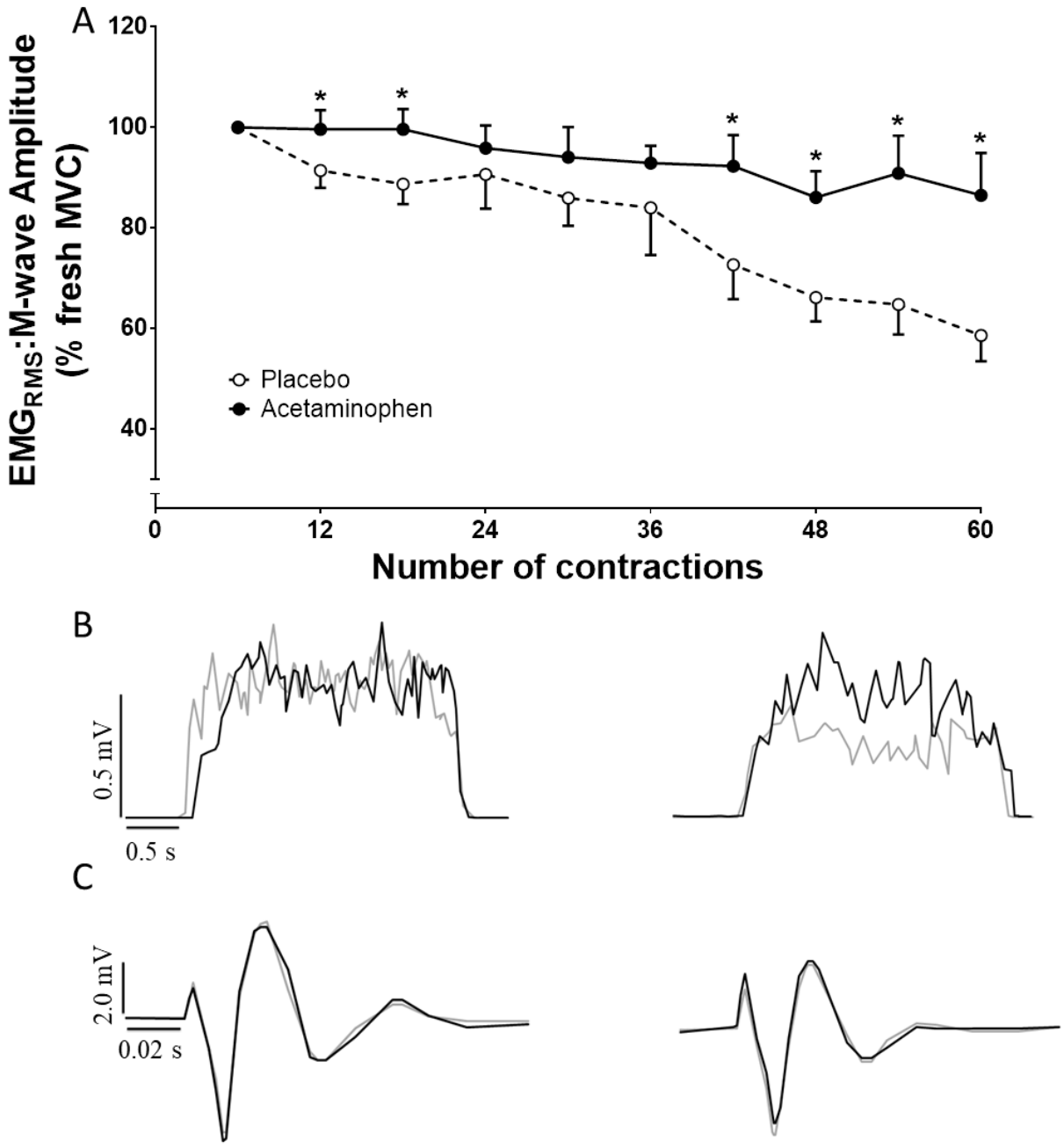

Figure 4.5. *Surface electromyography (EMG) responses (expressed relative to M-wave amplitude) during the 60 MVC test for placebo (clear circles) and acetaminophen (filled circles) trials. Mean ± SE EMG responses are presented in panel A with the EMG response from a representative individual presented in panel B, for the 5th and 55th contractions, respectively, for PL (grey line) and ACT (black line). Individual representative data for m-wave can be seen in panel C 6th and 54th contractions, respectively, for PL (grey line) and ACT (black line). *Significantly different from placebo (P<0.05).*

DISCUSSION

Consistent with our experimental hypotheses, the principal findings of this study were that acute ACT ingestion increased mean MVC torque, and increased CT and EMG amplitude during the latter stages of the 60 MVC protocol. These observations offer insights into the mechanisms by which ACT blunts fatigue development and suggest that the ergogenic effect of ACT ingestion may be linked to increases in CT and muscle activation during fatiguing skeletal muscle contractions.

In the present study, fatigue development and some of its underpinning mechanisms were assessed during the completion of 60 MVCs of the knee-extensors using the protocol described by Burnley (2009). Consistent with Burnley (2009), torque and pTw declined by 60% and ~55%, respectively, and VA declined to 59%, reflecting peripheral and central fatigue development. However, although neuromuscular fatigue was also manifest during the ACT trial, the acute ingestion of ACT increased the mean torque during the fatiguing 60 MVC protocol by ~4% compared to the PL condition. This finding is in line with previous observations that acute ACT ingestion can improve performance during whole body exercise in humans (Foster et al. 2014; Mauger et al. 2010a).

The attenuation of neuromuscular fatigue development following ACT ingestion was not accompanied by specific alterations in central fatigue (voluntary activation), peripheral fatigue (potentiated twitch, pTw) or peripheral neuromuscular excitability (M-wave) between the ACT and placebo trials. However, while EMG declined across the 60 MVC protocol in both conditions, it declined to a lesser extent with ACT (declined to ~87 %) compared to PL (declined to ~59%). This observation suggests that improved muscle activation might have contributed to the ergogenic effect of ACT ingestion. This interpretation is strengthened by our observation of a positive correlation between the inter-trial changes in EMG and torque (r = 0.85). Although ACT ingestion has previously been reported to increase cortico-spinal excitability at rest (Mauger & Hopker, 2013), the neuromuscular mechanisms for improved performance during exercise had not been explored in previous studies

reporting an ergogenic effect of ACT ingestion (Foster et al. 2014; Mauger et al. 2010, 2014). Therefore, by suggesting that ACT can improve muscle activation during repeated fatiguing skeletal muscle contractions, our findings extend previous observations by providing insight into the potential neuromuscular bases for the ergogenic effect of ACT ingestion.

It is possible that the effect of ACT may be linked to a sub-conscious neuromuscular alteration during exercise via a reduction in the magnitude of muscle afferent feedback. During exercise, ascending group III and IV muscle afferents discharge in response to noxious, metabolic and mechanical stimuli within the contracting skeletal muscles to regulate the sensation of pain, muscle activation and peripheral fatigue development (Amann et al. 2009, 2011; Blain et al. 2016; Hureau et al. 2016; McCord and Kaufman, 2010; Pollak et al. 2014). When the magnitude of group III and IV muscle afferent feedback is reduced, performance is compromised and pacing strategy is adversely impacted (Amann et al. 2009, 2011; Blain et al. 2016), despite enhanced muscle activation, as peripheral fatigue development is exacerbated. ACT administration, on the other hand, which seemingly acts predominantly through central processes to blunt pain sensation (Anderson, 2008; Graham et al. 2013; Smith, 2009; Toussaint et al. 2010), appears to have attenuated the decline in muscle activation without impacting peripheral fatigue development, culminating in blunted neuromuscular fatigue development in the current study. Taken together, these observations suggest that reducing, but not abolishing, pain sensation can attenuate neuromuscular fatigue development during exercise and/or that a higher magnitude of afferent feedback is needed to trigger a given pain sensation. However, the findings, whilst suggesting that improved maintenance of muscle activation might have contributed to the ergogenic effect of ACT ingestion, cannot differentiate between a sub-conscious alternation in neuromuscular control or a conscious ability to increase muscle recruitment via a reduction in pain sensation.

The increased muscle activation in the latter stages of the ACT trial compared to the placebo trial was accompanied by a higher mean torque over the final 12 MVCs, and therefore a higher CT (Burnley, 2009). The CT is an important

physiological threshold that is linked to neuromuscular fatigue development through influencing muscle metabolic homeostasis (Jones et al. 2008; Vanhatalo et al. 2016), systemic respiratory and acid-base profiles (Poole et al. 1988) and neuromuscular fatigue development (Burnley et al. 2012). During the 60 MVC protocol employed in the current study, there is a precipitous perturbation to skeletal muscle homeostasis, central and peripheral fatigue development, a decline in muscle activation and a hyperbolic reduction in torque that asymptotes at CT (Burnley, 2009; Burnley et al. 2010). By lowering pain sensation (Foster et al. 2014; Mauger et al. 2010), ACT might have permitted the attainment of a greater degree of intramuscular metabolic perturbation, thereby leading to improved exercise tolerance. However, since potentiated twitch force was not different between the ACT and PL trials, it appears that ACT ingestion did not result in greater peripheral fatigue development in this study. Instead, ACT ingestion enhanced muscle activation and increased CT over the latter stages of the 60 MVC protocol. Therefore, it is also possible that ACT ingestion lowered fatigue development through permitting greater muscle activation for a given degree of intramuscular metabolic perturbation.

The blunted neuromuscular fatigue development in this study occurred in association with increased muscle activation. However, it is surprising that the attenuation in neuromuscular fatigue development was not accompanied by changes in central (e.g., voluntary activation) or peripheral (e.g., potentiated twitch force) fatigue in this study. Indeed, since muscle activation and torque were increased, this might be expected to result in greater intramuscular metabolic perturbation and, by extension, greater peripheral fatigue development. Although we cannot exclude the possibility that ACT enhanced systemic physiological responses or skeletal muscle efficiency to mitigate this potential for greater peripheral fatigue development, we are not aware of any published evidence to support such effects.

Experimental Considerations

It is acknowledged that a limitation of the current study was that pain sensation were not directly assessed. Pain was not assessed due to the protocol requiring

the completion of 60 MVCs with a short (2 s) recovery period; asking subjects to rate pain sensation in this setting may have compromised their ability to focus on the exercise task and provide a true maximal effort during the experimental protocol. Previous studies have observed a higher power output for the same pain sensation, suggestive of a reduction in the sensation of pain during cycle ergometry exercise (Foster et al. 2014; Mauger et al. 2010). However, these studies used a higher ACT dose (~1.5 g) and a different exercise modality (cycling) compared to the current study. Therefore, we are unable to draw conclusions regarding pain sensation with ACT in the current study. It should also be acknowledged that in the face of a relatively small (~4%) improvement in torque, it is possible that the central and peripheral fatigue measurements in our study were not sensitive enough to detect small defects in central nervous system and peripheral muscle function. Moreover, while ACT increased EMG during the latter stages of the 60 MVC protocol compared to PL, which might be linked, in part, to increased muscle activation and central motor drive, VA was not impacted by ACT. This might have been a function of the continuous assessment of the EMG response and torque throughout the 60 MVC protocol, compared to every 6th MVC for VA, such that EMG might have provided a more complete picture of central fatigue development across the protocol. Recent research has also challenged the validity of VA estimated using the interpolated twitch technique as a measure of central fatigue (Neyroud et al. 2016), which might contribute to the disparity between the EMG-inferred central motor drive and the VA results. Accordingly, further research is required to resolve the underlying mechanisms for the ergogenic effect of ACT ingestion. In addition, although the current study is consistent with previous studies reporting an ergogenic effect of acute ACT consumption (Foster et al. 2014; Mauger et al. 2010, 2014), we do not advocate regular ACT use or exceeding a single dose of 1 g given the potent hepatotoxicity of ACT ingestion (Graham et al. 2013). Therefore, individuals wishing to explore the use of ACT to enhance exercise performance should do so infrequently, and with caution.

In conclusion, acute ACT ingestion increased the mean torque across 60 MVCs of the knee-extensors in agreement with earlier reports that ACT can attenuate neuromuscular fatigue development and improve exercise performance. Our

results extend these previous observations by providing novel insights into the mechanisms for the potential ergogenic effect of ACT ingestion. Specifically, the improved mean torque was accompanied by an increase in CT and greater muscle activation during the latter stages of the 60 MVC protocol. Therefore, ACT ingestion appears to attenuate fatigue development during repeated skeletal muscle MVCs by enabling a better preservation of muscle activation during exercise.

Table 4.2. Parameters of the 60 MVC test for placebo and acetaminophen

	Placebo						Acetaminophen					
	Total impulse	**Mean Torque**		**End-test torque (CT)**		**Curvature constant (W')**	**Total impulse**	**Mean torque**		**End-test torque (CT)**		**Curvature constant (W')**
Subject	(N·m·s)	(N·m)	(% MVC)	(N·m)	(% MVC)	(N·m·s)	(N·m·s)	(N·m)	(% MVC)	(N·m)	(% MVC)	(N·m·s)
1	19072	106.0	56.9	82.8	44.5	4162	25185	139.9	56.7	114.4	46.3	4589
2	26481	147.1	46.0	97.1	30.4	9008	28304	157.2	51.2	92.9	30.2	11591
3	17854	99.2	47.9	69.0	33.3	5427	23308	129.5	51.0	87.2	34.3	7617
4	28802	160.0	83.3	131.5	68.4	5124	28389	157.7	78.1	140.4	69.5	3123
5	21691	120.5	66.7	85.0	47.0	6385	23676	131.5	60.5	103.5	47.6	5044
6	24102	133.9	68.9	92.3	47.5	7495	25334	140.7	75.5	96.9	52.0	7899
7	23494	130.5	70.5	103.3	55.8	4894	25020	139.0	69.9	110.9	55.8	5063
8	26437	146.9	61.9	90.4	38.1	10166	29108	161.7	69.2	109.7	46.9	9353
9	19481	108.2	37.8	48.3	16.9	10794	18946	105.3	41.4	54.1	21.3	9205
10	21095	117.2	49.9	69.1	29.4	8662	22141	123.0	61.5	76.6	38.3	8356
11	20256	112.5	45.9	67.1	27.4	8186	26654	148.1	59.0	110.0	43.8	6849
12	15893	88.3	58.3	69.7	46.0	3349	16568	92.0	57.0	68.8	43.1	4178
Mean	**22,055**	**122.5**	**57.8**	**83.8**	**40.4**	**6,971**	**24,386***	**135.5***	**61.0***	**97.1***	**44.1***	**6,906**
SD	**3,885**	**22.0**	**13.7**	**22.7**	**14.7**	**2,432**	**3,792**	**21.1**	**10.7**	**23.2**	**12.5**	**2,537**

Mean torque, average torque achieved during the test; MVC, maximal voluntary contraction, End-test torque (e.g., CT), mean torque measured in the last 12 contractions of the 60 MVC test. *Significantly different from placebo (P<0.05).

Acetaminophen ingestion improves muscle activation and performance during a 3-min all-out cycling test

Original investigation

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Paul T. Morgan[1], Anni Vanhatalo[1], Joanna L. Bowtell[1], Andrew M. Jones[1] and Stephen J. Bailey[1,2]

[1]Sport and Health Sciences, College of Life and Environmental Sciences, University of Exeter, St. Luke's Campus, Heavitree Road, Exeter, EX1 2LU, UK.

[2]School of Sport, Exercise and Health Sciences, Loughborough University, Ashby Road, Loughborough, Leicestershire LE11 3TU, UK.

ABSTRACT

Purpose: Acute acetaminophen (ACT) ingestion has been shown to enhance cycling time-trial performance. The purpose of this study was to assess whether ACT ingestion enhances muscle activation and critical power (CP) during maximal cycling exercise. **Methods**: Sixteen active male participants completed two 3-min all-out tests against a fixed resistance on an electronically-braked cycle ergometer 60 min after ingestion of 1 g of ACT or placebo (maltodextrin, PL). CP was estimated as the mean power output over the final 30 s of the test and W′ (the curvature constant of the power-duration relationship) was estimated as the work done above CP. The femoral nerve was stimulated every 30 s to measure membrane excitability (M-wave) and surface electromyography (EMG_{RMS}) was recorded continuously to infer muscle activation. **Results:** Compared to PL, ACT ingestion increased CP (ACT: 297 ± 32 vs PL: 288 ± 31 W, $P<0.001$) and total work done (ACT: 66.4 ± 6.5 vs PL: 65.4 ± 6.4 kJ, $P=0.03$) without impacting W′ (ACT: 13.1 ± 2.9 vs PL: 13.6 ± 2.4 kJ, $P=0.19$) or the M-wave amplitude ($P=0.66$) during the 3-min all-out cycling test. Normalized EMG_{RMS} amplitude declined throughout the 3-min protocol in both PL and ACT conditions; however, the decline in EMG_{RMS} was attenuated in the ACT condition, such thats the EMG_{RMS} amplitude was greater in ACT compared to PL over the last 60 s of the test ($P=0.04$). **Conclusion**: These findings indicate that acute ACT ingestion might increase performance and CP during maximal cycling exercise by enhancing muscle activation.

Key words: Analgesic; critical power; electromyography; exercise performance; muscle activation; neuromuscular fatigue

INTRODUCTION

Fatigue is a complex, multi-factorial process that is linked to perturbations within the central nervous system and the contracting skeletal muscles (Enoka & Duchateau, 2008; Hureau et al. 2018). Recent studies suggest that fatigue development may be related, at least in part, to pain sensation (Astokorki & Mauger 2017a; s2017b; O'Leary et al. 2017). Acetaminophen (ACT) is a commonly used medicine for general pain relief. Ingestion of ACT lowers pain sensation by inhibiting the cyclooxygenase enzymes, which stimulate nociceptor discharge through the synthesis of prostaglandins (Graham et al. 2013; Jóźwiak-Bębenista & Nowak, 2014), and modulating serotoninergic, opioid and cannabinoid pathways that may contribute to the responsiveness of the corticospinal tract (Andersson et al. 2011; Graham et al. 2013; Ottani et al. 2006; Pickering et al. 2006, 2008). Acute ACT ingestion has been shown to enhance endurance exercise performance consistent with the notion that interventions that can modulate pain sensation have the potential to influence exercise performance (Foster et al. 2014; Mauger et al. 2010, Morgan et al. 2018). Indeed, similar to the effects of caffeine (O'Connor et al. 2004), Mauger et al. (2010) and Foster et al. (2014) have both previously reported enhanced exercise performance and/or work output at a given level of muscle pain following acute ACT ingestion. These results suggest that ACT reduces pain at a given absolute work rate and/or permits a higher work rate for an equivalent pain sensation.

In a recent study conducted within our lab, Morgan et al. (2018) reported an attenuated decline in skeletal muscle electromyography (EMG) amplitude, reflective of an increase in muscle activation, and an increased critical torque during a maximal intermittent single-leg knee extensor test following ACT ingestion. During cycling exercise, the power equivalent of the critical torque, the critical power (CP), represents an important threshold for oxidative metabolic control and exercise tolerance (Jones et al. 2010; Vanhatalo et al. 2011). Indeed, CP, which is the asymptote of the hyperbolic relationship between power output and time to exhaustion, reflects the highest work rate that can be sustained without a progressive loss of intramuscular and systemic homeostasis (Black et al. 2017; Poole et al. 1988; 2016; Vanhatalo et al. 2016),

and interacts with the curvature constant of this relationship, W′, to define exercise tolerance within the severe exercise intensity domain (Jones et al. 2010; Vanhatalo et al. 2011). Since CP is linked to muscle activation and neuromuscular fatigue development during exercise, as inferred from EMG responses (Burnley et al. 2012), and since ACT ingestion can concomitantly influence EMG responses and the critical torque (Morgan et al. 2018), ACT might also enhance CP by modulating aspects of central fatigue development during large muscle mass exercise. This potential blunting of central fatigue development could be mediated by inhibition of nociceptor sensitising prostaglandins (Graham et al. 2013; Jóźwiak-Bębenista & Nowak, 2014), enhanced efferent pathways (Andersson et al. 2011; Ottani et al. 2006) and/or enhanced corticospinal excitability (Mauger & Hopker, 2013), permitting an increased CP and thus improved endurance exercise performance.

Although the improvement in cycling performance that has been reported following ACT ingestion (Foster et al. 2014; Mauger et al. 2010) may also be linked to enhanced neuromuscular function and a higher CP, as observed during single leg exercise (Morgan et al. 2018), the exercise modality and the volume of skeletal muscle mass engaged are known to influence the degree of neuromuscular and peripheral fatigue development. Specifically, greater peripheral fatigue development has been observed at the same relative intensity during knee-extensor exercise compared to cycling and single vs. double leg exercise (Rossman et al. 2012, 2014). Therefore, further research is required to resolve the .mechanisms underpinning the potential ergogenic effect of ACT on larger muscle mass exercise such as cycling, which is more relevant for sports performance, requires further research.

The purpose of the present study was, therefore, to assess the effect of acute ACT ingestion on neuromuscular fatigue development and its potential underlying mechanisms during large muscle mass exercise. We tested the hypotheses that, compared to placebo (PL), acute consumption of 1 g of ACT would increase total work done, CP and muscle activation during a 3-min all-out cycling test.

METHODS

Participants

Sixteen trained male cyclists (mean ± SD: age 29 ± 9 y, height 1.79 ± 0.07 m, body mass 77 ± 8 kg, $\dot{V}O_{2peak}$ 60.8 ± 6.0 ml·kg^{-1}·min^{-1}, range: 52-77 ml·kg^{-1}·min^{-1}) provided written informed consent to participate in the present study, which was approved by the local Ethics Committee (Sport and Health Sciences, University of Exeter). All subjects participated in local cycling competitions. Trained individuals were selected as it has been shown that endurance training influences pain tolerance (O'Leary et al. 2017). After being informed of the experimental procedures and associated risks, all participants completed a medical health questionnaire, which was checked by a medical doctor, to ensure it was safe to consume ACT prior to performing exhaustive exercise. The questionnaire incorporated questions pertaining to: known allergies to medications, current intake of medication and prior use of ACT as well as any history of illnesses, cigarette use, alcohol consumption, illegal drug intake and chronic illnesses (personal and family history). None of the participants had a history of motor and/or neurological disorders or frequent chronic ingestion of pain relief medication (e.g., ACT, non-steroidal anti-inflammatory medication etc.). Participants were also advised to avoid ingestion of pain relief medication over the duration of the study and were provided with a list of prohibited medication(s). Participants were instructed to arrive at the laboratory in a rested and fully hydrated state, at least 3 h post-prandial, and to avoid strenuous exercise, and consumption of caffeine and alcohol in the 24 h prior to each testing session.

Experimental Design

Participants visited the laboratory on 5 occasions over a 5- to 6-week period with all tests conducted at a similar time of day (± 90 min). All tests were conducted on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands). On the first laboratory visit, participants performed a incremental ramp cycling test for the determination of the linear factor (as described below), gas exchange threshold (GET), peak aerobic power output and the peak oxygen uptake ($\dot{V}O_{2peak}$). During this initial laboratory visit, the seat and handlebar positions were adjusted for comfort and replicated for all

tests. The second and third laboratory visits were used to familiarise participants to the measurements and experimental protocol (as described below). During these visits (e.g., visits 2 and 3), participants completed a 3-min all-out cycling test to ensure the coefficient of variation for work done and CP between visits was <1% and that the criteria to ensure a valid test were fulfilled (Jones et al. 2010). For each 3-min test, achievement of $\dot{V}O_{2peak}$ (>95%), as verified by the $\dot{V}O_{2peak}$ achieved during the ramp incremental ramp tests, was an obligatory criterion for a valid test. In the one instance where these criteria were not fulfilled, the participant completed a further familiarisation trial prior to commencing the experimental trials. During these sessions, the settings and placement of EMG and peripheral nerve stimulation electrodes were recorded for each subject as a reference for electrode placement in subsequent experimental trials (see below for further details). These trials were not included in the subsequent data analysis. Participants then performed the fatiguing protocol under two experimental conditions: placebo (PL) and ACT. Experimental sessions were separated by 3-7 days.

Experimental protocol

All trials (visits 1-5) started with a standardised warm-up routine (10 min at 100-150 W, corresponding to <90% GET, followed by 5 min of passive rest) and testing of the optimal EMG electrode (for recording muscle activation), anode and cathode placement and stimulation intensity (for peripheral nerve stimulation). Single peripheral nerve stimulation pulses were manually triggered to determine the characteristics of the M-wave response to supra-maximal nerve stimulation. Neuromuscular function was assessed pre-, during- and post-trial (<10 s) as described below.

The experimental protocol comprised a 3-min period of unloaded pedalling at the participant's preferred cadence, followed by a 3-min all-out sprint, 60 min following ingestion of either PL (1 g maltodextrin) or 1 g ACT (visits 4-5). This timing was selected to coincide with the approximate attainment of the peak plasma [ACT] concentration (Anderson et al. 2008). The placebo was made from dextrose powder inserted into gelatine capsules designed to have an identical appearance and weight to ACT capsules but without the analgesic and

antipyretic effects. The order of trials for visits 4 and 5 were administered in a double-blind, randomised fashion using a counter-balanced cross-over experimental design. The 3-min all-out cycling protocol used in this study replicated the procedures described previously by Vanhatalo et al. (2007, 2008). The fixed resistance for the all-out sprint was set using the linear mode of the ergometer such that upon reaching their preferred cadence, the participants would achieve a power output equivalent to 50% of the difference between GET and $\dot{V}O_{2peak}$ (linear factor = 50%Δ power output/preferred cadence^2).

Measurements

Breath-by-breath pulmonary gas exchange

Throughout all laboratory tests, participants wore a mask connected to an impeller turbine transducer assembly (Cortex Metalyzer, Cortex, Leipzig, Germany). Inspired and expired gas volume and concentration signals were continuously sampled at 100 Hz. ThFo raFe analyser was calibrated before each test with gases of known concentration (O_2 15%, CO_2 4.5%), and a calibration syringe of known volume (3-L; Hans Rudolph, KS).

Electromyography

Neuromuscular function was assessed pre-, during- and immediately post each of the trials. Pre- and post-trial neuromuscular function was tested with the participant cycling at 80 rpm with a low resistance (20 W) as described below. Surface EMG activity was measured from *m*. vastus lateralis, *m*. vastus medialis, *m*. rectus femoris and *m*. biceps femoris muscles of the right leg to continuously record muscle activity during exercise using active bipolar bar electrodes with a single differential configuration (DE2.1, DelSys Inc, Boston, MA, USA). Bipolar electrodes were positioned over the muscle belly parallel to the longitudinal axis of each muscle (according to guidelines of the SENIAM (Surface Electromyography for the Non-Invasive Assessment of Muscles) project.). The placement of electrodes was considered optimal on achieving the largest and most reproducible M-wave signal from the *m*.vastus lateralis and *m*.vastus medialis whilst noting minimal activity in the *m*.bicep femoris. Placement of electrodes was optimised during each laboratory visit. Double-

sided adhesive tape and a hypoallergenic medical tape were used to ensure the EMG sensor stability for recording electrodes. The skin area underneath each EMG electrode was shaved, then exfoliated and cleaned with alcohol to minimise the skin impedance. The EMG signal was pre-amplified (1000 x), band-pass filtered (20–450 Hz, Bagnoli-8, DelSys Inc., Boston, Mass., USA), and then transferred to a computer with a sampling frequency of 2 kHz. EMG data were recorded continuously and digitised synchronously with 16 bit resolution via an A/D converter (±5 V range, CED 1401 power, Cambridge, UK). EMG was average rectified using the root mean square method (EMG_{RMS}). EMG_{RMS} throughout the trial was then normalised to the EMG signal during the first 30 s of the 3-min test to provide a percentage of the maximal signal. Finally, EMG_{RMS} was normalised to the local (closest) standardised M-wave amplitude and presented as a percentage of the maximal signal. In addition, M-wave amplitude was normalised by pre-exercise, resting values, and presented as a percentage. This method of normalizing the EMG trace to the M-wave may enable a more accurate assessment of changes in muscle activation that are likely occurring upstream of the neuromuscular junction (e.g., spinal and/or supraspinal in origin). The ground electrode was placed over the patella of the right leg in an 'electrically quiet' location to minimize extraneous noise.

Peripheral Nerve Stimulation

Electrical stimulation was applied using a constant current stimulator (Digitimer Stimulator DS7AH, Digitimer, Hertfordshire, UK). Initially, the crank angle at which peripheral nerve stimulation was to be delivered during the trials was determined for each subject as described by Black et al. (2017) and as performed by Sidhu et al. (2012). Stimulations were delivered at the identified crank angle specific to each trial (62 ± 7° relative to full knee extension, 180°) to align with maximal EMG_{RMS} amplitude. A custom written sequencer script triggered 3 single stimulations, independently, with at least 1 and up to 10 pedal revolutions between stimuli. During the 3-min cycling test, these stimulations were delivered every 30 s. M-waves were elicited in *m*.vastus lateralis and *m*.vastus medialis by supramaximal percutaneous electrical stimulation of the femoral nerve (200 µs duration), approximately 3–5 cm below the inguinal ligament in the femoral triangle. The cathode was systematically moved

vertically and horizontally and the amplitude of the muscle action potential (e.g., M-wave) was monitored to identify the optimal position of the cathode for attaining maximal peak-to-peak M-wave (M_{max}) amplitude. To determine the stimulation intensity, single stimuli were delivered in 20 mA step-wise increments from 100 mA until a plateau (e.g., M_{max}) in the M-wave was observed. A supramaximal pulse of 130% M_{max} current (Burke, 2002; Goodall et al. 2010; Neyroud et al. 2014) was applied during the exercise tests (mean stimulation intensity: 251 ± 48 mA). The procedures for determining optimal electrode placement and stimulation intensity were completed during each laboratory visit (visits 2-5).

Data Analysis

Data were analysed using a custom written script developed in Spike2 software (CED, Cambridge, UK). CP was estimated as the mean power output over the final 30 s of the test, and the W′ was estimated as the work done above the CP (Vanhatalo et al. 2007, 2008). Peak $\dot{V}O_2$ was determined as the highest 15-s interval (e.g., $\dot{V}O_{2peak}$). Total work was calculated as the area under the power-time curve. Peak power output attained in the 3-min test was defined as the maximal 1-s interval. The changes in power output, M_{Max} and EMG_{RMS}, were used to quantify neuromuscular fatigue development and changes in muscle activation. All neuromuscular parameters and power output were averaged across the protocol into 6 × 30-s bin averages. Estimates of CP and W′ were also used to predict the time taken to complete a range of total work done (W) targets (50, 75, 100, 125, 150, 175, 200, 225, 250, 500, 750, 1000 kJ) as previously described (Kelly et al. 2013) using equation (1):

$$T_{lim} = (W-W') / CP \qquad \text{(equation 1)}$$

Statistics

Paired-samples *t*-tests were used to compare the CP, W', total work done and cardiorespiratory responses between ACT and PL conditions. In addition, paired samples *t*-tests were used to assess parameters of neuromuscular function at task end between trials (e.g., M_{max} and EMG_{RMS}). The profiles of power output, M-wave amplitude and EMG_{RMS} before, during and after the 3-min test were

analysed using two-way ANOVAs (time × condition) with repeated measures (using 30 s averages; e.g., 6 time points) between PL and ACT. A two-way repeated-measures ANOVA was also used to assess differences in predicted performance times. Where the ANOVA revealed a significant interaction effect, post-hoc comparisons were completed using a Bonferroni correction. A Pearson's product moment correlation coefficient was used to determine the relationship between the change in EMG amplitude and the change in power output between conditions. A one-way ANOVA was used to assess differences in $\dot{V}O_{2peak}$ obtained during the incremental ramp test and both 3-min trials (PL and ACT). To assess the possibility of an order effect of trials, a paired samples *t-test* was conducted on total work done for visits 4 and 5. For calculation of effect size, partial eta squared (η^2) was used for omnibus tests. Cohen's *d* was used to calculate the effect size for paired *t*-tests and post-hoc comparisons. All statistical tests were performed both on % change and raw data. Where sphericity was violated, a Greenhouse Geisser correction factor was used. For all tests, results were considered statistically significant when $P<0.05$. Data are presented as mean ± SD, unless otherwise indicated. All statistical analyses were conducted using IBM SPSS Statistics version 24.

RESULTS

Mean $\dot{V}O_{2peak}$ measured in the ramp incremental test was 4.50 ± 0.41 $L \cdot min^{-1}$ (61 ± 6 $ml \cdot kg^{-1} \cdot min^{-1}$) and the peak aerobic power output was 393 ± 29 W. The GET occurred at 1.98 ± 0.26 $L \cdot min^{-1}$ and 152 ± 22 W. The $\dot{V}O_{2peak}$ achieved during the 3-min test following PL (4.51 ± 0.59 $L \cdot min^{-1}$, 60 ± 7 $ml \cdot kg^{-1} \cdot min^{-1}$) and ACT ingestion (4.53 ± 0.57 $L \cdot min^{-1}$, 61 ± 8 $ml \cdot kg^{-1} \cdot min^{-1}$) were not significantly different to the values achieved during the ramp incremental test ($P=0.77$).

3-min all-out cycling test

The $\dot{V}O_2$ profile during the 3-min test for PL and ACT conditions is shown in figure 5.1 (panel A). In addition, the mean power output profile for all participants (and differences in CP) during the 3-min all-out cycling test is shown in figure 5.1 (panel B) for the PL and ACT conditions. Panel C represents changes to power output throughout the duration of the 3-min test in

all trials and is provided in 30-s averages. During the PL trial, power output declined from 820 ± 139 W during the first 5 s of the test to 288 ± 31 W during the last 30 s of the 3-min test (P<0.0001, η^2=0.99; table 5.1). However, during the ACT trial, power output declined from 838 ± 127 W during the first 5 s of the test to 297 ± 32 W during the final 30 s of the test (table 5.1). There was a significant interaction effect (time × condition; P=0.04, η^2=0.26) with the mean power output in the 3-min cycling test being greater in ACT (368 ± 36 W) compared to PL (363 ± 36 W, P=0.007, d=0.13). CP (ACT: 297 ± 32 W vs. PL: 288 ± 31 W, P<0.0001, d=0.28) and total work done (ACT: 66.4 ± 6.5 kJ vs. 65.4 ± 6.4 kJ; P=0.03, d=0.15) was higher with ACT compared to PL (table 5.1; figure 5.2). However, there was no difference in peak power output (ACT: 838 ± 127 W vs. PL: 820 ± 139 W, P=0.10, d=0.16) or W' (ACT: 13.1 ± 2.9 vs. PL: 13.6 ± 2.4 kJ; P=0.19, d=0.20) during the 3-min cycling test between conditions. No order effect was observed between visit 4 and visit 5 for total work done (Visit 4: 65.8 ± 6.5 kJ vs. Visit 5: 66.0 ± 6.4 kJ; P=0.75, d=0.03).

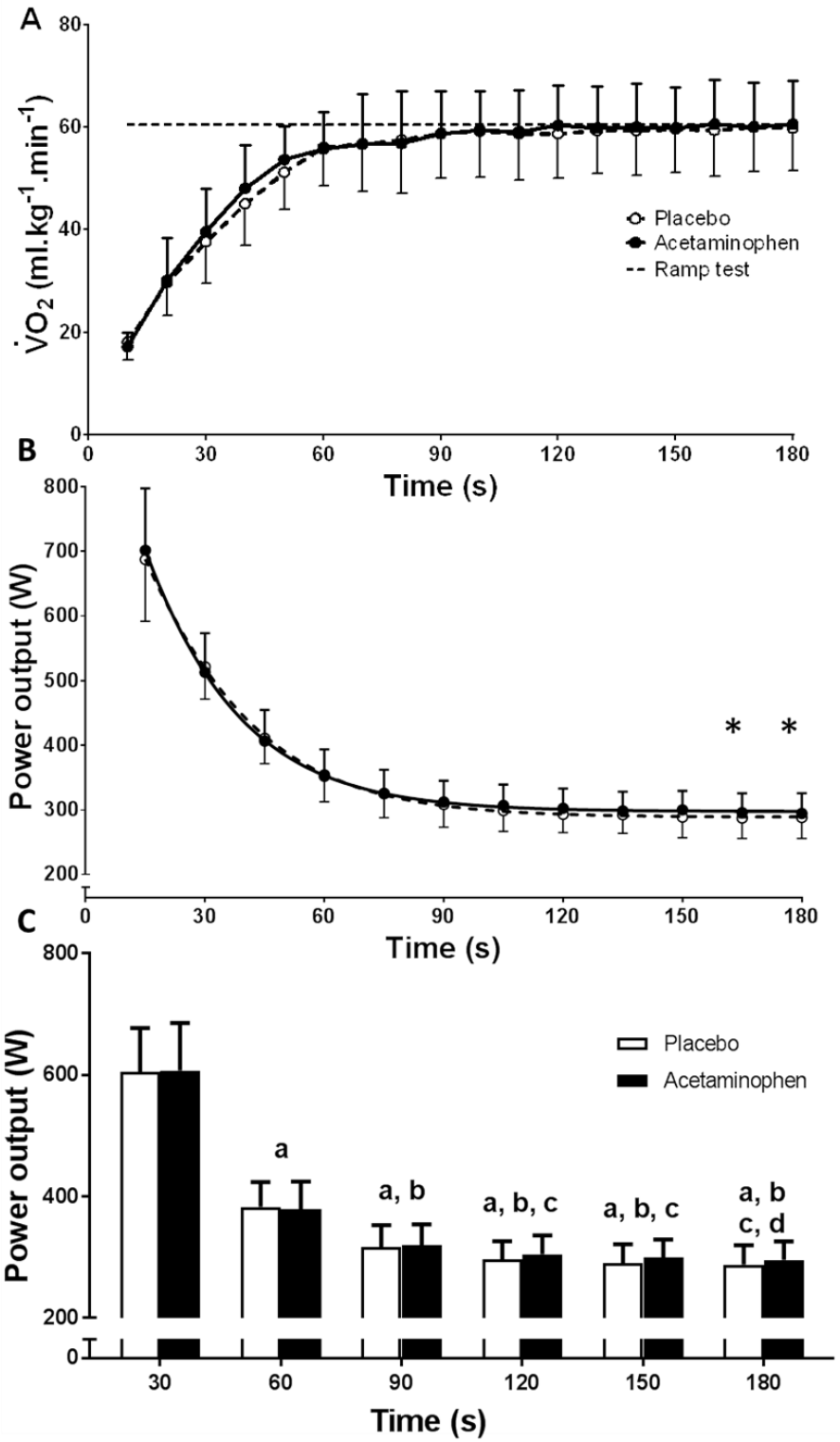

Figure 5.1. *Group mean ± SE $\dot{V}O_2$ during acetaminophen (ACT, filled circles) and placebo (PL, clear circles) is presented in panel A. The dashed line represents the $\dot{V}O_{2peak}$ attained in the incremental ramp test. Panel B illustrates the mean ± SE power output profile during the 3-min maximal cycling protocol for placebo (clear circles) and acetaminophen (filled circles) trials derived from*

*15 s averages. Note that after attainment of peak power output a few seconds into the test, power output falls over the first ~90-120 s before reaching stable values (the end-test power output; e.g., CP). CP is significantly elevated in the last 30 s of the ACT condition. Significant changes to power output over time (derived from 30 s averages) throughout the 3-min cycling test for both ACT and PL conditions are shown in panel C. *Significantly different from PL (e.g., main effect of condition); [a]significantly different from 30 s; [b]significantly different from 60 s; [c]significantly different from 90 s; [d]significantly different from 120 s (main effect of time, P<0.05).*

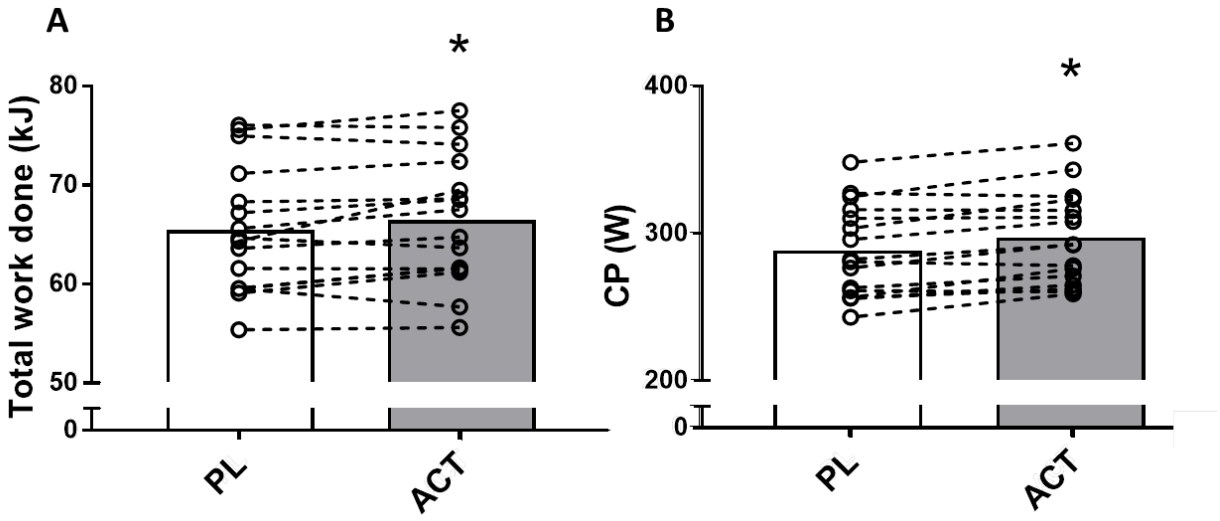

Figure 5.2. *Group mean total work done in the placebo (PL) and acetaminophen (ACT) conditions are shown in the open and closed bars, respectively (Panel A). Individual responses in the PL and ACT conditions are shown by the open circles and linked with dashed lines. *Significantly different from PL (P<0.05). Panel B represents the group mean critical power (CP) in the PL and ACT conditions in the open and closed bars, respectively. Individual responses in the PL and ACT conditions are shown by the open circles and linked with dashed lines.*

Table 5.1: *Performance and neuromuscular function parameters during the 3 min test following placebo and acetaminophen ingestion.*

	Placebo	**Acetaminophen**
3-min test performance		
Peak power output (W)	820 ± 139	838 ± 127
Mean power output (W)	363 ± 36	368 ± 36*
Total work done (kJ)	65.4 ± 6.4	66.4 ± 6.5*
CP (W)	288 ± 31	297 ± 32*
W' (kJ)	13.6 ± 2.4	13.1 ± 2.9
Neuromuscular function		
End-exercise M-wave amplitude (*m*VL, %)	84 ± 21	83 ± 22
End-exercise M-wave amplitude (*m*VL, mV)	3.94 ± 1.9	4.15 ± 1.60
End-exercise EMG_{RMS} amplitude (*m*VL, %)	54 ± 17	72 ± 18*
End-exercise EMG_{RMS} amplitude (*m*VL, mV)	0.09 ± 0.03	0.12 ± 0.03*

Data are presented as Mean ± SD. CP, critical power; W', the work done above CP; M-wave, amplitude of M-wave response; EMG, electromyography; *m*VL, vastus lateralis muscle *Significantly different from placebo (P<0.05).

When the CP and W′ were combined to predict the time required to complete fixed work targets between 50 and 1000 kJ, using equation 1, the ANOVA revealed a main effect by condition (P<0.0001, η^2=0.56) and an interaction effect (P<0.0001, η^2=0.86, table 5.2). Post-hoc analysis revealed that the performance times were lower in the ACT condition compared with the PL condition for all time-trials with the exception of the two shortest (e.g., 50 and 75 kJ), with the improvement ranging from 1.1% (100 kJ) to 3.0% (1000 kJ).

Table 5.2: *Predicted time to complete fixed work targets (50 – 1000 kJ) as calculated using the CP and W' estimates following placebo and acetaminophen ingestion.*

	Placebo	Acetaminophen
Fixed work targets (kJ)		
50 kJ	128 ± 17	126 ± 17
75 kJ	216 ± 25	211 ± 25
100 kJ	303 ± 34	296 ± 33*
125 kJ	391 ± 43	381 ± 42*
150 kJ	479 ± 52	466 ± 50*
175 kJ	567 ± 61	552 ± 59*
200 kJ	655 ± 70	637 ± 68*
225 kJ	743 ± 80	722 ± 76*
250 kJ	831 ± 89	807 ± 85*
500 kJ	1709 ± 182	1659 ± 172*
750 kJ	2588 ± 276	2510 ± 259*
1000 kJ	3466 ± 369	3362 ± 346*

Data are presented as Mean ± SD. kJ, kilojoules; s, seconds (predicted time to complete 'target work'); *Significantly different from placebo (P<0.05).

Neuromuscular Function

From pre to post exercise, there was a main effect for time on M-wave amplitude in the *m*.vastus lateralis (P=0.003, η^2=0.29, figure 5.3), which declined as the protocol progressed. However, there was no main effect by condition (P=0.66, η^2=0.01) or time × condition interaction effect (P=0.70, η^2=0.03). EMG_{RMS} in the *m*.vastus lateralis decreased from 94 ± 4% over the first 30 s to 54 ± 17% over the final 30 s of the 3-min all-out test in the PL trial (P<0.0001, η^2=0.50; figure 5.4). However, this decline in EMG_{RMS} was attenuated following ACT ingestion (from 92 ± 5 over the first 30 s to 72 ± 18% over the final 30 s of the 3-min all-out test), with there being a time × condition

interaction effect (P=0.04, η^2=0.23). Post-hoc analysis revealed EMG_{RMS} was elevated at 150 s (P=0.02, d=0.84) and 180 s (P=0.001, d=1.31) in ACT compared to PL (figure 5.4). There was a significant positive correlation between the change in EMG amplitude and the change in power production over the last 30 s of exercise between conditions (r=0.88, P=0.04, figure 5.5).

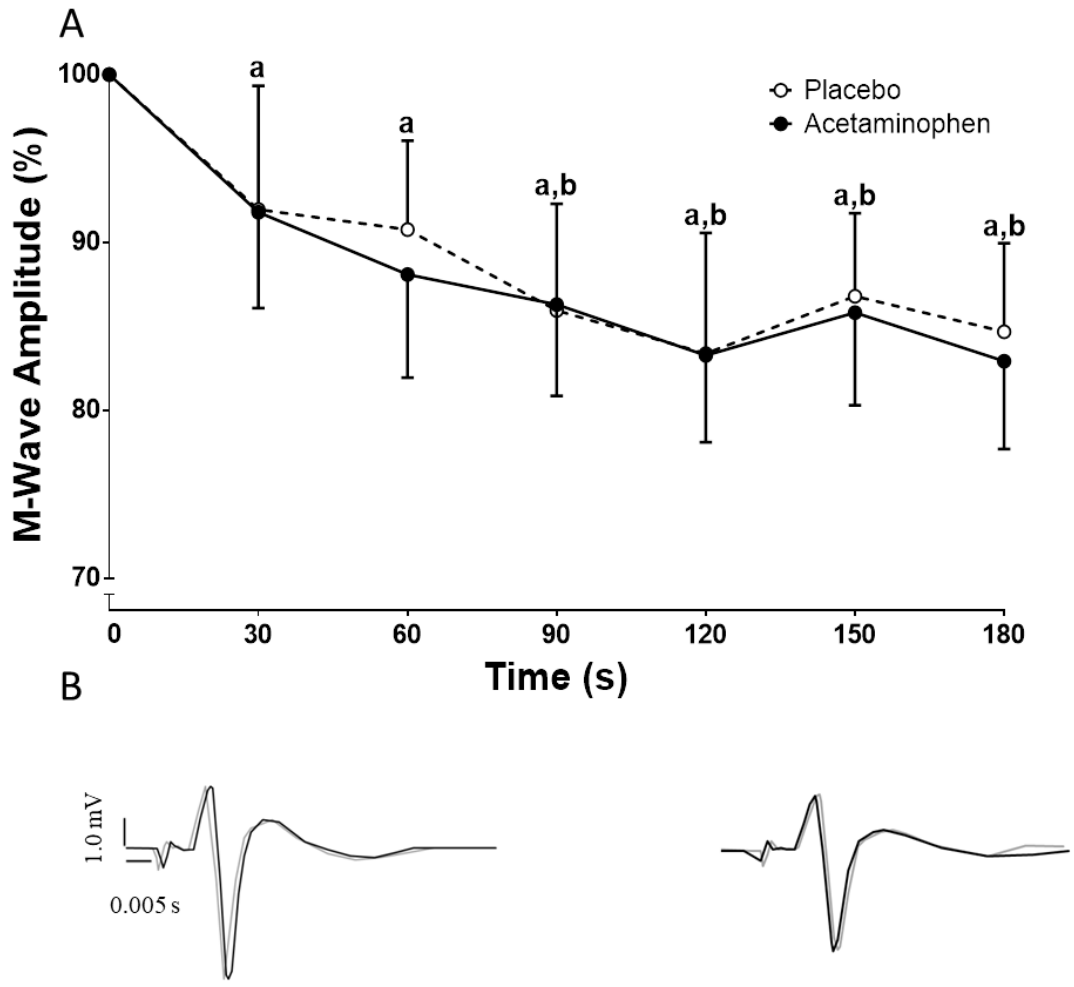

Figure 5.3. *M-wave amplitude responses in the m.vastus lateralis during the 3-min cycling test for placebo (clear circles) and acetaminophen (filled circles) trials. Mean ± SE M-wave responses are presented in panel A with the M-wave response from a representative individual presented in panel B, for PL (grey line) and ACT (black line), for the first 30 and final 30 s, respectively of the 3-min protocol. [a]significantly different from baseline; [b]significantly different from 30 s (P<0.05).*

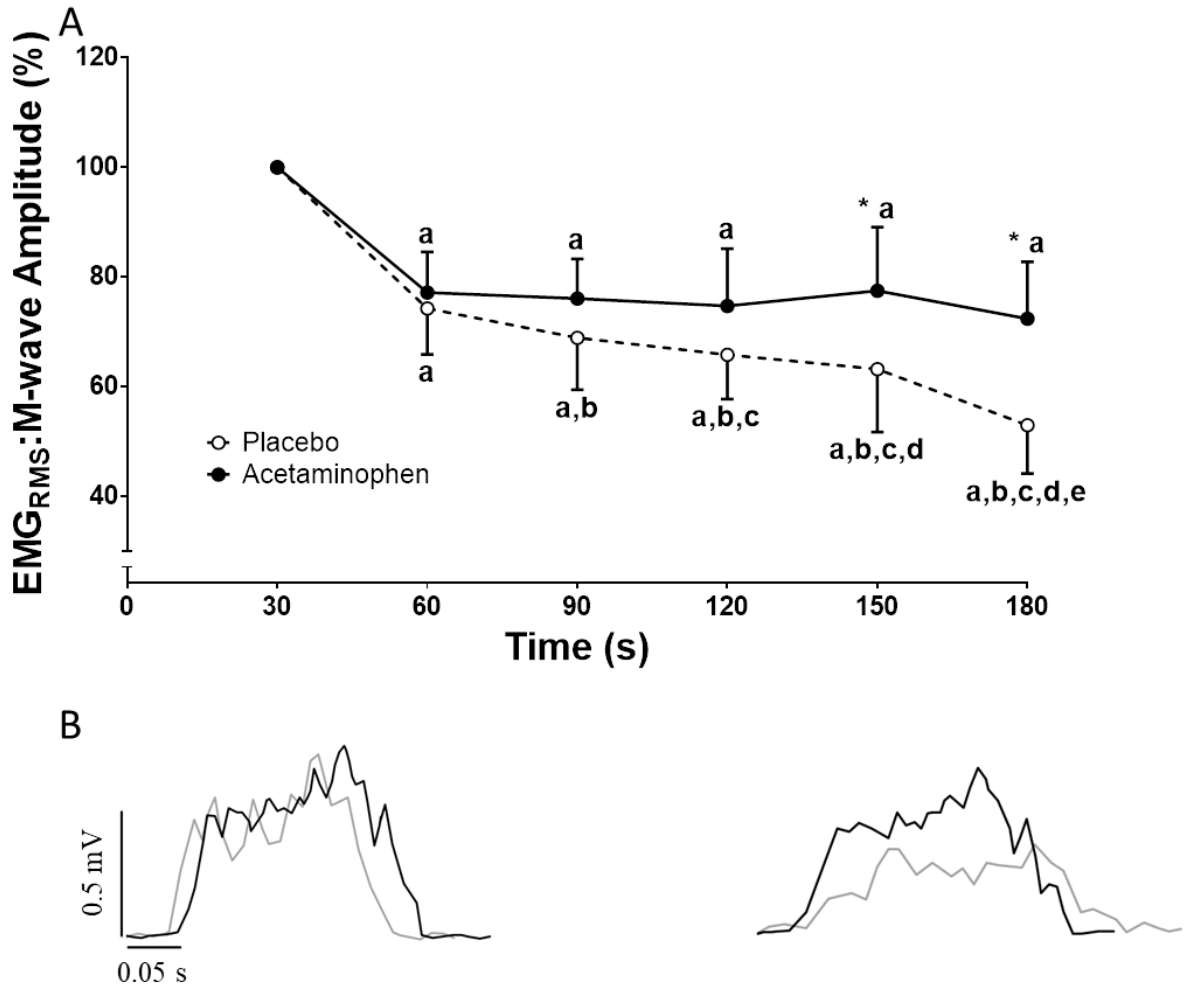

Figure 5.4. *Surface electromyography (EMG) responses (expressed relative to M-wave amplitude) in the m.vastus lateralis during the 3-min cycling test for placebo (clear circles) and acetaminophen (filled circles) trials. Mean ± SE EMG responses are presented in panel A with the EMG response from a representative individual presented in panel B, for PL (grey line) and ACT (black line), for the first 30 and final 30 s, respectively of the 3-min protocol. *Significantly different from placebo; [a]significantly different from 30 s; [b]significantly different from 60 s; [c]significantly different from 90 s; [d]significantly different from 120 s; [e]significantly different from 150 s (P<0.05).*

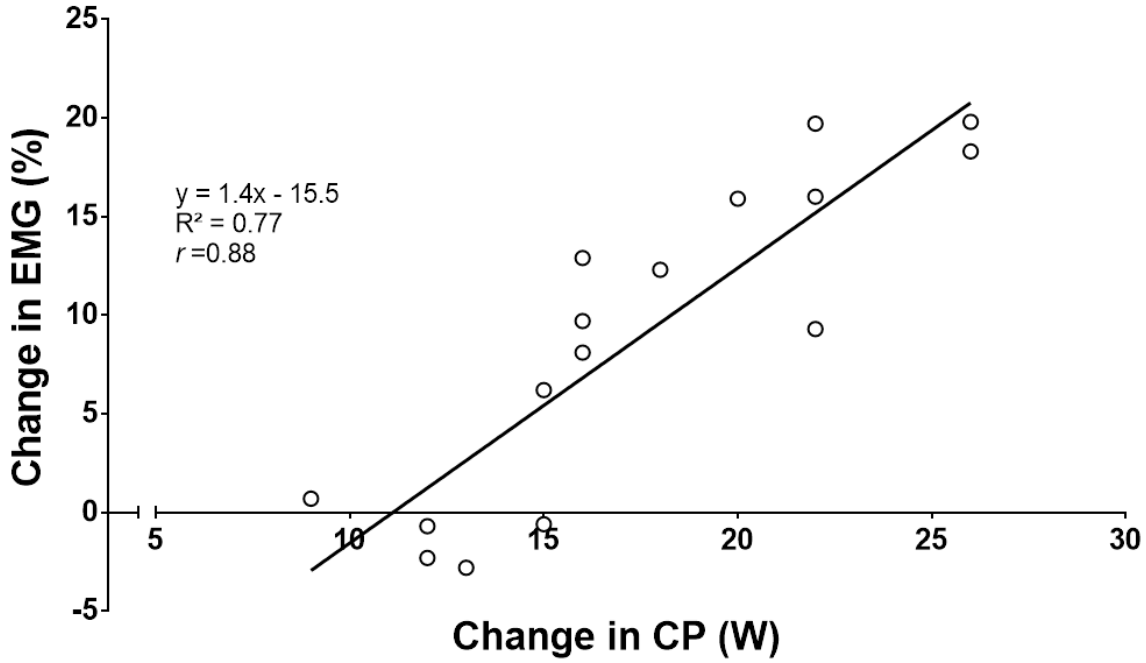

Figure 5.5. *Correlation between the change in electromyography amplitude (EMG, %) and the change in critical power (CP) between conditions (acetaminophen and placebo). The solid line represents the line of best fit.*

DISCUSSION

Consistent with our hypotheses, the principal original findings of this study were that acute ACT ingestion enhanced total work done and CP, and attenuated the decline in EMG amplitude, in trained individuals during a 3-min all-out cycling test. The ACT-induced increase in CP was predicted to translate into a 1-3% reduction in the time required to complete a range of target work cycling trials (100-1000 kJ). The results of this study provide some insight into the mechanisms by which ACT ingestion is ergogenic during large muscle mass exercise and suggest that enhanced performance following ACT ingestion is attributable, at least in part, to increases in CP and muscle activation.

Power-duration relationship

Our finding of an increase in total work done following acute ACT ingestion in the 3-min all-out cycling test is consistent with previous observations of enhanced exercise performance following acute ACT ingestion of similar doses (~1-1.5 g; Foster et al. 2014; Mauger et al. 2010, Morgan et al. 2018). In the present study, neuromuscular fatigue development was assessed during the

completion of a 3-min all-out cycling test to offer insight into the potential underlying mechanisms for the ergogenic effects of ACT ingestion. Consistent with our previous finding of a 4% increase in critical torque when utilising a single-limb knee-extension model (Morgan et al. 2018), CP achieved during a 3-min all-out cycling test was improved by ~3% following the acute ingestion of ACT in the present study. Moreover, and consistent with our previous findings (Morgan et al. 2018), W′ was not altered following ACT ingestion in the current study.

The potential practical significance of the 3% improvement in CP becomes clear when applied to an exercise performance scenario. An important practical application of the CP is that this parameter, in conjunction with W′, can be used to robustly predict cycling TT performance (Black et al. 2014, 2017; Burnley et al. 2012; Chidnok et al. 2013; Florence & Weir, 1997; Skiba et al. 2012; Smith et al. 1999). Accordingly, the influence of a given intervention on CP and W′ can be used to predict the effect that that intervention might have on endurance exercise performance. For example, although Kelly et al. (2013) reported no statistically significant increase in either CP (+1.4%) or W′ (+8.4%) following dietary nitrate supplementation, when the combined effect on these parameters was integrated, an improvement of 2-3% in cycling time-trial performance was predicted. Similarly, in the current study, endurance performance was predicted to be improved by ~1-3% following acute ACT ingestion in the work trial simulations (~5-60 min). Since this magnitude of performance enhancement following acute ACT ingestion exceeds 0.6%, which is suggested to be the smallest ‘worthwhile’ improvement in road TT cycling (Paton & Hopkins, 2006), our results suggest that acute ACT ingestion may enable a practically meaningful improvement in endurance exercise performance. It should also be noted that, although we did not directly assess the effect of acute ACT ingestion on cycling TT performance in the current study, the predicted 1-3% is similar to the empirically demonstrated 1.8% improvement in 10-mile cycling TT performance reported previously (Mauger et al. 2010).

Interestingly, improvements in exercise performance with acute ACT ingestion have been reported in trained participants in both the current study and in

previous studies (Mauger et al. 2010) despite evidence that endurance training increases pain tolerance (Jones et al. 2014; O'Leary et al. 2017) such that trained individuals are more likely to have a greater tolerance to pain (Janal et al. 1994; Tesarz et al. 2013). However, it should be stressed that, although the current and previous studies support an ergogenic effect of acute ACT consumption (Foster et al. 2014; Mauger et al. 2010, 2014; Morgan et al. 2018), regular ACT use, or exceeding a single dose of 1 g, is not recommended given the hepatotoxicity of ACT (Graham et al. 2013).

Neuromuscular function

In addition to influencing the degree of muscle metabolic perturbation and the trajectory of the $\dot{V}O_2$ slow component during exercise (Jones et al. 2008, 2010; Poole et al. 1988; Vanhatalo et al. 2011), CP is linked to muscle activation characteristics during exercise, as inferred from EMG responses, and represents a critical threshold for neuromuscular fatigue development (Burnley et al. 2012). Indeed, concomitant with our observation of an increased CP in the current study, the decline in EMG amplitude during the 3-min all-out test was attenuated in ACT compared to PL. These findings are strikingly similar to our recent study, which reported a blunted decline in the EMG amplitude and an increased critical torque during a 5-min maximal intermittent single-legged knee extension exercise task (Morgan et al. 2018). Together, these results suggest that improved maintenance of muscle activation contributes to the elevated CP and total work done following ACT ingestion. However, the blunting of neuromuscular fatigue development following ACT ingestion was not accompanied by improvements in peripheral muscle excitability, as inferred from measurements of M-wave amplitude between the ACT and PL trials, suggesting that this alteration occurred due to mechanisms upstream of the neuromuscular junction.

Our results support the notion that the ergogenic effect of ACT is principally mediated centrally (Anderson, 2008; Andersson et al. 2011; Graham et al. 2013; Mauger & Hopker, 2013; Ottani et al. 2006; Smith, 2009; Toussaint et al. 2010). However, while we are not aware of any evidence to suggest that ACT might influence peripheral muscle excitability (Mauger & Hopker, 2013), or that

interventions aimed at reducing inflammation improve performance during whole body exercise (e.g., Cleak, & Eston. 1992; Da Silva et al. 2015; Nosaka & Clarkson, 1996; Tokmakidis et al. 2003), we cannot exclude that peripheral factors that were not assessed in the current study, such as inflammation and/or alterations to muscle metabolism, may have contributed to the ergogenic effect of ACT. Moreover, due to the nature of cycling exercise, it is technically challenging to directly test cortical alterations via changes to voluntary activation using the interpolated twitch technique (Doyle-Baker et al. 2018).

Whilst we have previously investigated the contribution of central and peripheral factors to the improved performance following ACT ingestion in a small muscle mass model (Morgan et al. 2018), the mechanisms underpinning fatigue development, and therefore ACT's potential ergogenic effect, could differ for large muscle mass exercise (Rossman et al. 2012, 2014). We observed a strong correlation between the change in end-exercise EMG_{RMS} and the change in power output (e.g., CP) within the last 30 s of the 3-min cycling test (r=0.88) following ACT ingestion compared to placebo. However, the change in EMG_{RMS} was much larger than the change in CP. Although the mechanisms for this effect remain to be defined, this observation is in agreement, with Felippe et al. (2018). Specifically, these authors reported that, compared to placebo, caffeine ingestion increased mean power output by ~4% during a 4-km cycling test, resulting in a 2% reduction in time to complete the 4-km distance, alongside a ~17% increase in muscle recruitment (as inferred by EMG).

It is possible that, through lowering pain sensation (Foster et al. 2014; Mauger et al. 2010), ACT might have permitted the development of, and/or tolerance to, a greater degree of intramuscular metabolic perturbation beyond that required to evoke a 'critical' threshold of peripheral fatigue, thereby permitting improved exercise performance (Blain et al. 2016). Alternatively, since the effects of ACT are believed to be largely centrally mediated (Anderson, 2008; Graham et al. 2013; Smith, 2009; Toussaint et al. 2010), it is possible that ACT ingestion attenuated the development of central fatigue. A blunting in central fatigue development following ACT ingestion would be expected to permit enhanced central motor output, possibly through a reduction in inhibitory feedback via

cyclooxygenase inhibition and a resultant decline in the synthesis of prostaglandins.

The higher EMG_{RMS} during the latter stages of the 3-min all-out cycling test observed following ACT ingestion may also have been a consequence of enhanced excitability of the corticospinal tract (Mauger & Hopker, 2013). Greater corticospinal excitability following ACT ingestion, as inferred from a greater motor-evoked potential from cortical stimulation in the study of Mauger & Hopker (2013), may be linked to enhanced firing of motor units, and increased spinal excitability, as has been reported with caffeine consumption (e.g., Kalmar & Cafarelli, 2004; Walton et al. 2003). This is particularly pertinent as fatigue has been associated with impaired corticospinal excitability (Ross et al. 2010). Together, these effects on motor cortical and/or spinal excitability may explain the enhanced muscle activation and the subsequent greater amount of work performed with ACT ingestion in the current study. However, since cortical and peripheral contributions to fatigue development were not directly tested in this study, further research is required to resolve the underlying mechanisms for the ACT-mediated enhancement in muscle activation and performance during maximal exercise, particularly during locomotion which has the most relevance to sporting performance.

In conclusion, acute ACT ingestion increased total work done during a 3-min all-out cycling test in agreement with earlier reports of an ergogenic effect of ACT ingestion on cycling performance. The improved performance in the 3-min all-out test was accompanied by an increase in CP and better preservation of the EMG amplitude during the latter stages of the protocol. When the ACT-induced increase in CP was used to predict the effects of acute ACT ingestion on cycling performance, the estimated 1-3% improvement was in line with previous experimental observations. Therefore, our results extend previous reports by revealing that ACT ingestion improves performance concomitant with enhanced CP and muscle activation during a 3-min all-out cycling test. These observations provide insight into the ergogenic effect of ACT ingestion during large muscle mass exercise.

Contralateral fatigue during severe-intensity single-leg exercise: influence of acute acetaminophen ingestion

Original investigation

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Paul T. Morgan[1], Stephen J. Bailey[1,3], Rhys A. Banks[1], Jon Fulford[2], Anni Vanhatalo[1], and Andrew M. Jones[1]

[1]Sport and Health Sciences, College of Life and Environmental Sciences, University of Exeter, St. Luke's Campus, Heavitree Road, Exeter, EX1 2LU, UK.

[2]Peninsula NIHR Clinical Research Facility, College of Medicine and Health, Exeter, UK.

[3]School of Sport, Exercise and Health Sciences, Loughborough University, Ashby Road, Loughborough, Leicestershire LE11 3TU, UK.

ABSTRACT

Purpose: Acetaminophen (ACT) ingestion has been suggested to improve exercise performance by blunting central fatigue development. Since exhaustive single-leg exercise can lower time to task failure (T_{lim}) during subsequent exhaustive exercise in the contralateral limb by exacerbating central fatigue development, this study tested the hypothesis that ACT ingestion would be more effective at improving T_{lim} during single-leg exercise completed with, compared to without, prior contralateral fatigue. **Methods:** Fourteen recreationally-active men performed constant-load, single-leg, severe-intensity knee extensor exercise to exhaustion on the left (Leg_1) and right (Leg_2) legs without prior contralateral fatigue, and on Leg_2 immediately following Leg_1 ($Leg_{2\text{-}CONTRA}$). These tests were all completed following ingestion of 1 g ACT or maltodextrin (PL) capsules or no capsule ingestion (CON). Intramuscular phosphorous-containing metabolites (adenosine diphosphate and inorganic phosphate) and substrates (phosphocreatine), and muscle activation were assessed using ^{31}P-MRS and electromyography, respectively. **Results:** T_{lim} was not different between the Leg_{1ACT} and Leg_{1PL} conditions (402 ± 101 vs. 390 ± 106 s; $P>0.05$). There was also no difference in T_{lim} between $Leg_{2ACT\text{-}CONTRA}$ and $Leg_{2PL\text{-}CONTRA}$ (324 ± 85 vs. 311 ± 92 s; $P>0.05$), but T_{lim} was shorter in both these tests compared to Leg_{2CON} (385 ± 104 s; $P<0.05$). There were no differences in muscle activation, intramuscular phosphorous-containing metabolites and substrates or ratings of perceived exertion (RPE) between the Leg_{1ACT} and Leg_{1PL} or the $Leg_{2ACT\text{-}CONTRA}$ and $Leg_{2PL\text{-}CONTRA}$ conditions ($P>0.05$). **Conclusion:** These findings suggest that acute ACT ingestion does not alter RPE, muscle activation and metabolic perturbation, or T_{lim} during single-leg severe-intensity knee extensor exercise completed with or without prior exhaustive exercise in the contralateral limb.

Key words: Paracetamol, ^{31}P-magnetic resonance spectroscopy; non-local muscle fatigue; muscle activation; muscle metabolic; neuromuscular

INTRODUCTION

The mechanisms of exercise-induced fatigue can be attributed to processes within the central nervous system, termed central fatigue, and within the contractile elements of the working muscle, termed peripheral fatigue (*for review* Davis & Walsh, 2010, Enoka & Duchateau, 2008; Gandevia, 2001; Taylor et al. 2016). It is now recognised that peripheral and central fatigue development are interlinked, in part, via group III/IV muscle afferent feedback. Empirical support for a role of group III/IV muscle afferent feedback in modulating the mechanisms of neuromuscular fatigue is provided by reports that inhibiting group III/IV muscle afferent feedback, via lumbar intrathecal administration of fentanyl, lowers central fatigue development and results in increased skeletal muscle metabolic perturbation [greater and/or more rapid increases in adenosine diphosphate (ADP) and inorganic phosphate (Pi) accumulation and declines in phosphocreatine (PCr) and pH] and thus peripheral fatigue development (i.e., Amann et al. 2009, 2011; Blain et al. 2016; Broxterman et al. 2017a, b, 2018; Sidhu et al. 2014, 2017, 2018). Conversely, whilst the mechanisms for non-local muscle fatigue are not yet entirely understood (Halperin et al. 2015 for review), prior fatiguing single-limb exercise has been reported to accentuate central fatigue development and lead to lower peripheral fatigue development during subsequent fatiguing exercise in a contralateral or non-local (previously rested) muscle group, when group III/IV muscle afferent feedback would be expected to be elevated (Amann et al. 2013; Johnson et al. 2015; Halperin et al. 2014, 2015; Rattey et al. 2006; Sidhu et al. 2014). Based on these observations, whilst the locus of fatigue during high-intensity single-leg exercise is predominantly peripheral (i.e., Burnley et al. 2012), it is possible that a contralateral limb task may elevate the proportional contribution of central fatigue to impaired performance. However, the effect of prior fatiguing single-limb exercise on skeletal muscle metabolic perturbation during subsequent fatiguing exercise in a contralateral or non-local muscle group has yet to be investigated. Moreover, while lumbar intrathecal administration of fentanyl and prior fatigue of a contralateral or non-local muscle group can alter group III/IV muscle afferent feedback and the physiological bases of exercise-induced neuromuscular fatigue, the effect of such interventions on exercise performance is equivocal (i.e., Amann et al. 2009,

2011, 2013; Blain et al. 2016; Broxterman et al. 2017a, b, 2018; Johnson et al. 2015; Kennedy et al. 2015; Halperin et al. 2014, 2015; Rattey et al. 2006).

There is an emerging body of evidence to suggest that oral ingestion of acetaminophen (ACT) can blunt the development of exercise-induced neuromuscular fatigue and improve exercise capacity and/or performance (Foster et al. 2014; Mauger et al. 2010; Morgan et al. 2018a, b). It is generally accepted that the principal mechanism of action of ACT is the inhibition of cyclooxygenase, the enzyme that catalyses the synthesis of prostaglandins (Anderson, 2008). Since prostaglandins sensitize nociceptors (Rueff and Dray, 1993; Schaible et al. 2011), and since blocking cyclooxygenase attenuates group III/IV muscle afferent discharge during dynamic exercise (Hayes et al. 2006), this might account for reports of increased work output for the same level of perceived pain and exertion (Foster et al. 2014; Mauger et al. 2010) and elevated muscle activation (Morgan et al. 2018a, b) during exercise after ACT ingestion. Therefore, ACT administration might be ergogenic by reducing, but not abolishing, the net magnitude of group III/IV muscle afferent feedback, leading to a blunting of exercise-induced central fatigue. and improvement in exercise performance. This is particularly pertinent to note given the complex intensity and task specific behaviour of group III/IV muscle afferent feedback. Specifically, muscle afferent feedback has been shown to inhibit neural drive and facilitate cardiopulmonary function during maximal and sub-maximal exercise, respectively (i.e., Sidhu et al. 2017). Moreover, since ACT appears to attenuate exercise-induced neuromuscular fatigue by abating aspects of central fatigue development (Foster et al. 2014; Mauger et al. 2010; Morgan et al. 2018a, b), ACT might be more effective at lowering exercise-induced neuromuscular fatigue following prior exhaustive exercise in a contralateral limb, where the locus of fatigue is primarily central (Amann et al. 2013; Johnson et al. 2015; Halperin et al. 2014; Rattey et al. 2006), compared to unilateral exercise without prior exhaustive exercise in a contralateral limb, where the predominant locus of fatigue is more likely to be peripheral (Burnley et al. 2012). The effects of ACT ingestion on exercise-induced fatigue development and its underlying mechanisms following prior exhaustive exercise in a contralateral limb has yet to be investigated.

The purpose of this study was to assess the effects of ACT ingestion on exercise-induced neuromuscular fatigue and some of its underlying mechanisms during single-leg severe-intensity knee extensor exercise completed with and without prior exhaustive severe-intensity knee extensor exercise in the contralateral limb. It was hypothesised that: 1) prior exhaustive exercise would impair subsequent exercise tolerance in the contralateral limb by lowering muscle activation and the degree of muscle metabolic perturbation that could be attained; 2) ACT ingestion would enhance single-leg knee extensor exercise tolerance by increasing muscle activation (higher surface EMG) and permitting the attainment of a greater degree of muscle metabolic perturbation [lower muscle pH and PCr concentration ([PCr]) and higher ADP ([ADP]) and Pi ([Pi]) concentrations]; and 3) ACT ingestion would improve exercise tolerance to a greater extent with, compared to without, the completion of prior exhaustive exercise by the contralateral limb.

METHODS

Subjects

Fourteen active males volunteered to participate in this study (mean ± SD: age 23.8 ± 4.7 y, height 1.80 ± 0.10 m, body mass 81.6 ± 14.9 kg). All procedures were approved by the Ethics Committee of the Department of Sport and Health Sciences, University of Exeter. Subjects completed a health questionnaire, which was checked by a medical doctor, to ensure it was safe to consume ACT prior to performing exhaustive exercise. The questionnaire incorporated questions pertaining to: known allergies to medications, current intake of medication and prior use of ACT as well as any history of illnesses, cigarette and illegal drug use, alcohol consumption and chronic illnesses (personal and family history). Prior to each visit, subjects were required to refrain from caffeine (for at least 12 h), strenuous exercise and alcohol (for at least 24 h), analgesics and any form of anti-inflammatory drug (for the duration of the experimental trial) and to arrive in a fully rested, hydrated state. With the exception of these restrictions, subjects were instructed to maintain their usual diet and exercise regime during the study. All tests were performed at a similar time of day (± 2 h).

Pre-experimental procedures

Subjects visited the laboratory on twelve occasions over an 8-12 week period to complete the experimental testing, with a minimum of 72 h separating all tests (figure 6.1). The experimental testing incorporated 4 pre-experimental trials (visits 1-4) and 8 experimental trials (visits 5-12). *Visits 1-4* were completed within a replica of an MRI scanner (with no magnetic field present). Initially, subjects completed a single-limb incremental test on the left leg (*visit 1,* Leg_1) and right leg (*visit 2,* Leg_2) to exhaustion to establish the limb-specific work rates that would be applied in subsequent experimental visits (as described below). Following these preliminary tests, subjects completed a familiarisation session on *visits 3 and 4* which comprised a single-leg severe-intensity constant work rate (CWR) test to exhaustion with the left leg (Leg_1), a single-leg severe-intensity CWR test to exhaustion with the right leg (Leg_2), and a crossover limb test where the Leg_1 exhaustive protocol was repeated and immediately followed by the Leg_2 exhaustive protocol to assess contralateral fatigue in Leg_2. In these preliminary tests, the exhaustive Leg_1, Leg_2 and Leg_2 contralateral protocols were interspersed by 10 min of passive recovery to minimise the time burden on participants.

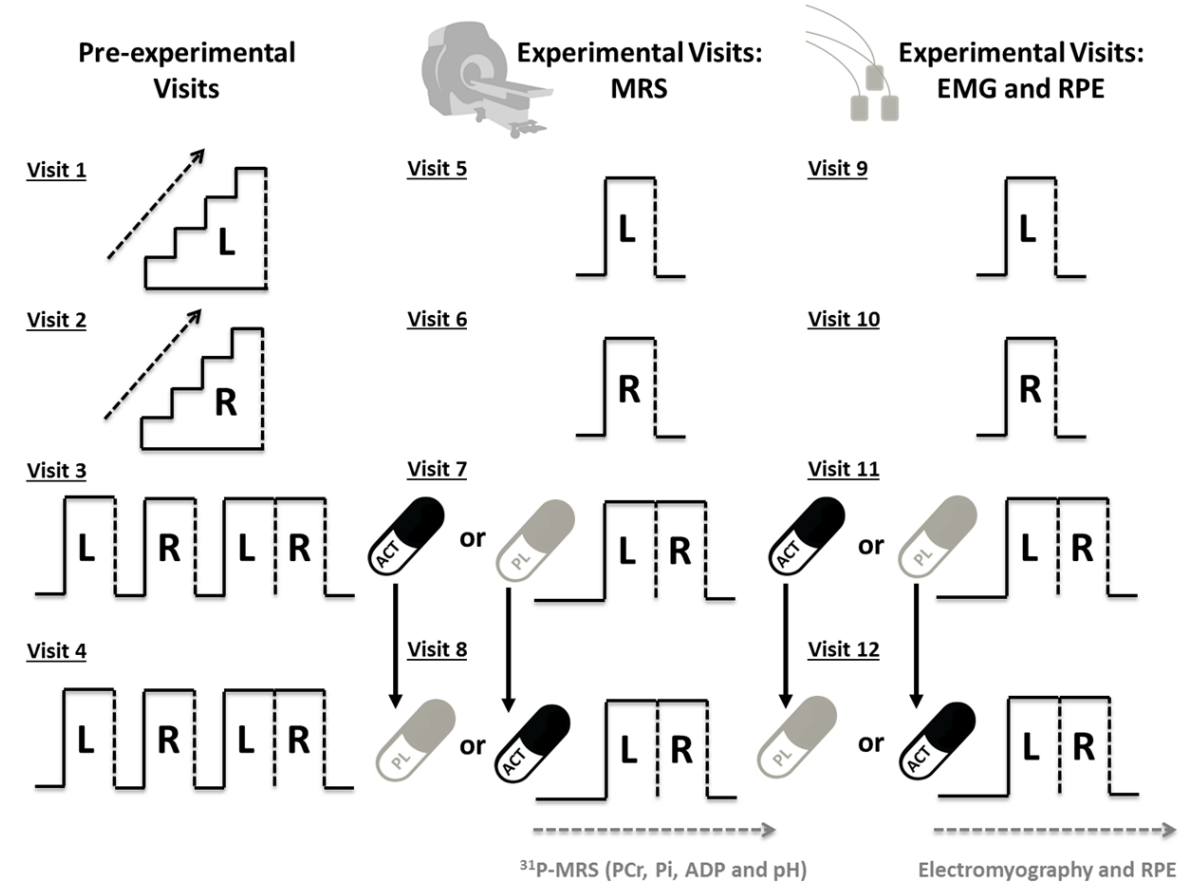

Figure 6.1. *Protocol schematic. Visits 1-4 were completed within a replica of the MRI scanner. Subjects completed a single-limb incremental test on the left leg (visit 1, Leg_1) and right leg (visit 2, Leg_2). Subjects then completed a familiarisation session on visits 3 and 4 which comprised a single-leg severe-intensity constant work rate (CWR) test to exhaustion with Leg_1, Leg_2 and a crossover limb test where the Leg_1 exhaustive protocol was repeated and immediately followed by the Leg_2 exhaustive protocol. During visits 5 and 6, subjects completed the Leg_1 and Leg_2 protocols, respectively, without oral consumption of any capsules. On visits 7 and 8, subjects commenced the crossover limb test, 45 mins following the consumption of 1 g maltodextrin (PL) and 45 mins following the consumption of 1 g ACT. Visits 5-8 were completed within the bore of an MRI scanner for assessment of intramuscular phosphorous substrates and metabolites and then replicated within a replica of the MRI scanner (visits 9-12) to assess muscle electromyography (EMG) and ratings of perceived exertion (RPE). The dashed lines represent the limit of tolerance (i.e., T_{lim}) for each limb, respectively.*

Experimental procedures

During *visits 5 and 6*, subjects completed the Leg_1 and Leg_2 protocols without oral consumption of any capsules (Leg_{1CON} and Leg_{2CON}, respectively). On *visits 7 and 8*, subjects completed the crossover limb tests described above, 45 mins following the consumption of 1 g maltodextrin (placebo, PL) to determine time to task failure (T_{lim}) values for Leg_1 (Leg_{1PL}) and Leg_2 contralateral ($Leg_{2PL\text{-}CONTRA}$), and 45 mins following the consumption of 1 g ACT, to determine T_{lim} values for Leg_1 (Leg_{1ACT}) and Leg_2 contralateral ($Leg_{2ACT\text{-}CONTRA}$). PL and ACT were administered in the form of 2 identically coloured pills. The placebo was made from maltodextrin powder inserted into gelatine capsules designed to have a similar appearance to ACT without inducing any analgesic or antipyretic effects. The oral consumption of PL and ACT ~45 min prior to commencing exercise was selected to broadly coincide with attainment of the peak plasma [ACT], which occurs ~60 min post ACT ingestion (Anderson et al. 2008; Forrest et al. 1982), at the onset of the $Leg_{2\text{-}CONTRA}$ exhaustive tests. The PL and ACT conditions were administered double-blind in a counterbalanced cross-over experimental design. *Visits 5-8* were completed within the bore of an MRI scanner for assessment of exercise-induced changes in intramuscular phosphorous-containing substrates and metabolites. *Visits 5-8* were replicated in *visits 9-12* within a replica of the MRI scanner (with no magnetic field present) to assess muscle electromyography (EMG) and ratings of perceived exertion (RPE).

Experimental set-up

Exercise tests were performed in a prone position within the bore of a 1.5 T superconducting magnet (Gyroscan Clinical Intera, Philips, The Netherlands) using a custom-built ergometer for the assessment of intra-muscular [PCr], [Pi], [ADP] and pH (*visits 5-8*) or within a replica of the MRI scanner for preliminary testing (*visits 1-4*) and the assessment of EMG and RPE responses (*visits 9-12*). Subjects' feet were fastened securely to padded foot braces using Velcro straps and connected to the ergometer load baskets via a rope and pulley system. The sprocket arrangement was such that when a bucket containing non-magnetic weights was attached, it provided a concentric-only resistive load, allowing for the performance of rhythmic knee-extension exercise. Single-leg

knee-extensions over a distance of ~0.22 m were performed continuously at a constant frequency which was set in unison with the magnetic pulse sequence (40 pulses min^{-1}) to ensure the quadriceps muscle was in the same phase of contraction during each magnetic resonance pulse acquisition. To prevent displacement of the quadriceps relative to the magnetic resonance spectroscopy (MRS) coil, Velcro straps were also fastened over the subject's thighs, hips and lower back.

Experimental protocol

To determine peak work rate (WR_{peak}) for each leg, subjects initially completed single-leg incremental knee-extensor exercise on *visits 1 and 2* until they were unable to continue the prescribed work rate, as described previously (Vanhatalo et al. 2011). The load for the initial increment was 4 kg and this was increased by 0.5 $kg \cdot min^{-1}$ thereafter until T_{lim}. T_{lim} was recorded when subjects were unable to sustain the required contraction frequency for 3 consecutive repetitions. Following these initial tests, subjects were familiarized with the different exercise tests that comprised the experimental testing protocol. During these visits, a limb-specific, high-intensity work rate (between 85% and 110% WR_{peak}), which was expected to elicit T_{lim} in approximately 5–8 min, was prescribed for each subject.

The experimental exercise protocol consisted of CWR, single-leg knee-extension exercise (94 ± 7% WR_{peak}) to T_{lim}. Initially, subjects completed single-leg knee-extension exercise for each limb individually over two separate laboratory visits. Subsequently, to investigate the influence of ACT on contralateral limb fatigue, subjects completed single-leg knee-extension exercise until exhaustion with Leg_1, followed consecutively (<3 s) by the identical task with the contralateral limb (i.e., Leg_2). These crossover limb tests to assess contralateral fatigue in Leg_2 were completed 60 min following the consumption of PL and ACT over two separate laboratory visits. For all trials, subjects received strong verbal encouragement to continue for as long as possible but no feedback was given on the elapsed time.

MRS measurements

^{31}P-MRS data were acquired every 1.5 s with a spectral width of 1,500 Hz and 1,000 data points. Phase cycling with four phase cycles was used, leading to a spectrum being acquired every 6 s. The subsequent spectra were quantified by peak fitting, using the AMARES fitting algorithm in the jMRUI (v3) software package. Absolute values of [PCr] and [Pi] concentrations were subsequently calculated via the ratio of PCr:adenosine triphosphate (ATP) and Pi:ATP assuming an ATP concentration of 8.2 mM . Intracellular pH was calculated using the chemical shift of the Pi spectra relative to the PCr peak. The ADP concentration was calculated as described by Kemp *et al*. (2001). In all cases, relative amplitudes were corrected for partial saturation resulting from the short repetition time relative to T1 relaxation time, via a spectrum consisting of 24 averages that was acquired with a TR of 20s prior to the beginning of exercise testing.

Electromyography

Throughout *visits 9-12*, muscle activity of the right and left *m.*vastus lateralis was recorded using active bipolar bar electrodes with a single differential configuration (DE2.1, DelSys Inc, Boston, MA, USA). Initially, the leg was shaved and cleaned with alcohol to minimize skin impedance. The electrodes were placed over the respective muscle bellies parallel to the longitudinal axis of each muscle (SENIAM guidelines). Double-sided adhesive tape and a hypoallergenic medical tape were used to ensure the EMG sensor stability. The position of the EMG electrodes was measured with respect to the location of the patella and the anterior superior iliac spine and marked with indelible ink to ensure placement in the same location on subsequent visits. The ground electrode was placed over the patella of the respective leg. The EMG signals were pre-amplified (1,000x), band-pass filtered (20–450 Hz, Bagnoli-8, DelSys Inc, Boston, MA, USA), and then transferred to a computer with a sampling frequency of 2 kHz. EMG data were recorded continuously and digitised synchronously with 16 bit resolution via an A/D converter (± 5 V range, CED 1401 power, Cambridge, UK) using Spike2 software (CED, Cambridge, UK). During these trials, ratings of perceived exertion (RPE) was measured at 2-min intervals from the onset of exercise using Borg's 6-20 scale (Borg, 1985).

Data Analysis

Baseline values for [PCr], [P_i], [ADP], and pH were defined as the mean values measured over the final 60 s of rest (i.e., prior to initiation of the severe-intensity exercise bout). Baseline values for Leg_2 during the crossover protocol (for both PL and ACT) were calculated during the final 60 s of exhaustive Leg_1 exercise. End-exercise values for these variables were defined as the mean values measured over the final 30 s of exercise. The changes (Δ) in [PCr], [P_i], [ADP] and pH across the protocol were then calculated as the difference between end-exercise and baseline values. [PCr], [P_i] and [ADP] were expressed as absolute concentrations and as a percentage change relative to resting baseline (i.e. 100%). The overall rate of change for [PCr], [P_i], [ADP] and pH was calculated as the difference between end-exercise and baseline values divided by T_{lim}. EMG was average rectified and normalised to the first 30 s of each trial (aEMG).

Statistics

Differences in T_{lim}, baseline and end-exercise aEMG and muscle [PCr], [Pi], [ADP], and pH between control limbs (i.e. Leg_1 vs. Leg_2) were assessed using paired samples *t*-tests. A two-way repeated measures ANOVA (time x condition) was employed to test for differences in the profiles of muscle PCr], [P_i], [ADP], and pH and aEMG (using 30 s mean values), and RPE (using 120 s mean values). Where the ANOVA revealed a significant main or interaction effect, post-hoc tests were completed using a Bonferroni correction. For calculation of effect size, partial eta squared (η^2) was used for omnibus tests. Cohen's *d* was used to calculate the effect size for paired *t*-tests and post-hoc comparisons. Where sphericity was violated, a greenhouse-geisser correction factor was used. For all tests, results were considered statistically significant when $P<0.05$. Data are presented as means ± SD unless otherwise indicated. All statistical analyses were conducted using IBM SPSS Statistics version 24.

RESULTS

There was no difference in T_{lim} during the Leg_{1CON} (396 ± 105 s) and Leg_{2CON} (385 ± 104 s) protocols ($P>0.05$, $d=0.10$, figure 6.2). Moreover, there were no differences in [PCr], [P_i], [ADP], pH (table 6.1, figure 6.3), aEMG amplitude

(table 6.2, figure 6.5) and RPE (figure 6.6) between Leg_{1CON} and Leg_{2CON} at any time (all $P>0.05$). Compared to Leg_{2CON}, T_{lim} was reduced by 19% when Leg_2 was preceded by a CWR test to exhaustion in Leg_1 following the consumption of PL (Leg_{2CON}: 385 ± 104 s vs. $Leg_{2PL\text{-}CONTRA}$: 311 ± 92 s, $P<0.01$, $d=0.76$, figure 6.2).

Effect of ACT on single-limb exercise tolerance and contralateral limb fatigue

There was no difference in T_{lim} between the Leg_{1CON} (396 ± 105 s), Leg_{1ACT} (402 ± 101 s) and Leg_{1PL} (390 ± 106 s) conditions ($P>0.05$, $\eta^2=0.07$, figure 6.2). Both $Leg_{2PL\text{-}CONTRA}$ and $Leg_{2ACT\text{-}CONTRA}$ T_{lim} were significantly lower compared to Leg_{2CON} ($P<0.05$, $\eta^2=0.71$, figure 6.2). However, there was no difference in T_{lim} between $Leg_{2PL\text{-}CONTRA}$ and $Leg_{2ACT\text{-}CONTRA}$ (311 ± 92 s vs. 324 ± 85 s, respectively, $d=0.15$, $P>0.05$, figure 6.2).

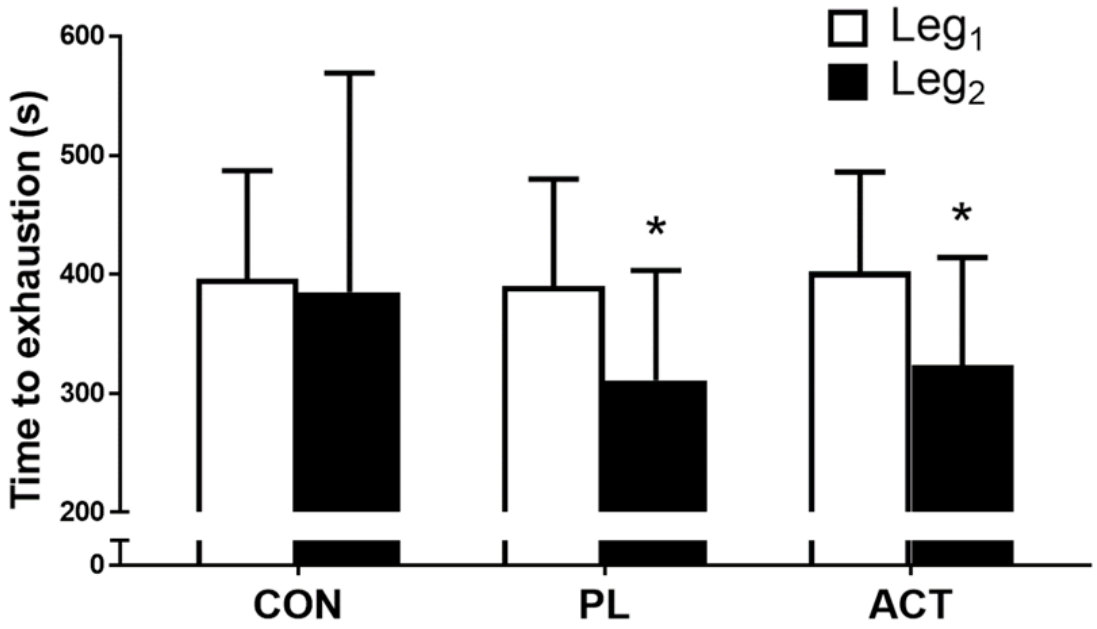

Figure 6.2. *Exercise tolerance (time to exhaustion, s) in Leg_{1CON}, Leg_{2CON}, Leg_{1PL}, $Leg_{2PL\text{-}CONTRA}$, Leg_{1ACT} and $Leg_{2ACT\text{-}CONTRA}$ conditions. Data are presented as mean ± SD *significantly different from Leg_{1CON}, Leg_{2CON}; Leg_{1PL} and Leg_{1ACT} ($P<0.05$).*

Muscle metabolic measurements

The [PCr], [Pi], [ADP] and pH profiles are illustrated in figure 6.3 for Leg_{1PL} and Leg_{1ACT} and in figure 6.4 for Leg_{2CON}, $Leg_{2PL\text{-}CONTRA}$ and $Leg_{2ACT\text{-}CONTRA}$,

respectively. There were no significant differences in [PCr], [Pi], [ADP] or pH measured at any time points between Leg_{1CON} and Leg_{2CON} (P>0.05, table 6.1, figure 6.3). Similarly, there were no differences in end-exercise [PCr] (Leg_{2CON}: 44 ± 8, $Leg_{2PL\text{-}CONTRA}$: 45 ± 7, $Leg_{2ACT\text{-}CONTRA}$: 44 ± 8%, η^2=0.13), [ADP] (Leg_{2CON}: 1024 ± 401, $Leg_{2PL\text{-}CONTRA}$: 980 ± 316, $Leg_{2ACT\text{-}CONTRA}$: 978 ± 312%, η^2=0.09) and pH (Leg_{2CON}: 6.83 ± 0.15, $Leg_{2PL\text{-}CONTRA}$: 6.83 ± 0.20, $Leg_{2ACT\text{-}CONTRA}$: 6.80 ± 0.15, η^2=0.05) between the Leg_{2CON}, $Leg_{2PL\text{-}CONTRA}$ and $Leg_{2ACT\text{-}CONTRA}$ conditions (P>0.05, table 6.1, figure 6.4). However, end-exercise [Pi] was significantly lower in $Leg_{2PL\text{-}CONTRA}$ and $Leg_{2ACT\text{-}CONTRA}$ compared to Leg_{2CON} (Leg_{2CON}: 588 ± 177, $Leg_{2PL\text{-}CONTRA}$: 459 ± 110, $Leg_{2ACT\text{-}CONTRA}$: 460 ± 109%, P=0.04, η^2=0.89, table 6.1, figure 6.4). Baseline [PCr] was significantly higher (PCr: 100 ± 0 vs. 93 ± 5 vs. 93 ± 4%, P<0.0001, η^2=3.04), and [Pi] and [ADP] were significantly lower, in Leg_{2CON} when compared to $Leg_{2PL\text{-}CONTRA}$ and $Leg_{2ACT\text{-}CONTRA}$, respectively (Pi: 100 ± 0 vs. 125 ± 24 vs. 126 ± 23%, P<0.01, η^2=2.13; ADP: 100 ± 0 vs. 200 ± 78 vs. 201 ± 77%, P<0.01, η^2=2.55, table 6.1, figure 6.4). The rate of change for [Pi] (0.05 ± 0.01 vs. 0.05 ± 0.02 vs. 0.05 ± 0.02 mmol/s, P>0.05, η^2=0.10), [PCr] (-0.06 ± 0.02 vs. -0.06 ± 0.04 vs. -0.06 ± 0.03 mmol/s, P>0.05, η^2=0.11), [ADP] (0.15 ± 0.09 vs. 0.17 ± 0.10 vs. 0.15 ± 0.09 µmol/s, P>0.05, η^2=0.17) and pH (P>0.05, η^2=0.08) was not different between the Leg_{2CON}, $Leg_{2PL\text{-}CONTRA}$ and $Leg_{2ACT\text{-}CONTRA}$ conditions, respectively.

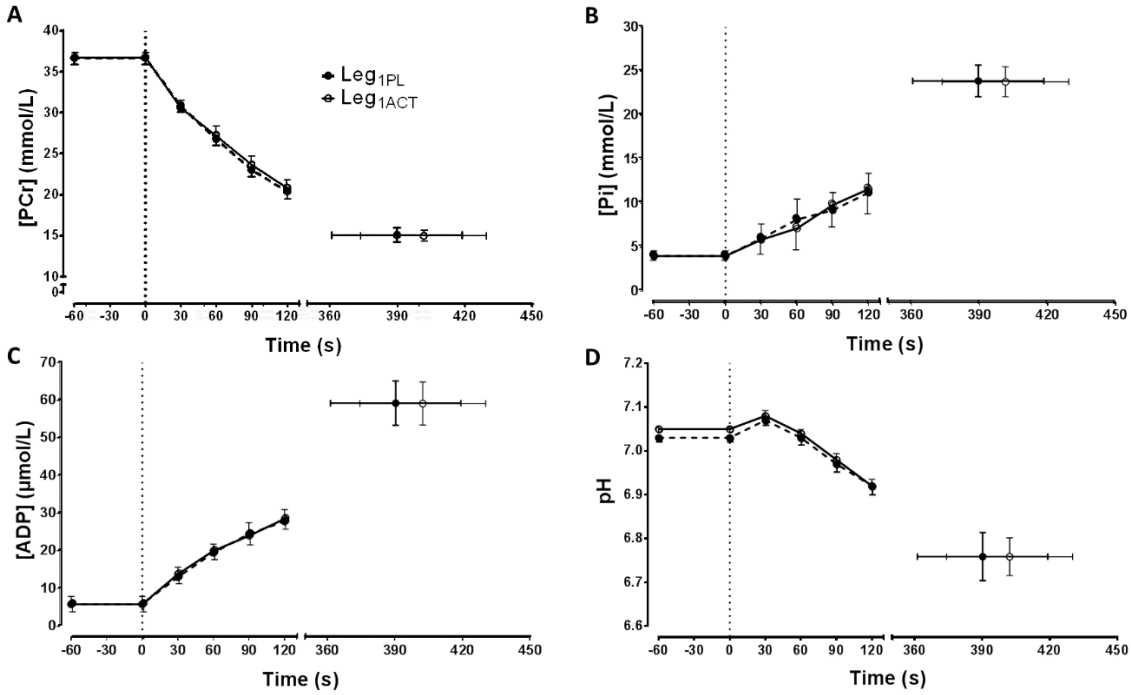

Figure 6.3. *Intramuscular phosphocreatine concentration ([PCr]; panel A), inorganic phosphate concentration ([Pi]; panel B), adenosine diphosphate ([ADP]; panel C) and pH (panel D) during severe-intensity, single-limb knee-extensor exercise in the left leg following PL ingestion (Leg_{1PL}, filled circles) and ACT (Leg_{1ACT}, clear circles) ingestion. Data are expressed as group mean ± SE.*

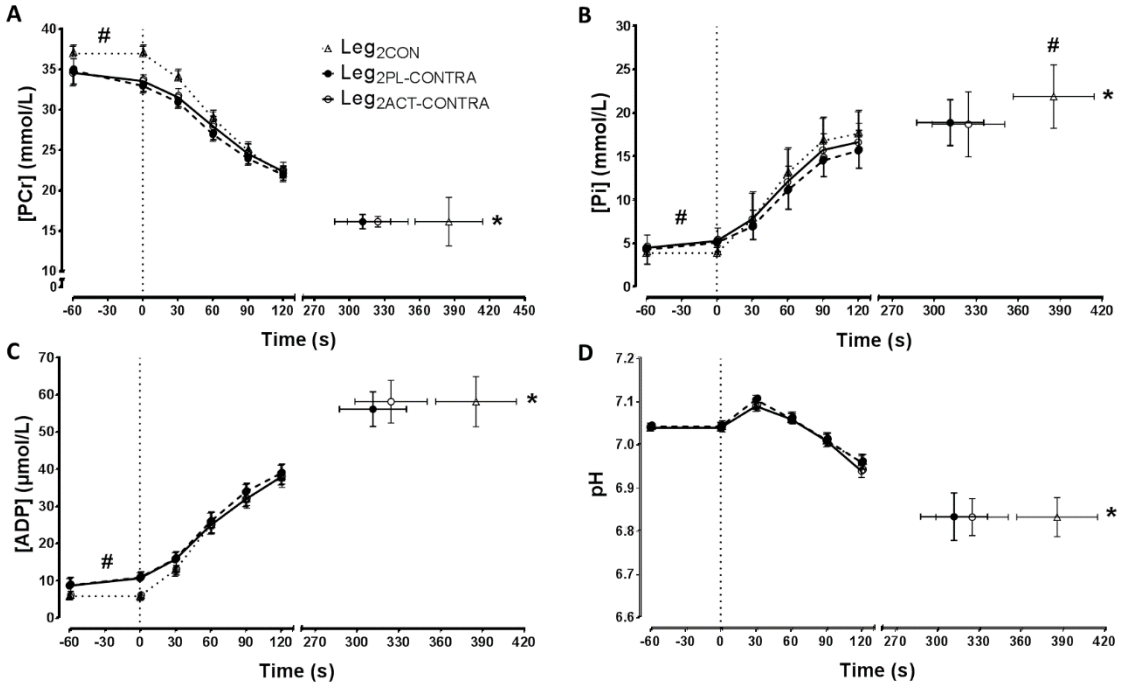

Figure 6.4. *Intramuscular phosphocreatine concentration ([PCr]; panel A), inorganic phosphate concentration ([Pi]; panel B), adenosine diphosphate ([ADP]; panel C) and pH (panel D) during severe-intensity, single-limb knee-extensor exercise in the right control leg (Leg$_{2CON}$, open triangles) and in the right leg following prior exhaustive exercise in the left leg after PL ingestion (Leg$_{2PL-CONTRA,}$ filled circles) and ACT (Leg$_{2ACT-CONTRA,}$ clear circles) ingestion. Data are expressed as group mean ± SE. *T_{lim} significantly different from Leg$_{2PL-CONTRA}$ and Leg$_{2ACT-CONTRA}$ (P<0.05); #significantly different from Leg$_{2PL-CONTRA}$ and Leg$_{2ACT-CONTRA}$ (P<0.05).*

Electromyography (n=10)

aEMG amplitude of *m*. vastus lateralis rose significantly from the first minute of exercise to end-exercise in all conditions (figure 6.5; P<0.01, η^2=3.8). However, there were no differences in aEMG between Leg$_{1CON}$, Leg$_{1PL}$ and Leg$_{1ACT}$ at T_{lim} (Leg$_{1CON}$: 229 ± 54, Leg$_{1PL}$: 224 ± 43, Leg$_{1ACT}$: 238 ± 51%, P=0.69, η^2=0.09, table 6.2, figure 6.5). End-exercise aEMG in Leg$_{2CON}$ was also similar to Leg$_{2PL-CONTRA}$ and Leg$_{2ACT-CONTRA}$, respectively (Leg$_{2CON}$: 234 ± 52, Leg$_{2PL-CONTRA}$: 226 ± 58, Leg$_{2ACT-CONTRA}$: 242 ± 52%, P=0.69, η^2=0.20, table 6.2, figure 6.5). However, absolute aEMG was elevated at the start of Leg$_{2PL-CONTRA}$ and Leg$_{2ACT-CONTRA}$

exercise when compared to Leg_{2CON} (Leg_{2CON}: 0.04 ± 0.02, $Leg_{2PL\text{-}CONTRA}$: 0.05 ± 0.02, $Leg_{1ACT\text{-}CONTRA}$: 0.05 ± 0.02 mV, $P<0.05$, $\eta^2=0.58$, table 6.2, figure 6.5).

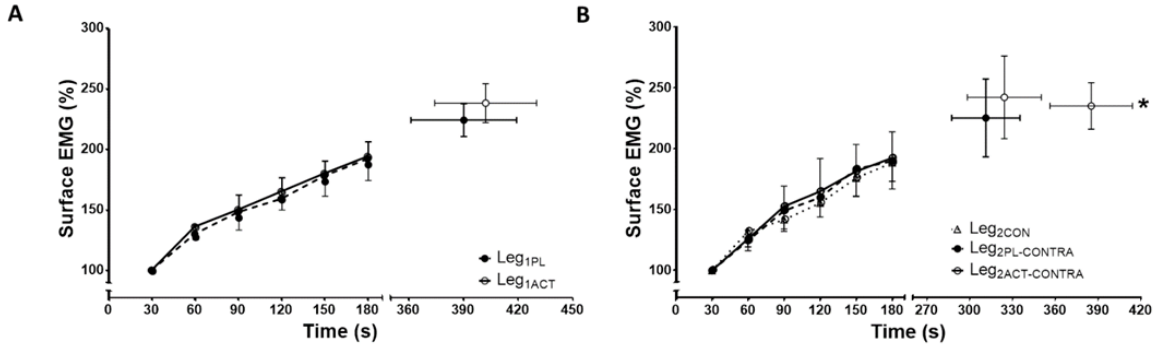

Figure 6.5. *Surface electromyography (EMG) of the m.vastus lateralis muscle during severe-intensity, single-limb knee-extensor exercise in Leg_{1PL} (filled circles) and Leg_{1ACT} (clear circles) (panel A), and in the right control leg (Leg_{2CON}, open triangles), and in Leg_2 following prior exhaustive exercise in Leg_1 after PL ($Leg_{2PL\text{-}CONTRA}$, filled circles) and ACT ($Leg_{2ACT\text{-}CONTRA}$, clear circles) ingestion (panel B). Mean values for average rectified EMG (aEMG) during each muscle contraction were calculated and averaged over each 30-s period. Data are expressed as group mean ± SE relative to the first 30 s of each trial. *T_{lim} significantly different from $Leg_{2PL\text{-}CONTRA}$ and $Leg_{2ACT\text{-}CONTRA}$ ($P<0.05$).*

Ratings of perceived exertion

RPE increased in all trials following the onset of exercise (figure 6.6). However, despite the prior ingestion of ACT, there were no differences in reported RPE between Leg_{1CON}, Leg_{1PL} and Leg_{1ACT} at any time point ($P>0.05$, $\eta^2=0.08$, figure 6.6). The rate of rise and end-exercise RPE was also similar during the Leg_{2CON} trial compared with the $Leg_{2PL\text{-}CONTRA}$ and $Leg_{2ACT\text{-}CONTRA}$ trials ($P>0.05$, $\eta^2=0.18$). However, at the onset of exercise, RPE was significantly higher in $Leg_{2PL\text{-}CONTRA}$ and $Leg_{2ACT\text{-}CONTRA}$ when compared to Leg_{2CON} ($P<0.05$, $\eta^2=0.55$, figure 6.6). Specifically, during the first 2 min of exercise, there was a 14% and 13% elevation in RPE in $Leg_{2PL\text{-}CONTRA}$ and $Leg_{2ACT\text{-}CONTRA}$, respectively, compared to Leg_{2CON} ($P<0.05$). There were no differences in RPE at any time points between $Leg_{2PL\text{-}CONTRA}$ and $Leg_{2ACT\text{-}CONTRA}$ ($P>0.05$, $\eta^2=0.21$, figure 6.6).

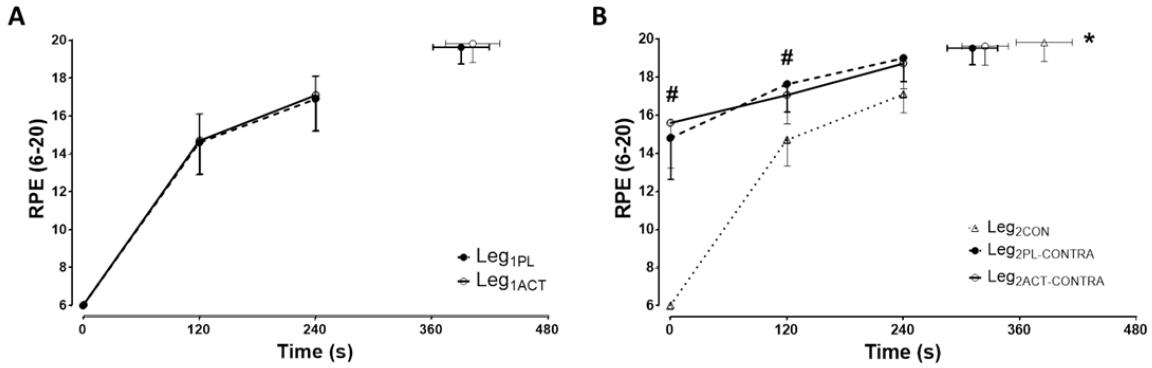

Figure 6.6. *Ratings of perceived exertion (RPE) during severe-intensity, single-limb knee-extensor exercise of the left leg in Leg_{1PL} (filled circles) and Leg_{1ACT} (clear circles) (panel A), in the right control leg (Leg_{2CON}, open triangles), and in the right leg following prior exhaustive exercise in the left leg after PL ingestion ($Leg_{2PL\text{-}CONTRA}$, filled circles) and ACT ($Leg_{2ACT\text{-}CONTRA}$, clear circles) ingestion (panel B). Data are expressed as group mean ± SE. *T_{lim} significantly different from $Leg_{2PL\text{-}CONTRA}$ and $Leg_{2ACT\text{-}CONTRA}$ ($P<0.05$); #RPE significantly different from Leg_{2CON} ($P<0.05$).*

Table 6.1. Muscle metabolic responses in Leg_{1CON}, Leg_{1PL}, Leg_{1ACT}, Leg_{2CON}, $Leg_{2PL\text{-}CONTRA}$ and $Leg_{2ACT\text{-}CONTRA}$ conditions.

	Leg_{1CON}	Leg_{1PL}	Leg_{1ACT}	Leg_{2CON}	$Leg_{2PL\text{-}CONTRA}$	$Leg_{2ACT\text{-}CONTRA}$
Muscle metabolic response						
[PCr]						
Baseline PCr (%)	100 ± 0	100 ± 0	100 ± 0	100 ± 0	92 ± 5*	93 ± 4*
PCr at 120 s (%)	70 ± 8	70 ± 8	71 ± 47	71 ± 8	62 ± 9*	63 ± 7*
End-exercise PCr (%)	42 ± 9	41 ± 9	41 ± 8	44 ± 8	45 ± 7	44 ± 8
Rate of change PCr (mmol/s)	-0.06 ± 0.01	-0.06 ± 0.03	-0.06 ± 0.02	-0.05 ± 0.03	-0.06 ± 0.04	-0.06 ± 0.03
[Pi]						
Baseline Pi (%)	100 ± 0	100 ± 0	100 ± 0	100 ± 0	125 ± 24*	126 ± 23*
Pi at 120 s (%)	310 ± 66	313 ± 71	306 ± 62	312 ± 66	316 ± 70	318 ± 64
End-exercise Pi (%)	590 ± 149	590 ± 137	594 ± 156	588 ± 177	459 ± 110*	460 ± 109*
Rate of change Pi (mmol/s)	0.05 ± 0.02	0.05 ± 0.02	0.05 ± 0.02	0.05 ± 0.02	0.05 ± 0.02	0.05 ± 0.02
[ADP]						
Baseline ADP (%)	100 ± 0	100 ± 0	100 ± 0	100 ± 0	200 ± 78*	201 ± 77*
ADP at 120 s (%)	404 ± 161	415 ± 183	400 ± 148	412 ± 170	538 ± 176	516 ± 154*
End-exercise ADP (%)	1028 ± 386	1036 ± 421	1046 ± 409	1024 ± 401	980 ± 316	978 ± 312
Rate of change ADP (µmol/s)	0.15 ± 0.08	0.15 ± 0.09	0.14 ± 0.07	0.15 ± 0.09	0.17 ± 0.10	0.15 ± 0.09
pH						
Baseline pH	7.04 ± 0.01	7.03 ± 0.02	7.05 ± 0.04	7.04 ± 0.03	7.04 ± 0.03	7.05 ± 0.02
pH at 120 s	6.96 ± 0.09	6.94 ± 0.07	6.92 ± 0.08	6.95 ± 0.08	6.93 ± 0.10	6.94 ± 0.08
End-exercise pH	6.77 ± 0.18	6.76 ± 0.15	6.76 ± 0.16	6.83 ± 0.15	6.83 ± 0.20	6.80 ± 0.15

PL, placebo; ACT, acetaminophen; EMG, electromyography; PCr, Phosphocreatine; Pi, Inorganic Phosphate; ADP, Adenosine diphosphate; *significantly different from $Leg_{2CON,,}$ $P<0.05$

Table 6.2. Electromyography (EMG) responses of *m.* vastus lateralis in Leg_{1CON}, Leg_{1PL}, Leg_{1ACT}, Leg_{2CON}, $Leg_{2PL\text{-}CONTRA}$ and $Leg_{2ACT\text{-}CONTRA}$ conditions.

	Leg_{1CON}	**Leg_{1PL}**	**Leg_{1ACT}**	**Leg_{2CON}**	**Leg_{2PL}-CONTRALATERAL**	**Leg_{2ACT}-CONTRALATERAL**
Neuromuscular function						
Baseline EMG_{RMS} amplitude (mV)	0.04 ± 0.01	0.04 ± 0.02	0.04 ± 0.02	0.04 ± 0.02	0.05 ± 0.02*	0.05 ± 0.02*
End-exercise EMG_{RMS} amplitude (%)	229 ± 54	224 ± 43	238 ± 51	234 ± 52	226 ± 58	242 ± 52
EMG_{RMS} amplitude at 120 s (%)	150 ± 27	160 ± 25	166 ± 26	158 ± 29	155 ± 32	158 ± 34

PL, placebo; ACT, acetaminophen; EMG, electromyography; RMS, root mean square; *significantly different from Leg_{2CON}, $P<0.05$. Data are from 10 subjects.

DISCUSSION

The principal original finding of this study was that, while time to task failure was shorter during severe-intensity single-leg knee extensor exercise after the completion of prior fatiguing exercise in the contralateral limb, this effect was not mitigated by acute ACT ingestion. Moreover, there was no difference in the rates of change and end-exercise values for skeletal muscle activation (via EMG), metabolic perturbation (via ^{31}P-MRS) and perceived exertion (via RPE) during exercise after prior contralateral leg fatigue following ACT and PL ingestion. There were also no differences in time to task failure (T_{lim}), skeletal muscle activation, metabolic perturbation and RPE during single-leg exercise without the completion of prior fatiguing exercise in the contralateral limb following ACT and PL ingestion. These findings do not support our experimental hypotheses and suggest that acute 1 g ACT ingestion does not improve time to task failure, skeletal muscle activation, metabolic perturbation or RPE during single-leg severe-intensity knee extensor exercise completed with or without prior fatiguing exercise by the contralateral limb. Collectively, these results contribute to our understanding of fatigue development during exercise, performed with or without prior contralateral limb exercise, and in the presence or absence of analgesia elicited by ACT ingestion.

In the present study, T_{lim} in the $Leg_{2PL\text{-}CONTRA}$ protocol was shorter than the Leg_{2CON} protocol, indicative of an earlier task failure after completing exhaustive exercise in the contralateral limb compared to no prior fatiguing contralateral limb exercise. This observation is consistent with some (i.e., Amann et al. 2013; Doix et al. 2013; Halperin et al. 2014; Halperin et al. 2014; Kennedy et al. 2013; Rattey et al. 2006; Takahashi et al. 2011), but not all (i.e., Elmer et al. 2013; Grabiner & Owings, 1999; Regueme et al. 2007; Todd et al. 2003; Zijdewind et al. 1998), previous studies reporting greater fatigue development after prior contralateral or non-local muscle fatigue. While the neuromuscular bases of contralateral fatigue development have yet to be resolved, there is evidence to suggest that greater central fatigue makes an important contribution to this phenomenon (Amann et al. 2013). In the current study, RPE was higher at baseline and over the initial stages of the $Leg_{2PL\text{-}CONTRA}$ test compared to the Leg_{2CON} test, leading to an earlier attainment of peak RPE and T_{lim}, consistent

with previous observations (i.e., Amann et al. 2013) and the notion that afferent feedback may contribute to increased pain and effort sensation (Amann et al. 2011; Gagnon et al. 2012). Amann and colleagues (2013) also previously reported a lower EMG response at task failure and reduced peripheral fatigue development after prior contralateral leg fatigue. Although the EMG amplitude was not different at task failure in the current study between the Leg_{2CON} and $Leg_{2PL\text{-}CONTRA}$ tests, baseline EMG was elevated in the $Leg_{2PL\text{-}CONTRA}$ condition, presumably due to isometric stabilisation, leading to the earlier attainment of the same peak EMG amplitude. The greater muscle activation in the non-exercising contralateral limb during the baseline 'resting' period in the $Leg_{2PL\text{-}CONTRA}$ condition was accompanied by lower muscle [PCr], and higher muscle [Pi] and [ADP] compared to the Leg_{2CON} condition. Since there were no differences in muscle [PCr] and [ADP] at T_{lim}, and since the rates of change in [PCr] and [ADP] were not different between the Leg_{2CON} and $Leg_{2PL\text{-}CONTRA}$ tests, the muscle [PCr] nadir and [ADP] peak were attained earlier in the $Leg_{2PL\text{-}CONTRA}$ test. These observations cohere with reports that the end-exercise values of muscle [PCr], [ADP] and pH are consistent both when several bouts of exhaustive exercise of differing duration are completed within the severe-intensity domain (Vanhatalo et al. 2010) and when T_{lim} is altered via prior passive heating of the legs (Bailey et al. 2012) or by hyperoxic gas inhalation (Black et al. 2017; Vanhatalo et al. 2010). Interestingly, however, and despite a higher baseline muscle [Pi] in the $Leg_{2PL\text{-}CONTRA}$ condition compared to the Leg_{2CON} condition, muscle [Pi] was lower at the point of task failure in the $Leg_{2PL\text{-}CONTRA}$ test. These novel observations suggest that the ergolytic effect of prior contralateral fatigue may be related, at least in part, to a limitation in the attainment of peak intramuscular [Pi].

It is unclear why prior contralateral limb fatigue limited the attainment of peak [Pi] in the $Leg_{2PL\text{-}CONTRA}$ condition compared to the Leg_{2CON} condition, whereas the peak [ADP] and the nadir in pH and [PCr] were not different between these conditions. However, our observations of a limited peak perturbation of muscle [Pi], but not pH, [PCr] and [ADP], when group III/IV muscle afferent feedback would be expected to be enhanced via prior contralateral fatigue (Amann et al. 2013), are in accord with studies from another group who observed greater

peak perturbation of muscle [Pi], but not pH, [PCr] and [ADP], when muscle group III/IV afferent feedback was abolished via lumbar intrathecal administration of fentanyl (Blain et al. 2016; Broxterman et al. 2017b, 2018). Taken together, these complementary observations suggest that intramuscular phosphorous-containing metabolites and substrates do not respond in a uniform manner to manipulations in skeletal group III/IV muscle afferent feedback and that muscle [Pi] might be a more sensitive marker of muscle afferent feedback and peripheral fatigue development. However, it should be acknowledged that, since inter-test variability is greater for contracting skeletal muscle [Pi] than for pH, [PCr] and [ADP] (Edwards et al. 2012), further research is required to verify these observations as this variation may explain these differences.

Although the completion of prior single-leg fatiguing exercise lowered T_{lim} during subsequent exercise in the contralateral limb in the current study, there were no differences between the $Leg_{2ACT\text{-}CONTRA}$ and $Leg_{2PL\text{-}CONTRA}$ conditions in T_{lim}, RPE, or muscle activation and phosphorous-containing metabolites and substrates. Similarly, and also in contrast to our hypothesis, acute ACT ingestion did not alter T_{lim}, RPE, or muscle activation, pH, [PCr], [ADP] or [Pi], during single-leg severe-intensity knee extensor exercise completed without prior fatiguing exercise in the contralateral limb, with these responses being similar between the Leg_{1CON}, Leg_{1PL} and Leg_{1ACT} conditions. These findings conflict with reports that acute ACT consumption can improve exercise performance by increasing pain tolerance (Foster et al. 2014; Mauger et al. 2010) and muscle activation (Morgan et al. 2018a, b). The lack of an ergogenic effect of ACT administration in the current study might be due to differences in the ACT administration procedure compared to previous studies reporting improved performance and delayed neuromuscular fatigue development (Foster et al. 2014; Mauger et al. 2010; Morgan et al. 2018a, b). In the present study, ACT was ingested 45 min prior to the start of the Leg_{1ACT} test, which immediately transitioned into the $Leg_{2ACT\text{-}CONTRA}$ protocol that was the primary focus of the current study. Since peak plasma [ACT] is attained ~60 min post oral ACT ingestion (Anderson et al. 2008; Forrest et al. 1982), we elected to administer ACT in the current study such that peak plasma [ACT] was expected to coincide with the onset of the $Leg_{2ACT\text{-}CONTRA}$ protocol rather than the Leg_{1ACT}

protocol. This might account for the lack of an ergogenic effect of ACT during the Leg_{1ACT} protocol compared to other studies that administered ACT 60 min prior to the performance trial (Foster et al. 2014; Mauger et al. 2010; Morgan et al. 2018a, b). Therefore, we cannot exclude the possibility that earlier ACT ingestion (Foster et al. 2016), at the same or a greater dose (Foster et al. 2014; Mauger et al. 2010), might have resulted in improved single-leg severe-intensity exercise tolerance. However, it should also be noted that differences between participants (i.e., training status, familiarity with the exercise task, different responses to analgesic medication) may have also contributed to the lack of ergogenic effect observed. Future research should continue to explore these potential explanations.

In addition to differences in the ACT dosing procedure, the lack of an ergogenic effect of ACT administration in the current study might be linked to the nature of the fatiguing exercise test administered. In the present study, subjects completed continuous single-leg severe-intensity (~95% WR_{peak}) knee extensor exercise until task failure with no pre-determined end point (i.e., an 'open loop' exercise test). This differs from situations in which ACT ingestion has been reported to be ergogenic, such as completion of a fixed distance (16.1-km) time-trial (Mauger et al. 2010), a fixed number of maximal effort repetitions (Foster et al. 2014; Morgan et al. 2018a), or a fixed duration of maximal effort (Morgan et al. 2018b), all of which have a predetermined end point (i.e., a 'closed loop' exercise task). Moreover, since exercise-induced pain sensation is positively associated with exercise intensity (Astokorki & Mauger, 2017; Cook et al. 1997), and since ACT ingestion is suggested to be ergogenic by mitigating pain sensation (Foster et al. 2014; Mauger et al. 2010), this might account for the improved exercise performance reported during maximal-intensity exercise following ACT ingestion (Foster et al. 2014; Morgan et al. 2018a, b), and the lack of improvement in performance in the longer duration, continuous severe-intensity exercise test employed in the current study. With regard to contralateral fatigue development, we cannot exclude the possibility that ACT might have been effective at attenuating the effects of prior single-leg fatigue on T_{lim} during subsequent exercise had a greater degree of contralateral fatigue been attained. For example, T_{lim} was lowered by 19% in $Leg_{2PL\text{-}CONTRA}$

compared to Leg_{2CON} in the current study, whereas Amann and colleagues (2013) reported a much larger (49%) reduction in T_{lim} following contralateral limb fatigue, which would have provided greater scope for an ergogenic effect with ACT ingestion. Moreover, since RPE is higher and T_{lim} is shorter at the same relative exercise intensity when a larger muscle mass is recruited (Rossman et al. 2012), it is possible that ACT ingestion might have improved T_{lim} during exercise after prior fatigue had a larger muscle mass been recruited in either the initial or the subsequent fatiguing exercise task. Further research is required to assess the exercise settings in which ACT administration is more or less likely to be ergogenic (and its mechanistic underpinnings).

In conclusion, the completion of prior single-leg fatiguing exercise compromised exercise tolerance during subsequent exercise in the contralateral leg. This ergolytic effect of prior contralateral limb fatigue was accompanied by elevated RPE, muscle activation and [ADP], and lower [PCr], at baseline leading to the earlier attainment of peak (RPE, muscle activation and [ADP]) or nadir (muscle [PCr]) values in these variables, and the attainment of a submaximal end-exercise [Pi]. However, acute ACT ingestion was not effective at lowering RPE, increasing muscle activation or intramuscular perturbation or enhancing T_{lim} during single-leg severe-intensity exercise completed with or without prior fatigue in the contralateral leg. These findings do not support an ergogenic effect of analgesia, at least using the ACT administration and exercise testing procedures employed in the current study.

Acute ibuprofen ingestion does not attenuate fatigue during maximal intermittent knee extensor or all-out cycling exercise

Original investigation

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Paul T. Morgan, Anni Vanhatalo, Joanna L. Bowtell, Andrew M. Jones and Stephen J. Bailey[1]

[1]Sport and Health Sciences, College of Life and Environmental Sciences, University of Exeter, St. Luke's Campus, Heavitree Road, Exeter, EX1 2LU, UK.

[2]School of Sport, Exercise and Health Sciences, Loughborough University, Ashby Road, Loughborough, Leicestershire LE11 3TU, UK.

Chapter 7: Acute ibuprofen ingestion does not attenuate fatigue during maximal intermittent knee extensor or all-out cycling exercise

ABSTRACT

Purpose: Recent research suggests that acute consumption of pharmacological analgesics can improve exercise performance, but the ergogenic potential of ibuprofen (IBP) administration is poorly understood. This study tested the hypothesis that IBP administration would enhance maximal exercise performance. **Methods**: In one study, 13 physically active males completed 60 × 3-s maximum voluntary contractions (MVC) of the knee extensors interspersed with a 2-s passive recovery period, on two occasions, with the critical torque (CT) estimated as the mean torque over the last 12 contractions (part A). In another study, 16 active males completed two 3-min all-out tests against a fixed resistance on an electrically-braked cycle ergometer with the critical power (CP) estimated from the mean power output over the final 30-s of the test (part B). All tests were completed 60 min after ingesting maltodextrin (placebo, PL) or 400 mg of IBP. Peripheral nerve stimulation was administered at regular intervals and electromyography was measured throughout. **Results**: For part A, mean torque (IBP: 60 ± 12 vs. PL: 58 ± 14% of pre-exercise MVC) and CT (IBP: 41 ± 16 vs. PL: 40 ± 15% of pre-exercise MVC) were not different between conditions (P>0.05). For part B, end-test power output (IBP: 292 ± 28 W vs PL: 288 ± 31 W) and work done (IBP: 65.9 ± 5.9kJ vs PL: 65.4 ± 6.4kJ) during the 3-min all-out cycling tests were not different between conditions (all P>0.05). For both studies, neuromuscular fatigue declined at a similar rate in both conditions (P>0.05). **Conclusion**: Acute ingestion of 400 mg IBP does not improve single-leg or maximal cycling performance in healthy humans.

Key words: Electromyography; neuromuscular fatigue; non-steroidal anti-inflammatory drugs; single leg exercise; whole-body cycling exercise

Chapter 7: Acute ibuprofen ingestion does not attenuate fatigue during maximal intermittent knee extensor or all-out cycling exercise

INTRODUCTION

Ibuprofen (IBP), a non-steroidal anti-inflammatory drug (NSAID) predominantly used to treat pain and reduce inflammation and fever, is considered safe for oral ingestion within healthy populations at the standard therapeutic dose (Albert & Gernaat, 1984; García-Martín et al. 2004). Consequently, utilisation of analgesic (e.g., pain relieving) and anti-inflammatory medication, such as IBP, has emerged as a popular pre-competition strategy amongst elite athletes attempting to enhance athletic performance (Alaranta et al. 2008; Corrigan & Kazlauskas, 2003; Da Silva et al. 2015; Gorski et al. 2011; Huang et al. 2006). It is believed that NSAIDs act centrally and peripherally to reduce the perception of pain and tissue inflammation, respectively (Fridén & Lieber, 1992). The therapeutic effect of NSAIDs in treating inflammation and pain has largely been ascribed to inhibited synthesis of prostaglandins (e.g., PGE_2, Fridén & Lieber, 1992). Specifically, IBP limits the metabolism of arachidonic acid, a precursor for the synthesis of prostaglandins, by inhibiting the enzyme, cyclooxygenase (Albert & Gernaat, 1984).

Following the onset of muscle contractions, changes in contraction-induced mechanical stimuli and noxious chemicals (including an increased PGE_2 release) activate and/or sensitise molecular receptors located on the terminal end of group III and IV nerve fibers, contributing to an increased sensation of muscle pain during exercise (McCord & Kaufman, 2010; O'Connor & Cook, 1999; Pollak et al. 2014). The activation of these receptors appears to play a role in neuromuscular fatigue development through modulating both central and peripheral fatigue. Indeed, when the ascending projection of group III and IV muscle afferents is attenuated via intrathecal fentanyl administration, central motor drive is increased (as inferred via electromyography, EMG) and peripheral fatigue development is expedited (Amann et al. 2009, 2011; Blain et al. 2016). Therefore, it is possible that an intervention that is able to reduce the magnitude of afferent feedback, such as NSAID administration, may attenuate the decline in skeletal muscle activation during intense exercise and thus improve exercise performance (Amann & Calbet, 2008; Morgan et al. 2018a; Morgan et al. 2018b). Indeed, elevating the magnitude of muscle afferent

feedback is known to impair endurance exercise capacity (e.g., Amann et al. 2013).

Acute consumption of analgesics (e.g., acetaminophen, ACT) has been shown to improve exercise performance (Foster et al. 2014; Mauger et al. 2010; Morgan et al 2018a; Morgan et al. 2018b). Mauger et al. (2010) administered an acute dose of 1.5 g ACT to trained cyclists and reported a 2% improvement in 16.1-km time-trial (TT) performance. Moreover, an acute dose of ACT has been shown to improve exercise tolerance in the heat (Mauger et al. 2014), repeated sprint performance (Foster et al. 2014), repeated maximal voluntary contraction (MVC) performance of the knee extensors (Morgan et al. 2018a) and maximal cycling performance (Morgan et al. 2018b). However, while these studies suggest that oral administration of a commercially-available pharmaceutical analgesic can delay fatigue development and improve exercise performance (Foster et al. 2014; Mauger et al. 2010; Morgan et al 2018a, b), the ergogenic effects of IBP are unclear despite widespread use of IBP as a putative performance aid (Cleak, & Eston. 1992; Nosaka & Clarkson, 1996).

In contrast to the effects of ACT consumption on exercise performance, there is currently very limited published research on NSAID and NSAID-like compounds during exercise when muscle damage is not present. Despite the prevalence of its use amongst athletic populations, it is unclear whether IBP will provide an ergogenic benefit to performance in a 'fresh' state. Other NSAID-like compounds have elicited no ergogenic effect on endurance performance (e.g., aspirin, Cook et al. 1997; ginger, Black & O'Connor, 2008). In contrast, enhanced aerobic exercise performance following caffeine ingestion has been related, at least in part, to altered pain perception and enhanced endurance performance (e.g., Gonglach et al. 2016). In addition, whilst exercise has been shown to increase PGE_2 release (Trappe et al. 2001; Nowak & Wennmalm, 1978; Wilson & Kapoor, 1993; Viinikka et al. 1984), the extent to which it plays an important role, especially following interventions aimed at reducing PGE_2 synthesis, during endurance exercise in the absence of muscle damage is unclear.

The purpose of this study was to test the hypotheses that, compared to a placebo, acute consumption of 400 mg IBP would increase total work done, and reduce the rate of fatigue development by enabling a better maintenance of muscle activation during exercise. Two studies are presented using two different types of exercise: a 5-min single-leg intermittent MVC test and a 3-min maximal whole body cycling test to provide a more comprehensive understanding of the ergogenic potential of IBP.

METHODS

Participants

Two independent groups of thirteen recreationally-active males (mean ± SD: age 31 ± 7 years, height 1.76 ± 0.08 m, body mass 75 ± 11 kg), and sixteen recreationally-active males (mean ± SD: age 29 ± 9 years, height 1.79 ± 0.07 m, body mass 77 ± 8 kg, $\dot{V}O_{2peak}$ 60.8 ± 7.8 $ml \cdot kg^{-1} \cdot min^{-1}$), volunteered for the first (A) and second (B) part of this study, respectively. All participants provided written, informed consent to participate in this study, which was approved by the Sport and Health Sciences Ethics Committee at the University of Exeter. After being informed of the experimental procedures and associated risks, all participants completed a medical health questionnaire to ensure it was safe for them to consume IBP prior to performing exhaustive exercise, due to the potential contraindications associated with IBP ingestion. Participants were not consumers of any 'pain relief' or anti-inflammatory medication (prescription or non-prescription) over the course of the study. None of the participants had a history of motor or neurological disorders. Participants were instructed to arrive at the laboratory in a rested and fully hydrated state, at least 3 h post-prandial, and to avoid strenuous exercise and refrain from consuming caffeine and alcohol in the 24 h preceding each testing session. Participants were also instructed to consume their habitual diet and continue normal training activities for the experimental period. For part A and B, participants recorded their diet and physical activity for 7 d prior to the first experimental visit and then replicated this for all remaining experimental visits, respectively.

Experimental Design

Both protocols (part A and B) followed identical experimental designs to previous research conducted within our laboratory (Morgan et al. 2018a, 2018b). For part A (Morgan et al. 2018a), participants visited the laboratory on three occasions over a 3-4 week period with tests being conducted on an isokinetic dynamometer (Biodex System 3, Shirley, NY, USA). For part B (Morgan et al. 2018b), participants visited the laboratory on five occasions over a 5-6 week period and tests were conducted on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands). Experimental tests were separated by at least 7, but no more than 9, days (part A) and for at least 72 h (part B), and were completed at a similar time of day (± 90 mins). The first laboratory visits for each study were used to familiarise participants to the measurements and experimental protocols described below. Subsequently, participants performed the fatiguing protocol (s) under two conditions *(see 'Experimental Protocol')*: placebo (PL) and IBP.

Experimental Protocol A (60 MVC protocol)

The isokinetic dynamometer was initially adjusted so that the axis of rotation of the lever arm was in line with the lateral epicondyle of the right femur. Participants were seated with the hip and knee joints at relative angles of 155° and 90°, respectively. The remainder of the chair settings were recorded (during familiarisation) and replicated in all subsequent tests to ensure identical body position throughout the experimental trials. Inelastic padded Velcro straps were fastened at the ankle, quadriceps, hip and shoulders to maintain a stable body position.

Following the familiarisation trial, visits 2 and 3 were completed in a double-blind, randomised fashion using a cross-over experimental design. 1000 mg of maltodextrin (placebo, PL) or 400 mg IBP (combined with 600 mg maltodextrin) was ingested orally, 60 minutes prior to the exercise bout such that the start of the exercise trial was expected to coincide with peak plasma [ibuprofen] concentration (Janssen & Venema, 1985). For IBP, 2 identical capsules containing 200 mg ibuprofen and 300 mg maltodextrin each were ingested. The placebo was made from dextrose powder inserted into 2 gelatine capsules (500

mg in each capsule) designed to have a similar appearance and the weight to IBP capsules. The trials started with a standardised isometric warm-up routine (10 isometric contractions for 3 s at 50% of pre-exercise MVC as measured during familiarisation testing) and testing of the optimal EMG electrode, anode, and cathode placement and stimulation intensity for peripheral nerve stimulation. The experimental protocol consisted of 60 brief MVCs (3 s contraction, 2 s rest), in response to a visual prompt to 'go' and 'relax', accompanied by the same verbal instructions from the experimenter. Every 6th contraction was accompanied by peripheral nerve stimulation during and 1 s post MVC (as described for pre-trial measurements below). Participants were not made aware of the time or the number of MVCs that had elapsed during the protocol and were instructed to continue to perform maximal contractions throughout.

Experimental protocol B (3-min cycling test)

For the 3-min cycling protocol, participants initially completed an incremental ramp test to exhaustion for the determination of gas exchange threshold (GET), linear factor, peak aerobic power output and peak oxygen uptake $\dot{V}O_{2peak}$ as previously described (Black et al. 2014; Morgan et al. 2018b). The fixed resistance for the 3-min cycling protocol was set using the linear mode (e.g., linear factor) of the ergometer such that on reaching their preferred cadence, the participants would achieve a power output equivalent to 50% of the difference between GET and $\dot{V}O_{2peak}$ (linear factor = 50%Δ peak aerobic power output/preferred cadence^2). During this visit, the seat and handlebar positions were adjusted for comfort and replicated for all tests. During the second and third laboratory visits, participants completed a 3-min all-out test with these serving as familiarization trials to the experimental protocol as described below and to ensure the coefficient of variation for work done and critical power (CP) between visits was <1%. Participants then performed the 3-min all-out cycling test under two conditions: placebo (PL) and IBP.

The experimental protocol consisted of a 3-min period of unloaded pedalling at each participant's preferred cadence (85-100 rpm), followed by a 3-min all-out sprint, 60 min following ingestion of either placebo (1000 mg maltodextrin) or

400 mg of IBP. The order of trials on visits 4 and 5 were administered in a double-blind, randomised fashion using a cross-over experimental design. The 3-min all-out cycling protocol used in this study replicated the procedures described previously by Vanhatalo et al. (2007, 2008).

Neuromuscular function (Parts A and B)

For part A and B, neuromuscular function was assessed pre-, during- and post-trial (< 10 s). Single peripheral nerve stimulation pulses were manually triggered at rest to determine pre-exercise neuromuscular function, namely the characteristics of the M-wave response (M-wave amplitude; M_{max}) to supra-maximal nerve stimulation, voluntary activation (for part A only) and potentiated twitch torque (pTw, for part A only). During MVCs, peripheral nerve stimulation pulses were triggered to occur as soon as a peak torque was achieved (typically 1.5 s into a 3 s contraction) and were each separated by a 45 s rest period. The stimuli were also delivered 1-s after the cessation of the contraction to provide a resting pTw. Identical measurements were repeated as soon as possible (< 10s) after the fatiguing exercise to determine post-exercise neuromuscular function (see figure 7.1).

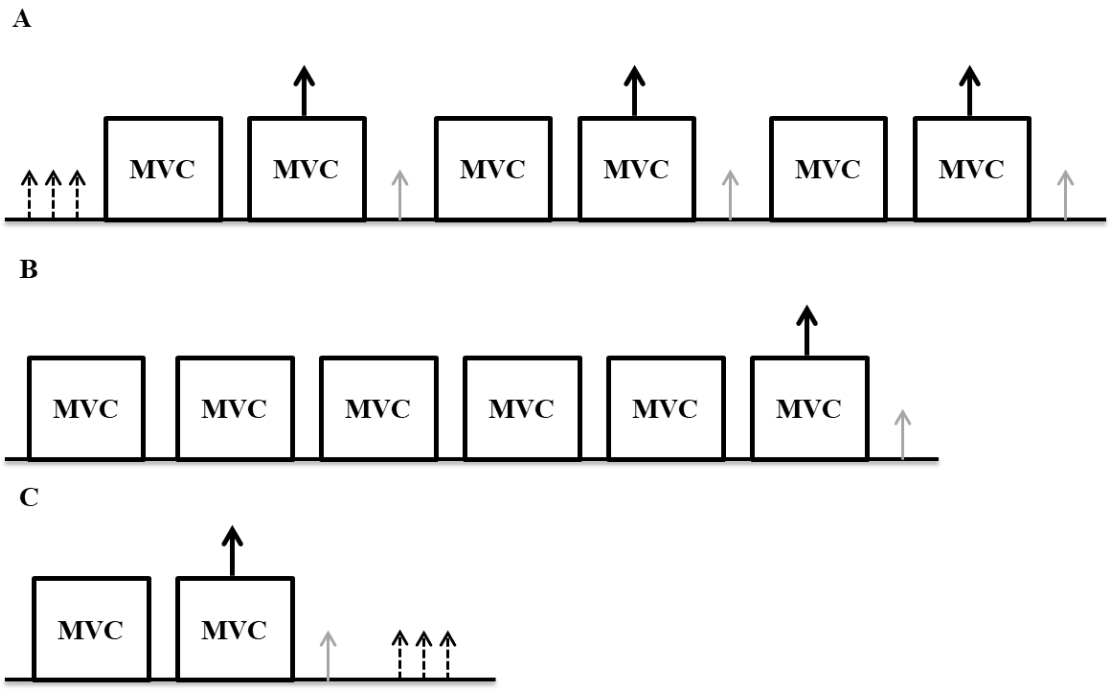

Figure 7.1. *Schematic of the procedures used prior to (panel a), during (panel b) and within 10 s following (panel c) the 60 maximal isometric voluntary contraction (MVC) protocol (part A). 10 s separated each single pulse stimulation administered at rest (small dashed arrows). A, C 45 s rest period separated maximal efforts (MVCs). Single pulse stimuli were administered during peak force production of MVCs (large solid arrow) and immediately (<1-s) post MVCs (small grey arrows). B: 60 MVC protocol of the knee extensors. The figure presents a period of 30 s which is repeated sequentially for 5 min. Each MVC was held for 3 s and interspersed by a 2 s passive recovery period. Every 6th MVC was accompanied by single pulse stimuli administered during peak force production (large solid arrow) and immediately following (<1-s) post MVCs (small grey arrows). This cycle was repeated 10 times such that the protocol spanned 5 minutes requiring the completion of 60 MVCs. Surface electromyography (EMG) was measured throughout.*

Torque (Part A)

For part A, knee-extensor torque from the Biodex isokinetic dynamometer was sampled at 1000 Hz and low-pass filtered at 40 Hz, before being displayed on a

wide screen monitor using Spike2 (CED, Cambridge, UK). Torque was expressed throughout as a percentage (%) of initial pre-exercise MVC.

Breath-by-breath pulmonary gas exchange (Part B)

For the 3-min maximal cycling protocol, participants wore a face mask connected to an impeller turbine transducer assembly (Cortex Metalyzer, Cortex, Leipzig, Germany). Inspired and expired gas volume and concentration signals were continuously sampled at 100 Hz. The analyser was calibrated before each test with gases of known concentration, and a calibration syringe of known volume (3-L; Hans Rudolph, KS, USA).

Electromyography (Parts A and B)

For parts A and B, surface EMG activity was recorded from *m.*vastus lateralis, *m.*vastus medialis and *m.*rectus femoris of the quadriceps and *m.*biceps femoris of the hamstring of the right leg using active bipolar bar electrodes in a single differential configuration (DE2.1, DelSys Inc, Boston, MA, USA). The electrodes were placed over the respective muscle bellies (SENIAM guidelines). Double-sided adhesive tape and a hypoallergenic medical tape were used to ensure the EMG sensor stability. The skin area underneath each EMG electrode was shaved, then exfoliated and cleaned with alcohol to minimise the skin impedance. The EMG and torque signals were pre-amplified (1,000 x), band-pass filtered (20–450 Hz, Bagnoli-8, DelSys Inc, Boston, MA, USA), and then transferred to a computer with a sampling frequency of 2 kHz. EMG and torque data were recorded continuously and digitised synchronously with 16 bit resolution via an A/D converter (± 5 V range, CED 1401 power, Cambridge, UK). EMG was average rectified using the root mean square method (EMG_{RMS}). EMG_{RMS} was then normalised to the pre-exercise maximum (or maximal EMG signal) and the local M-wave amplitude (closest time point measure of the M-wave) in order to exclude any changes to the EMG trace to changes in local excitability. The ground electrode was placed over the patella of the right leg.

Peripheral Nerve Stimulation (Parts A and B)

Electrical stimulation was applied with a constant current stimulator (Digitimer Stimulator DS7A, Digitimer, UK) for the assessment of M-waves (parts A and B)

and potentiated twitch force (part B). M-waves were elicited by supramaximal percutaneous electrical stimulation of the femoral nerve (200 μs duration). The cathode was placed over the femoral nerve in the inguinal fossa, approximately 3–5 cm below the inguinal ligament in the femoral triangle. The cathode was systematically moved vertically and horizontally and the amplitude of the muscle action potential (e.g., M-wave) was monitored to identify the optimal position of the cathode for attaining maximal peak-to-peak M-wave amplitude. Once the M-wave was elicited, the maximum amplitude (peak-to-peak) of the M-wave was determined (M_{max}) for the vastus lateralis and vastus medialis. To determine the stimulation intensity, single stimuli were delivered in 20 mA step-wise increments from 100 mA until plateaus in quadriceps pTw (part A only) and M-wave were observed. To ensure a supramaximal response, the current was increased by an additional 30% (mean ± SD current = 214 ± 66 mA; 251 ± 46 mA, part A and B, respectively). The average M_{max} was obtained from 3 stimuli, with ~8-10 s separating each pulse at rest. For the 3-min maximal cycling protocol (part B), single peripheral nerve stimulation pulses were manually triggered at 'rest' (defined as 80 rpm at 20 W) to determine pre-exercise neuromuscular function. Initially, the crank angle at which peripheral nerve stimulation was to be delivered during the trials was determined for each subject as described by Black et al. (2017) and as performed by Sidhu et al. (2012). Peripheral nerve stimulation pulses were triggered to coincide with maximal muscle activation around the crank cycle (typically around 50-60º from top-dead centre) 3 times, randomly, during a 10 s period using a custom written sequencer script. Identical measurements were repeated every 30 s in the all-out sprint.

Data Analyses

Data were analysed using a custom written script developed in Spike2 software (CED, Cambridge, UK). For part A, mean torque for each 3 s contraction during the 60 MVC protocol was determined as the mean value over a 1-s period which approximated the plateau level of the highest torque (e.g., 500 ms before and after the peak torque). The pTw was calculated as the peak torque achieved following the single pulse delivered 1-s post-MVC. The twitch torque superimposed onto the peak force production of the MVC (sTw) was calculated

as the increment in torque immediately following the pulse during MVCs. The end-test torque (e.g., critical torque, CT) during the 60 MVC test was defined as the mean of the last 12 contractions (e.g., the last 60 s; Burnley, 2009; Morgan et al. 2018a). The torque-impulse was calculated as the area under the torque-time curve by accumulating the time integral of each MVC (3 s).

Voluntary activation (VA, %) was calculated using the interpolated twitch method from peripheral nerve stimulation (Merton, 1954; Goodall et al. 2010). Specifically, the increment in torque evoked during the MVCs was expressed as a fraction of the amplitude of the potentiated twitch produced with the same stimulus in the relaxed muscle post-MVC. The level of voluntary drive was then quantified as a percentage: [1 – evoked torque (superimposed on voluntary torque, sTw)/ (mean control evoked response, pTw) × 100] (e.g., Allen at al. 1998). The changes in voluntary torque and pTw, were used to assess global fatigue and peripheral fatigue, respectively with VA, M_{Max} and EMG_{RMS} used to assess central fatigue. The maximal EMG was taken from the first MVC during the 60 MVC task and compared to the last MVC at task end. The neuromuscular parameters extracted from the three sets of maximal contractions completed post-exercise were tested for statistical differences between sets of contractions and then compared to the first set of MVCs completed pre-exercise (Doyle-Baker et al. 2018; Froyd et al. 2013; Pageaux et al. 2015). Neuromuscular function was also measured for each of the stimulated contractions during the exercise and normalised to the corresponding pre-exercise values at 100% MVC.

For the 3-min cycling protocol (part B), the end-test power (e.g., CP) during the 3-min test was defined as the mean of the last 30 s (Vanhatalo et al. 2007; 2008). The W′ was calculated as the area above the CP from the power-time curve. Power output was recorded second-by-second, and the peak power was determined as the highest 1-s value. The changes in power output, M_{Max} and EMG_{RMS}, were used to quantify neuromuscular fatigue development and changes in muscle activation. Peak $\dot{V}O_2$ was determined as the highest value recorded in a 15-s interval. The achievement of $\dot{V}O_{2peak}$ was an essential

criterion for a valid 3-min test. All neuromuscular parameters and torque were averaged across the protocol using 30-s bin averages.

Statistics

Paired-samples *t*-tests were used to compare the mean torque, total work done, CT, W' and pTw between IBP and PL in part A. In addition, paired-samples *t*-tests were used to compare the total work done, CP, W' and cardiorespiratory responses between IBP and PL obtained from the 3-min maximal cycling test. For the 60 MVC test, the profiles of VA and Ms-wave amplitude were analysed using two-way ANOVAs with repeated measures (using 12 contraction averages; e.g., 6 time points). In addition, a two-way ANOVA with repeated measures (condition × work rate) was used to assess differences in end exercise $\dot{V}O_2$ and the profiles of EMG and M-wave amplitude. Normalised EMG_{RMS} were analysed using two-way ANOVAs with repeated measures using 30-s means (e.g., 10 time points). Where sphericity was violated, Greenhouse-Geisser correction factor was used. For all tests, results were considered statistically significant when $P<0.05$. Data are presented as means ± SD unless otherwise indicated. All statistical analyses were conducted using IBM SPSS Statistics version 23.

RESULTS

Part A (60 MVC test)

The mean MVC torque achieved prior to the 60 MVC protocol was 232 ± 47 and 230 ± 55 N·m for PL and IBP, respectively. VA of the knee extensors achieved during the preliminary MVCs was 88 ± 7 and 89 ± 6% for PL and IBP, respectively. Baseline MVC and VA were not different between conditions (P>0.05). The profile for mean torque during each contraction across all participants for the 60 MVC protocol is illustrated in figure 7.2a. During the PL trial, torque declined from a peak of 99 ± 3% MVC (relative to pre-exercise MVC) during the first contraction to 40 ± 15% MVC during the last 12 contractions ($P<0.01$, table 7.1, figure 7.2a). During the IBP trial torque declined from a peak of 99 ± 3% to 41 ± 16% MVC. The mean torque (relative to pre-exercise MVC) achieved across the 60 MVCs not different with IBP (60 ± 13%, 87.1 ± 22.2 N·m,) compared to PL (58 ± 14%, 83.8 ± 22.7 N·m, $P=0.39$). There

was no difference in CT (PL: 40 ± 15% vs. IBP: 41 ± 16%. *P*=0.74), W' (PL: 6971 ± 2432 vs. IBP: 7231 ± 3570 N·m·s; *P*=0.69) or total impulse, the surrogate measure of total work done (PL: 22,055 ± 3,885 vs. IBP: 22,919 ± 3,394 N·m·s; *P*=0.26) between the PL and IBP conditions.

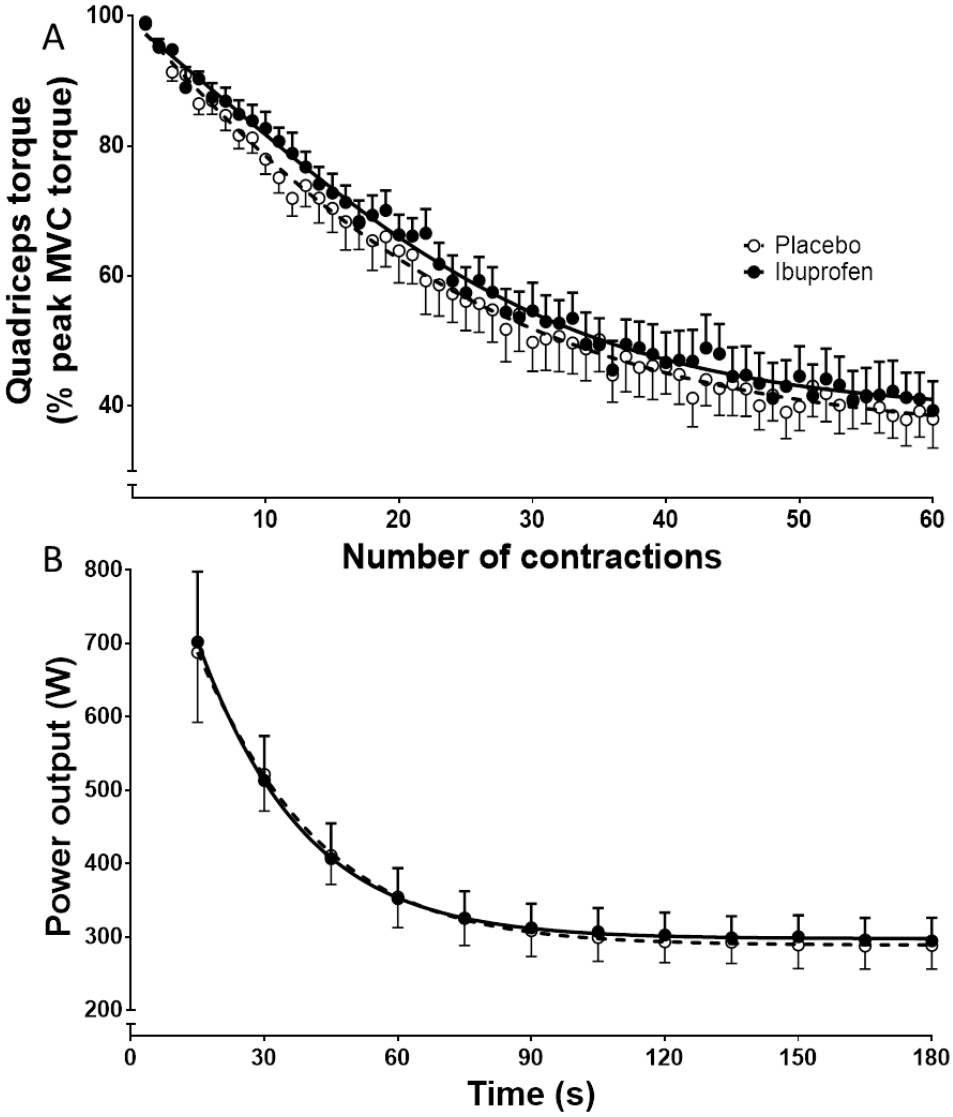

Figure 7.2. *The torque profile during the 60 maximal contractions for placebo (PL, clear circles) and ibuprofen (IBP, filled circles) trials in protocol (a) is demonstrated in panel A. The torque during all contractions was normalized to a control maximal voluntary contraction (MVC) performed before the test commenced. Note that torque falls over the first ~150 s before reaching stable values between 240 and 300 s (the end-test torque; last 12 MVCs). Panel B illustrates the mean ± SE power output profile during the 3-min maximal cycling*

protocol for placebo (clear circles) and ibuprofen (filled circles) trials. Note that power output falls over the first ~120-150 s before reaching stable values (the end-test power output; e.g., CP).

Table 7.1: Performance and neuromuscular function parameters of the 60 MVC (protocol A) and 3-min all-out cycling (protocol B) tests following placebo and ibuprofen ingestion.

	Placebo (PL)	**Ibuprofen (IBP)**
Protocol A		
Performance		
Peak MVC (N·m)	232 ± 47	230 ± 55
Mean torque (% MVC)	58 ± 14	60 ± 13
Total Impulse (N·m·s)	22055 ± 3885	22919 ± 3394
Critical torque, CT (% MVC)	40 ± 15	41 ± 16
W' (N·m·s)	6971 ± 2432	7231 ± 3570
Neuromuscular function		
Change in pTw (N·m)	44 ± 27	43 ± 25
End-exercise pTw (N·m)	30 ± 21	31 ± 23
End-exercise VA (%)	59 ± 19	60 ± 18
End-exercise M-wave amplitude (%)	96 ± 14	95 ± 12
End-exercise EMG amplitude (%)	59 ± 17	64 ± 20
Protocol B		
Performance		
Peak power output (W)	820 ± 139	816 ± 131
Total Work Done (kJ)	65.4 ± 6.4	65.9 ± 5.9
Critical power, CP (W)	288 ± 31	292 ± 28
W' (kJ)	13.6 ± 2.4	13.7 ± 2.8
Neuromuscular function		
End-exercise M-wave amplitude (%)	84 ± 21	83 ± 18
End-exercise EMG amplitude (%)	54 ± 17	57 ± 14

MVC, maximal voluntary contraction; CT, critical torque measured in the last 6 contractions; CP, critical power measured in the last 30 s of the 3-minute maximal cycling test; pTw, potentiated twitch force; VA, voluntary activation measured using the interpolated twitch method technique; EMG, electromyography; N·m, newton metres; N·m·s, newton metres per second; ms, milliseconds.

Part A (Neuromuscular Function)

Alongside the decline in voluntary force (figure 7.2a), pTw (figure 7.3a), VA (figure 7.3b), EMG_{RMS} (figure 7.3c) and M_{Max} (figure 7.3d) were also reduced as protocol A progressed (main effect of time, all $P<0.01$). There were no differences between PL and IBP in pTw, VA, EMG amplitude or M-wave amplitude at any time point (all $P>0.05$). pTw declined from 63 ± 14 to 30 ± 21 N·m and from 68 ± 17 to 31 ± 23 N·m, VA declined from 89 ± 8 to 59 ± 19% and from 88 ± 7 to 60 ± 18%, EMG declined from 99 ± 4 to 59 ± 17% and 100 ± 2 to 64 ± 20% (from first 6 to last 6 contractions) and M-wave amplitude declined from 100 ± 1 to 96 ± 14% and 100 ± 1 to 95 ± 12% in the PL and IBP conditions, respectively ($P<0.05$), with no differences between PL and IBP for any of these variables ($P>0.05$).

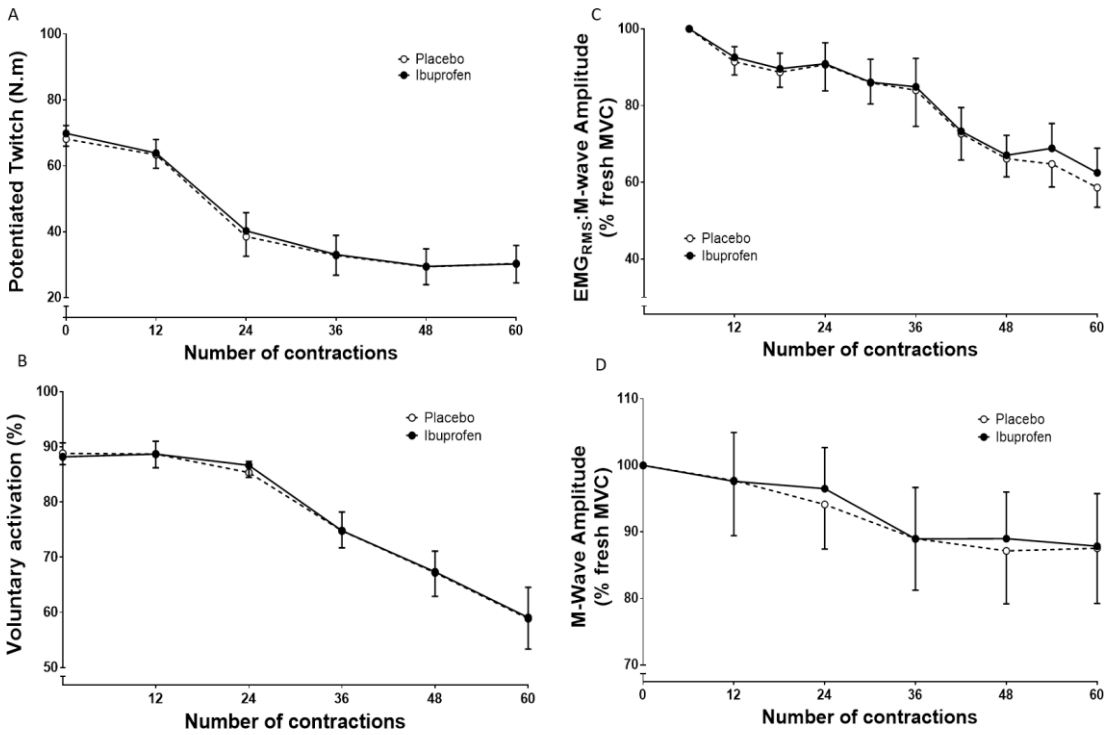

Figure 7.3. *Mean ± SE potentiated twitch (A), voluntary activation (B), and EMG amplitude (C) and M-wave amplitude (D) responses during the 60 MVC test for placebo (clear circles) and ibuprofen (filled circles) trials for protocol a.*

Part B (3-min cycling test)

The mean power output profile for all participants during the 3-min all-out cycling test is shown in figure 7.2b for the PL and IBP conditions. During the PL trial, power output declined from 820 ± 139 W to 288 ± 31 W during the last 30 s of the 3-min test (P<0.01; table 7.1, figure 7.2b). During the IBP trial, power output declined from 816 ± 131 W to 292 ± 28 W during the last 30 s of the 3-min test. There were no differences in CP (PL: 288 ± 31 vs. IBP: 292 ± 28 W, P=0.11), total work done (PL: 65.4 ± 6.4 vs. IBP: 65.9 ± 5.9 kJ, P=0.11) or W′ (PL: 13.6 ± 2.4 vs. IBP: 13.7 ± 2.8 kJ, P=0.84) between conditions.

Part B (Neuromuscular Function)

Similar to the 60 MVC protocol, alongside the decline in power output, there was a progressive decline in M-wave (P<0.01, figure 7.4b) and EMG amplitudes (P<0.01, figure 7.4a). The profiles of the M-wave and EMG amplitudes were not altered following IBP ingestion at any time point (both P>0.05). Using 30 s mean values, EMG decreased from 94 ± 4 to 54 ± 17% and from 96 ± 6 to 57 ± 14% (figure 7.4a) in PL and IBP, respectively.

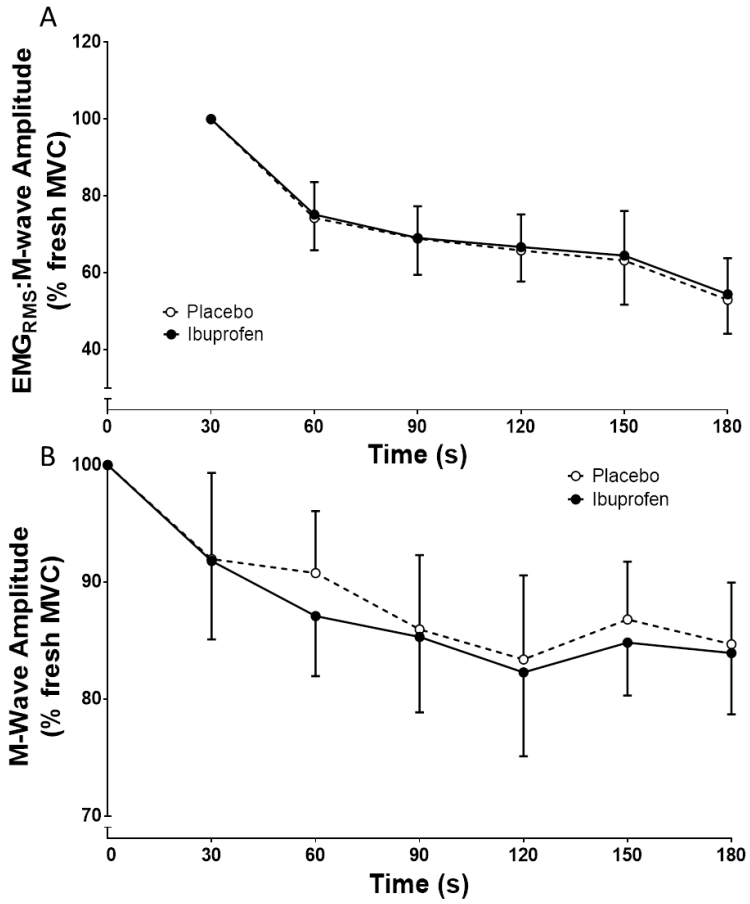

Figure 7.4. Mean ± SE M-wave amplitude (A) and EMG amplitude (B) responses during the 3-minute maximal cycling exercise for placebo (clear circles) and ibuprofen (filled circles) trials for protocol b.

DISCUSSION

Contrary to our experimental hypotheses, the principal findings of this study were that acute ingestion of 400 mg of IBP in a 'fresh' state had no effect on fatigue development or neuromuscular function during either a 5-min single-leg intermittent MVC test or a 3-min maximal cycling test. Across both protocols, power output and torque declined, and neuromuscular fatigue was evident in both the IBP and PL conditions. However, there were no differences in CT or CP, total work done or neuromuscular fatigue markers between the IBP and PL conditions. These findings do not support the acute ingestion of IBP as a strategy to blunt fatigue development during exercise in healthy, recreationally active adults.

Effects of acute IBP ingestion on exercise performance

In contrast with our previous findings of improved CT and CP following the acute ingestion of ACT (Morgan et al. 2018a; Morgan et al. 2018b), these variables were not improved in the current study following the acute ingestion of IBP. However, our findings in the current study are in line with some previous observations that IBP ingestion does not improve performance during whole body exercise in humans (e.g., Cleak, & Eston. 1992; Da Silva et al. 2015; Nosaka & Clarkson, 1996; Tokmakidis et al. 2003). Previous studies that have reported improved exercise performance following IBP administration have typically assessed exercise performance following muscle damage. Nonetheless, while there is some evidence to suggest that the increases in muscle soreness, pain, damage and contractile dysfunction after contraction-induced muscle damage can be attenuated following NSAID administration (Ebbeling & Clarkson, 1989; Hasson et al. 1993; Pizza et al. 1999; Tokmakidis et al. 2003), there is also evidence that NSAID administration does not impact these variables after muscle damage is induced (Da Silva et al. 2015; Donnelly et al. 1990; Grossman et al. 1995; Tokmakidis et al. 2003; VanHeest et al. 2002). Taken together, these observations suggest that the ergogenic potential of administering IBP, a purported analgesic and anti-inflammatory agent (Fridén & Lieber, 1992), is limited without prior induction of muscle damage, and even when muscle damage is induced, and inflammation and pain sensation are correspondingly increased, the effect of IBP ingestion on exercise performance is equivocal.

One explanation for the lack of performance improvement following IBP ingestion in our study may be the reduced ability to regulate the distribution of effort (e.g., pacing strategy) in the maximal protocols employed in response to a potential modulation of pain. For example, Mauger et al. (2010) reported that pain` sensation was reduced (e.g., a greater power output was possible for the same pain sensation) and cycling time trial performance was enhanced following ingestion of ACT. Similarly, Gonglach et al. (2016) reported that manipulating pain perception via caffeine ingestion lead to higher power outputs when participants were asked to pace their effort based upon pain perception. Therefore, we cannot exclude the possibility that IBP consumption could be

ergogenic in situations wherein pacing strategy is self-selected such as during longer duration endurance exercise.

Another possible explanation for the absence of an ergogenic effect of IBP in our study is that inhibiting cyclooxygenase may induce secondary effects that may limit the cardiopulmonary response to exercise including a reduction in exercise-induced hyperaemia (Bradford et al. 2007; Schrage et al. 2004). If skeletal muscle perfusion was impaired following IBP ingestion as a function of cyclooxygenase inhibition (Albert & Gernaat, 1984), this could have negated any potential ergogenic effects following IBP ingestion. However, given that performance was not enhanced during either smaller (single-leg exercise) or larger (double leg cycling) muscle mass exercise following acute IBP ingestion in the current study, and that skeletal muscle perfusion is less likely to be a limiting factor for performance in a single leg model (Joyner & Casey, 2015), this seems unlikely.

Effects of acute IBP ingestion on neuromuscular function

The ingestion of 400 mg of IBP was not associated with attenuation of neuromuscular fatigue development as estimated with peripheral muscle excitability (part A and B), voluntary activation (part A), potentiated twitch (part A) or EMG amplitude (part A and B), consistent with the lack of change in exercise performance. We previously investigated the influence of acute ingestion of 1 g of ACT on neuromuscular function during exercise and reported that, whilst EMG declined across the 3-min cycling protocol in both conditions, EMG declined to a lesser extent in the ACT (~72 %) compared to the PL (~54 %) condition (Morgan et al. 2018b). The magnitude of this effect was remarkably similar to the results we obtained during a 60 MVC protocol completed in a single leg exercise model (ACT: 87% vs. PL: 59 %, Morgan et al. 2018a). This observation suggests that improved maintenance of muscle activation contributed to the ergogenic effect of ACT (Morgan et al. 2018a). It is of interest, therefore, that similar effects were not evident following IBP ingestion. We are not aware of any research to suggest that ACT acts as a more potent stimulus to reduce pain sensation or alter muscle activation. However, it is possible that a combination of the higher ACT dose administered

(1000 mg vs 400 mg for IBP), ACT's potential as an antipyretic (Foster et al. 2014) and differences in pharmacokinetics (Anderson, 2008; Albert & Gernaat, 1984) may have contributed to the disparate effects of IBP in our current study and ACT in our previous studies (Morgan et al. 2018a; Morgan et al. 2018b) on exercise performance and neuromuscular fatigue development. It is also pertinent to note that ACT ingestion has been reported to increase corticospinal excitability at rest, a factor which may contribute to its ergogenic potential (Mauger & Hopker, 2013).

Experimental considerations

Whilst our study contributes to a further understanding of the effect of IBP on exercise-induced fatigue and some of its underlying mechanisms, there are some limitations that require consideration. Firstly, it is acknowledged that we did not measure pain sensation or biomarkers of inflammation. Pain was not assessed due to the protocols requiring the completion of maximal exercise. Asking participants to rate pain sensation may have compromised their ability to focus on the exercise task and to provide a true maximal effort. The effect of IBP on exercise performance when pacing is permitted, and the individual can adjust their pacing strategy in response to potential differences in pain sensation, warrants further investigation. In addition, the extent to which prostaglandin synthesis during endurance exercise plays a role in pain perception in the absence of muscle damage requires further consideration. It is also important to point out that individuals intending to ingest IBP need to consider its potential side effects and be aware that it could impair the adaptive response to exercise (Schoenfeld et al. 2012) and aspects of the beneficial remodelling of skeletal muscle to exercise training (Mikkelsen et al. 2009). Therefore, individuals wishing to explore the use of pain relievers to enhance exercise performance should do so infrequently, with caution, and at the recommended therapeutic doses.

In conclusion, acute ingestion of IBP did not attenuate the decline in neuromuscular function or improve CT or CP during a 60 MVC protocol of the knee extensors or a 3-min maximal cycling test. Therefore, our results indicate that IBP ingestion does not attenuate neuromuscular fatigue development,

during either single-limb or whole-body cycling exercise, and do not support IBP ingestion as an ergogenic aid.

CHAPTER 8
GENERAL DISCUSSION

The overall aim of this thesis was to investigate the effect of acutely consuming the standard therapeutic dose of non-specific COX-inhibitors (i.e., ACT, IBP) on neuromuscular fatigue development and performance during a variety of different exercise tasks. In the first experimental chapter (chapter 4), a well-controlled single-leg model was employed to assess the effect of acute ACT consumption on performance (total impulse), the parameters of the torque-time relationship (CT and W') and neuromuscular fatigue development during 60 MVCs of the knee extensors. The findings from this study revealed that ACT consumption increased total impulse, and CT and EMG amplitude over the latter stages of the test. Subsequently, the second experimental chapter (chapter 5) assessed whether these findings could be replicated during a 3-min maximal cycling bout when a larger muscle mass was recruited, which would have greater implications for 'real-world' exercise performance. Consistent with study 1, acute ACT consumption increased total work done, and CP and EMG amplitude over the later stages of the test. The third experimental chapter (chapter 6) assessed whether acute ACT consumption could improve performance during single-leg severe-intensity knee extensor exercise after prior fatigue of the contralateral limb, where afferent feedback and central fatigue development are exacerbated, and whether the potential ergogenic effect of ACT ingestion in this setting would be greater than the same exercise completed without prior contralateral fatigue. In this study, ACT did not improve performance during severe-intensity single-leg knee extensor exercise completed with or without prior fatigue of the contralateral limb. Finally, the fourth experimental chapter (chapter 7) assessed whether acute IBP consumption could improve performance during the maximal-intensity single-leg and cycling exercise protocols that were administered in studies 1 and 2. In contrast with ACT ingestion, IBP ingestion did not alter performance in either of these protocols. Collectively, these four original research studies advance understanding of the effects of non-specific COX-inhibitors on exercise performance, and the bases of neuromuscular fatigue development during exercise. This chapter (chapter 8) will summarise the main findings of this

thesis, place these findings in context within the existing literature, and outline the novel contributions, and potential limitations and implications of the work conducted in this thesis.

8.1 Main findings

8.1.1 ACT and small muscle mass exercise

It is believed that the effects of ACT ingestion are predominantly mediated by central factors (Anderson, 2008; Andersson et al. 2011; Graham et al. 2013; Jóźwiak-Bębenista & Nowak 2014; Smith 2009; Ottani et al. 2006; Pickering et al. 2006; 2008; Toussaint et al. 2010). Specifically, ACT is known to prevent the metabolism of arachidonic acid into PGs, by non-selectively blocking COX enzymes and reducing nociceptor discharge (and thus the magnitude of group III/IV muscle afferent feedback), which provides analgesic and antipyretic effects. The analgesic effect of ACT has also been attributed to the potentiation of descending serotoninergic pathways (Pickering et al. 2006; 2008), and the modulation of opioid and cannabinoid receptors (Graham et al. 2013; Ottani et al. 2006). These mechanisms likely interact to lower pain sensation following acute ACT ingestion by increasing the nociceptive stimuli required to evoke a given pain sensation. ACT may also help to maintain cortical excitability, enhance muscle activation and/or reduce cortical inhibition by modulating pain sensation (e.g., Dubé & Mercier 2011). Evidence also exists to suggest that reducing group III/IV afferent feedback by fentanyl administration attenuates intracortical inhibition and thus the development of central fatigue (Hilty et al. 2011). However, it should also be acknowledged that acute ACT ingestion may improve exercise performance independent of its effect on pain (and effort) sensation by, for example, enhancing corticospinal excitability and thus alleviating the development of central fatigue (Mauger & Hopker, 2013),

Given the association between the sensation of pain and exercise performance (i.e., Astokorki & Mauger, 2017a; 2017b; Cook et al. 1997), recent investigations have attempted to assess the effect of acute ACT ingestion on performance. ACT ingestion has previously been shown to improve cycling TT performance (Mauger et al. 2010), exercise tolerance during cycling in the heat (Mauger et al. 2014) and repeated sprint cycling performance (Foster et al.

2014). Specifically, acute consumption of ACT has been shown to improve exercise performance by increasing pain tolerance/lowering pain sensation (e.g., Foster et al. 2014; Mauger et al. 2010; 2014) and reducing thermal stress in the heat (Mauger et al. 2014). However, it is important to note that for the studies presented in this thesis, performance would not be expected to be enhanced by improved thermoregulation as they were all short-duration and performed in temperate conditions. More recently, data has also been presented to suggest ACT may reduce exercise-induced pain during the latter stages of sprint interval treadmill running but without influencing performance (Park et al. 2016), possibly highlighting a task (and/or individually) specific effect of ACT on performance and/or that the effects of ACT ingestion on performance and pain sensation can be dissociated in certain exercise settings. It is also possible that in the study by Park et al. (2016) that, because afferent feedback is '*permitted*' to accumulate to a higher magnitude in exercise tasks where less muscle mass is engaged (i.e., Rossman et al. 2012; 2014), a higher degree of neuromuscular fatigue development is observed in cycling exercise (compared to running), affording a higher ability for ACT to influence exercise performance (Park et al. 2016). We also speculate that, during treadmill running, the regulation of work output is likely more restricted and involves more conscious regulation and awareness in altering speed than in cycling exercise (or running which is not constrained to a treadmill) which is freely controllable and may help explain the lack of observed effects in the study by Park and colleagues (2016).

In addition, Chapter 4 sought to assess the ergogenic potential of ingesting an acute dose of ACT (1 g) during a 5 min single-leg maximal intermittent knee-extension test. This approach was adopted as it afforded the opportunity to robustly assess performance, and neuromuscular, peripheral and central fatigue development to examine some of the underpinning mechanisms for the ergogenic effect of ACT ingestion (Chapter 4). In addition to investigating the neurophysiological bases of a potential improvement in exercise performance following ACT ingestion, this study assessed how ACT ingestion impacted the parameters of the torque-time relationship, CT and W′. Therefore, the purpose of this initial study was to test the efficacy of acute ACT consumption to improve

total work (impulse), CT and muscle activation and the rate of neuromuscular fatigue development, using a controlled experimental model.

Consistent with previous research using a similar design (Burnley, 2009; Broxterman et al. 2017; 2018; Fulford et al. 2013), this study demonstrated that torque (-60%), pTw (-30%), EMG (-41%) and VA (-59%) declined during the 60 MVC protocol affirming neuromuscular, peripheral and central fatigue development, respectively (Chapter 5). We also demonstrated that mean torque (61 ± 11 vs. 58 ± 14% pre-exercise MVC) and end-test torque, reflective of CT (44 ± 13 vs. 40 ± 15% pre-exercise MVC), were greater following ACT compared to PL ingestion. VA and pTw declined at a similar rate in both conditions. However, the decline in EMG was attenuated in the ACT trial with EMG being significantly greater compared to PL from 210 s onwards (end exercise EMG 87 ± 28 vs. 59 ± 17%), suggesting that improved muscle activation likely contributed to the ergogenic effect of ACT ingestion. This interpretation was strengthened by our observation of a *strong* positive correlation between the inter-trial changes in EMG and performance (r=0.85). Our findings are in line with previous observations that acute ACT ingestion can improve performance during whole body exercise (Foster et al. 2014; Mauger et al. 2010). Specifically, Mauger et al. (2010) demonstrated a similar ~2% improvement (vs. 3% improvement in total impulse, Chapter 4) in 10-mile TT cycling performance following a 1.5 g dose of ACT. However, our observations offer novel insights into the mechanisms for the ergogenic effect of ACT reported previously (Foster et al. 2014; Mauger et al. 2010) by demonstrating a 28% increase in end-exercise EMG (measure of muscle activation) and a 4% improvement in CT, which reflects the maximal sustainable rate of oxidative metabolism, represents a critical threshold for neuromuscular fatigue development (Burnley et al. 2012) and has been identified as a significant predictor of endurance performance (Jones & Vanhatalo, 2017 *for review*). Furthermore, this increase in CT occurred without influencing the W', the curvature constant of the hyperbolic power-duration (or torque-time) relationship. Our findings are in contrast with previous work using an identical experimental model (i.e., 60 MVC test) but with intrathecal fentanyl to abolish pain and afferent feedback, where no improvement in CT was observed

(Broxterman et al. 2018). In addition, whilst the abolition of afferent feedback accelerated the rate of ATP cost per contraction, an initial increase in the ATP cost of contraction (in the first minute of exercise) was not observed which coincided with a significant increase in force production, perhaps providing further support of a ‘lag-time’ in afferent feedback and/or that muscle afferent feedback protects a metabolic reserve related to W' rather than the CT (Broxterman et al. 2018). However, whilst cardiopulmonary function was not limiting in this mode of exercise, the use of ACT causes a reduction, as opposed to abolition, of afferent feedback which may have important consequences for maintaining skeletal muscle efficiency (Broxterman et al. 2018).

As we observed no differences in our main measures of peripheral (i.e., pTw) and/or central (i.e., VA) fatigue, we were unable to identify the precise mechanism for the ergogenic effect of ACT ingestion on muscle activation. It is possible that ACT ingestion enhanced cortical function (Dubé & Mercier, 2011; Mauger & Hopker, 2013) and this, potentially alongside a reduction in group III/IV muscle afferent feedback, permitted further muscle activation (i.e., Mauger et al. 2011). Since there was no difference in peripheral membrane excitability (i.e., M-wave) between conditions, the enhanced EMG amplitude following ACT ingestion might have been linked to processes upstream of the neuromuscular junction (i.e., spinal and/or supraspinal in origin). This postulate is strengthened by observations of ACT enhancing spinal and supraspinal excitability at rest (Mauger & Hopker, 2013) and attenuating corticospinal inhibition during exercise (Pageaux & Mauger, 2018). In addition, previous research suggests that acute pain increases corticospinal inhibition (Dubé & Mercier, 2011), which would prevent optimal activation of the motor cortex during voluntary movement and impair muscle function and exercise performance. In further support for a role of ACT ingestion in supressing central fatigue development, other methods of reducing group III/IV afferent feedback have been linked to reduced intracortical inhibition (e.g., Hilty et al. 2011) and enhanced spinal and supraspinal excitability (e.g., Sidhu et al. 2014; 2017) which would be expected to increase voluntary neural drive during exercise (Amann et al. 2009; 2011; Blain et al. 2016; Hilty et al. 2011; Sidhu et al. 2014; 2017).

Since ACT ingestion improved performance in study 1, and this effect would have been expected to be a function of reduced inhibitory feedback, improved cortical function and greater peripheral fatigue development, it was surprising that the attenuation in neuromuscular fatigue development was not accompanied by changes in central (i.e., VA) or peripheral (i.e., pTw) fatigue markers. Since muscle activation and torque were increased, this might be expected to result in greater intramuscular metabolic perturbation and, by extension, greater peripheral fatigue development. Although we cannot exclude the possibility that ACT enhanced systemic physiological responses or skeletal muscle efficiency to mitigate this potential for greater peripheral fatigue development, we are not aware of any published evidence to support such effects. It should, however, be acknowledged that in the face of a relatively small (< 5%) improvement in torque, it is possible that our indirect measures of central and peripheral fatigue in this study were not sensitive enough to detect potentially small changes in CNS and peripheral muscle function. Moreover, while ACT increased EMG during the latter stages of the 60 MVC protocol compared to PL, which might be linked to increased muscle activation and CMD, VA was not impacted by ACT. This might have been a function of the continuous assessment of the EMG response and torque throughout the 60 MVC protocol, compared to every 6th MVC for VA, such that EMG might have provided a more ‘complete picture’ of central fatigue development across the protocol.

According to previous work (e.g., Foster et al. 2014; Mauger et al. 2010), we would have also expected pain and effort sensation to be similar in the ACT trial despite an increase in work output. However, it is acknowledged that a limitation of this study was that pain sensation was not assessed. This was due to the protocol requiring the completion of 60 MVCs with a short (2 s) recovery period such that asking participants to rate their pain sensation would likely have compromised their ability to focus on the exercise task to provide a true maximal effort. In addition, previous work has highlighted the difficulties with measuring exercise-induced pain and accurately dissociating this from perceived exertion (Astokorki & Mauger, 2017a), further complicating pain assessment during such exercise tasks.

8.1.2 ACT and large muscle mass exercise

Chapter 5 was completed to investigate whether the effect of ACT on performance in a small muscle mass model reported in chapter 4 was transferable to a larger muscle mass model. Therefore, a standard therapeutic dose of ACT (1 g) was, again, administered but prior to assessing performance in an experimental model with improved translation to whole body exercise performance by conducting whole-body cycling exercise during a 3-min all-out test (Chapter 5). This is important since the exercise modality (more specifically, the systemic challenge) employed, and volume of skeletal muscle mass engaged, is known to influence the extent, and underpinning mechanisms, of neuromuscular fatigue development (i.e., Rossman et al. 2012; 2014). The femoral nerve was stimulated every 30 s to measure peripheral membrane excitability and surface EMG was recorded continuously to measure muscle activation using novels methods during locomotion. Although this experimental model offered greater translation to exercise performance, assessments of neuromuscular function were restricted to on-bike measurements as the recovery of neuromuscular function has been shown to occur rapidly following the completion of exercise (Carroll et al. 2017). In addition, given the task-specific nature of fatigue, assessment of neuromuscular function on a dynamometer during an isometric contraction (as traditionally adopted when assessing fatigue) would have provided an invalid assessment of neuromuscular function (rendering the measurements of limited use) and so was avoided in this study.

Compared to PL, ACT ingestion increased end-test power, reflective of CP (297 ± 32 vs. 288 ± 31 W), and total work done (66.4 ± 6.5 vs. 65.4 ± 6.4 kJ) without impacting W' (13.1 ± 2.9 vs. 13.6 ± 2.4 kJ) or the M-wave amplitude (end-exercise: 4.15 ± 1.60 vs. 3.94 ± 1.9 mV) during a 3-min all-out cycling test. These findings demonstrated a similar improvement in exercise performance (total work and CP) compared to chapter 4 but in a group of trained individuals (group mean $\dot{V}O_{2peak}$: >60 $ml \cdot kg^{-1} \cdot min^{-1}$). In addition, EMG declined throughout the 3-min protocol in both the PL and ACT conditions; however, the decline in EMG was attenuated in the ACT condition, with the EMG amplitude being greater compared to PL over the last 60 s of the test (end-exercise EMG 72 ±

18 vs. 54 ± 17%). These observations were consistent with those reported in chapter 4. Our observation of no difference in M-wave amplitude in chapters 4 and 5 is also in agreement with previous findings that peripheral membrane excitability is not altered at rest following ACT ingestion (Mauger & Hopker, 2013). Moreover, and also in agreement with chapter 4, CP (exercise-modality equivalent of CT) was increased without influencing the W' following ACT ingestion. This is an important finding as when the parameters of the power-duration relationship are known (i.e., CP and W'), they can be used to robustly predict TT performance (e.g., Black et al. 2014; Poole et al. 2016 *for review*). Indeed, in agreement with the magnitude of improvement (2%) in TT performance empirically demonstrated by Mauger et al. (2010), the ACT-induced increase in CP was predicted to translate into a 1-3% reduction in the time required to complete a range of target work cycling trials (100-1000 kJ). Interestingly, improvements in exercise performance with acute ACT ingestion have been reported in trained participants in this chapter (Chapter 5) and in the previous study by Mauger et al. (2010) despite evidence that endurance training increases pain tolerance (i.e., O'Leary et al. 2017) and that trained individuals are more likely to have a greater tolerance to pain (Janal et al. 1994; Tesarz et al. 2013) and rely less on afferent signals to regulate (or '*pace*') work output (Mauger, 2014; Tucker, 2009). This demonstrates the clear potential for ACT (and by extension, pain suppression) to enhance performance in trained individuals as well as providing further support for pain having a role in regulating exercise performance by utilising a 'metabolic reserve' (e.g., Foster et al. 2014; Mauger, 2014; Mauger et al. 2010; 2011). Indeed, it has recently been suggested that pain sensation arising from exercise may play a combined role in the regulation of work output and preservation of a metabolic reserve by the central nervous system (Mauger, 2014).

8.1.3 ACT and contralateral fatigue

Based on the findings from chapters 4 and 5 , and other studies assessing the effect of ACT ingestion on performance (Foster et al. 2014; Mauger et al. 2010) and neuromuscular function (Dubé & Mercier, 2011; Mauger & Hopker, 2013), as well as studies reporting enhanced cortical function during fatiguing exercise after reducing group III/IV afferent feedback (i.e., Amann et al. 2009; 2011;

Blain et al. 2016; Hilty et al. 2011; Sidhu et al. 2014; 2017), we postulated that the mechanisms responsible for the ergogenic effect and enhanced muscle activation of ACT was a blunting of central fatigue. The development of non-local muscle fatigue is thought to be predominantly centrally mediated (see Halperin et al. 2015 *for review*). Indeed, despite the mechanisms of contralateral limb fatigue still remaining entirely unknown, the exacerbation of central fatigue during a contralateral limb task has been suggested to be mediated by increased group III/IV muscle afferent feedback (e.g., Amann et al. 2013) leading to a ~49% reduction in T_{lim} during a contralateral limb task. Amann et al. (2013) suggested that the reduced exercise tolerance after prior contralateral fatigue was achieved by elevating perceived exertion (i.e., RPE) and "restricting CMD" (inferred from a reduced end-exercise EMG amplitude despite a similar rate of rise during the exercise task), preventing further peripheral fatigue development (i.e., reduced pTw). Collectively, considering these observations, it was proposed that the premature restriction of CMD was likely due to participants reaching their 'sensory tolerance limit', a hypothetical construct that might coincide with an individually specific degree of peripheral fatigue and associated intramuscular metabolic milieu, at an earlier time point due to afferent feedback being elevated at the end of the prior leg exercise task (Amann et al. 2013). Since the circulatory and ventilatory responses during such small muscle mass exercise are well within the respective maximal capacities, the authors concluded that the any impact of muscle afferents on performance during such a task was likely independent of the established role of muscle afferent feedback in regulating peripheral hemodynamic responses during whole-body exercise.

Since the prevailing mechanism by which ACT ingestion is purported to be ergogenic is through a blunting of central fatigue development (e.g., Foster et al. 2014; Mauger et al. 2010; Mauger & Hopker, 2013; Chapters 4-5), chapter 6 sought to test the hypotheses that ACT ingestion would attenuate contralateral limb fatigue and that this effect would be greater compared to short-duration unilateral severe-intensity exercise in which fatigue development is predominantly peripherally mediated (i.e., Froyd et al. 2013; Matkowski et al. 2011). To test this hypothesis, participants performed CWR single-leg severe-

intensity knee extensor exercise to exhaustion with one leg and consecutively with the contralateral rested limb, 60 mins after ingesting PL or 1 g of ACT. This study used ^{31}P-MRS to assess whether acute ACT ingestion permitted the attainment of a greater degree of perturbation to intramuscular phosphorous substrates and metabolites during exercise to provide novel insight into the mechanisms by which acute ACT ingestion might be ergogenic. We observed a 19% reduction in T_{lim} during severe-intensity exercise in the contralateral limb ($Leg_{2CONTRA}$) after completing prior severe-intensity knee extensor exercise to exhaustion in the opposite limb (Leg_1) following PL ingestion ($Leg_{2PL\text{-}CONTRA}$) compared to no prior fatigue in Leg_1 (Leg_{2CON}) (Leg_{2CON}: 385 ± 104 vs. $Leg_{2PL\text{-}CONTRA}$: 311 ± 92 s). In addition, ACT ingestion did not improve T_{lim} in Leg_1 (i.e., without prior contralateral fatigue, Leg_{1ACT}) compared to the PL condition (Leg_{1PL}) (Leg_{1PL}: 390 ± 106 vs. Leg_{1ACT}: 402 ± 101 s). Moreover, ACT ingestion did not improve T_{lim} in Leg_2 following prior contralateral fatigue in Leg_1 ($Leg_{2ACT\text{-}CONTRA}$) compared to the PL condition ($Leg_{2PL\text{-}CONTRA}$) ($Leg_{2ACT\text{-}CONTRA}$: 324 ± 85 vs. $Leg_{2PL\text{-}CONTRA}$: 311 ± 92 s). There were also no changes in intramuscular phosphorous substrates and metabolites, ratings of perceived exertion (i.e., RPE) or EMG amplitude after ACT ingestion compared to PL ingestion in the severe-intensity single-leg knee extensor exercise tests completed with or without prior contralateral fatigue. Therefore, these findings did not support our experimental hypotheses and, instead, suggest that acute ACT ingestion does not alter T_{lim} during single-leg constant work rate severe-intensity knee extensor exercise.

Although ACT ingestion did not blunt the effect of prior contralateral fatigue, our findings offer some novel insights into the potential mechanisms for the ergolytic effect of prior contralateral limb fatigue. This is important since the existence of contralateral limb fatigue is equivocal and the mechanisms of its existence are poorly understood (see Halperin et al. 2015 *for review*). Our observations of compromised exercise performance after prior contralateral limb fatigue are consistent with some (i.e. Amann et al. 2013; Doix et al. 2013; Halperin et al. 2014; Halperin et al. 2014; Kennedy et al. 2013; Rattey et al. 2006; Takahashi et al. 2011), but not all (i.e. Elmer et al. 2013; Grabiner & Owings, 1999; Regueme et al. 2007; Todd et al. 2003; Zijdewind et al. 1998), previous studies

reporting enhanced fatigue development after prior contralateral or non-local muscle fatigue. However, the magnitude of change was not consistent in the contralateral limb across the group (range +1.6 to -48.5%), potentially highlighting an individually specific conscious and/or sub-conscious response to sensory input. In addition, the similar end-exercise metabolic perturbation in $Leg_{2PL\text{-}CONTRA}$ (i.e., PCr, ADP, pH) is not in agreement with Amann et al. (2013) who demonstrated reduced peripheral fatigue development at T_{lim}. These findings from Amann et al. (2013) are consistent with an afferent-feedback mediated model whereby afferent feedback from the prior leg exercise contributes to the earlier attainment of the 'sensory tolerance limit' and reduced T_{lim} in the contralateral limb. Interestingly, however, and despite the higher baseline muscle [Pi] in the Leg_{2PL} contralateral conditions compared to the Leg_{2CON} condition, muscle [Pi] was lower at the point of task failure in the former compared to the latter. This observation, however, is in accord with studies from another group who observed greater peak perturbation of muscle [Pi], but not pH, [PCr] and [ADP], when group III/IV muscle afferent feedback was abolished via lumbar intrathecal administration of fentanyl (Blain et al. 2016; Broxterman et al. 2017b; 2018). Taken together, these complementary observations suggest that intramuscular phosphorous metabolites do not respond in a uniform manner to manipulations in skeletal type III/IV muscle afferent feedback and that muscle [Pi] might be a more sensitive predictor of muscle afferent feedback, peripheral fatigue development and T_{lim}. Our findings compliment recent research which implicates metabolites (specifically [Pi] and $[H^+]$), to play a more critical role in peripheral fatigue development (Blain et al. 2016; Nielsen et al. 2014).

The lack of an effect of ACT ingestion on single limb or contralateral limb performance in this study was an interesting finding and conflicts with recent reports that acute ACT consumption can improve exercise performance by increasing pain tolerance (Foster et al. 2014; Mauger et al. 2010) and muscle activation (Chapters 4-5). However, these disparate findings might be due to differences in the ACT administration procedure compared to previous studies reporting improved performance and delayed neuromuscular fatigue development following ACT ingestion (Foster et al. 2014; Mauger et al. 2010;

Chapters 4-5). Indeed, ACT was ingested 45 min prior to the start of the Leg_{1ACT} test, which immediately transitioned into the Leg_2 contralateral protocol that was the primary focus of the current study. Since peak plasma [ACT] is attained ~60 min post oral ACT ingestion (Anderson et al. 2008; Forrest et al. 1982), we elected to administer ACT in the current study such that peak plasma [ACT] was expected to coincide with the onset of the $Leg_{2ACT\text{-}CONTRA}$ protocol rather than the Leg_{1ACT} protocol. This might account, in part, for the lack of an ergogenic effect of ACT ingestion during the Leg_{1ACT} protocol compared to other studies that acutely administered ACT ~60 min prior to the performance trial (Foster et al. 2014; Mauger et al. 2010; Chapters 4-5). This postulate is supported by recent observations that plasma [ACT] might actually peak 80-100 min post ingestion (Foster et al. 2016). Therefore, we cannot exclude the possibility that earlier ACT ingestion (Foster et al. 2016) at the same or a greater dose (Foster et al. 2014; Mauger et al. 2010) than consumed in the current study, might have elicited improved single-leg severe-intensity exercise tolerance. The lack of effect might also be explained partially by the low dose (1 g) of ACT not acting as a sufficient stimulus to attenuate group III/IV muscle afferents in this specific task (i.e. CWR single-leg sub-maximal exercise). However, it is also pertinent to note that since ACT ingestion does not selectively inhibit PG production at the location where production of these pain provoking stimuli is greatest, ACT ingestion might not provide an appropriately targeted approach to sufficiently reduce afferent feedback during single-leg sub-maximal exercise. In addition, whilst we observed a 19% reduction in exercise tolerance of the contralateral limb, this was not comparable to the magnitude of effect (~49%) reported by Amann et al. (2013). Therefore, the scope for mitigating the ergolytic effect of prior contralateral fatigue was lower in our study compared to that of Amann et al. (2013) which might have accounted for the lack of an effect of ACT ingestion in our study.

In addition to differences in the ACT dosing procedure, the lack of an ergogenic effect of ACT administration in the current study might be linked to the fatiguing exercise test administered. In the present study, participants completed continuous single-leg severe-intensity (~95% WR_{peak}) knee extensor exercise until task failure. Since exercise-induced pain sensation is positively associated

with exercise intensity (Astokorki & Mauger, 2017a; Cook et al. 1997), and since ACT ingestion is suggested to be ergogenic by mitigation pain sensation (Foster et al. 2014; Mauger et al. 2010) this might account for the improved exercise performance during all-out maximal-intensity exercise following ACT ingestion (Foster et al. 2014; Chapters 4-5), and the lack of improvement in performance in the continuous severe-intensity submaximal exercise test employed in the current study. Moreover, the fatiguing exercise protocol administered in the current study, which was a fixed-work-rate test continued until task failure with no pre-determined end point (i.e., open loop), differed from the completion of a fixed-distance (16.1-km) TT (Mauger et al. 2010) or a set number of maximal (all-out) effort repetitions (Foster et al. 2014; Chapter 4) or duration of maximal (all-out) effort (Chapter 5) with a predetermined end point (i.e., closed loop). Since the development of exercise-induced pain is correlated with performance in closed loop tasks (Astokorki and Mauger, 2017a) and pharmacological interventions that attenuate, but not completely abolish, exercise-induced pain appear to be more effective at enhancing performance in closed loop tasks, this could explain the lack of an ergogenic effect of ACT ingestion in the current study compared to previous studies (Foster et al. 2014; Mauger et al. 2010; Chapters 4-5).

8.1.4 Factors determining the efficacy of ACT ingestion

The acute consumption of ACT was associated with improved performance in some (chapters 4-5) but not all (chapter 6) experimental chapters in this thesis. A consistent observation in this thesis was that performance was enhanced when muscle activation, assessed via EMG amplitude, was better preserved (Chapters 4-5), while performance was not enhanced in exercise settings where muscle activation was not improved compared to the placebo condition (chapter 6). As pain likely needs to be elevated before any impact on performance is observed following an analgesic intervention (i.e., ACT), it is plausible to suggest that the effect we observed on CT and CP in experimental chapters 4 and 5, may have been a consequence of elevated pain during the latter portion of the test (i.e., when CT and CP are calculated) which is absent in the former (when W′ is calculated). These observations may also be explained by the ‘lag time’ of afferent feedback (particularly mechanosensitive group IV afferents)

(Ulmer, 1996). However, whilst this seems unlikely to fully explain our observations, whether the effect of ACT is simply an artefact of the respective tests, requires further investigation (see 8.4 Directions for future research). Importantly, the exercise tests used in these chapters have been validated and are sensitive to improvements in endurance performance, with the CT and CP representing a critical threshold for metabolic and neuromuscular control (Burnley, 2009; Burnley et al. 2012; Vanhatalo et al. 2008). Moreover, we observed strong correlations between the inter-trial changes in EMG and performance (and CT and/or CP) in experimental chapter 4 (r=0.85) and 5 (r=0.88) which lends further support to the notion that performance enhancement following ACT ingestion is associated with an increase in muscle activation and CT and/or CP. This improvement in muscle activation following ACT ingestion may arise from mechanisms dependent and independent of pain sensation and may also be linked to conscious (i.e., reduced pain sensation) and subconscious (i.e., reduced intracortical inhibition and enhanced cortical excitability) processes. Specifically, in addition to observations of enhanced muscle activation (Chapters 4-5), ACT has also been shown to alter pain and effort sensation during cycling exercise (Foster et al. 2014; Mauger et al. 2010; 2014). ACT may also alleviate the development of central fatigue in the absence of pain by enhancing corticospinal excitability (Mauger & Hopker, 2013) and attenuating intracortical inhibition associated with reduced group III/IV afferent feedback (Hayes et al. 2006, COX-inhibitor; Hilty et al. 2011, fentanyl administration) as well as helping to maintain cortical excitability and/or reduce cortical inhibition in the presence of acute pain (e.g., Dubé & Mercier, 2011) and thus voluntary activation. It is also possible that by reducing the magnitude of group III/IV muscle afferent feedback (via reducing PG synthesis), the development of central fatigue may be slowed by attenuating intracortical inhibition during fatiguing exercise (Hilty et al. 2011, fentanyl administration).

However, whilst our observation of improved performance following ACT ingestion is in agreement with previous research implicating altered pain and effort sensation during exercise (Foster et al. 2014; Mauger et al. 2010; 2014), it should be noted that these studies used a higher dose of ACT (~1.5 g) than those presented in this thesis (chapters 4-6). Indeed, a study by Burtscher et al.

(2013) failed to observe any effect of a 500 mg dose of ACT during running exercise in the heat. In contrast to findings presented in these studies (Foster et al. 2014; Mauger et al. 2010; 2014), not all research has observed improvements in exercise performance following ACT ingestion (using a similar dose) when pain sensation is reduced (Chapter 6; Park et al. 2016). Indeed, whilst Park et al. (2016) observed reductions in exercise-induced pain during the latter stages of a sprint interval running protocol with ACT ingestion, no effect was observed on performance between groups. It has, however, previously been suggested that exercise-induced pain (and thus reducing pain sensation) is a moderator of *'permissible'* exercise intensity (i.e., Mauger, 2014). This may be more important during tasks whereby there is an ability to self-regulate or 'pace' effort such as in a TT scenario or when continued 'maximal' effort and/or maximal level of exertion is required from the participant (i.e., in closed loop tasks) and may help partly explain the absence of effect observed in chapter 6 whereby the intensity of exercise was dictated by the experimenter (i.e., in open loop tasks). In addition, these observations may also be explained by the inter-study differences in the relative intensity of exercise (i.e., maximal, chapters 4-5 vs. sub-maximal, chapter 6). It was surprising, however, that following prior contralateral limb fatigue, which, seemingly, increases central fatigue development (i.e., Amann et al. 2013), ACT ingestion did not enhance performance despite previous studies reporting its ability to potentially increase corticospinal excitability (i.e., Mauger & Hopker, 2013), attenuate afferent feedback (Hayes et al. 2006) and increase intracortical facilitation (i.e., Hilty et al. 2011, fentanyl), important components of central fatigue development. However, it should be noted that establishing a direct causative role for group III/IV feedback to regulate exercise performance is highly complex and, as with the mechanisms underpinning the existence of non-local muscle fatigue, evidence to support such an effect is equivocal (Amann et al. 2012; 2015; Halperin et al. 2015 *for review*). The inter-study differences in the effects of acute ACT ingestion in this thesis might also be explained, at least in part, by inter-individual responses in the effect of analgesic medication (Cregg et al. 2013 *for review*), pain sensation during exercise (i.e., Mogil, 1999) and ACT pharmacokinetics and pharmacodynamics (i.e., Foster et al. 2016).

Finally, whilst we are not aware of any evidence to support altered pharmacokinetics and pharmacodynamics of ACT after prior fatiguing exercise, we cannot discount that the prior exercise may have influenced the metabolism of ACT in the contralateral limb and thus altered its pharmacodynamics in chapter 6. Moreover, although recent research suggests that the attainment of peak plasma [ACT] is achieved >60 mins post ACT ingestion (Foster et al., 2016), performance and muscle activation were enhanced in chapters 4 and 5 when ACT was ingested 60 min prior to these fatigue exercise tasks. However, it is possible that the relative importance of peak [ACT] and/or peak pain suppression following ACT consumption might differ between the exercise tests administered (i.e., maximal vs. submaximal exercise; without vs. without prior existence of contralateral limb fatigue) and differences in training status (i.e., trained vs. untrained) across chapters 4-6. Given the disparate effect of contralateral limb fatiguing exercise, as evidenced by a range of +1.6 to -48.5% in chapter 6, and as reported in numerous previous studies (see Halperin et al. 2015 *for review*), the magnitude of group III/IV muscle afferent feedback and the conscious and sub-conscious responses to a given degree of group III/IV muscle afferent feedback, are likely to be highly variable between individuals. Therefore, further investigation is required to improve understanding of the specific role of group III/IV muscle afferents in exercise-induced neuromuscular fatigue, particularly since it is not currently possible to accurately quantify the magnitude of group III/IV muscle afferent feedback.

8.1.5 IBP and performance

Having determined the effect of ACT on small (chapter 4) and large muscle mass maximal exercise (chapter 5), and severe-intensity single limb exercise completed with and without prior fatiguing contralateral exercise (chapter 6), it was important to investigate the effect of other non-specific COX-inhibitors, such as IBP, on performance and neuromuscular function to determine whether these effects were specific to ACT (chapter 7). Indeed, whilst research suggests that acute consumption of pharmacological analgesics can improve exercise performance (Foster et al. 2014; Mauger et al. 2010; Chapters 4-5), the ergogenic potential of IBP administration is poorly understood, particularly when muscle damage is not present, or inflammation is not elevated above baseline

levels (see Chpater 7). This is an important research question to address as IBP is regularly consumed by athletes to enhance performance (Alaranta et al. 2008; Corrigan & Kazlauskas, 2003; Da Silva et al. 2015; Gorski et al. 2011; Huang et al. 2006). In a similar fashion to ACT, IBP limits the metabolism of arachidonic acid by inhibiting the COX enzymes, and thus the synthesis of PGs (Albert & Gernaat, 1984). However, IBP, a non-steroidal anti-inflammatory drug, is known to act both centrally and peripherally to respectively reduce the sensation of pain and tissue inflammation, whereas ACT is thought to act predominantly centrally (Fridén & Lieber, 1992). It would, therefore, be of interest to determine whether IBP had any additive effects on performance beyond what has been demonstrated by ACT ingestion (e.g., Foster et al. 2014; Mauger et al. 2010; Chapters 4-5). Chapter 7, therefore, tested the efficacy of a standard therapeutic dose of IBP (400 mg) to improve performance and neuromuscular function using the same protocols as administered in studies 1 and 2 (chapters 4-5) to facilitate a direct comparison between these COX inhibitors. However, in contrast to our previous observations following ACT ingestion mean torque (IBP: 60 ± 12 vs. PL: 58 ± 14% of pre-exercise MVC) and end-test torque (IBP: 41 ± 16 vs. PL: 40 ± 15% of pre-exercise MVC) were not different between the IBP and PL conditions when completing 60 MVCs of the knee extensors. Similarly, end-test power output (IBP: 292 ± 28 vs. PL: 288 ± 31 W) and work done (IBP: 65.9 ± 5.9 vs. PL: 65.4 ± 6.4 kJ) during the 3-min all-out cycling tests were not different between the IBP and PL conditions. Neuromuscular fatigue markers also developed at a similar rate in both exercise tests in the PL and IBP conditions (i.e., pTw, VA, M-wave, EMG). Importantly, these findings were observed despite the participants being the same as those who took part in experimental chapters 4 and 5, respectively, whereby we observed improved performance following ACT consumption.

The lack of an ergogenic effect of IBP ingestion in chapter 7 is in line with previous observations that IBP ingestion does not improve performance during whole body exercise in humans (i.e., Cleak, & Eston. 1992; Da Silva et al. 2015; Nosaka & Clarkson, 1996; Tokmakidis et al. 2003). In addition, previous studies that have reported improved exercise performance following IBP administration have typically assessed exercise performance following muscle damage, the

findings of which are equivocal (Da Silva et al. 2015; Ebbeling & Clarkson, 1989; Hasson et al. 1993; Pizza et al. 1999; Tokmakidis et al. 2003). Despite anecdotal evidence of athletes consuming IBP to enhance performance, to our knowledge, no studies had assessed its efficacy to improve performance in a 'fresh' state. Whilst a recent study demonstrated IBP improved swimming performance during a TTE test in rats (Lima et al. 2016), these observations have not been reproduced in humans. Taken together, the findings from chapter 7 suggest that the ergogenic potential of administering 400 mg IBP is limited. We are not aware of any research to support ACT as a more potent drug to alter muscle activation and performance compared to IBP; however, a combination of the higher dose (1000 mg vs 400 mg), the potential of ACT as an antipyretic (Foster et al. 2014; Mauger et al. 2014), and the differences in the pharmacokinetics (Anderson, 2008; Albert & Gernaat, 1984), may have contributed to the lack of performance improvement following IBP ingestion in this thesis. In addition, whilst the mechanisms of action of ACT and IBP are thought to be similar, more recently it has been suggested that ACT may also act to inhibit 'COX-3', an enzyme thought to be expressed in the cortex (Roos et al. 2002) as well as to modulation of serotonergic pathways and opioid and cannabinoid receptors (*see Chapter 2: Literature review*). Indeed, as ACT can pass the blood brain barrier, it's possible that central inhibition of COX (i.e., via ACT) is more effective that peripheral inhibition (i.e., via IBP), particularly in enhancing performance. Indeed, it would seem that ACT cerebrospinal concentrations track pain suppression better than plasma concentrations (Anderson, 2008). However, such speculation requires further investigation. Nonetheless, it is likely that the use of IBP is more important for pain associated with inflammation (Olesen et al. 2012). In addition, the role of inflammation during exercise and the use of anti-inflammatory medication as a performance aid are not clear. In contrast, it is possible to speculate that NSAIDs may actually impair exercise performance via an increase in gastrointestinal stress. Indeed, currently, our data do not support the use of IBP to enhance exercise performance.

8.1.6 Exercise-induced pain

Previous studies have reported a higher power output for the same level of pain sensation at a given work output during cycle ergometry exercise, suggestive of a reduction in the sensation of pain (i.e., Foster et al. 2014; Mauger et al. 2010). Therefore, pain sensation was not a primary focus of this thesis. Instead this thesis focused on the physiological neuromuscular bases for any ergogenic effect of commonly consumed pain relief medications (ACT and IBP), as this had received limited research attention prior to the completion of this thesis. Whilst acute ACT ingestion may improve exercise performance irrespective of its well-known effect on pain sensation by, for example, altering neuromuscular function in the absence of pain (i.e., Mauger & Hopker, 2013), ACT may also help to maintain cortical excitability and/or reduce cortical inhibition in the presence of acute pain (i.e., Dubé & Mercier 2011). Though the focus of this thesis was to assess the efficacy of COX-inhibitors on performance and its neurophysiological basis, it is acknowledged that a limitation of the current line of investigations was that pain sensation, *per se*, was not directly assessed (perceived exertion was only assessed in chapter 6). As we have alluded to within the experimental chapters (Chapters 4, 5 and 7), asking the subjects to rate pain (and effort) sensation in this setting may have compromised their ability to focus on the exercise task and provide a true maximal effort, which would have confounded our primary outcome variable.

As work output has previously been shown to increase for the same level of pain sensation during closed loop tasks following ACT ingestion (i.e., Foster et al. 2014; Mauger et al. 2010), the assessment of exercise-induced pain may have been most insightful in experimental chapters 4 and 5. However, whilst an intervention that reduces pain during exercise has the potential to enhance performance during closed-loop tasks (i.e., Foster et al. 2014; Mauger et al. 2010), when pain sensation is completely abolished during such exercise tasks pacing strategy is adversely impacted, peripheral fatigue development is exacerbated and cardiopulmonary function is impaired (i.e., Amann et al. 2009). In contrast, if the analgesic stimulus is too low to significantly reduce PG synthesis (i.e., low vs. high ACT dose), this will likely not have a sufficient impact on alleviating the sensitisation of nociceptors via the use of COX-

inhibitors. Together, these observations offer important considerations regarding the efficacy of ACT, and other interventions that can modulate exercise-induced pain and group III/IV muscle afferent feedback, to impact exercise performance. Specifically, it appears that reducing but not abolishing pain and type III/IV muscle afferent feedback may be an important consideration for optimising exercise performance. Interestingly, this effect can also be achieved by sprint interval training (O'Leary et al. 2017), perhaps suggesting a non-pharmacological alternative.

Whilst not a direct assessment of perceived pain, it is important to note that we observed no differences in RPE in chapter 6 following ACT ingestion (1 g) as well as no improvement in performance during an open loop task. It should be noted, however, that exercise-induced pain (i.e., a muscle 'burn' or ache) and perceived exertion and/or effort (i.e., effort to drive a muscle and/or the relative level of exertion associated with this effort), whilst having considerable similarities, can be dissociated (Astokorki & Mauger, 2017a; Groslambert et al. 2006; O'Connor & Cook, 2001; Pageaux et al. 2015; Pageaux, 2016). In addition, it should also be noted that a recent study has also suggested that perceptions of effort and perceptions of exertion may differ (Peñailillo et al. 2018), an opinion in which there is no consensual agreement. Whilst, by definition, RPE is a measure of perceived exertion, throughout this thesis we use 'exertion' and 'effort' interchangeably such that it is likely they represent similar (or identical) sensations. However, more research is required for understanding the development of such perceptions during exercise. Whilst we cannot draw specific conclusions regarding pain sensation with ACT in this study (chapter 6), the lack of effect on performance is, perhaps, in support of the likelihood that pain sensation is not an important determinant of performance in this type of task (i.e., closed loop, single-leg sub-maximal CWR exercise). However, the discrepancies in our observations might also be explained, at least in part by individually specific responses to analgesic medication (Cregg et al. 2013 *for review*) and the specific timing of the attainment of peak pain suppression following consumption of an acute dose of ACT (i.e., Foster et al. 2016).

8.1.7 Novel insights into the mechanisms underpinning the ergogenic effects of non-specific COX-inhibitors

The findings from this thesis offer insights into the potential for non-specific COX inhibitors to influence performance and neuromuscular fatigue development during different forms of exercise. The original findings from this work suggest that acute ACT ingestion, but not IBP ingestion, might have important implications for improving exercise performance. However, this effect may be specific to maximal-intensity closed-loops tasks (or, at least, tasks whereby there is an ability to regulate work output and muscle activation) and not submaximal severe-intensity, open-loop exercise. In addition, this ergogenic effect was consistently linked to increased muscle activation and, therefore, it seems that this is an important mechanism and determinant of the efficacy of the ingestion of COX-inhibiting agents. Indeed, acute consumption of 1 g of ACT, but not 400 mg of IBP, increased muscle activation and CP (or CT), and attenuated fatigue development during maximal-intensity knee extensor and cycle ergometry exercise. However, acute ACT ingestion did not influence performance during continuous severe-intensity exercise completed with or without prior fatigue of the contralateral limb where central fatigue would be expected to be elevated. Whilst we were unable to isolate the precise mechanism responsible for the ergogenic effect of ACT in chapters 4 and 5, it is likely that the ACT-induced increase in muscle activation (inferred from EMG) occurred due to mechanisms upstream of the neuromuscular junction as we observed no differences in M-wave characteristics. In addition, given that metabolic changes at CP determine the nociceptive signal, ACT likely elevates the measurable CP by reducing pain for a given nociceptive signal and/or influencing the degree of voluntary activation. Indeed, it is plausible to suggest that ACT may permit *tolerable* exercise closer to the metabolic CP. Therefore, CP should not be simply considered as a metabolic boundary but an important performance parameter which amalgamates all factors that contribute to endurance performance. Ultimately, it is likely that a combination of conscious (i.e., reduced pain sensation) and sub-conscious (i.e., attenuated central fatigue, enhanced corticospinal excitability) mechanisms relating to both afferent and efferent pathways contribute to the ergogenic effect of ACT by enhancing muscle activation and CP (or CT). The original research findings

presented in this thesis have advanced knowledge into the effect of acute consumption of non-specific COX-inhibitors on neuromuscular fatigue and exercise performance, making a novel contribution to understanding of the development of exercise-induced fatigue. In combination with the existing body of evidence available, we propose a multi-faceted hypothetical approach attempting to explain the mechanisms responsible for the ergogenic effect of ACT ingestion, as shown in figure 8.1.

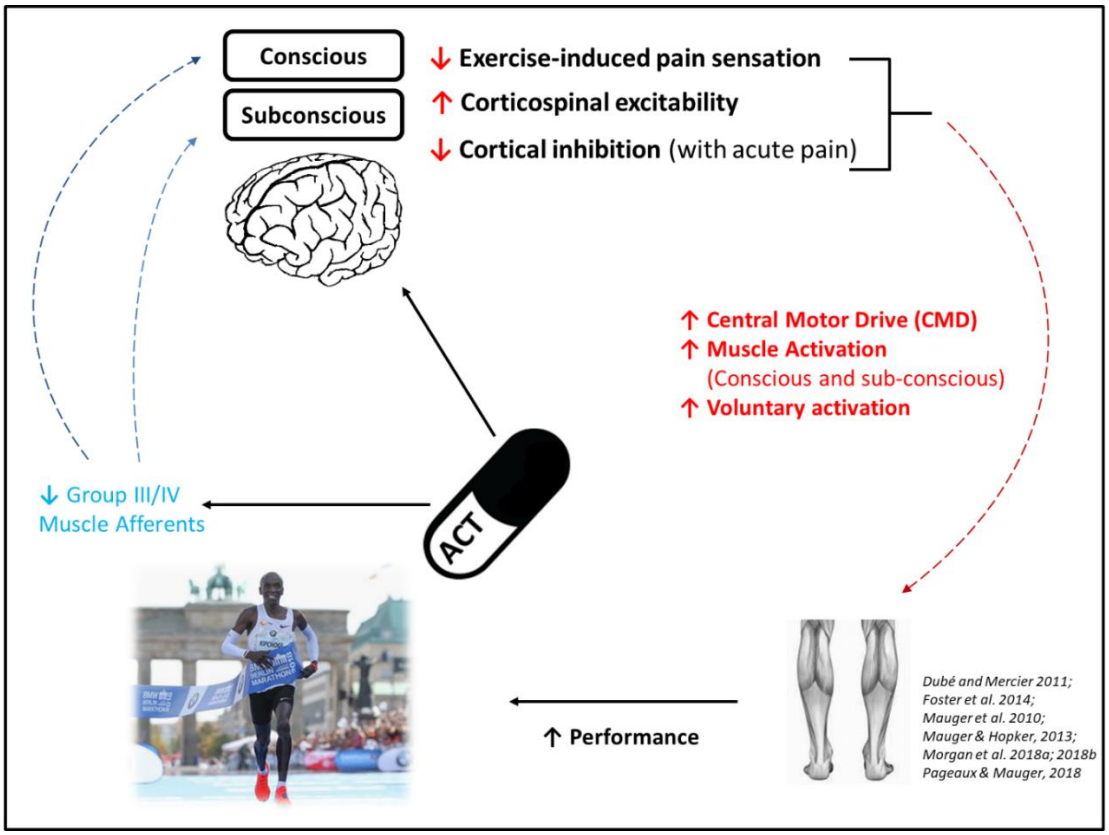

Figure 8.1. *Proposed multi-faceted approach in explaining the mechanisms responsible for the ergogenic capability of an acute dose of acetaminophen (ACT) consumed prior to performing intense exercise under 'normal' conditions. The analgesic and antipyretic properties of ACT ingestion are likely to combine to enhance exercise performance via a number of different conscious and sub-conscious mechanisms. The performance improvement is likely manifest differently in different types of exercise. The multi-faceted effects of ACT likely relate to: 1) reduced pain-sensation (i.e., conscious increase in muscle activation, reduced corticospinal inhibition, utilisation of metabolic reserve); 2). COX-inhibition (i.e., reduced afferent feedback; reduced corticospinal inhibition); 3) Serotonergic and cannabinoid pathways (i.e., enhanced corticospinal excitability; modulating efferent pain pathways).*

8.2 Implications for the applied practitioner and researcher

This thesis has highlighted the ergogenic potential of acute ACT consumption and some of the underpinning mechanisms by which ACT ingestion can favourably enhance exercise performance (chapters 4-5). This novel and original information has the potential to benefit applied practitioners and researchers wishing to use COX inhibitors in the future. However, this thesis

has also highlighted that the ergogenic effect of ACT ingestion appears to be specific to the task (chapter 6 vs. chapters 4-5) and that acute IBP ingestion is unlikely to enhance exercise performance in a 'fresh' state (chapter 7). Based on the findings presented in this thesis, researchers should ensure that ACT ingestion is omitted immediately before exercise trials and should obtain a history on participants' use of ACT prior to completing experimental tests to avoid confounding effects on exercise performance measures (*see appendix 9.1 for pre-screening questionnaire*). For trained individuals, ACT (and/or the suppression but not abolishment of pain) may act as a means to optimise exercise performance in events whereby small margins dictate success by utilising a 'metabolic reserve' which, under normal conditions, is preserved by the central nervous system (Mauger, 2014). However, whilst the current thesis is consistent with previous studies reporting an ergogenic effect of acute ACT consumption, it is pertinent to note that we do not advocate regular ACT use (or chronic use during a training period) or exceeding a single dose of 1 g given the potent hepatotoxicity of ACT ingestion (Yoon et al. 2016). Therefore, individuals wishing to explore the use of ACT to enhance exercise performance should do so infrequently, and with caution. In addition, as we observed no effect of IBP, we do not support its efficacy and thus individuals should reconsider utilising IBP to optimise performance particularly when inflammation is not elevated above baseline levels. It is also important to note that individuals intending to use IBP need to consider its potential side effects and be aware that it could impair aspects of the adaptive response to exercise training (Mikkelsen et al. 2009; Schoenfeld et al. 2012). Indeed, as ACT and IBP ingestion have been implicated in changes in molecular responses regulating the activation and synthesis of certain proteins (e.g., satellite cell activity, translational signalling and lipid mediator response), this may have the potential to impair exercise training adaptations (i.e., D'Lugos et al. 2018; Markworth et al. 2014; Mikkelsen et al. 2009; Schoenfeld et al. 2012; Trappe et al. 2011; Trappe et al. 2002). It is also worth highlighting that ACT and IBP can cause gastrointestinal complications and severe bleeding under certain circumstances (Andrade et al. 1998).

As a more sustainable method to obtain analgesic, antipyretic and ergogenic effects similar to ACT ingestion, athletes and researchers should consider consuming and investigating foods and the use of training interventions (i.e., high-intensity sprint interval training, O'Leary et al. 2017) that might provide such effects. Further research is required to identify and test such alternatives (*see section 8.4 below*). Finally, the extensive use of these drugs for performance enhancement certainly raises significant ethical concerns that need to be addressed. Overall, whilst we support the efficacy of ACT (but not IBP) to enhance exercise performance, we encourage continued greater awareness among coaches and athletes on the potential adverse effects of these drugs. However, fundamentally, the world anti-doping association (WADA) classifies ACT as a legal substance for sports performance, irrespective of the moral considerations. Indeed, and perhaps unsurprisingly, throughout this series of experimental investigations we have received increasing social media attention as well as attracted attention from publicists within cycling wishing to question our moral justification for assessing the efficacy of these drugs on exercise performance. However, it should be noted that the current advice suggests that ACT use during exercise, where such robust controls are not in place, must not be advocated. This is particularly pertinent given the risk of interaction with factors that affect performance (as well as the interactions with other medications) is not well known (Esh et al. 2017 *for brief review*).

8.2.1 Safety and ethical considerations

ACT and IBP ingestion are known to be safe at *standard* therapeutic doses (1 g and 400 mg per dose up to a maximum of 4 g and 1.6 g per day, respectively). Specifically, whilst consumption of ACT may present risk to clinical populations (e.g., Aminoshariae & Khan, 2015; Hinz & Brune, 2012), ACT is considered one of the safest non-opioid analgesics at therapeutic doses within *healthy* individuals (Bunchorntavakul & Reddy, 2013; Graham et al. 2013; Toussaint et al. 2010). Nevertheless, the use of analgesic, antipyretic and anti-inflammatory drugs to favourably enhance exercise performance is a highly controversial topic and raises significant ethical concerns. Individuals wishing to explore the use of these medications for performance should also consider the

contraindications and their potential side-effects. An additional concern is that relatively little is known of the possible side-effects of *chronic* ingestion of these drugs. It is pertinent to note, however, that despite the many difficulties with achieving ethical approval in conducting studies investigating the ergogenic capabilities of such drugs, doses of ≥1.5 g have been safely utilised in exercise science research (i.e., Foster et al. 2014; Mauger et al. 2010) and acute doses of up to 2 g have been used within research outside exercise science in both healthy and clinical populations without adverse side effects (i.e., Benson, 1983; Dippel et al. 2001, 2003; Forrest et al. 1979; Skoglund & Pettersen, 1991). In addition, studies have also demonstrated peak plasma [ACT] following a standard therapeutic dose to be substantially lower than concentrations typically associated with a risk of toxicity (Bunchorntavakul & Reddy, 2013) or damage to the liver (Prescott, 1983). To reduce the likelihood of any adverse effect, we used a questionnaire throughout this thesis to identify any 'at risk' populations which was checked and signed off by a medical doctor (*see questionnaire in appendix, 9.1*).

It is particularly pertinent to note, however, that whilst there are anecdotal accounts of many athletes using these drugs to optimise performance, not all studies have observed performance improvements. Therefore, athletes should strongly consider the risk of potential side-effects (especially with IBP ingestion given the increase risk of possible adverse side effects and the lack of effect on performance observed), particularly when the doses are prolonged and/or above the standard therapeutic dosage. In addition, as pain during exercise serves as a protective sensation that helps safely regulate exercise performance (Mauger, 2014 *for brief review*), participants wishing to ingest analgesics to boost performance should consider the risks associated with the exercise task. Specifically, as a result of reducing pain sensation, athletes are likely to be at a greater risk of tissue damage and injury. This is particularly important for athlete populations who likely exercise at close to their true 'physiological limit' and therefore the use of ACT could prove harmful and put these individuals at risk. Indeed, when exercise-induced pain is completely abolished via intrathecal fentanyl administration, peripheral fatigue develops beyond 'critical levels' than with intact 'pain feedback', subsequently impairing

performance and causing ambulatory problems for up to 2 days after exercise (i.e., Amann et al. 2009).

In a recent review on the use of analgesics during exercise, Lundberg & Howatson (2018) raised some interesting concerns on the chronic dampening of the pain response. Specifically, that use of ACT might lead to athletes returning to training and/or competition prematurely, thereby increasing the likelihood for injury or tendency for reoccurrence of previous injuries (Lundberg & Howatson, 2018). In addition, whilst there have been recent reports strongly advocating that ACT should be added to the WADA doping list (Lippi & Sanchis-Gomar, 2014), this is a complicated issue as many athletes consume analgesic and anti-inflammatory medications to therapeutically control pain in order to compete. These ethical issues associated with ingestion of analgesic and anti-inflammatory medication should continue to be addressed.

8.3 Experimental limitations

An overriding theme of this thesis was to better understand the potential neurophysiological alterations following ACT consumption and how this subsequently influenced exercise performance. Whilst this thesis has presented some novel findings to improve our understanding of the exercise settings in which, the mechanisms by which, ACT ingestion might be ergogenic, some limitations should be acknowledged. Firstly, we were unable to locate the precise mechanism of enhanced exercise performance after ACT ingestion, as might have been achieved had we used techniques such as TMS. The use of TMS in the exercise sciences requires more standardised protocols to be developed and validated in a range of exercise tasks to improve its validity and reliability, particularly during exercise. There have, however, been some excellent developments in this area (i.e., *see recent reviews* Goodall et al. 2014; Taylor & Gandevia, 2001). Further research using TMS should be conducted to provide a comprehensive assessment of the spinal and supraspinal fatigue contributions following ACT ingestion (*see section 8.4 on the directions for future research*). In addition, we were likely restricted with the sensitivity of our measures in identifying small differences in central and/or peripheral function (see *chapter 4* for brief discussion) and the measuring of pain sensation (see

8.1.6 'Exercise-induced pain'). To help inform future research, techniques should be developed to overcome the difficulty with measuring muscle afferent feedback, particularly during exercise. To do so would significantly improve understanding of the central regulation of exercise performance and knowledge of the mechanistic bases of the ergogenic effect of ACT (chapter 6). In addition, it is acknowledged that PGs were not measured during this thesis as their synthesis is well established following ACT consumption. However, future research on the effect of ACT should consider investigating the inter-relationships between physiological and performance alterations and PG synthesis to better understand the mechanisms for the ergogenic effect of ACT ingestion. Finally, it would have been of interest to assess exercise performance in chapters 4 and 5, by ensuring participants were aware of the contractions/time elapsed (and contractions/time remaining). Indeed, future research should attempt to closely replicate competition (condition and participant group) as, despite adding inherent variability to outcome measures, ecological validity of research is maximized. Indeed, elite athletes demonstrate distinct differences in physiology as well as psychological drives, suggesting that the mechanisms in 'real-life' sporting situations when compared to the majority of research (conducted amongst non-elite populations out of competition), likely differ. Further research should consider these limitations as well as the limitations associated with animal and isolated muscle models.

8.4 Directions for future research

Whilst the result of this thesis has provided insight into the efficacy of acute consumption of non-specific COX-inhibitors, further research is required to explore the exercise settings in which COX-inhibitors are more or less effective at enhancing exercise performance. This is particularly pertinent given the complex, task-dependent and multi-faceted nature of exercise-induced fatigue and exercise performance. This is further complicated by evidence suggesting that the ergogenic effect of ACT is also likely multi-faceted (see figure 8.1), individual and task-dependent. In addition, whilst a potential ergogenic effect of ACT is now recognised in certain exercise settings, the *precise* physiological mechanisms by which ACT is ergogenic has yet to be resolved. Specifically, further research is required to ascertain the underlying mechanisms for the

ACT-mediated enhancement in muscle activation and performance (associated with an increased CT or CP) during maximal exercise observed in chapters 4 and 5. Whilst we speculate that this effect may be mediated, in-part, by reducing group III/IV muscle afferent feedback, support for this theory has only been provided by using a COX-inhibitor in cats (Hayes et al. 2006) and/or using fentanyl in humans (i.e., Hilty et al. 2011), and the findings on performance are equivocal.

Further consideration should also be placed around the safe and optimal dose, and timing of intake (i.e., peak plasma [ACT] vs. peak pain suppression) to maximise the ergogenic effect of ACT ingestion. As referred to throughout this thesis, the use of TMS has previously suggested that acute consumption of ACT may enhance corticospinal excitability at rest (i.e., Mauger & Hopker, 2012). However, this effect needs to be assessed during exercise in a controlled setting with minimal disruption to the task. Recent studies have attempted to design an ergometer to measure neuromuscular fatigue throughout the whole motor pathway during cycling with minimal alterations to the demands of the task (i.e., Doyle-Baker et al. 2018). Such methods continue to be improved and are likely to enhance research aiming to improve the understanding of the mechanistic bases for enhanced performance following ACT ingestion, and other potential ergogenic aids as well as our understanding of exercise-induced fatigue. From a research perspective in the exercise sciences, the use of TMS requires some development, particularly its application during locomotion. In addition, given that the use of surface EMG has been criticised in identifying differences in neural drive (i.e., Martinez-Valdes et al. 2018), using TMS (and electroencephalography, EEG) during exercise may be of vital importance to advance the field of exercise-induced fatigue and the central regulation of exercise performance. Therefore, a further comprehensive measurement of alterations to spinal and supraspinal fatigue mechanisms following ACT ingestion is required.

In this thesis we also demonstrated that acute IBP consumption is not beneficial to performance during small and large muscle mass exercise, despite being reported to exhibit a similar effect on PG synthesis compared to ACT at the

whole-body level. However, we were restricted by the relatively low-dose of IBP (400 mg) compared to ACT (1000 mg) due to the contraindication associated with higher doses of IBP. It is possible that IBP may be ergogenic at higher doses; however, testing this this would be unethical considering the potential side effects and contraindications associated with IBP toxicity. It is important to note, though, that for ACT (1000 mg) and IBP (400 mg), these are the effective doses that can be taken without serious side effects and that, as they are different drugs, the quantity of dose cannot be directly compared. In addition, it would be interesting to assess whether the effect of non-specific COX-inhibitors on performance is population-specific (i.e., greater or lesser effects are manifest in trained or untrained individuals, chronic users and clinical populations).

As discussed throughout this thesis, it is possible that ACT *'permits'* an individual to exercise close to their 'true' physiological limit. This suggests that, following ACT ingestion, participants are able to exercise at a higher boundary of their metabolic CP (or exercise modality equivalent). It is, therefore, plausible to suggest that either pain is perhaps not normally tolerable at CP and/or that the use of ACT allows participants to 'utilise' a previously unused 'metabolic reserve'. It is also possible that peripheral fatigue development is unchanged, but that ACT permits exercise at a 'critical' level of peripheral fatigue for longer. Future research should combine muscle metabolic measurements with multiple severe-intensity trials to estimate CP to provide clarity here. In addition, given the apparent ability for analgesics to enhance performance and the ability of exercise training to elevate pain tolerance, it would be of interest as to whether 'pain training' improves exercise performance independent of a physical training programme.

Ultimately, to advance the field of exercise-induced fatigue, rather than assessing fatigue as a central vs. peripheral debate, researchers should continue to attempt to understand the complex interplay between direct physiological impairments (i.e., within the central nervous system and within the contractile elements of peripheral muscle), conscious and sub-conscious regulatory mechanisms as well as the relative contributions from each, respectively, in regulating work output to avoid the risk of a 'muscle-less' model

to explain exercise-induced fatigue development. Indeed, whilst much of this thesis has focussed on the potential central limiting factors to performance and T_{lim}, it is important to note that peripheral fatigue has a profound effect on force-producing capacity and, by extension, the regulation of performance. Finally, as we have stipulated throughout, we do not advocate chronic use of ACT (or IBP) or exceeding a single dose of 1 g to enhance performance given the potent hepatotoxicity of ACT ingestion. However, such promising data supports the potential for safer means to inhibit COX enzyme activity to enhance performance. Future research should explore this potential.

8.5 Conclusion

Acute consumption of the recommended therapeutic dose of ACT (1 g), but not IBP (400 mg) enhanced performance during maximal-intensity single-leg and cycling exercise in healthy participants concomitant with an increase in muscle activation and critical torque (and critical power). However, ACT ingestion did not improve T_{lim}, RPE, muscle activation or the perturbation to intramuscular phosphorus substrates and metabolites during severe-intensity single-leg knee extension exercise completed with or without prior fatigue of the contralateral leg. Therefore, our findings suggest that acute consumption of the non-specific COX inhibitor, ACT, can improve performance, but that the ergogenic efficacy of ACT may be task-dependent, and more likely to be beneficial during maximal-intensity exercise. These original findings improve understanding of exercise-induced fatigue development, the ergogenic potential of ingesting non-specific COX inhibitors and some of the underpinning mechanisms responsible for these effects.

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SCHOOL OF SPORT AND HEALTH SCIENCES

St. Luke's Campus
University of Exeter
Heavitree Road
Exeter
EX1 2LU
United Kingdom
Web address: shs.exeter.ac.uk/
Tel: +44 (0)1392 722896 / 722884
Email: shs-office@exeter.ac.uk

Appendix Questionnaire

Study: Influence of acetaminophen and ibuprofen ingestion on parameters of the power-duration relationship.

Pre-Screening Questionnaire for Participants

	Please initial box **Yes / No**
I. Are you allergic to any medications? If yes, please list the drug(s) and the reaction it causes when you take it? ----------	☐ ☐
II. Do you have any previous illnesses (including asthma, heart disease, stroke, diabetes, high blood pressure, operations etc.)? If yes, please write them below, along with when you had them ----------	☐ ☐
III. Are you currently on any medication? If yes, please list all the drugs you take (including both prescribed and off-the-shelf). ----------	☐ ☐
IV. Have you previously used acetaminophen (paracetamol) and/or ibuprofen? If yes, have you experienced any side effects? ----------	☐ ☐
V. Have you got a history of cigarette use (inc. timing, quantity and type, if any)? ----------	☐ ☐
VI. Do you drink alcohol? If yes, how much do you drink per week and have you ever drunk excessively? ----------	☐ ☐

VII. Have you ever used illegal drugs? If yes, please describe (this, like all of your answers to this questionnaire, is completely confidential) ☐ ☐

VIII. Do/have you suffered from any of the below? Check All That Apply To You. History of: ☐ ☐

Alcohol-related Diseases eg cirrhosis	
Asthma	
Cardiac problems i.e. angina, heart attack or heart rhythm disturbances like atrial fibrillation (AF)	
Cerebrovascular disease i.e. stroke or TIA	
Connective tissue disorders	
Crohn's disease	
Heart failure	
Hypertension	
Kidney problems	
Liver Disease	
Peripheral arterial disease	
Stomach bleeds or stomach ulcers	
Ulcerative colitis	

IX. Have you a family history of any of the below? Check All That Apply To You. ☐ ☐

Asthma	
Cardiac problems i.e. angina, heart attack or heart rhythm disturbances like AF	
Cerebrovascular disease i.e. stroke or TIA	
Connective tissue disorders	
Crohn's disease	
Heart failure	
Hypertension	
Kidney problems	
Liver Disease	
Peripheral arterial disease	
Stomach bleeds or stomach ulcers	
Ulcerative colitis	

If you have answered **YES to one or more questions:**

You should consult with your doctor to clarify that it is safe for you to take part in this study at this current time and in your current state of health. Medical advice will also be sought via the University.

If you have answered **NO to all questions:**

It is reasonably safe for you to participate in this study, gradually building up your performance from your current ability level.

I have read, understood and accurately completed this questionnaire. I confirm that I am voluntarily engaging in the above research, and my participation involves a small risk of injury.

Signature

Print name

Date

Having answered YES to one of the above, I have sought medical advice and my GP has agreed that I may take part in the above study.

Signature

Date

Note: This clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the above questions.

_____	_____	_____
Name of Participant	Date	Signature
_____	_____	_____
Researcher	Date	Signature
_____	_____	_____
Name of Medical Professional	Date	Signature