THE EFFECTS OF NEUROMUSCULAR FATIGUE ON THE COMPLEXITY OF ISOMETRIC TORQUE OUTPUT IN HUMANS

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Abstract

The temporal structure, or complexity, of torque output is thought to reflect the adaptability of motor control and has important implications for system function, with high values endowing greater adaptability in response to alterations in task demand. The aim of this thesis was to investigate the effect of neuromuscular fatigue on the complexity of isometric muscle torque output. It was hypothesised that neuromuscular fatigue would lead to a reduction in the complexity of muscle torque output, as measured by approximate entropy (ApEn), sample entropy (SampEn) and the detrended fluctuation analysis (DFA) α scaling exponent. The first experimental study (Chapter 4) demonstrated that muscle torque complexity was significantly reduced during both maximal and submaximal intermittent fatiguing contractions, with the values at task failure indicative of increasingly Brownian noise (DFA $\alpha > 1.50$). It was subsequently shown in the second study (Chapter 5) that this reduction in complexity occurred exclusively during contractions performed above the critical torque. It was next demonstrated, in the third study (Chapter 6), that pre-existing fatigue significantly reduced torque complexity and time to task failure, but still resulted in consistent values of complexity at task failure regardless of the time taken to reach that point. In the fourth study (Chapter 7) caffeine ingestion was found to slow the rate of reduction in torque complexity with fatigue, seemingly through both central and peripheral mechanisms. Finally, in the fifth study (Chapter 8) eccentric exercise decreased the complexity of torque output, with values only recovering to baseline levels after 24 hours recovery, in comparison to only 10 minutes recovery following isometric exercise. These results demonstrate that torque complexity is significantly perturbed by neuromuscular fatigue. This thesis has thus provided substantial evidence that the complexity of motor control during force production becomes less complex, and that muscles become less adaptable, with neuromuscular fatigue.

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Symbols, Abbreviations and Definitions

[ ]	**Concentration** (mM)
$[\ ]_i$	**Intracellular concentration** (mM)
ANOVA	**Analysis of variance**
ApEn	**Approximate entropy**
arEMG	**Average rectified EMG**
ATP	**Adenosine triphosphate**
Ca^{2+}	**Calcium**
CK	**Creatine kinase**
CO_2	**Carbon dioxide**
CP	**Critical power** (W)
CT	**Critical torque** (N·m)
CV	**Coefficient of variation**
DFA	**Detrended fluctuation analysis**
EMG	**Electromyography / electromyogram** (mV)
H^+	**Hydrogen ion**
K^+	**Potassium**
Hz	**Hertz**
ICC	**Intraclass coefficient**
kg	**Kilograms**
kJ	**Kilojoules**
m	**Metres**
mA	**Milliamps**
M-wave	**Compound muscle action potential**
mM	**Millimolar**
mV	**Millivolts**
MVC	**Maximal voluntary contraction**
O_2	**Oxygen**
PCr	**Phosphocreatine**
pH	**Logarithmic scale used to express acidity and alkalinity of solutions**
P_i	**Inorganic phosphate**

P_o	**Maximal isometric tension**
RPE	**Rating of perceived exertion**
R-R interval	**Electrocardiographic cardiac interval**
SampEn	**Sample entropy**
sEMG	**Surface electromyography**
SD	**Standard deviation**
V_{max}	**Maximum shortening velocity**
$\dot{V}O_{2max}$	**Maximal oxygen uptake** ($L \cdot min^{-1}$; $mL \cdot min^{-1}$; $mL.kg^{-1}.min^{-1}$)
W	**Watts**
W'	**Curvature constant of the power-time relationship** (J; kJ)

Publications

The following publications and communications are a direct consequence of this work.

Pethick, J., Winter, S.L. and Burnley, M. (2015). Fatigue reduces the complexity of knee extensor torque fluctuations during maximal and submaximal intermittent isometric contractions in man. Journal of Physiology, **593,** 2085-2096.

Pethick, J., Winter, S.L. and Burnley, M. (2016). Fatigue-induced loss of knee extensor torque complexity during isometric muscle contractions occurs exclusively above the critical torque in man. American Journal of Physiology, **310,** R1144-1153.

Communications

Pethick, J., Winter, S.L. and Burnley, M. (2015). Fatigue-induced loss of knee extensor torque complexity during isometric muscle contractions occurs exclusively above the critical torque in man. (Abstract).
Endurance Research Conference, University of Kent.

Pethick, J., Winter, S.L. and Burnley, M. (2016). Loss of torque complexity during fatiguing submaximal isometric knee extensions in man is slowed by caffeine ingestion. (Abstract).
Biomedical Basis of Elite Performance, Nottingham.

Pethick, J., Winter, S.L. and Burnley, M. (2016). Prolonged depression of knee extensor torque complexity following eccentric contractions of the knee extensors in man. (Abstract).
Physiology 2016, Dublin.

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I would also like to thank all those who volunteered to participate in my experimental studies. I realise the things I asked you to do were not the most pleasant (particularly the femoral nerve stimulation!), so I really appreciate your commitment and effort. Without you, these studies would not have been possible. I would especially like to thank the two people who took part in all five of the studies; just one was more than enough for most people!

Chapter 1 – Introduction

In 1905, Albert Einstein discovered noise, publishing a seminal paper in the Annalen der Physik outlining the principle that particles move according to Brownian molecular motion (Einstein, 1905). This discovery solved one of the greatest issues in the history of science, providing proof of the existence of atoms. In the century since that monumental discovery, noise has gone on to permeate and influence virtually every field of science and technology (Cohen, 2005).

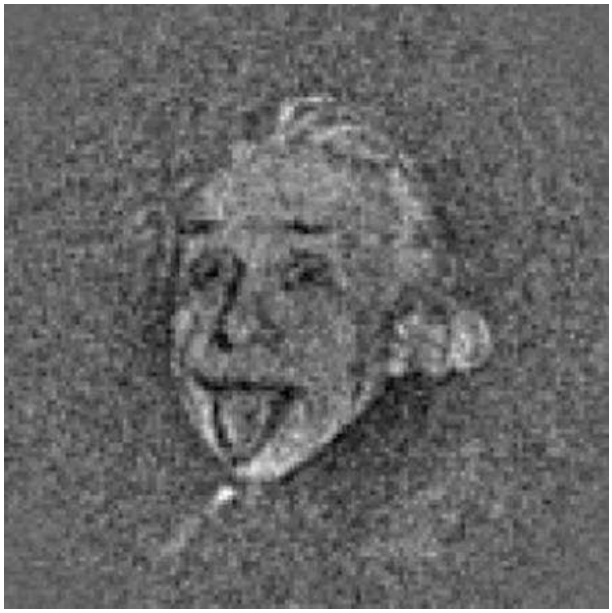

Figure 1.1. A familiar photograph of Albert Einstein emerges from 1000 images of pure white noise. From Shatsky et al., 2009.

However, over the last 111 years the essential contribution that noise makes to numerous physical and biological systems has frequently, and unjustly, been overlooked (Sejdíc and Lipsitz, 2013). Indeed, when it comes to physiology and sporting performance, noise is often regarded and defined as unwelcome, disturbing the balance of a system and degrading performance (Slifkin and Newell, 1998). As such, attempts are often made to reduce or even remove noise altogether, through the use of methods such as filtering, averaging and repeated trials (Ivanov et al., 2001; Lipsitz, 2002). In recent years, though, it has started to become recognised that noise in physiological systems is beneficial (Goldberger and West, 1987a), and that rather than being a problem, noise can, in fact, play a constructive, functional role (Faisal et al., 2008).

What is noise?

Noise is traditionally thought of as unwanted variability in a signal; a viewpoint confirmed by the Oxford English Dictionary, which defines noise as "random or irregular fluctuations which interfere with or obscure a signal". From a scientific point of view, noise is a stochastic process with specific spectral characteristics (Sejdíc and Lipsitz, 2013). Stochastic processes are the probabilistic counterpart to deterministic processes. Deterministic processes are governed by a set of equations that determine exactly how a system will behave over time; while stochastic processes demonstrate indeterminacy, and are at least partially random (Kaplan et al., 1991; Pincus and Goldberger, 1994). Whilst many different stochastic processes exist, the three most common types are white noise, pink noise and Brownian noise.

White noise (Figure 1.2A and B), also known as $1/f^0$ noise, is a stochastic process characterised by equal energy over all frequencies (Sejdíc and Lipsitz, 2013). Its name derives from an analogy with the frequency spectrum of white light, in that its power spectral density is the same at all frequencies. White noise can be thought of as having no memory; it is a serially uncorrelated, random signal, in which current and future values are unpredictable and independent of past values.

Pink noise (Figure 1.2C and D), also known as 1/f noise, is a non-stationary random process characterised by equal energy per octave, and has a power spectral density roughly inversely proportional to frequency (Sejdíc and Lipsitz, 2013). It combines the influence of past events on the future, with the influence of random events (Keshner, 1982). Originally observed as low-frequency noise in vacuum tubes (Johnson, 1925), pink noise has since been observed in a variety of diverse fields, from economics (Baillie, 1996) to music (Voss and Clarke, 1975) to numerous physiological systems and outputs (Lipsitz and Goldberger, 1992; Manor and Lipsitz, 2012).

Brownian noise (Figure 1.2E and F), also known as $1/f^2$ noise, can be mathematically defined as the integral of white noise and has a power spectral density inversely proportional to its frequency squared, and is characterised by greater energy at lower frequencies (Sejdíc and Lipsitz, 2013). It is named not after the colour brown, but after the botanist Robert Brown, who observed Brownian motion in the seemingly erratic

movements of pollen in water. Brownian noise can be thought of as being characterised by a relatively smooth output and by a long-term memory (Goldberger et al., 2002).

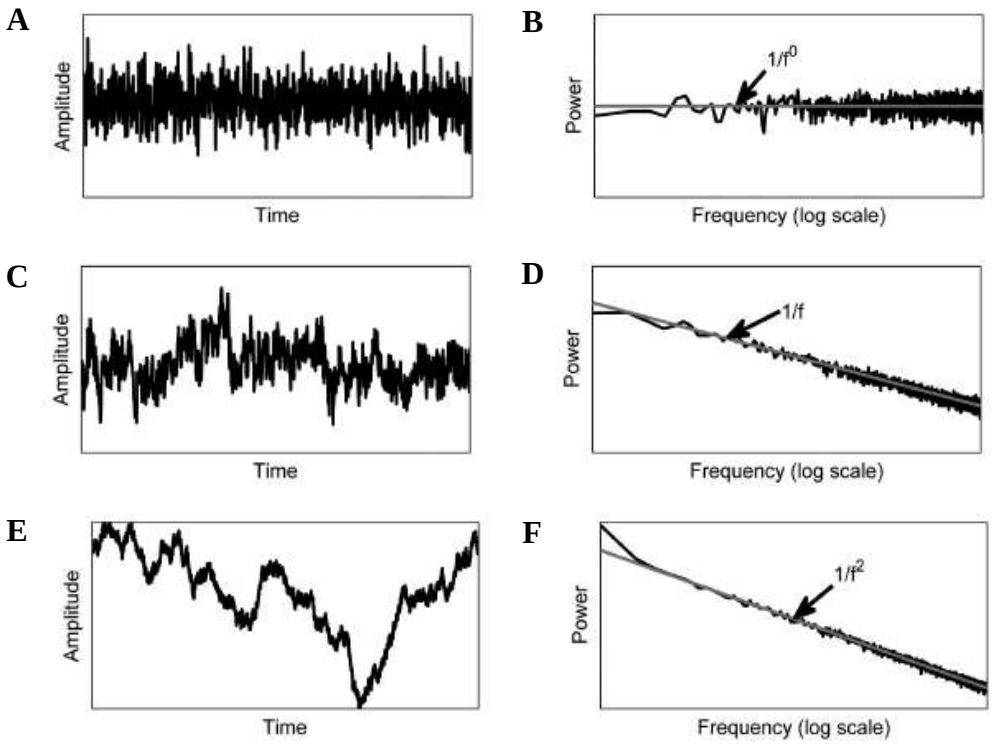

Figure 1.2. White noise (A), the power spectral density of white noise (B); pink noise (C), the power spectral density of pink noise (D); Brownian noise (E), and the power spectral density of Brownian noise (F). From Sejdíc and Lipsitz, 2013.

Noise and variability in sports performance

In sports performance, variability often serves as a measure of success in realising task goals (Slifkin and Newell, 1998). Noise and high variability are typically thought of as being indicative of inconsistent and poor performance; while the absence of noise and variability are thought of as necessary for successful performance. Athletes spend years of their lives practicing; seeking to achieve consistency and to minimise noise and variability in their actions. Some sports (e.g. archery, biathlon, darts and shooting) even reward competitors for their consistency in meeting task requirements (Slifkin and Newell, 1998; Lakie, 2010).

It is now starting to be recognised, however, that noise and variability are not only omnipresent and unavoidable in sporting performance, but are also functional (Davids et al., 2004). It has been demonstrated that elite athletes do not exhibit motor invariance, even after years of practice (Bauer and Schöllhorn, 1997; Davids et al., 2004) and that

outcome consistency does not require movement consistency (Bartlett et al., 2007). It has, instead, been suggested that highly skilled performers are able to exploit variability and that variability can reflect subconscious compensatory measures. Variability in the co-ordination patterns of skilled performers may, therefore, provide an adaptive mechanism to potential external (e.g. environmental) perturbations, may reduce injury risk and may facilitate performance (Holt et al. 1995; Bartlett et al., 2007; Stergiou and Decker, 2011).

Noise and variability in physiology

The principle of homeostasis has been a dominant tenet in physiology for approaching a century (Goldberger, 1991). First introduced as a concept by Claude Bernard in 1865, the term homeostasis was coined and popularised by the American physiologist W.B. Cannon, and describes the observation that an organism operates to reduce variability in its physiological processes in order to maintain a constant internal environment (Cannon, 1929; Goldberger, 2001; Que et al., 2001).

Homeostatic systems can be defined as seeking constant outputs, and can be characterised by three essential features (Davis, 2006). The first feature of a homeostatic system is that it has a set-point that defines, precisely, the output of the system (Davis, 2006). The second feature is feedback; any perturbation to the system will be sensed, compared with the set-point and adjusted back to that set-point (Turrigiano, 2007). The third feature is precision; homeostatic systems will precisely re-adjust back to their set-point, with sensors providing information on the exact deviation, allowing effectors to act via a negative feedback loop (Davis, 2006). Based on these features, it would be expected that any variability or noise in a homeostatic system would be immediately minimised or eliminated (Goldberger, 1991).

The notion of the physiological steady state implied by homeostasis has, however, been complicated over the last 25 years by the observation that many physiological processes, under basal conditions, are characterised by a great deal of intrinsic variability (Goldberger et al., 1990; Lipsitz, 2002; West, 2006). For example, numerous studies have demonstrated that the normal healthy heart rate (Figure 1.3) is characterised by fluctuations and variability that violate the constancy expected from an unperturbed homeostatic process (Yamamoto and Hughson, 1994; Peng et al., 1995a; Goldberger et

al., 2002). Such variability is indicative of stochastic processes, can be modelled as 1/f or pink noise (Peng et al., 1995b), and has also been demonstrated across many other physiological systems and processes, including the normal electroencephalogram, hormone level and gait pattern (Goldberger et al., 1990; Goldberger et al., 1991; Lipsitz, 2002; Hausdorff, 2007).

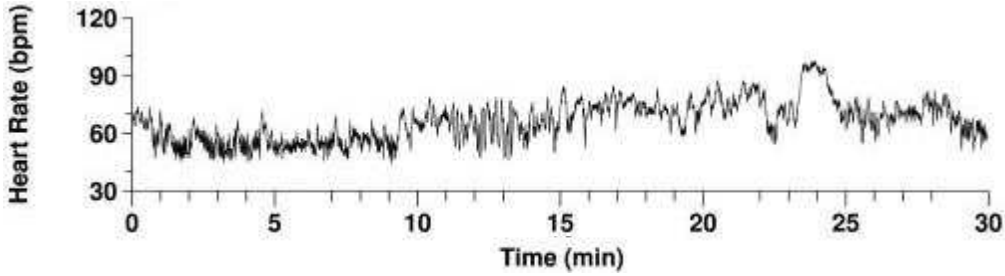

Figure 1.3. Normal resting heart rate is far from homeostatic and is characterised by seemingly random fluctuations. From Goldberger et al. 2002.

The observation that many physiological systems are in constant flux and exhibit intrinsic variability, even at rest, has provided an interesting challenge to the classic concept of homeostasis. Indeed, with continuous fluctuations appearing to characterise normal, healthy function in so many physiological processes, the traditional view of variability and noise being unwanted comes into question. It would seem, therefore, that homeokinesis, defined as the ability of an organism functioning in a variable external environment to maintain a highly organised internal environment fluctuating within acceptable limits, is a more appropriate term (Yates, 1982; Que et al., 2001).

A new view of noise and variability

Noise and variability are now starting to become recognised as integral parts of physiological systems, rather than being detrimental to performance and needing to be minimised. It has been suggested that there is an optimum amount of variability and noise within physiological systems (Figure 1.4), which functions as an essential ingredient and confers adaptability to the system (Manor et al., 2010; Sejdíc and Lipsitz, 2013). Deviations either side of this optimal variability, caused by perturbations such as ageing, disease and fatigue, are associated with reduced function (Figure 1.4; Que et al., 2001). Thus, the traditional view that too much variability is detrimental to performance still

holds, but it also seems that a lack of variability can be equally detrimental (Vaillancourt and Newell, 2002).

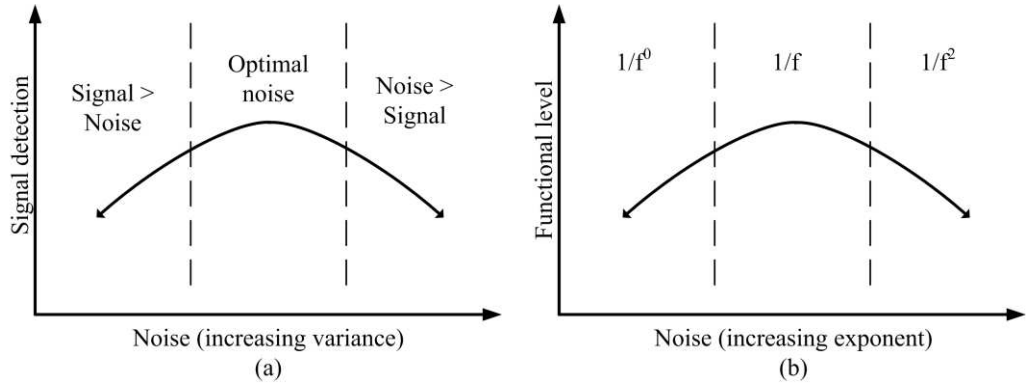

Figure 1.4. Noise can have a beneficial effect or can degrade system performance. $1/f^0$ represents white noise; 1/f represents pink noise; and $1/f^2$ represents Brownian noise. From Sejdíc and Lipsitz, 2013.

Due to its recent recognition as functional, interest in the noise and variability of physiological systems and their outputs has been increasing. Research suggests that any perturbation to the system, be it chronic or acute, has the potential to disrupt the functional, adaptive baseline levels of noise and variability. Such changes have frequently been observed across a wide range of systems in the chronic perturbation that is ageing (Kaplan et al., 1991; Ho et al., 1997; Vaillancourt et al., 2001). It is postulated that these changes could have important implications for the system's adaptability, functionality and performance in a wide variety of settings (Manor and Lipsitz, 2012).

Noise and variability in neuromuscular fatigue

Variability is a common feature of human movement, with contracting muscles producing neither a smooth nor steady force (Contessa et al., 2009). For close to a century it has been known that neuromuscular fatigue exacerbates this variability (Binet, 1920); greatly increasing its amplitude (Hunter et al., 2004) and decreasing the quality of the contraction (Missenard et al., 2009). The purpose of the present thesis is to utilise relatively new metrics in the quantification of noise and variability to investigate the complexity and fractal scaling of motor output during neuromuscular fatigue. The central themes of the following review of literature are: 1) the characteristics, measurement and mechanisms of neuromuscular fatigue; and 2) complexity and fractal scaling, and their applications to human physiology.

Chapter 2 – Review of Literature

Part I – Neuromuscular fatigue and related concepts

2.1 – Fatigue

It has been recognised by perceptive observers since antiquity that intensively exercised muscles show a progressive decline in performance, a phenomena physiologists term "neuromuscular fatigue" (Allen et al., 2008a). There are many different definitions of neuromuscular fatigue. It can broadly be defined as a reduction in the maximal voluntary force or power generating capacity of a muscle or muscle group (Gandevia, 2001; Allen et al., 2008a; Enoka and Duchateau, 2008). This definition, it could be argued, is incomplete. It fails to elucidate the reason for the decrement in performance and fails to reflect its transient nature. Perhaps, then, a more complete definition of neuromuscular fatigue is "a loss in the capacity for developing force and/or velocity of a muscle, resulting from muscle activity under load and which is reversible by rest" (NHLBI, 1990). This definition is reflective of the fact that neuromuscular fatigue is induced by and progresses during exercise, and starts to recover on the cessation of exercise (Gandevia, 2001; Taylor and Gandevia, 2008); and also distinguishes it from muscle weakness or damage (Fitts, 1994; Vøllestad, 1997; Allen et al., 2008a). Neuromuscular fatigue is also distinct from exhaustion, which is defined as an inability to maintain exercise at a given target force/intensity (Vøllestad et al., 1988)

The causes of neuromuscular fatigue are complex; it is not a simple process with a single cause. Multiple processes in the nervous system and muscle contribute to the development of neuromuscular fatigue, many of which begin at the onset of muscle contraction (Enoka and Duchateau, 2008; Taylor and Gandevia, 2008). The causes of neuromuscular fatigue are task dependent, and differ depending on the type of exercise, the intensity of exercise (e.g. maximal or submaximal) and the duration of the exercise bout (Fitts, 1994; Allen et al., 1995a; Taylor and Gandevia, 2008). Understanding the aetiology of neuromuscular fatigue is of great importance, as the development of fatigue often leads to significant reductions in muscle and whole body performance (Hill, 1925; Merton, 1954; Stephens and Taylor, 1972; Sahlin and Seger, 1995; Amann and Dempsey, 2008), which can profoundly restrict performance in occupational duties (such as

firefighting and soldiery), recreational sports and professional sports (McKenna and Hargreaves, 2007).

Characteristics of neuromuscular fatigue

As previously mentioned, neuromuscular fatigue can be defined as "a loss in the capacity for developing force and/or velocity, resulting from muscle activity under load and which is reversible by rest" (NHLBI, 1990). This definition highlights two important characteristics of neuromuscular fatigue, reduced force generating capacity and reduced shortening velocity, and hints at a third, namely a reduction in power output, the product of force multiplied by shortening velocity (Allen et al., 1995a; Allen et al., 2008a).

The loss in the force generating capacity of a muscle has been demonstrated across a wide range of experimental conditions. It is most evident during a sustained maximal voluntary contraction (MVC; Figure 2.1A), in which force steadily declines and neuromuscular fatigue is observed from the start (Merton, 1954; Bigland-Ritchie et al., 1978; Bigland-Ritchie et al., 1982; Bigland-Ritchie et al., 1983a). During such contractions, lasting about a minute, force falls to approximately one-third to one-half of the initial value (Stephens and Taylor, 1972; Bigland-Ritchie et al., 1983a). During intermittent maximal or submaximal contractions, the decline in force generating capacity is less readily apparent and force can be maintained at a target intensity for a longer period of time (Figure 2.1B). Under such conditions, neuromuscular fatigue gradually develops and exhaustion eventually manifests as the inability to continue the contraction(s) at the original intensity, with the target force often becoming greater than the force that can be produced by a maximal effort (Bigland-Ritchie et al., 1985; Bigland-Ritchie et al., 1986a; Taylor et al., 2000). The rate of development of neuromuscular fatigue during submaximal contractions is dependent on the contraction intensity, with higher intensity contractions leading to more rapid development (Dolmage and Cafarelli, 1991). There is also some dependence on the muscle or muscle group tested; muscles with a greater proportion of fast twitch, type II fibres (e.g. the quadriceps) fatigue more quickly than those with predominantly slow twitch, type I fibres (e.g. the soleus; Bigland-Ritchie et al., 1986a; Harridge et al., 1996).

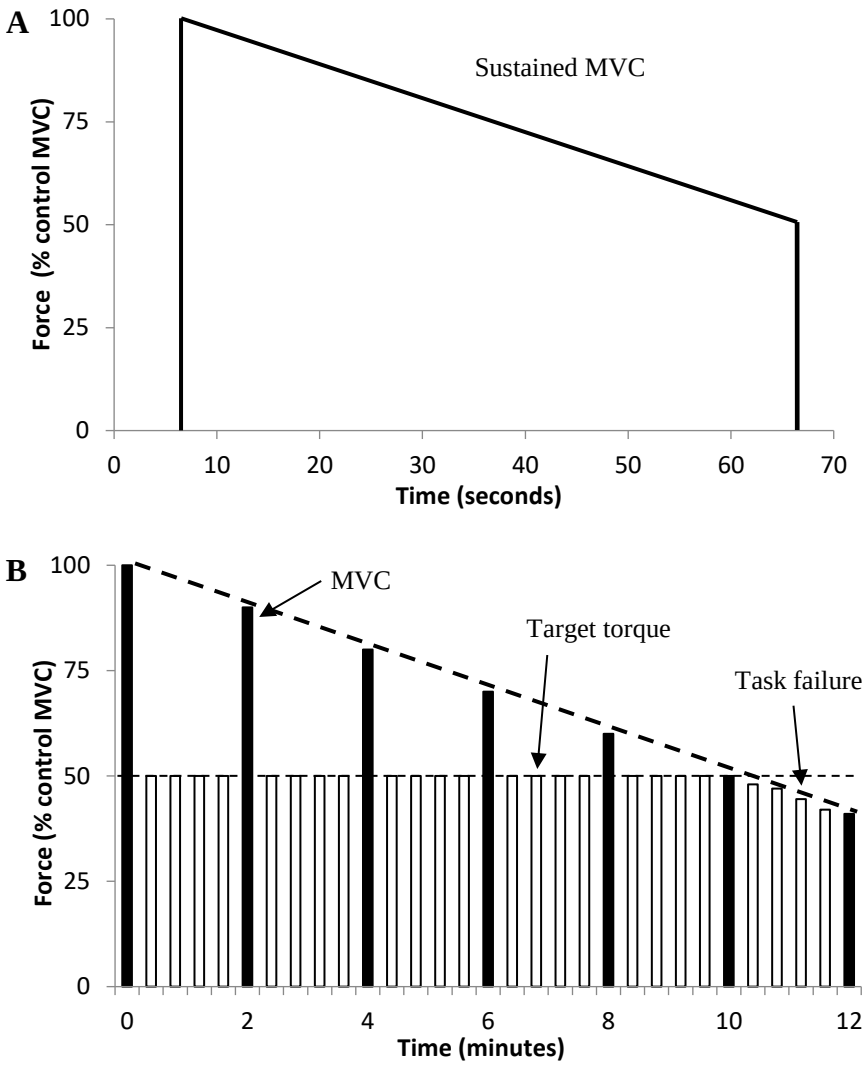

Figure 2.1. Schematic representations of the reduced muscular performance seen from the start of a sustained MVC (A), and the delayed reduction of muscular performance seen during intermittent submaximal contractions interspersed with MVCs (B). Note the different time scales on the two graphs. Adapted from Vøllestad, 1997.

Fatigue-induced reductions in shortening velocity have been studied less extensively than the decrement in force generating capacity, probably due to the greater technical difficulty in its measurement (Allen et al., 1995a). Reductions in shortening velocity are of physiological importance, as most voluntary muscle activity is used for generating movements requiring shortening against various loads (Hill, 1927; Wilkie, 1950). The reduced shortening velocity brought about by neuromuscular fatigue can be described in terms of a decrease in maximal shortening velocity (i.e. the shortening velocity at zero load; Edman and Mattiazzi, 1981; De Ruiter and De Haan, 2000; Jones et al., 2006) or by increased curvature of the concentric part of the force-velocity relationship (Figure 2.2; De Ruiter et al., 1999; De Ruiter and De Haan, 2000; De Ruiter et al., 2000).

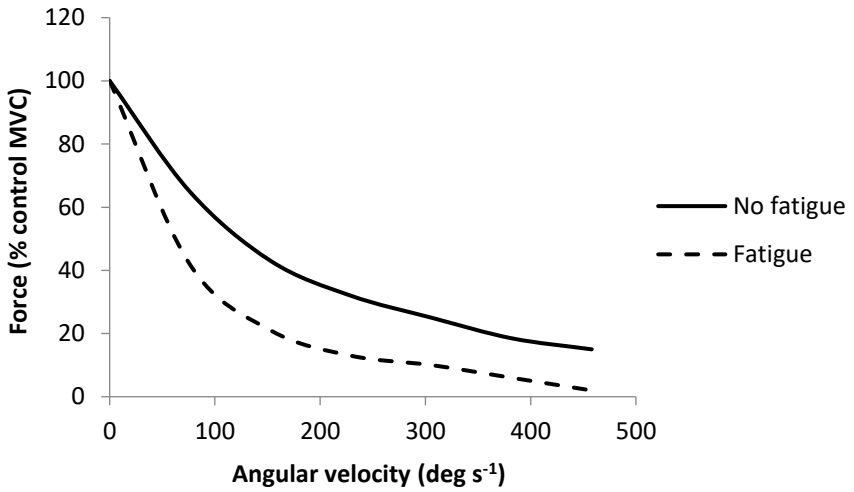

Figure 2.2. Schematic representation of the increased curvature of the force-velocity relationship with neuromuscular fatigue in the adductor pollicis in situ. Adapted from De Ruiter et al. 2000.

Interestingly, in contrast to in situ human muscle, results from isolated frog and mouse muscle fibres demonstrate decreased curvature of the force-velocity relationship with neuromuscular fatigue (Edman and Mattiazzi, 1981; Westerblad and Lännergren, 1994), which would act to preserve muscle power. Such differences between in situ human muscle and isolated mammalian fibres have been attributed to the lower than normal physiological temperatures typically used when examining isolated fibres (De Ruiter and De Haan, 2000).

A reduction in the capacity to generate force and a reduced shortening velocity combine to result in a decrease in their mathematical product, mechanical power (Allen et al., 1995a; Allen et al., 2008a). With neuromuscular fatigue, not only is there a reduced capacity to generate power, but maximum power is produced at progressively lower shortening velocities as neuromuscular fatigue develops (Figure 2.3; De Haan et al., 1989; Westerblad and Lännergren, 1994; De Ruiter and De Haan, 2000). Neuromuscular fatigue typically induces a power decrement approximately twice the extent of the decrement seen in isometric force production (James et al., 1995; Jones et al., 2006). Decreased force generating capacity has a large impact on power output during movements that require high forces, while reduced shortening velocity becomes more important as the speed of the movement increases (Allen et al., 1995a; Allen et al., 2008a).

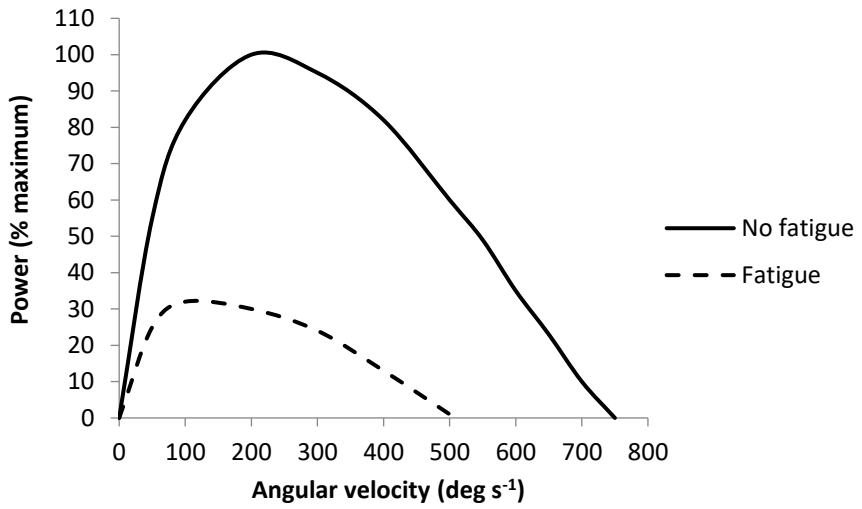

Figure 2.3. Schematic representation of the decreased power output and lower velocity of maximum power generation with neuromuscular fatigue in the adductor pollicis in situ. Adapted from De Ruiter et al. 1999.

2.2 - Models and measurement of neuromuscular fatigue

Decades of study on neuromuscular fatigue have utilised a wide variety of models, protocols and assessment methods to elucidate its characteristics and mechanisms (Vøllestad, 1997; Allen et al., 2008a). The model used to assess neuromuscular fatigue can range from the study of individual muscle fibres to the whole body, and is of great importance, as different models can be used to provide information about the specific mechanism (i.e. the physiological processes) involved in neuromuscular fatigue or to assess performance changes (i.e. functional relevance; Vøllestad, 1997; Cairns et al., 2005). The measures used to assess neuromuscular fatigue have traditionally been reductions in force, torque, shortening velocity or power, though techniques such as electrical stimulation and electromyography can also provide important information and have been frequently applied (Binder-Macleod and Snyder-Mackler, 1993; Vøllestad, 1997; Millet et al., 2012).

Skinned muscle fibres

Skinned muscle fibres, in which the surface membrane has been either chemically or mechanically removed, have been widely used in the study of neuromuscular fatigue (Lamb, 2002; Allen et al., 2008a). Studies of skinned muscle fibres involve the fibre being attached to a force transducer and immersed in experimental buffers (Pate et al., 1995;

Coupland et al., 2001; Debold et al., 2004). This allows the measurement of maximal isometric tension (P_o) and maximum shortening velocity (V_{max}). The composition of the experimental buffers varies, and can be changed from an environment reflective of the normal intracellular milieu to one reflecting the metabolic changes that have been shown to occur during fatigue. Two metabolic changes postulated to be of the greatest importance to the decline of maximum force during neuromuscular fatigue have been studied extensively using skinned fibres: acidosis, due to an increased concentration of hydrogen ions ($[H^+]$); and increased inorganic phosphate ($[P_i]$), due to breakdown of phosphocreatine (PCr; Allen et al., 1995a; Westerblad et al., 2002).

The main advantage of using skinned fibres is that they provide the most direct way to assess the cellular mechanisms of fatigue (Westerblad et al., 2002); that is, they allow the intracellular solution to be completely specified, making it possible to examine the effects of changing individual metabolites (though it must be noted that it may be an interaction of metabolites, rather than an individual metabolite, that causes fatigue), and they allow metabolic and ionic changes with fatiguing contractions to be studied in isolation (Pate et al., 1995; Allen et al., 2008a). Skinned fibres also make it possible to study myofibrillar properties, sarcoplasmic reticulum release and uptake, and action potential/calcium (Ca^{2+}) release coupling (Posterino et al., 2000; Allen et al., 2008a). Furthermore, both animal (Fryer et al., 1995; Pate et al., 1995; Dutka and Lamb, 2000) and human fibres (Stienen et al., 1996; Tonkonogi et al., 1998; Bottinelli et al., 1999) can be used. However, the disadvantages of skinned preparations are that they may lose important intracellular constituents, the relevant metabolites to study must first be identified using other models, and it could be argued that conclusions drawn from studies on single skinned fibres are not relevant to the fatigue experienced by humans during various types of exercise (Westerblad et al., 2000; Westerblad et al., 2002; Allen et al., 2008a). In response to this latter criticism, available data indicate that the mechanisms of fatigue are qualitatively similar across diverse experimental models, ranging from single fibres up to exercising humans (Allen et al., 1995a).

Single muscle fibres

Isolated single muscle fibres, dissected from whole muscles, are another useful model for investigating neuromuscular fatigue. Typically, single fibres are subject to tetanic stimulation (Westerblad and Allen, 1991; Westerblad et al., 1993; Chin and Allen, 1996)

with plate electrodes, so that the action potential is generated simultaneously at multiple points along the fibre, which eliminates longitudinal transmission of the action potential as a possible mechanism of neuromuscular fatigue (Allen et al., 2008a). Stimulation is typically supramaximal (i.e. above that required to elicit a maximal response) and continues until force reaches a value approximately 30% of its initial value (Figure 2.4; Westerblad et al., 1993; Chin et al., 1997; Chin and Allen, 1998). This method allows the measurement of P_o, V_{max}, the force-velocity relationship (from which mechanical power can be calculated) and the rates of force development and relaxation (Lännergren and Westerblad, 1989; Westerblad et al., 1997).

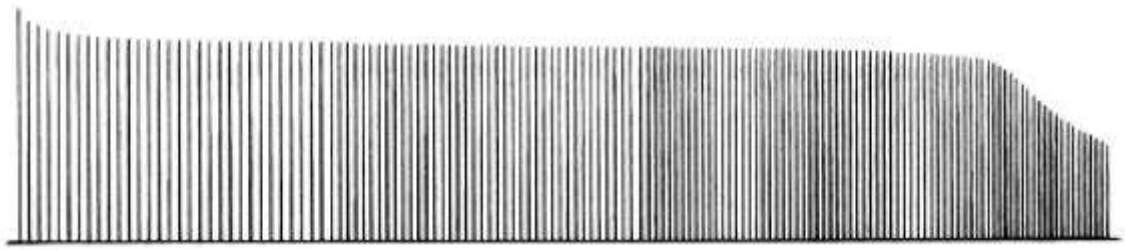

Figure 2.4. A typical force record produced by tetanic stimulation in an isolated muscle fibre. From Lännergren and Westerblad, 1991.

The advantages of working with single fibres are that there is only one type of fibre present, changes in force with ionic and metabolic changes can be correlated, fluorescent measurements of ions, metabolites and membrane potential is possible, and extracellular drugs, ions and metabolites can be easily and rapidly applied (Allen et al., 2008a). However, single fibres do not survive well and are prone to damage at normal physiological temperatures (Lännergren and Westerblad, 1987), likely due to production of reactive oxygen species (Moopanar and Allen, 2005). This has meant that a great deal of research on single muscle fibres has been conducted at low, un-physiological temperatures, at which it is becoming increasingly clear that muscle responds differently (Pate et al., 1994). It must be noted, though, that more recent work has been conducted at higher temperatures, more representative of those found in the body (Westerblad et al., 1997; Nelson and Fitts, 2014). Further disadvantages of using single fibres are the technically difficult dissection and the absence of changes in oxygen tension (O_2) and potassium (K^+) concentration, which may contribute to neuromuscular fatigue (Allen et al., 2008a).

Isolated whole muscle

Many studies on neuromuscular fatigue have been performed on isolated whole muscles to measure force and $[Ca^{2+}]_i$ (Westerblad et al., 2000; Allen et al., 2008a). As with experiments on single muscle fibres, the whole muscles are bathed in a solution mimicking that found in vivo, and are subject to tetanic stimulation at supramaximal currents (Dahlstedt et al., 2000). Arguably the only advantages of using isolated whole muscles are that it is simpler than a single fibre experiment and the dissection is much less technically demanding than that required for a single or skinned muscle fibre (Allen et al., 2008a).

The main issue with using isolated whole muscles is the absence of vascular perfusion (Barclay, 2005; Allen et al., 2008a), which means the muscle's metabolic requirements must be met by diffusion of O_2 from the preparation's outer surface. In vivo, the distance O_2 must travel from the blood vessels to the centre of muscle cells is no more than a few tens of micrometres; in isolated preparations, the distances O_2 must diffuse are typically 10- to 100-fold greater (Barclay, 2005). As O_2 is consumed as it diffuses through an isolated muscle, if the rate of consumption is high relative to the rate of supply, an anoxic core will develop in the centre of the muscle (Hill, 1928; Barclay, 2005; Allen et al., 2008a). This anoxic core develops more rapidly at 35 °C than at 20 °C (Barclay, 2005), making testing at normal physiological temperatures problematic. A further issue is the fact that K^+, H^+, carbon dioxide (CO_2) and lactate all accumulate in the extracellular space. Until a diffusion gradient sufficient to allow diffusion out of the preparation develops, the concentrations of extracellular K^+, H^+, CO_2 and lactate will be substantially higher in the centre of the muscle (Allen et al., 2008a).

Muscle in vivo

The "gold standard" model for assessing neuromuscular fatigue is the intact perfused muscle under central control (Allen et al., 2008a). By studying neuromuscular fatigue in this way, all physiological mechanisms are present, and fatigue can be caused by either central and/or peripheral mechanisms. Furthermore, it allows an extensive range of exercise protocols to be applied.

As neuromuscular fatigue is primarily characterised and defined by a loss in the capacity for generating force (Bigland-Ritchie et al., 1982; Bigland-Ritchie et al., 1986a; NHLBI, 1990; Gandevia, 2001), its reliable assessment is highly dependent upon our ability to measure force generating capacity (Bigland-Ritchie et al., 1983a; Vøllestad, 1997). In vivo studies on humans frequently use isometric MVCs to assess force generating capacity, as they provide an easy and direct assessment of neuromuscular fatigue, which is readily observed from the start of the contraction (Vøllestad, 1997; Cairns et al., 2005). It must be noted, though, that while numerous studies of neuromuscular fatigue have used MVCs as their assessment measure, normal voluntary locomotor activity is intermittent and characterised by low motoneuron firing rates that result in only partial summation of force (Bigland-Ritchie et al., 1985; Vøllestad, 1997; Cairns et al., 2005). With this in mind, many researchers choose to use submaximal and/or intermittent contractions at varying percentages of MVC, with the contractions terminated when the participant can no longer maintain a target force (Bigland-Ritchie et al., 1985; Bigland-Ritchie et al., 1986a; Taylor et al., 2000).

Due to the functional importance of generating power during typical daily movements (Allen et al., 1995a; Allen et al., 2008a), measurement of the ability to generate power is also of importance. Changes in power output with fatiguing exercise can easily be measured using cycle ergometry or running (Sargeant et al., 1981; McCartney et al., 1983). Peak power is attained within a few seconds of the commencement of exercise and gradually tails off. During cycling, for example, power output typically falls by approximately 50% and reaches a steady value within three minutes, with this value equating to the critical power (Vanhatalo et al., 2007; Vanhatalo et al., 2008). Power output is also commonly assessed using isokinetic dynamometers, which allow a multitude of different muscles to have their performance measured at different contraction velocities and joint angles (Baltzopoulos and Brodie, 1989; Kannus, 1994).

Electromyography

Electromyography is a frequently used tool in the in vivo study of neuromuscular fatigue. Changes in the electromyogram (EMG) were first used to investigate neuromuscular fatigue in the 1950s (Cifrek et al., 2009), and are now one of the most widely used indirect indices of neuromuscular fatigue (Vøllestad, 1997). There are two types of EMG: surface

EMG (sEMG) and intramuscular EMG; with sEMG being the most commonly used, due to the fact it is non-invasive, with signals being recorded from the skin surface (Filligoi and Felici, 1999; Cifrek et al., 2009). The signal from the sEMG is the instantaneous algebraic summation of the electrical contributions made by active motor units (Farina et al., 2004; Sung et al., 2010). The characteristics of the sEMG, such as its amplitude and power spectrum, are dependent on the timing of the motor unit action potentials and the membrane properties of the muscle fibres, meaning that the sEMG is reflective of both the central and peripheral properties of the neuromuscular system (Farina et al., 2004).

The amplitude of the sEMG signal is related to the recruitment and discharge rates of active motor units (Solomonow et al., 1990; Farina et al., 2004). Because of this relation, the sEMG amplitude is often used to index the level of activation provided by the spinal cord, though due to factors including subcutaneous tissue thickness, electrode location and distribution of motor unit conduction velocities, there remains a mismatch between output from the spinal cord and the sEMG amplitude (Farina et al., 2002; Farina et al., 2004). During maximal isometric contractions, the sEMG amplitude falls progressively, in parallel with the fall in force (Figure 2.5; Bigland-Ritchie et al., 1979; Bigland-Ritchie et al., 1983a; Bigland-Ritchie et al., 1983b), which suggests that sEMG is a good marker for fatigue (Vøllestad, 1997). In contrast, during submaximal contractions, the sEMG amplitude rises gradually, as additional muscle fibres are recruited in an attempt to maintain the same contraction intensity (Viitasalo and Komi, 1977; Bigland-Ritchie et al., 1985).

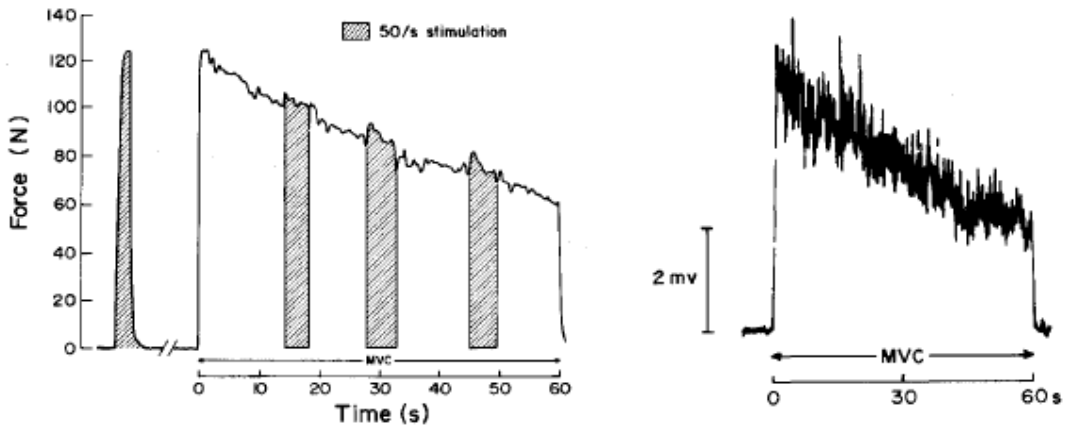

Figure 2.5. Parallel decrease in force and EMG during a sustained maximal voluntary contraction. The force trace is shown on the left and the EMG trace on the right. From Bigland-Ritchie et al., 1983a.

Another measure derived from the sEMG is the power spectrum of the signal, which provides a representation of the contributing frequencies to the signal (Seely and Macklem, 2004). The power spectrum is obtained by Fourier transformation of the raw signal (Cifrek et al., 2009); with the median frequency, the frequency dividing the spectrum into two equal halves, then typically being calculated (Vøllestad, 1997; Filligoi and Felici, 1999; Ravier et al., 2005). With neuromuscular fatigue, the frequency spectrum shifts towards lower values during both maximal (Petrofsky and Lind, 1980) and submaximal contractions (Moxham et al., 1982; Krogh-Lund and Jørgensen, 1991). These shifts in the power spectrum are associated with changes in conduction velocity in the muscle fibres (Lindström et al., 1970; Bernardi et al., 1999) and have been used to infer changes in motor unit recruitment (Farina et al., 2004).

Electrical stimulation

While voluntary movements and contractions serve as the first-choice method for the study of neuromuscular fatigue in vivo, they can be limited by participant motivation (Kroemer and Marras, 1980; Gandevia, 2001; Marras and Davis, 2001). To overcome this limitation, voluntary contractions are often accompanied by electrical stimulation, either at rest or superimposed during the contraction (Vøllestad, 1997; Cairns et al., 2005; Millet et al., 2012). This stimulation can be at the level of the muscle or peripheral nerve, and combined with recordings of force and/or EMG, provides a non-invasive insight into neuromuscular fatigue (Millet et al., 2011; Millet et al., 2012). In recent years, magnetic stimulation of the muscle, nerve or motor cortex has also been applied (Polkey et al., 1996; Taylor et al., 1996; Taylor and Gandevia, 2001). Though such magnetic stimulation serves to minimise participant discomfort, it is less commonly used than electrical stimulation (Millet et al., 2012).

During voluntary movements, the major determinant of the strength of a contraction is descending drive from the motor cortex (Taylor, 2009a). Increased descending drive results in the recruitment of more motor units, in an orderly fashion from small to large (Henneman et al., 1965; Henneman et al., 1974), with larger motor units producing greater force (Milner-Brown et al., 1973; Jones et al., 2004). An electrical stimulus delivered to the muscle or nerve bypasses this descending drive and directly activates the motor units, thus producing a contraction known as a twitch (Millet et al., 2012).

Interestingly, it has been suggested that electrical stimulation can reverse the order of motor unit recruitment, with larger motor units, which are typically characterised by reduced impedance and faster rates of action potential conductance, being recruited first (Feiereisen et al., 1997). Evidence for such a phenomenon is, however, limited (Gregory and Bickel, 2005; Bickel et al., 2011). As the intensity of electrical stimulation increases, the number of motor units activated increases, leading to an increase in force (Adams et al., 1993).

Based on the relationship between increasing stimulation intensity and the number of activated motor units, electrical stimulation protocols typically begin with a gradation of stimulus intensity, in order to establish the optimal stimulation intensity; that is, the intensity required for complete spatial recruitment of the muscle group of interest (Adam and De Luca, 2005; Neyroud et al., 2014). For some researchers, this occurs when a further increase in stimulation intensity results in no further addition of force (Merton, 1954; Adam and De Luca, 2005; Søgaard et al., 2006) and for others when a plateau in the compound muscle action potential (M-wave) is reached (Hamada et al., 2000; Sidhu et al., 2009; Klass et al., 2012). It is argued, though, that adequate assessment of central and peripheral fatigue requires a stimulus greater than the optimal stimulation intensity (Neyroud et al., 2014). With neuromuscular fatigue, the motor unit axonal excitation threshold increases due to sustained and/or repeated firing (Kernell and Monster, 1982; Butler et al., 2003), meaning that the optimal stimulation intensity may not be sufficient to recruit all motor units, and peripheral fatigue may be overestimated (Neyroud et al., 2014). It is thus recommended that a supramaximal intensity of at least ~120% of the optimal stimulation intensity be used for the assessment of central and peripheral fatigue (Löscher et al., 1996; Sidhu et al., 2009; Neyroud et al., 2014).

As previously mentioned, electrical stimulation can be applied at the level of the muscle (e.g. quadriceps) or peripheral nerve (e.g. peroneal nerve, femoral nerve) in an effort to recruit motor units (Hultman et al., 1983; Millet et al., 2011). The most commonly used method is to place the stimulating electrodes on the skin over the belly of the muscle (Bickel et al., 2011). While this method produces less pain than nerve stimulation (Forrester and Petrofsky, 2004), it is only possible with superficial muscles and is complicated by subcutaneous fat and connective tissue (Mesin and Merletti, 2008; Bickel et al., 2011). Stimulation directly over the nerve trunk is also frequently used. It has been

suggested that applying stimulation in this way generates a greater proportion of contraction from central pathways, which could minimise the fatigue associated with electrical stimulation (Bergquist et al., 2012). However, not all muscles can be activated by nerve stimulation, due to either the nerve not being superficial enough (Millet et al., 2012), or due to the nerve evoking responses from both the agonist and antagonist muscles, as is the case when nerve stimulation is applied to the musculocutaneous nerve to evoke a motor response from the elbow flexors (Allen et al., 1998).

Aside from stimulation intensity and electrode location, a further consideration when using electrical stimulation is the type of stimulus delivered, and whether this takes the form of a single stimulus, a doublet (two stimuli with a very short inter-pulse interval) or a tetanus (multiple, rapid stimulations per second). The type of stimulus delivered has a large influence on muscle performance, affecting force production, fatigability and what can be measured (Bickel et al., 2011). A single stimulus, for example, results in a twitch and allows the measurement of both mechanical (i.e. force) and electromyographic (i.e. M-wave) responses (Millet et al., 2012). A doublet also produces a twitch, but is only used to investigate mechanical properties, as the second stimulation interferes with the M-wave. Doublets do, however, augment the peak force produced by stimulation (Burke et al., 1970; Karu et al., 1995), a phenomenon termed a “catchlike property”, and are the optimal type of stimulation for the investigation of central and peripheral fatigue (Place et al., 2007). Tetanic stimulation, in contrast to single stimuli and doublets, results in more prolonged contractions that can be either unfused or fused, depending on the frequency of stimulation. Greater frequencies result in increased force production and smoother, more fused contractions (Binder-Macleod et al., 1995; Gregory et al., 2007).

One of the most frequently utilised electrical stimulation techniques in the study of neuromuscular fatigue is the twitch interpolation technique (Figure 2.6), introduced by Merton in 1954. The principle of twitch interpolation is to stimulate, supramaximally, the muscle or nerve during a voluntary contraction, in order to add an additional action potential to those produced voluntarily (Merton, 1954; Belanger and McComas, 1981). Any motor units not recruited or not firing fast enough to produce maximal force will be activated by this additional action potential, evoking a twitch-like increase in force (Taylor, 2009a; Gandevia et al., 2013). As voluntary force increases, more motor units are recruited and are firing faster; thus, the interpolated twitch should be smaller,

indicating greater voluntary activation of the muscle (Shield and Zhou, 2004; Taylor, 2009a; Gandevia et al., 2013). Voluntary activation is calculated as the ratio of the increment in force as a result of stimulation during a contraction to that of the force produced by a subsequent stimulation at rest (Belenger and McComas, 1981; Behm et al., 1996).

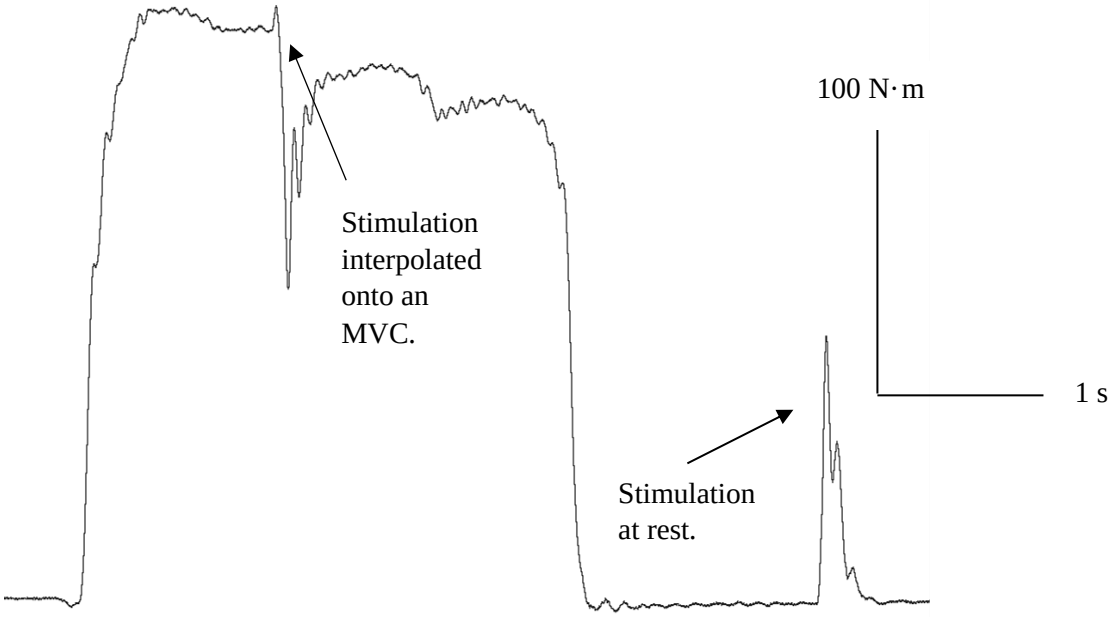

Figure 2.6. An interpolated twitch during an MVC of the knee extensors, followed by stimulation at rest. In this case, voluntary activation is approximately 95%.

The principle of twitch interpolation is based on and supported by an inverse relationship between the amplitude of the twitch evoked by stimulation during the contraction and the voluntary force at the moment of stimulation (Taylor, 2009a). Such relationships have been demonstrated for a variety of muscle groups, including the triceps surae, tibialis anterior, quadriceps, biceps brachii and adductor pollicis (Belanger and McComas, 1981; Dowling et al., 1994; Allen et al., 1995b; Behm et al., 1996). Twitch interpolation thus provides a reliable way to quantify voluntary activation, and how much of a muscle's possible force is not engaged by voluntary drive, in many muscle groups (Herbert and Gandevia, 1999; Taylor, 2009a).

While the underlying principle of twitch interpolation is sound, the technique is, nonetheless, subject to many practical pitfalls (Taylor, 2009a). Electrical stimulation can result in higher, lower or similar forces to maximal voluntary contractions, due to the fact that stimulation may activate antagonists and may fail to activate synergists, which are often activated during voluntary contractions (Taylor, 2009a; Taylor, 2009b).

Furthermore, accurate estimation of voluntary activation requires a linear relationship between superimposed twitch amplitude and the force of voluntary contraction (Shield and Zhou, 2004), while in reality non-linearities exist (Belanger and McComas, 1981); and also requires stimulation during a plateau in force (Gandevia, 2001), which is often difficult to attain, particularly during an MVC (Gandevia, 2001) and when neuromuscular fatigue develops. Other criticisms levelled at the twitch interpolation technique are a low sensitivity near maximal contraction intensities, and mismatches between calculated voluntary activation and predicted maximal torque capacity, leading to overestimates of the former and underestimates of the latter (De Haan et al., 2009).

Despite the above limitations, the twitch interpolation technique is generally held to be a valid measure of a muscle's voluntary activation, and is sufficient to reveal changes in activation as a result of intervention and pathology (Allen et al., 1995b; Behm et al., 1996; Taylor, 2009a). In the study of neuromuscular fatigue, the twitch interpolation technique has been used to infer a progressive impairment in voluntary activation, as demonstrated by a reduction in voluntary force and an increase in the interpolated twitch size as contractions progress (Bigland-Ritchie et al., 1983a; Bigland-Ritchie et al., 1983b; McKenzie et al., 1992; Gandevia et al., 1996). This is reflective of a failure to obtain optimal force from the muscle voluntarily and represents a failure in sustaining the recruitment and discharge frequency of motor units (Gandevia et al., 2013).

2.3 - Mechanisms of neuromuscular fatigue

For muscles to contract, there is a complex chain of events (Figure 2.7), leading from the higher centres of the brain, via the spinal cord and motoneurons to the muscle fibres themselves, and finishing with the individual cross-bridges generating force (Gandevia, 2001; Jones et al., 2004; Allen et al., 2008a). Neuromuscular fatigue and impaired muscle function can occur through failure at any one of these steps (Kent-Braun, 1999; Jones et al., 2004; Millet et al., 2012).

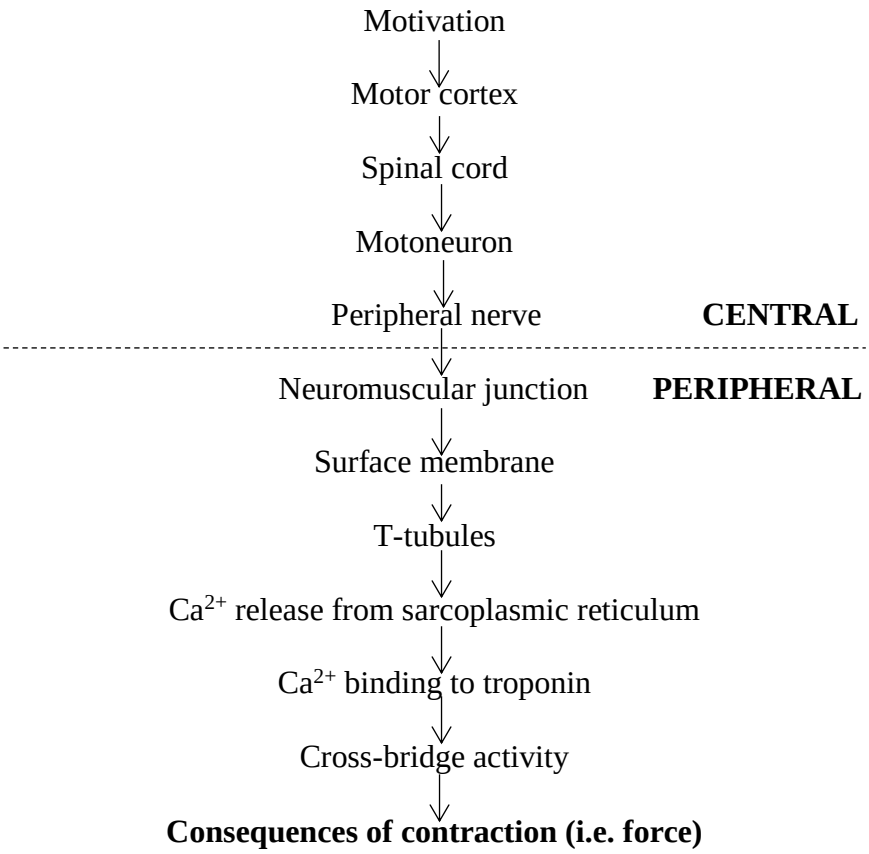

Figure 2.7. Chain of command leading from motivation to the generation of force by the cross-bridges, showing the central and peripheral sites where failure could occur. From Jones et al., 2004.

The failure of processes occurring proximal to the neuromuscular junction is termed central fatigue (Gandevia, 1998; Gandevia, 2001; Taylor and Gandevia, 2008); while the failure of processes occurring at or distal to the neuromuscular junction is termed peripheral fatigue (Gandevia, 2001; Taylor and Gandevia, 2008). It is likely that both central and peripheral mechanisms contribute to the reduced physical performance associated with neuromuscular fatigue in all cases, irrespective of the task (Kent-Braun, 1999; Schillings et al., 2003; Nordlund et al., 2004; Søgaard et al., 2006; Burnley et al., 2012). However, the extent to which central and peripheral mechanisms are involved and which mechanism predominates is very much task dependent (Enoka and Stuart, 1992); depending on exercise intensity (Eichelberger and Bilodeau, 2007; Yoon et al., 2007; Place et al., 2009), exercise duration (Behm and St-Pierre, 1997; Schillings et al., 2003), whether the exercise is sustained or intermittent (Duchateau and Hainaut, 1985; Bilodeau, 2006) and the age and sex of the participants (Hunter et al., 2009b; Griffith et al., 2010).

Central fatigue

As previously mentioned, central fatigue refers to processes occurring proximal to the neuromuscular junction (i.e. within the central nervous system), and can be defined as a progressive exercise-induced reduction of voluntary activation of the muscle (Blomstrand, 2001; Gandevia, 2001; Nordlund et al., 2004; Taylor and Gandevia, 2008). Central fatigue is typically assessed using the twitch interpolation technique during an MVC (Merton, 1954; Behm et al., 1996; Gandevia, 2001) and is demonstrated by an increase in the force evoked by nerve stimulation during such a maximal voluntary effort (Merton, 1954; Belanger and McComas, 1981; McKenzie et al., 1992; Taylor and Gandevia, 2008). If extra force is evoked by motor nerve stimulation during an MVC, this is reflective of some motor units not being voluntarily recruited or not firing fast enough to produce fused contractions (Herbert and Gandevia, 1999). Such an increase in force due to a superimposed twitch with the progression of fatigue signifies that processes proximal to the site of stimulation are contributing to the loss of force (Taylor and Gandevia, 2008).

Central fatigue is easily demonstrated using maximal contractions. Such contractions have four main advantages: they test the entire motor pathway, the task for the nervous system is maximal throughout the contraction, all motoneurons should be recruited and firing at high rates from the start, and fatigue develops within seconds (Taylor and Gandevia, 2008). Gandevia et al. (1996) observed that during a two-minute maximal elbow flexion, voluntary force began to fall immediately and the superimposed twitch evoked by motor cortex stimulation increased, signifying reduced capacity to fully recruit all motor units and thus the development of central fatigue. Other studies have confirmed the presence of central fatigue during sustained and intermittent maximal contractions of the adductor pollicis (Bigland-Ritchie et al., 1983a), elbow flexors (McKenzie and Gandevia, 1991; Taylor et al., 2000), diaphragm (McKenzie and Gandevia, 1991; McKenzie et al., 1992), ankle plantar- and dorsi-flexors (Kawakami et al., 2000; Nordlund et al., 2004) and quadriceps (Bigland-Ritchie et al., 1978; Place et al., 2007; Burnley, 2009). Comparison of sustained and intermittent maximal contractions has revealed that decreases in voluntary activation occur sooner and are more pronounced during sustained contractions (Bilodeau, 2006). It must be noted, though, that the majority of neuromuscular fatigue arising from maximal contractions is attributed to peripheral

processes (Bigland-Ritchie et al., 1983a; Hunter et al., 2006; Søgaard et al., 2006; Taylor and Gandevia, 2008).

Central fatigue is also present during sustained and intermittent submaximal contractions at low percentages of MVC, and, indeed, at such intensities its extent is substantial, accounting for up to 40% of the force loss (Behm and St-Pierre, 1997; Søgaard et al., 2006; Taylor and Gandevia, 2008). The extent of central fatigue during submaximal contractions can be assessed by introducing brief MVCs and twitch interpolation at regular intervals during the contraction(s) (Bigland-Ritchie et al., 1986a; Søgaard et al., 2006; Smith et al., 2007). Using this method, studies have shown that sustained contractions at <30% MVC result in pronounced decreases in voluntary activation in the first dorsal interosseous, elbow flexors and triceps surae (Löscher et al., 1996; Søgaard et al., 2006; Eichelberger and Bilodeau, 2007). A decrease in voluntary activation in the elbow flexors was also observed by Smith et al. (2007), through motor point and cortical stimulation, for a sustained contraction at only 5% MVC, indicating that the development of central fatigue does not require high levels of motor cortical output or the recruitment of a large proportion of the motoneuron pool (Taylor and Gandevia, 2008).

Further evidence for the presence of central fatigue during submaximal contractions comes from studies in which measurements of participant's ratings of perceived exertion (RPE) were taken. Søgaard et al. (2006) observed that the RPE required to maintain a weak target contraction increased disproportionately to the target force and EMG relative to their maxima. During a 40-minute elbow flexion at 15% MVC, participants reported their RPE to rise from ~2 (mild) to ~7.5 (very large), from a maximum of 10; whereas target force and EMG of the muscles only increased to 30% and 35%, respectively, of that recorded during a brief MVC. A similar mismatch between perceived exertion and motor output was observed by Smith et al. (2007) during a sustained contraction at only 5% MVC of the elbow flexors. Such mismatches between perceived exertion and motor output are strongly suggestive of significant impairment of central processes during submaximal contractions.

While there are some common features of the central fatigue caused by maximal and submaximal contractions, there are also critical differences (Taylor and Gandevia, 2008). During submaximal contractions, the fall in voluntary force has a greater supraspinal

component than during maximal efforts, meaning a greater proportion of the force loss is due to less than optimal motor cortex output (Gandevia, 2001). Submaximal contractions, despite their less intense nature, also require longer to recover voluntary activation after the end of a fatiguing protocol (Behm and St-Pierre, 1997; Taylor and Gandevia, 2008). For high-intensity contractions, recovery occurs within two to three minutes (Baker et al., 1993; Taylor et al., 2000), but takes >10 minutes and even up to an hour after prolonged weak contractions (Baker et al., 1993; Søgaard et al., 2003; Place et al., 2004). The difference in recovery time could be due to continued firing of small diameter muscle afferents (Taylor and Gandevia, 2008; Kennedy et al., 2013) or reflex inhibition of motor cortex excitability (La Pera et al., 2001; Taylor and Gandevia, 2008).

Potential mechanisms of central fatigue

The slowing of motor unit firing rates has been measured during both maximal (Bigland-Ritchie et al., 1983b; Rubinstein and Kamen, 2005) and submaximal (Garland et al., 1997; Carpentier et al., 2001; Thomas and del Valle, 2001) fatiguing contractions, and appears to be of significance to the development of central fatigue. Initially, it was thought that such changes in firing rates may match muscle contractile properties to preserve maximum force output, a hypothesis known as “muscle wisdom” (Marsden et al., 1983). However, more recent evidence suggests that this hypothesis does not hold. Rather than protecting against fatigue, decreased motor unit firing rates enhance fatigue (Fuglevand and Keen, 2003), with the mechanisms causing this slowing of firing rates being intrinsic to central fatigue (Taylor and Gandevia, 2008). It is recognised that three kinds of actions on the motoneuron pool could lead to a slowing of motor unit firing rates: a decrease in excitatory input, an increase in inhibitory input, or a decrease in the responsiveness of the motoneurons through a change in their intrinsic properties (Davis and Bailey, 1997; Taylor and Gandevia, 2008). It is likely that all three of these actions occur during central fatigue.

The testing of motoneuron excitability during fatiguing maximal contractions has shown that the slowing of motor unit firing rates is not solely due to a decrease in excitatory input (Taylor and Gandevia, 2008). Both Butler et al. (2003) and Martin et al. (2006) observed decreases in the cervicomedullary motor evoked potential, the size of which reflects motoneuron excitability (Gandevia et al., 1999; Petersen et al., 2002), during

sustained MVCs of the elbow flexors, which suggested that the motoneurons had become less responsive to synaptic input. Indeed, other studies have demonstrated that repetitive activation may decrease the responsiveness of motoneurons to synaptic input (Spielmann et al., 1993; Peters and Fuglevand, 1999). Initially motoneurons fire repetitively, but over time some motoneurons slow their firing rate and others stop firing completely (Spielmann et al., 1993; Peters and Fuglevand, 1999). Evidence for this is limited in humans (Nordstrom et al., 2007), though Johnson et al. (2004) demonstrated that if humans are given feedback that allows them to voluntarily activate a single motor unit at a constant rate, the longer the unit is activated the more drive is required to maintain its firing frequency. This required increase in excitatory input is demonstrated through increased sEMG, indicating that other motor units have been recruited or are firing more, and providing strong evidence that repetitive activation makes motoneurons less responsive to synaptic input (Taylor and Gandevia, 2008).

Changes in inputs to motoneurons also occur during fatiguing contractions (Gandevia, 2001; Taylor and Gandevia, 2008). Muscle spindle afferents decrease their discharge and firing during sustained isometric contractions, and it has been argued that this will lead to a progressive disfacilitation of α-motoneurons (Macefield et al., 1991; Gandevia, 1998; Nordlund et al., 2004), which predominate within motor pools and innervate force-generating extrafusal muscle fibres (Burke et al., 1977). It has also been suggested that fatiguing contractions bring about a reflex inhibition of the α-motoneurons mediated by small diameter group III and IV afferents originating from the fatigued muscle (Bigland-Ritchie et al., 1986b; Garland, 1991; Garland and Kaufman, 1995; Gandevia, 1998). Small diameter muscle afferents are sensitive to noxious mechanical and chemical stimuli, so that some increase firing with the accumulation of metabolites in fatigued muscle (Rotto and Kaufman, 1998; Kaufman et al., 2002; Li and Sinoway, 2002). The action of fatigue sensitive afferents on motoneurons is contentious, though, with a recent study finding inhibition of elbow extensor motoneurons but facilitation of elbow flexor motoneurons (Martin et al., 2006).

It has also been demonstrated that descending drive from the motor cortex may become suboptimal for force production, and is of importance during both maximal (Gandevia et al., 1996; Taylor et al., 2000; Hunter et al., 2006) and submaximal contractions (Duchateau et al., 2002; Smith et al., 2007). This does not necessarily mean that

descending drive decreases during fatiguing contractions; it could remain the same or even increase, but may simply become less effective at driving the motoneurons, which have become less responsive to input (Taylor and Gandevia, 2008). Studies using transcranial magnetic stimulation of the motor cortex have found increases in the size of superimposed twitches, indicating that some motor cortical output remains un-utilised during maximal efforts (Löscher and Nordlund, 2002; Hunter et al., 2006). Thus, there is a failure at the supraspinal level to generate maximum motor cortical output, despite the fact that the on-going output is not sufficient to drive the motor units maximally (Taylor and Gandevia, 2008).

While much research has focused on the involvement of the motor pathways in central fatigue and the evidence for this seems plausible, it is not the only proposed central mechanism of fatigue. Many researchers advocate a role for brain neurotransmitters and central monoamines (Romanowski and Grabiec, 1974; Newsholme, 1986). It is proposed that changes in plasma amino acid concentrations could play a role in central fatigue by influencing the synthesis, concentration and release of neurotransmitters, such as serotonin and dopamine, in the brain (Newsholme, 1986; Blomstrand, 2001; Roelands and Meeusen, 2010). The effect of this would be to reduce motivation and drive to the motor cortex. Support for these hypotheses is, however, equivocal.

The principal neurotransmitter thought to be involved in central fatigue is serotonin (Newsholme, 1986), which is involved in the control of arousal, sleepiness and mood (Davis et al., 2000; Blomstrand, 2001; Meeusen et al., 2006). During exercise, the plasma concentration of free tryptophan, a precursor of serotonin in the brain, increases (Davis et al., 1992; Blomstrand et al., 1997). This is due to a release of fatty acids from adipose tissue, the increase in the concentration of which causes tryptophan to be displaced from its loose binding to albumin (Curzon et al., 1973; Davis et al., 2000). The displacement of tryptophan from albumin, combined with the uptake of branched-chain amino acids by muscle, results in a marked increase in the plasma ratio of free-tryptophan to branched-chain amino acids (Blomstrand et al., 1988; Blomstrand et al., 1997). As tryptophan can only cross the blood-brain barrier in its unbound or free form, an increase in the plasma ratio of free-tryptophan to branched chain amino acids results in the transport of tryptophan into the brain (Davis et al., 2000). The subsequent synthesis and release of serotonin may augment lethargy and loss of drive, resulting in a reduction in motor unit

recruitment (Newsholme, 1986; Blomstrand, 2001; Meeusen et al., 2006; Roelands and Meeusen, 2010).

Initial studies in rats provided some support for this serotonin hypothesis, demonstrating that exercise leads to an increase in the synthesis and metabolism of serotonin in the brain (Barchas and Freedman, 1963; Blomstrand et al., 1989). In humans, several subsequent studies in which brain serotonin activity was increased by agonists found that fatigue occurred sooner and ratings of perceived exertion were higher when compared with the administration of a placebo (Wilson and Maughan, 1992; Davis et al., 1993). However, other studies using similar agonists have since found no difference in performance compared to controls or a placebo (Meeusen et al., 2001; Parise et al., 2001; Roelands et al., 2009), and further studies using nutritional interventions designed to lower serotonin have shown no benefit on performance in either rats (Verger et al., 1994) or humans (Varnier et al., 1994; Blomstrand et al., 1995; Van Hall et al., 1995). Such results suggest that serotonin is, in fact, not a key factor in the development of central fatigue.

As brain function is dependent on the interaction of a number of systems, it is unlikely that a single neurotransmitter is responsible for central fatigue (Davis and Bailey, 1997; Meeusen et al., 2006). Thus, Davis and Bailey (1997) extended Newsholme's (1986) original serotonin hypothesis to include dopamine. Their revised hypothesis was that the ratios of serotonin and dopamine interact; a high ratio of serotonin to dopamine in the brain is associated with feelings of tiredness and lethargy, accelerating the onset of fatigue, whereas a low ratio is associated with improved performance, through the maintenance of motivation and arousal. However, Meeusen et al. (1997) found the administration of L-DOPA, a dopamine precursor, had no effect on submaximal time to fatigue; and Piacentini et al. (2004) found no difference in time-trial performance between administration of a dopamine reuptake inhibitor and a placebo. Evidence for a positive effect of increased dopamine has since been demonstrated in several studies, but only for exercise in the heat. Watson et al. (2005) and Roelands et al. (2008) both found dopamine reuptake inhibitors to significantly improve exercise performance, compared to a placebo, in 30 °C heat, but not in temperate conditions (18 °C). Importantly, the dopamine reuptake inhibitors enabled participants to maintain greater power with the same perception of effort during exercise in the heat. A further study by Tumilty et al. (2011) found a similar effect for tyrosine, a dopamine precursor, compared with a placebo for exercise in 30 °C

heat. Based on such findings, it could be argued that dopamine does play a role in central fatigue, perhaps through modulation of subjective ability to tolerate exercise, though only during exercise in the heat

It must be noted that changes in descending drive and changes in neurotransmitters are not mutually exclusive potential mechanisms of central fatigue. There is likely some interaction between the two. This is particularly apparent in the case of the centrally acting ergogenic aid caffeine, whose performance enhancing effect is believed to be as an antagonist of adenosine (Fredholm et al., 1999). Adenosine has several functions within the central nervous system that involve inhibition of excitatory neurotransmitters, and thus has the potential to alter α-motoneuron excitability and decrease the firing of central neurons (Fredholm et al., 1999). Caffeine has been demonstrated to increase spinal excitability, the net effect of membrane properties and the summation of all synaptic input to α-motoneurons (Walton et al., 2003; Kalmar et al., 2006).

Peripheral fatigue

Peripheral fatigue, as mentioned previously, refers to processes that occur at or distal to the neuromuscular junction (i.e. within the muscle itself), and that lead to a reduction in force production (Gandevia, 2001; Nordlund et al., 2004; Taylor and Gandevia, 2008). As with central fatigue, peripheral fatigue can be assessed through electrical stimulation (Behm et al., 1996; Gandevia, 2001), and is characterised by a reduction in torque in response to stimulation at rest following an MVC (Bigland-Ritchie et al., 1986a; Kent-Braun, 1999; Taylor and Gandevia, 2008; Place et al., 2007). This stimulation technique has been used to examine the development of peripheral fatigue after sustained contractions (Bigland-Ritchie et al., 1978) and during periods of relaxation in repetitive, intermittent contractions (Lloyd et al., 1991; Schillings et al., 2005).

Peripheral fatigue has been observed, to some extent, during intermittent and sustained submaximal contractions at low percentages (<35%) of MVC (Fuglevand et al., 1993b; Bilodeau et al., 2001). Søgaard et al. (2006) observed that peripheral fatigue progressively developed during a 43-minute 15% MVC elbow flexion, as evidenced by a gradual decrease in the MVCs interspersed throughout the contraction and in the decreased amplitude of the twitch evoked by stimulation of the resting muscle. Similarly,

Burnley et al. (2012) observed a progressive, slow development of peripheral fatigue during an hour of intermittent contractions of the quadriceps at 29% MVC, as evidenced by a gradual decrease in twitch amplitude following doublet stimulation between contractions. Of further note are the findings of Smith et al. (2007), who observed development of peripheral fatigue for a 70-minute elbow flexion at only 5% MVC. In these studies, peripheral fatigue developed alongside central fatigue, though its extent was not as substantial as that of central fatigue, which Smith et al. (2007) estimated to account for two-thirds of the decrement in maximal torque.

Peripheral fatigue is more evident and substantial during high-intensity submaximal contractions. Bigland-Ritchie et al. (1986a) observed that intermittent contractions at 50% MVC performed until the limit of endurance (i.e. task failure) resulted in a 50% decline in MVC force; a decline in parallel with the resting force decrement from electrical stimulation. They observed no central fatigue and attributed their findings purely to failure of the muscle contractile apparatus. It is likely, though, that this absence of central fatigue was due to use of single electrical stimuli superimposed on their MVCs. More recent studies, such as Burnley et al. (2012) which used paired stimuli, have clearly demonstrated a role for both peripheral fatigue and central fatigue in high-intensity submaximal contractions. Indeed, sustained (Yoon et al., 2007; Goodall et al., 2010) and intermittent (Eichelberger and Bilodeau, 2007; Burnley et al., 2012) contractions above 40% MVC have been found to induce both central and peripheral fatigue, with the greatest effect on the reduction in force and the greatest contribution to task failure in such cases coming from peripheral fatigue.

Similar results have also been found during sustained (Gandevia et al., 1996; Kent-Braun, 1999; Schillings et al., 2003; Hunter et al., 2006) and intermittent maximal contractions (Duchateau and Hainaut, 1985; Kawakami et al., 2000; Nordlund et al., 2004). Following five minutes of intermittent MVCs, Burnley (2009) reported a 71% decrease in voluntary torque and a 53% reduction in torque in response to stimulation at rest between contractions, indicating significant peripheral fatigue. Other studies have found similar substantial effects of peripheral fatigue, with Schillings et al. (2003) deducing that 89% of the voluntary force decline observed during a two-minute sustained MVC was due to peripheral fatigue, and that this peripheral contribution was most pronounced during the

first minute of contraction, with central factors starting to play more of a role during the second minute.

The difference between the predominance of peripheral fatigue for high-intensity contractions and central fatigue for low-intensity contractions has been partially attributed to perfusion and blood flow. In intact individuals, when force exceeds ~50% MVC, high intramuscular pressure restricts blood flow to the contracting muscle (Sadamoto et al., 1983) and muscle circulation can completely collapse (Barcroft and Millen, 1939). Consequently, continuous maximal contractions may occur in ischaemic muscle. Such ischaemia does not allow recovery of muscle fibres during contractions and this maximises the fatigue occurring within the muscle (Taylor et al., 2000). During low-intensity contractions, blood flow is less compromised, and the muscle fibres fatigue more slowly, which may allow changes within the central nervous system to play a larger role in the loss of force generating capacity (Taylor et al., 2000). More recent research has, however, suggested that circulation can be compromised and tissue reoxygenation stopped at only 25% MVC during contractions of the knee extensors (De Ruiter et al., 2007).

Potential mechanisms of peripheral fatigue

As with central fatigue, the exact mechanisms of peripheral fatigue are yet to be fully elucidated. The primary factors thought to be responsible for the decreased muscle function are metabolite accumulation (Allen et al., 1995a; Westerblad et al., 2002; Allen et al., 2008a) and energy (glycogen) depletion (Nielsen et al., 2011; Ørtenblad et al., 2013), both of which cause inhibition of the contractile process, excitation-contraction coupling failure and reduced excitability of neuromuscular transmission. The metabolites believed to be involved are H^+ and P_i, both of which increase in concentration (Westerblad et al., 2002); K^+, the interstitial concentration of which increases and the intracellular concentration of which decreases (McKenna et al., 2008); and Ca^{2+}, which can be affected by other metabolites and by glycogen depletion (Allen et al., 2008b; Ørtenblad et al., 2013).

The traditional explanation of peripheral fatigue has been lactic acid accumulation (Fitts, 1994; Westerblad et al., 2002). During intense exercise, the anaerobic breakdown of

glycogen leads to an intracellular accumulation of lactic acid, which dissociates to lactate and H^+ (Westerblad et al., 1998). The resulting acidosis, due to the increased [H^+], can cause a decrease in pH from its resting value to as low as 6.2 (Metzger and Fitts, 1987; Wilson et al., 1988; Cady et al., 1989a). It was thought that such pronounced acidosis had a detrimental effect on the rate of cross-bridge attachment (Westerblad and Allen, 1993) and reduced shortening velocity (Metzger and Moss, 1987; Chase and Kushmerick, 1988). Recent evidence, however, has suggested that the negative effects of increased [H^+] have been considerably overestimated and that it may actually have an ergogenic effect (Nielsen et al., 2001; Cairns, 2006; Lamb and Stephenson, 2006; Allen et al., 2008b).

Initial evidence supported the assertion that increased [H^+] was the cause of peripheral fatigue. Studies utilising high-intensity sustained and intermittent contractions found strong temporal correlations between the accumulation of H^+ and a decline in force production (Miller et al., 1988; Cady et al., 1989a; Kent-Braun, 1999). Furthermore, studies utilising skinned skeletal muscle fibres contracting under conditions mimicking the pH decrease observed during neuromuscular fatigue demonstrated that decreased pH caused a decrease in both P_o and V_{max} of comparable magnitude to that observed during neuromuscular fatigue in vivo (Metzger and Moss, 1987; Cooke et al., 1988; Pate and Cooke, 1989).

More recent evidence, however, has emerged to challenge the traditionally accepted depressing role of increased [H^+]. Firstly, in humans the temporal correlation between impaired contractile function and reduced pH is not always present (Westerblad et al., 2002; Allen et al., 2008a), and force often recovers more rapidly than pH after fatiguing contractions (Miller et al., 1988). Sahlin and Ren (1989) found that following exhaustive exercise at 66% MVC, two-minutes of recovery was sufficient for force to return to pre-test values but that pH still remained at a low level, similar to that at the end of exercise. Thus, it seems that the temporal correlation between decreased pH and decreased force may be coincidental, rather than causal. Secondly, it appears that there is a temperature dependence of the role of [H^+] in skinned fibre studies. Initial skinned fibre studies were conducted at low experimental temperatures (≤15 °C); significantly below the normal physiological range for mammalian muscle (Pate et al., 1995), due to the fact that the fibres are prone to damage at physiological temperatures (Lännergren and Westerblad,

1987). More recent studies have used "temperature-jump" protocols to enable mechanical measurements to be made on skinned fibres at temperatures better approximating physiological conditions, namely 30 °C (Pate et al., 1994). These studies, along with other studies on intact single fibres, have shown that at physiological temperatures acidosis has little effect on isometric tension or shortening velocity (Figure 2.8; Ranatunga, 1987; Adams et al., 1991; Pate et al., 1995; Westerblad et al., 1997).

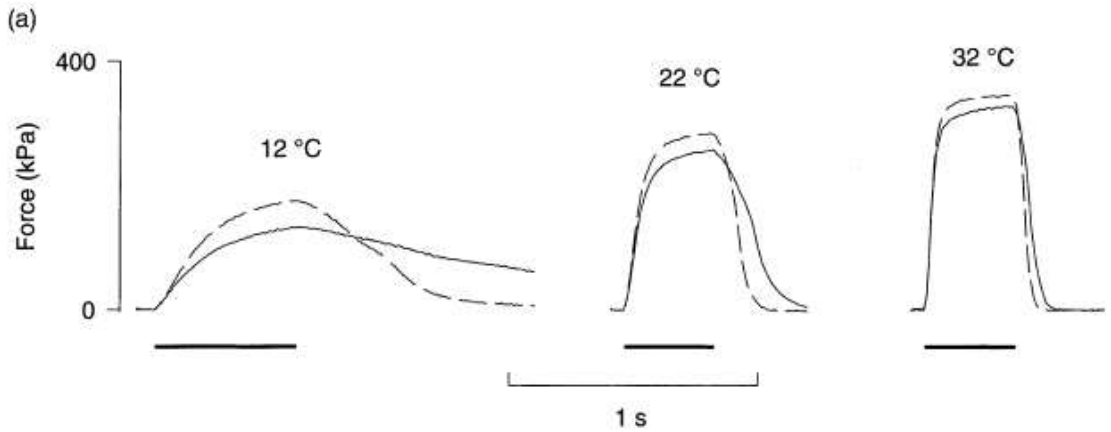

Figure 2.8. The effect of acidosis on contractile function becomes smaller as temperature is increased towards that found in muscle. Dashed lines indicate control conditions; solid lines indicate a decrease of approximately 0.5 pH units. From Westerblad et al. (1997).

The evidence presented above seems to discount increased [H^+] and the consequent acidosis as the cause of depressed cross-bridge function and decreased shortening velocity seen in neuromuscular fatigue. Indeed, further studies have demonstrated that acidosis may actually have a protective effect against fatigue. Bruton et al. (1998) observed that decreasing the pH from 7.1 to ~6.7 in single intact mouse muscle fibres seemingly slowed the onset of fatigue. It has also been suggested that increased intracellular acidity can help increase cytoplasmic [Ca^{2+}] and the consequent activation of the contractile process, due to the sarcoplasmic reticulum Ca^{2+} pump binding and resequestering less Ca^{2+} at an acidic pH (Lamb et al., 1992; Lamb and Stephenson, 2006). Decreased intracellular pH has also been shown to counter the inhibitory effect raised extracellular [K^+] has on action potentials (Nielsen et al., 2001).

With increased [H^+] unlikely to be the cause of the impaired muscle function seen with neuromuscular fatigue, attention has turned to other metabolites, with P_i, which accumulates as PCr is broken down, receiving increasing support (Allen and Westerblad, 2001; Westerblad et al., 2002). Studies in intact muscles have shown that the resting myofibrillar [P_i] is 1-5 mM (Kushmerick et al., 1992) and that this can rise to 30-40 mM

during intense contractions (Cady et al., 1989a). It was initially thought that increased [P_i] could act by hindering cross-bridge transition from low- to high-force binding states, meaning that as [P_i] increases fewer cross-bridges would be in a high-force state and consequently force would be reduced (Westerblad et al., 2002; Allen et al., 2008a). It has also become apparent that increased [P_i] could influence Ca^{2+} in several ways (Fryer et al., 1995; Balog et al., 2000; Westerblad and Allen, 2002).

In line with the hypothesis that fewer cross-bridges would be in high-force states as [P_i] increases, studies using skinned skeletal muscle preparations have shown that increased [P_i] depresses force at saturating [Ca^{2+}] (Cooke and Pate, 1985; Pate and Cooke, 1989; Millar and Homsher, 1990). As with the effects of increased [H^+], though, there appears to be a temperature dependence, with studies conducted nearer or at normal physiological temperatures showing that such negative effects of increased [P_i] are diminished (Dantzig et al., 1992; Coupland et al., 2001; Debold et al., 2004). Other studies using different models have, however, found evidence suggesting that [P_i] influences cross-bridge function. Several studies conducted on mouse soleus and fast-twitch Xenopus muscle fibres have found that reducing [P_i] results in an increase in cross-bridge force production (Phillips et al., 1993; Bruton et al., 1996; Bruton et al., 1997). Further support for the contention of an influence on cross-bridge function comes from studies on genetically modified mice completely lacking creatine kinase (CK; Dahlstedt et al., 2000; Dahlstedt et al., 2001), which found that such mice had elevated myoplasmic [P_i] and markedly lower force at rest than wild-type mice. It seems, therefore, that increased myoplasmic [P_i] does decrease force production through direct action on the cross-bridge cycle.

In recent years, it has become evident that increased [P_i] may also influence muscle function by acting on Ca^{2+}. Ca^{2+} plays a key role in skeletal muscle activation, with an increase in free myoplasmic [Ca^{2+}] being the trigger for contraction (Westerblad et al., 2000; Allen et al., 2008b). Increased [P_i] has been shown to decrease the maximal Ca^{2+}-activated force and decrease Ca^{2+} sensitivity, such that higher concentrations of Ca^{2+} are required to achieve the same level of contractile activation (Millar and Homsher, 1990; Martyn and Gordon, 1992). Interestingly, it has been shown that the inhibitory effect P_i has on myofibrillar Ca^{2+} sensitivity is greater at temperatures approaching physiological in vivo values than at lower temperatures (Debold et al., 2006). Increased [P_i] also slows sarcoplasmic reticulum Ca^{2+}-ATPase and may inhibit ATPase driven sarcoplasmic

reticulum Ca^{2+} reuptake (Dawson et al., 1978; Stienen et al., 1993; Duke and Steele, 2000). In the long term, this may substantially reduce the amount of Ca^{2+} available for release, resulting in reduced tetanic $[Ca^{2+}]_i$ (Westerblad et al., 2000).

The greatest effect increased $[P_i]$ is believed to have on Ca^{2+} is through entering the sarcoplasmic reticulum, resulting in calcium phosphate precipitation and decreasing the amount of Ca^{2+} available for release (Allen and Westerblad, 2001; Westerblad et al., 2002; Debold et al., 2004). This possibility was first raised by Fryer et al. (1995), who found exposure of a skinned fibre to a 50 mM concentration of myoplasmic P_i caused a sustained depression in sarcoplasmic reticulum Ca^{2+} release. This study provided the first evidence, albeit indirect, that P_i can reach a concentration in the sarcoplasmic reticulum high enough to exceed the threshold for calcium phosphate precipitation (Westerblad et al., 2002). Subsequent studies have extended this finding to skinned fibres with intact excitation-contraction coupling (Dutka et al., 2005) and to single fibres (Westerblad and Allen, 1996; Kabbara and Allen, 1999). Further support for this calcium phosphate mechanism comes from the finding that the decline in tetanic intracellular free $[Ca^{2+}]$ is delayed when P_i accumulation is prevented by inhibition of the CK reaction (Dahlstedt et al., 2000; Dahlstedt and Westerblad, 2001). It seems, therefore, that as well as its direct effect on cross-bridge kinetics, increased $[P_i]$ also has a negative influence on Ca^{2+} handling.

A further potential cause of peripheral fatigue is extracellular K^+ accumulation. During exercise, K^+ is released from the intracellular space to the extracellular space of contracting muscles and accumulates in the interstitium of contracting muscles (Fitts and Balog, 1996; Juel et al., 2000; Sejersted and Sjøgaard, 2000). Resting values for interstitial $[K^+]$ are typically around 4 mM (Green et al., 1999; Nordsborg et al., 2003) and can increase to 11-13 mM during intense exercise (Juel et al., 2000; Nordsborg et al., 2003). In vitro studies have shown that such concentrations are high enough to depolarise the resting membrane potential by ~10-15 mV, an amount sufficient enough to have a detrimental effect on action potential propagation and which can significantly depress force (Cairns et al., 1995; Fitts and Balog, 1996). However, some observations do not support the proposed role of increased $[K^+]$ in peripheral fatigue. Firstly, it has been demonstrated that lactic acid (which, as previously mentioned, accumulates during exercise) increases the $[K^+]$ at which force depression occurs by almost 2 mM (Pedersen

et al., 2003; Pedersen et al., 2004; De Paoli et al., 2007); and secondly, the interstitial [K^+] only reaches a critical level near the end of fatiguing exercise, especially in the presence of lactic acid, suggesting that most of the decrease in force may not be related to changes in interstitial [K^+] alone (McKenna et al., 2008). However, given the key role of K^+ in action potential propagation, it is nonetheless possible that changes in K^+, in conjunction with other metabolites, contribute to fatigue (McKenna et al., 2008).

The depletion of energy stores is another candidate mechanism for peripheral fatigue in both humans and other species (Pernow and Saltin, 1971; Lacombe et al., 2001). It has been known for more than half a century that our ability to exercise is seriously compromised when our glycogen stores are reduced to low levels, even when there is an abundance of other fuel sources (Ørtenblad et al., 2013). There is a close relationship between muscle glycogen content and endurance capacity, and an inability to continue exercise when glycogen stores are exhausted (Bergström et al., 1967; Hermansen et al., 1967). The most recognised theory concerning muscle glycogen levels and impaired contractile function is that glycogen is an essential substrate and its depletion reduces the rate at which adenosine triphosphate (ATP) can be regenerated (Ørtenblad et al., 2013). It is now known that muscle glycogen content may also affect excitation-contraction coupling and Ca^{2+} handling (Stephenson et al., 1999; Allen et al., 2008a).

Using rat and toad muscle fibres, it has been demonstrated that when recovery from glycogen depleting exercise occurs in the absence of glucose, glycogen does not recover and in a subsequent exercise bout fatigue occurs more rapidly and there is reduced tetanic intracellular free [Ca^{2+}] (Chin and Allen, 1997; Kabbara et al., 2000; Helander et al., 2002). In humans, Duhamel et al. (2006) observed that glycogen depleting exercise followed by four days of a low carbohydrate diet resulted in markedly reduced muscle glycogen at the initiation of a subsequent exercise bout and quicker deteriorations in sarcoplasmic reticulum Ca^{2+} release in comparison with a high carbohydrate diet between the exercise bouts. A similar study by Ørtenblad et al. (2011) demonstrated that providing carbohydrate during recovery from fatiguing exercise increased muscle glycogen content and normalised sarcoplasmic reticulum vesicle Ca^{2+} release, whereas both muscle glycogen and Ca^{2+} release remained low in the absence of carbohydrate. These data, obtained from studies on both single fibres and exercising humans, indicate that glycogen has a mechanistic role in sarcoplasmic reticulum Ca^{2+} release. It has been speculated that

depletion of muscle glycogen, and its subsequent effect on Ca^{2+}, may be the mechanism responsible for peripheral fatigue at low (<40% MVC) contraction intensities (Burnley et al., 2012).

Further studies using mechanically skinned muscle fibres, in which global ATP and other metabolites (such as PCr) can be kept high and constant, have shown that low glycogen content is associated with irreversible force depression during repeated tetanic contractions (Stephenson et al., 1999; Barnes et al., 2001). However, a more recent study by Nielsen et al. (2009), which used electron microscopy to provide an estimate of the subcellular localisation of glycogen, observed that endurance time (measured as the number of tetanic contractions required to induce a 50% reduction in force) correlated only with depletion of glycogen located within the myofibrils (i.e. intramyofibrillar glycogen). The results from these skinned fibre studies indicate that muscle glycogen content influences muscle contractile function under conditions of high and constant global myoplasmic ATP. The finding that glycogen affects excitation-contraction coupling despite constant global ATP argues against a direct metabolic effect of low glycogen levels at the whole cell level, though does not exclude a role of glycogen on compartmentalised energy transfer in the cell (Ørtenblad et al., 2013).

Interaction between peripheral and central fatigue

It has recently been proposed than an interaction between peripheral and central factors acts to regulate performance and restrict excessive development of peripheral fatigue (Amann and Dempsey, 2008; Amann, 2011). This hypothesis states that metabosensitive group III and IV muscle afferent fibres within the working muscle relate exercise-induced metabolic perturbations (i.e. peripheral fatigue) to the central nervous system (Kaufman et al., 2002; Light et al., 2008), which then adjusts central motor drive based on this inhibitory afferent feedback (Figure 2.9; Amann and Dempsey, 2008; Amann et al., 2009; Amann, 2011). It is proposed that such a feedback loop serves to limit voluntary drive (i.e. increases central fatigue) and restricts the development of peripheral fatigue beyond a critical threshold (Amann et al., 2006; Amann et al., 2013).

This hypothesis was based on numerous observations that the inability to continue exercise (i.e. exhaustion) often coincides with a specific and severe degree of peripheral

fatigue, regardless of the exercise protocol or rate of change of muscle metabolites (Hogan and Welch, 1984; Amann et al., 2006; Amann et al., 2007; Burnley et al., 2010). Based on this evidence it was postulated that a critical threshold for peripheral fatigue exists, beyond which an individual would never voluntarily perform high-intensity exercise (Amann, 2011). In order for an individual to never exceed this critical threshold, exercise is either voluntarily terminated once it is reached, or exercise intensity is reduced, via reducing central motor drive.

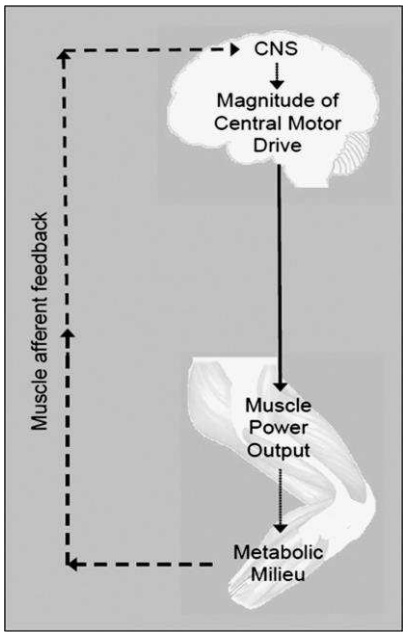

Figure 2.9. Schematic representation of the interaction between peripheral and central fatigue. From Amann and Dempsey (2009).

The first experimental test of this hypothesis was by Amann and Dempsey (2008). They compared a 5k cycling time-trial performed in a fresh state with time-trials performed with pre-existing moderate and severe levels of peripheral fatigue, brought about by prior cycling to exhaustion at 67 and 83% of peak power, respectively, and assessed via significant decreases in the quadriceps potentiated twitch response. It was observed that pre-existing fatigue had a significant negative effect on performance and a substantial dose dependent inverse effect on power output and central motor drive, as estimated via quadriceps EMG. Specifically, it was observed that the higher the level of pre-existing peripheral fatigue, the lower the average central motor drive was throughout the time-trial. Furthermore, the level of peripheral fatigue at the end of each time-trial was identical, despite the marked differences in the levels at the start and the marked differences in exercise performance. Though providing support for the hypothesis, a

limitation of the study was that the pre-fatiguing exercise may have elicited central fatigue through non-peripheral mechanisms (Nybo and Secher, 2004). Subsequent studies have overcome this limitation of using pre-fatiguing voluntary exercise by using electrical stimulation to induce peripheral fatigue; finding lower EMG during pre-fatigued trials and identical levels of peripheral fatigue between conditions at the end of exercise (Gagnon et al., 2009; Amann et al., 2013; Hureau et al., 2014).

More compelling evidence for the hypothesis comes from a recent study in which the central projection of lower limb afferents was pharmacologically blocked. Amann et al. (2009) accomplished this through the intrathecal injection of fentanyl, an opioid analgesic, to selectively block the projection of ascending afferent pathways without affecting motor nerve activity or maximal force output. Compared to a placebo condition, central motor drive, as estimated via EMG, was significantly higher throughout the first half of the time trial, leading to a significantly higher power output. Following completion of the time-trials, peripheral fatigue was ~44% greater in the fentanyl trial compared to the placebo trial, and was so severe that participants reported ambulatory problems for several hours afterwards. These findings indicated that in the absence of afferent feedback, caused by the fentanyl injection, the magnitude of central motor drive was uncoupled from the metabolic milieu of the working muscles, and the central nervous system thus did not act to limit the development of peripheral fatigue.

Peripheral fatigue and the associated afferent feedback to the central nervous system is one of only several potential mechanisms influencing central motor drive and exercise performance, with other potential mechanisms including changes in neurotransmitter activity, cerebral heat accumulation and inadequate substrate availability (Nybo and Secher, 2004; Amann, 2011). The evidence above does, though, appear to suggest that afferent feedback from working muscles may limit performance by exerting an inhibitory effect on central motor output. This has led to the suggestion that peripheral fatigue is a tightly regulated variable during exercise (Hureau et al., 2014).

2.4 – Critical Power

One of the most enduring relationships across the history of physical endeavour is that between power and time. Across millennia, the performance of soldiers, labourers, recreational and elite athletes has been constrained by this power-time relationship (Whipp et al., 1998; Jones et al., 2010). It is now appreciated that this power-time relationship has strong mathematical and physiological foundations (Hill and Lupton, 1923; Monod and Scherrer, 1965; Moritani et al., 1981; Jones et al., 2010).

From a historical perspective, scientific investigation to mathematically model the relationship between power and time to exhaustion began in the 1920s with the work of the celebrated English physiologist A.V. Hill. Initial observations by Hill and Lupton (1923) demonstrated a strong relationship between exercise intensity and the tolerable duration of exercise. Hill (1925) went on to construct velocity-time curves from world records for runners, swimmers, rowers and cyclists, and it is noteworthy that current world records also fit velocity-time curves similar to those Hill constructed.

Some years later, Monod and Scherrer (1965) observed that when a series of fatiguing constant work rate isometric contractions were performed to the limit of tolerance, a hyperbolic relationship emerged (Equation 1; Figure 2.10A). They defined two key parameters: the critical power (CP), which is the power asymptote of the hyperbolic relationship; and what has come to be known as W', the curvature constant of the relationship. Knowing that the total work performed is given by the product of power and time, this relationship can also be expressed in a linear form (Equation 2; Figure 2.10B); where the slope is the CP and the intercept is W' (Morton, 2006). The equations are:

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

$$W = W' + CP \cdot T_{lim} \qquad \text{Equation 2}$$

where T_{lim} is the time to exhaustion, W' is the curvature constant, P is the power output of the task, CP is the asymptote referred to as the “critical power,” and W is the work performed. Mathematically the two equations are equivalent.

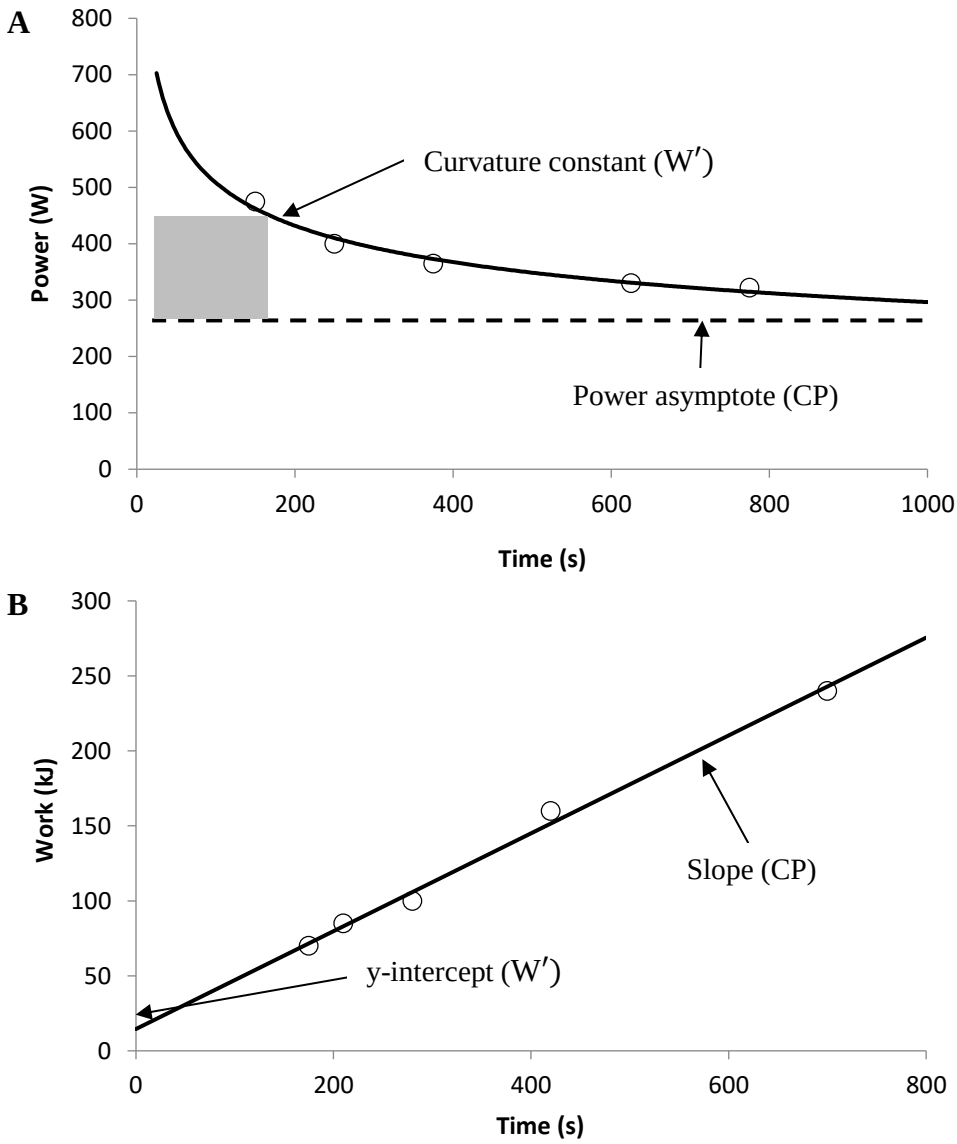

Figure 2.10. The hyperbolic power-time model of critical power (A), and the linear work-time model of critical power (B). Adapted from Jones et al., 2010.

Monod and Scherrer (1965) initially defined the CP, somewhat vaguely, as the "maximum rate of work that a muscle or muscle group can keep up for a very long time without fatigue". It was later suggested that the CP may equate to the highest sustainable rate of metabolism that occurs at a similar work rate to the maximal lactate steady state (Poole et al., 1988); though the CP has subsequently been found to be significantly higher than the maximal lactate steady state (Pringle and Jones, 2002; Dekerle et al., 2003). The CP is now defined as the asymptote of the relationship between power output and time to task failure (Jones et al., 2010). Monod and Scherrer (1965) considered the CP to be partly dependent on muscle blood flow, and thus oxygen delivery, based on their observation of improved performance during intermittent compared with continuous contractions. It is now known that the CP is determined unequivocally by oxidative function (Moritani et

al., 1981; Jones et al., 2008; Vanhatalo et al., 2010). W' is representative of a finite amount of work that can be performed above the CP, so that the magnitude of this work capacity remains the same regardless of the work rate (Moritani et al., 1981; Poole et al., 1988; Jones et al., 2010). The precise determinants of W' are unclear, though it appears to be related to the breakdown of PCr and glycogen during exercise, the accumulation of metabolites (such as H^+ and P_i), and a fall in pH (Fitts, 1994; Miura et al., 1999; Miura et al., 2000; Jones et al., 2008). It has also been postulated that W' is intrinsically related to the $\dot{V}O_2$ slow component during whole-body exercise in which $\dot{V}O_{2max}$ constrains the increase in $\dot{V}O_2$ (Murgatroyd et al., 2011; Vanhatalo et al., 2011).

Following on from Monod and Scherrer's initial modelling, which was based on isometric contractions, Moritani et al. (1981) extended the CP concept to large muscle mass exercise capable of fully taxing the cardiorespiratory system. The power-time relationship, and the parameters of CP and W', have subsequently been found to describe exercise tolerance across a wide range of species, including horses (Lauderdale and Hinchcliff, 1999) and mice (Billat et al., 2005). In humans, the concept has been proved to be robust and to describe exercise tolerance for different types and intensities of muscle contraction protocols (e.g. isometric and isotonic; Monod and Scherrer, 1965; Jones et al., 2008; Burnley et al., 2012), and for a range of exercise modalities, including cycling (Moritani et al., 1981; Hill, 2004), rowing (Kennedy and Bell, 2000; Hill et al., 2003), running (Hughson et al., 1984; Fukuba and Whipp, 1999), swimming (Wakayoshi et al., 1992) and even wheelchair propulsion (Arabi et al., 1999).

Research has also demonstrated that both CP and W' can be manipulated and are sensitive to interventions designed to increase or decrease exercise tolerance. The CP can be increased by continuous endurance training (Gaesser and Wilson, 1988; Jenkins and Quigley, 1992), high-intensity interval training (Poole et al., 1990; Vanhatalo et al., 2008) and inspiration of hyperoxic gas (Vanhatalo et al., 2010); and can be decreased by inspiration of hypoxic gas (Moritani et al., 1981). W' can be increased by high-intensity strength and sprint training (Jenkins and Quigley, 1993) and creatine supplementation (Smith et al., 1998; Miura et al., 1999); and can be decreased by glycogen depletion (Miura et al., 2000) and prior high-intensity exercise (Heubert et al., 2005; Vanhatalo and Jones, 2009). Interestingly, it appears that W' tends to decrease as a result of interventions that serve to increase the CP (Jenkins and Quigley, 1992; Vanhatalo et al., 2008).

Measurement of critical power

Traditionally, determination of CP first involved a $\dot{V}O_{2max}$ test, and then between three and seven trials, at intensities between 50 and 110% $\dot{V}O_{2max}$, designed to elicit a range of exhaustion times between 2 and 15 minutes (Gaesser and Wilson, 1988; Pringle and Jones, 2002; Hill, 2004; Vanhatalo et al., 2007). For determination of critical torque (CT), typically five trials to exhaustion at intensities between 35 and 60% MVC have been used (Burnley, 2009; Burnley et al., 2012). These methods of establishment necessitate repeated laboratory visits, and have arguably limited the use of the CP/CT concept for diagnostic and research purposes (Vanhatalo et al., 2007; Jones et al., 2010). In recent years, the use of prolonged “all-out” exercise to determine the CP and W' in a single session has been investigated.

Burnley et al. (2007) demonstrated that a distinct plateau in power output, at approximately one-third of peak power, can be attained within three minutes of all-out cycling against a fixed resistance. This power was subsequently shown to be synonymous with the CP determined from the traditional method of multiple tests to exhaustion (Figure 2.11; Vanhatalo et al., 2007). Similar agreement between end-test muscle performance and the CT has also been found for isometric contractions. Burnley (2009) demonstrated that five-minutes of intermittent maximal contractions of the quadriceps were required to achieve a plateau in torque; with this plateau found to be not significantly different from the traditionally obtained CT. This experiment provided an analogue to the three-minute all-out cycling test and supports the contention that all-out exercise leads to the eventual attainment of critical power, regardless of the modality of exercise (Jones et al., 2010). The five-minute test derived in this study also provides a useful maximal protocol for the assessment of global, central and peripheral fatigue during intermittent contractions.

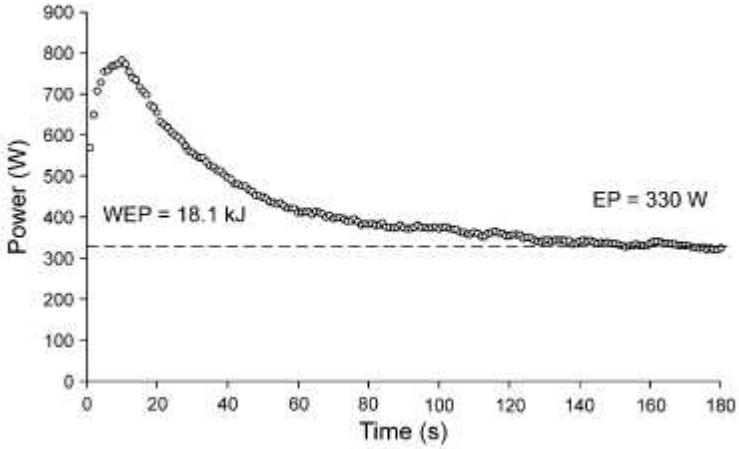

Figure 2.11. Determination of CP using a three-minute all-out test. Dashed line indicates independently calculated CP. From Vanhatalo et al. (2007).

Exercise below, at and above the critical power

Theoretically, exercise performed below the CP should be fatigueless and task failure should not occur (Moritani et al., 1981), though in reality, such sustained exercise would eventually become limited by factors such as substrate depletion, hyperthermia and motivation (Poole et al., 1988; Jones et al., 2008). In contrast, exercise performed above the CP requires utilisation of W' at a rate dependent on exercise intensity until it is expended, at which point exercise cannot be continued at the same intensity (Jones et al., 2008; Burnley, 2009). As work rate is increased further above the CP, W' will be expended more rapidly (Monod and Scherrer, 1965; Jones et al., 2008), the rate of accumulation of metabolites associated with neuromuscular fatigue will be greater (Fitts, 1994; Westerblad and Allen, 2003) and task failure will occur at a sooner and predictable time (Monod and Scherrer, 1965; Burnley, 2009).

Given the supposedly limitless potential for sustained exercise below the CP, compared with the gradual progression towards task failure above the CP, it would seem reasonable to suggest that the physiological responses to exercise below and above the CP will differ. Poole et al. (1988) were the first to investigate whether this was the case, comparing running at the CP (~80% $\dot{V}O_{2max}$) with running 5% above the CP. At CP, $\dot{V}O_2$ demonstrated a pronounced slow component rise before levelling off after several minutes, while blood [lactate] increased from resting values of ~1 mM to stabilise at 5-6 mM. In contrast, at 5% above CP, $\dot{V}O_2$ rose inexorably to $\dot{V}O_{2max}$ and blood [lactate]

continued to rise until the subject was no longer able to continue the task, a point which occurred earlier than during running at the CP. Based on these results, it can be concluded that the CP demarcates the upper boundary of the heavy intensity exercise domain and the lower boundary of the severe intensity exercise domain, the domain in which all power outputs lead to $\dot{V}O_{2max}$; and that it also represents the highest metabolic rate at which $\dot{V}O_2$ and blood [lactate] can be stabilised.

Further differences between exercise below and above the CP were noted by Jones et al. (2008), who used ^{31}phosphorus magnetic resonance spectroscopy to examine metabolic responses in the quadriceps during single-leg knee extensions. During exercise at 10% below CT, stable values for [PCr], [P_i] and pH were attained after three minutes of a 20-minute exercise bout. However, during exercise at 10% above CT, [PCr] and pH continued to fall and [P_i] continued to rise throughout exercise (which was terminated earlier than in the trial below CT), reaching values typically observed at the termination of high-intensity exhaustive exercise. This study provided evidence that regulators of oxidative metabolism exhibit the same intensity domain specific behaviours near the CP as the systemic responses previously established by Poole et al. (1988), and, along with several other studies (Burnley et al., 2010; Vanhatalo et al., 2010), evidence that it is not possible to attain a metabolic steady state during exercise above the CP. Additionally, the metabolic perturbations and loss of muscle homeostasis observed (i.e. increased [P_i] and decreased pH) with exercise above the CP suggested a link with peripheral fatigue (Westerblad et al., 2002).

Burnley et al. (2012) cemented a link between exercise above the CP/CT and peripheral fatigue, concluding that rather than representing a threshold for fatigueless and fatiguing exercise intensities, the CP/CT actually represents a critical threshold for peripheral fatigue development. Using intermittent isometric quadriceps contractions, they found differences in the development of central and peripheral fatigue below and above the CT. Contractions below the CT led to a modest decrease in MVC and a modest increase in EMG, though at the end of exercise there was still a significant reserve in both MVC torque and EMG amplitude. In contrast, contractions above the CT led to a progressive fall in MVC torque until task failure, at which point a maximal contraction was required to attain the target force, and a progressive rise in EMG to produce a value at task failure not different from that produced during a fresh MVC. Decreases in voluntary activation

and the potentiated doublet were present in both the trials below and above CT, indicating the presence of both central and peripheral fatigue for all intensities of contraction. However, it was observed that the rates of change for MVC torque and potentiated doublet torque were disproportionately large above compared to below the CT, indicating that peripheral fatigue is greater and its rate of development quicker during contractions above the CT. The implications of this are that the rate of fatigue development below the CT could not be predicted from the same indexes as above the CT, and that fatigue development increases suddenly when the CT is exceeded and does not increase proportionately as torque requirements increase.

Part II – Physiological complexity

2.5 – Non-linear dynamics and complexity

The field of non-linear dynamics is typically closely associated with physics, mathematics and chaos theory. In contrast to linear systems, which are simple, proportionate and can be considered as the sum of their parts (Goldberger, 1996; Goldberger, 2006), non-linear systems are characterised by a lack of proportionality, with small changes having striking, unpredictable effects, thus limiting the ability to predict their long-term behaviour (Goldberger, 1996; Peng et al., 2009). Non-linear systems are regarded as complex, chaotic and unpredictable; attributes that make of great interest to scientists. Over the last quarter of a century, this interest has spread from physics and mathematics to medicine and physiology, and to the study of one of the most complex dynamical systems in all of nature: the human body.

Physiological systems are inherently complex (Goldberger, 1996; Peng et al., 2009), both in their structure and function, as well as spatially and temporally (Goldberger, 1996; Eke et al., 2002; Seely and Macklem, 2004; Manor et al., 2010). The outputs generated by complex physiological systems are non-stationary (i.e. they are not stable) and non-linear (i.e. they are not directly proportional to their input; Goldberger, 1996; Eke et al., 2002; Goldberger et al., 2002); in other words, the outputs of physiological systems exhibit constant fluctuations, even under resting conditions (Goldberger et al., 1990; Lipsitz, 2002). In recent years, it has come to be recognised that such fluctuations in physiological outputs are not noise, as was initially thought (Cannon, 1929). Instead, these fluctuations contain meaningful structural richness, that is to say information, over multiple temporal and spatial scales (Lipsitz and Goldberger, 1992; Costa et al., 2002; Goldberger et al., 2002; Manor et al., 2010). The complexity of these physiological outputs exceeds that of the most challenging systems in the physical world and defies understanding based on traditional mechanistic and reductionist models (Peng et al., 2009). As such, physiologists have turned to the fields of non-linear dynamics and statistical physics in the search of new concepts and metrics to help explain and quantify this complexity. One of the most useful and frequently applied of these concepts is fractals (Mandelbrot, 1977; Eke et al., 2002; Lipsitz, 2004).

Fractals

The concept of fractals is rooted in, and most frequently associated with, mathematics and geometry. In the late 19th and early 20th century, mathematicians would generate complex geometric structures from simple objects (the initiator), such as straight lines, triangles or cubes, by applying a simple rule of transformation (the generator) in an infinite number of iterative steps (Eke et al., 2002). The resulting complex structures, examples of which include the von-Koch curve, Sierpinski triangle and Menger sponge (Figure 2.12; Peitgen et al., 1992; Bassingthwaighte et al., 1994), all display a self-similarity; that is, they maintain their characteristic form regardless of the scale used to examine them (Glenny et al., 1991; Eke et al., 2002).

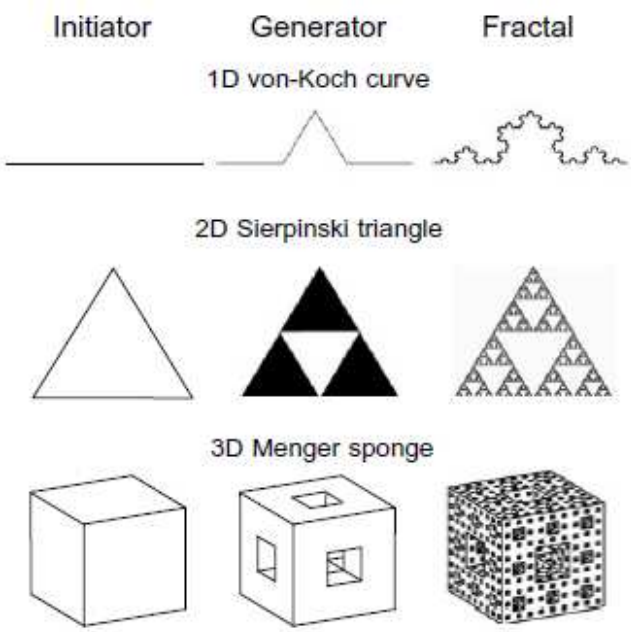

Figure 2.12. Examples of geometric fractals. The von-Koch curve, Sierpinski triangle and Menger sponge are all generated by repetitively applying a single rule of transformation, the generator, to a simple object, the initiator, in an infinite number of iterative steps. From Eke et al., 2002.

These geometric structures lay dormant until the late 20th century, when the Polish-born mathematician Benoit Mandelbrot realised that they represented a suitable geometry to describe the complex shapes and forms of nature (Mandelbrot, 1983; Eke et al., 2002). Mandelbrot (1977) coined the term “fractal” from the Latin fractus, the past participle of the verb frangere, “to break,” in order to describe the ever-finer fragments that appear as objects and processes are viewed at ever higher magnifications and to emphasise the fragmented character of the objects (Bassingthwaighte, 1988; Bassingthwaighte et al.,

1994; Eke et al., 2002). He went on to define fractals as geometric objects with self-similarity on multiple measurement scales (Mandelbrot, 1983); a definition Goldberger (1996) later simplified to objects composed of sub-units (and sub-sub-units, etc.) that resemble the structure of the overall object.

Fractals have subsequently come to be defined by a set of four characteristic properties: self-similarity, scaling, the fractal dimension and statistical properties (Bassingthwaighte et al., 1994; Liebovitch, 1998; Eke et al., 2002):

1. The self-similarity fractals exhibit can either be geometric or statistical. Geometrically self-similar objects are those whose pieces are smaller, exact replicas of the whole object (Mandelbrot, 1983; Eke et al., 2002), such as those illustrated in Figure 2.12. Statistically self-similar objects are "kind of like" the whole; the statistical properties of the pieces are proportional to the statistical properties of the whole (Bassingthwaighte et al., 1994).
2. Because of self-similarity, features at one resolution are related to features at other resolutions. Scaling refers to how the measured values depend on the resolution used to make the measurement (Bassingthwaighte et al., 1994; Liebovitch, 1998); thus, the length measured at finer resolutions will be longer as it contains finer features. Self-similarity determines the scaling relationship and can lead to power-law scaling (Bassingthwaighte et al., 1994; Eke et al., 2002).
3. The fractal dimension gives a quantitative assessment of self-similarity and scaling, describing how many new pieces similar to the whole object are revealed as the resolution is made finer (Bassingthwaighte et al., 1994; Liebovitch, 1998).
4. The statistical properties of fractals include the fact that fractal processes may not have a mean or variance. For the mean, as more data are analysed, rather than converging to a single value, the mean continues to increase towards an ever-larger value or decreases to an ever-smaller value (Bassingthwaighte et al., 1994; Liebovitch, 1998). For the variance, self-similarity means that small irregularities are reproduced as larger irregularities at larger scales, thus as more data are analysed these larger irregularities increase the variance, which then becomes infinite (Bassingthwaighte et al., 1994; Liebovitch, 1998).

Complexity and fractals in nature and physiology

Mandelbrot (1967; 1977; 1983) was the first to realise that fractals could not only be used to describe complex geometric structures, but also the complex shapes and forms of nature. The classic example he proposed is that of a coastline (Mandelbrot, 1967). If the length of a coastline is measured using a low-resolution photograph, a unique value will be obtained. However, if the image is magnified, new details, such as bays, inlets and peninsulas not previously seen, are exposed and accurate measurement now requires a smaller scale, and the length of the measurement becomes greater (Mandelbrot, 1967; Gitter and Czerniecki, 1995). Each magnification imposes greater detail, meaning the process of measurement can be carried out indefinitely, as the characteristic wrinkles and wiggles of the coastline are never resolved, no matter the magnification or scaling (Bassingthwaighte et al., 1994; Gitter and Czerniecki, 1995; Liebovitch, 1998). Other examples of fractal forms found in nature include branching trees, billowing clouds, coral reefs and the Roman cauliflower (Mandelbrot, 1983; Sugihara and May, 1990; Glenny et al., 1991; Aon et al., 2000; Goldberger et al., 2002; Hardstone et al., 2012).

From a physiological point of view, many complex anatomic structures exhibit fractal-like geometry (Bassingthwaighte et al., 1994; Goldberger, 1996; Liebovitch, 1998). Such structures are fractal if their small-scale form appears similar to their large scale form (Goldberger and West, 1987a; Glenny et al., 1991; Bassingthwaighte et al., 1994); though unlike the geometric fractals developed by mathematicians, which can also be termed exact fractals, these anatomic structures are statistical fractals, with their self-similarity manifested in the power-law scaling of the parameters characterising their structures at different scales of observation (Liebovitch, 1998; Eke et al., 2002).

The most studied fractal-like structure in the body is the bronchial tree (Figure 2.13), the generation of which can be viewed as an iterative process, much like that of the generation of a mathematical fractal such as the von-Koch curve (Lefèvre, 1983; Mandelbrot, 1983; West et al., 1986; Nelson and Manchester, 1988; Goldberger et al., 1990; Nelson et al., 1990; Glenny et al., 1991). The trachea initially undergoes a bifurcation to form the left and right bronchi, and subsequent generations are formed by a repetitive bifurcation of the most distal airways (Lefèvre, 1983). Other examples of fractal-like anatomic structures in the body are the arterial and venous trees (Goldberger et al., 1985; West and

Goldberger, 1987; Bassingthwaighte et al., 1994), the arteries and veins in the retina (Family et al., 1989; Mainster, 1990; Masters, 2004), the His-Purkinje conduction system (Goldberger et al., 1985; Goldberger and West, 1987a), and the dendrites in the nervous system (Smith et al., 1989; Caserta et al., 1990). These self-similar anatomic structures appear to subserve several common fundamental physiological functions: they enable rapid and efficient dissemination across complex, spatially distributed systems (Weibel, 1991; Goldberger, 1996; Goldberger et al., 2002), they minimise the energy required for system function (Murray, 1926; Glenny, 2011); and they minimise construction error, since they rely on an iterative mechanism (Goldberger and West, 1987a; Goldberger and West, 1987b).

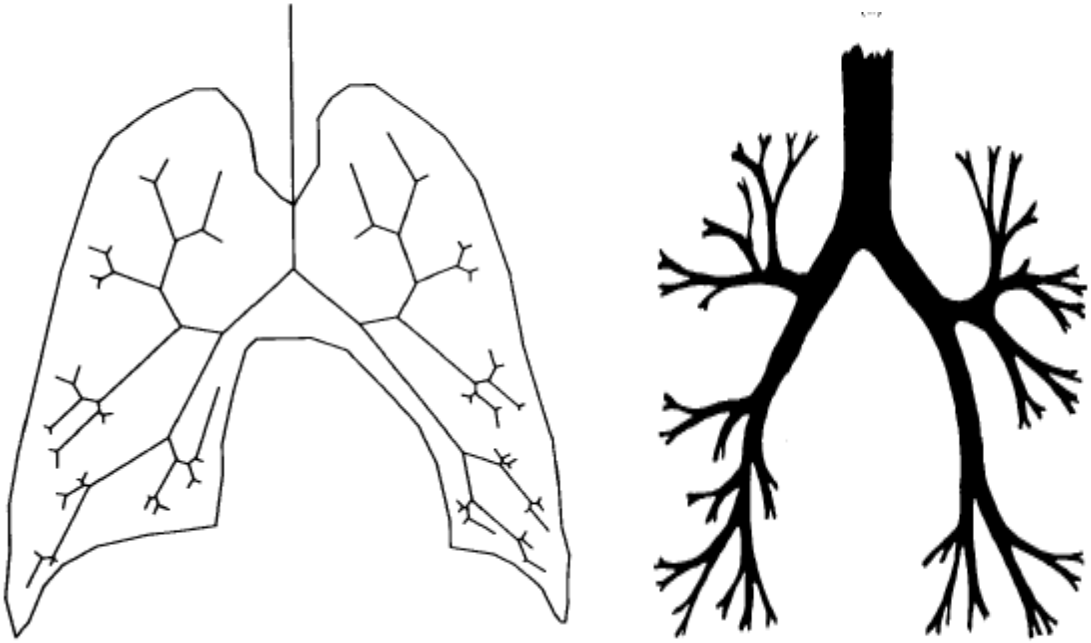

Figure 2.13. A simulation of the bronchial tree based on repeated applications of a branching rule (left), and a silhouette of the airways from a chest X-ray (right). Note the good agreement of the simulation with the actual structure. From Nelson and Manchester, 1988.

The fractal concept can be applied not only to anatomic structures, but also to various physiological processes (Goldberger, 1996; Goldberger et al., 2002). A process is fractal if a variable, as a function of time, undergoes characteristic changes that are similar regardless of the time interval over which the observations are made (Glenny et al., 1991). Fractal-like processes are thus said to generate irregular fluctuations across multiple time-scales (Goldberger, 1996; Goldberger et al., 2002). Heart rate is a good example of a fractal-like process (Figure 2.14; Yamamoto and Hughson, 1994; Peng et al., 1995a); with healthy heart rate traces exhibiting irregularity on different time scales that is not

visually distinguishable, but is statistically self-similar (Goldberger, 1996; Goldberger et al., 2002). Other fractal-like physiological processes include blood pressure (Marsh et al., 1990; Wagner and Persson, 1994), ion channel kinetics (Liebovitch et al., 1987; Liebovitch and Tóth, 1990), respiration (Bruce, 1996; Frey et al., 1998), renal blood flow (Wagner and Persson, 1995), gait (Hausdorff et al., 1995; Delignières and Torre, 2009) and muscle force output (Slifkin and Newell, 1999; Svendsen and Madeleine, 2010).

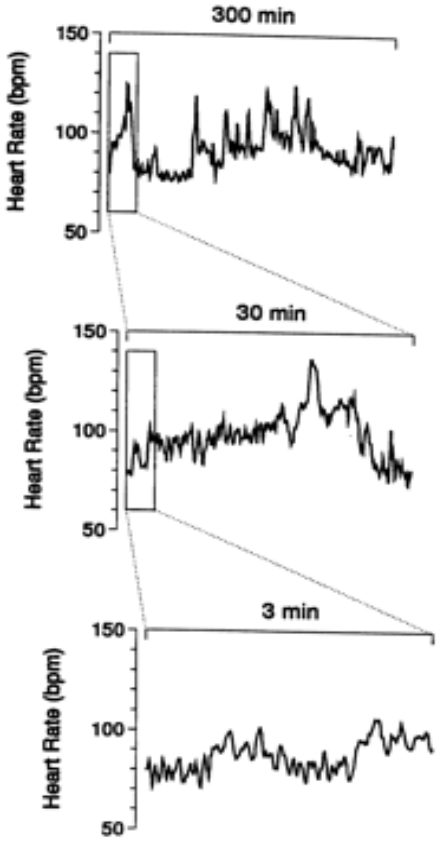

Figure 2.14. A fractal-like process, such as heart rate, generates fluctuations on different time scales that are statistically self-similar. From Goldberger, 1996.

2.6 – Measuring complexity and fractals

An important methodological challenge is how to measure the complexity and irregularity in the physiological processes exhibiting fractal-like properties. Typically, fluctuations in physiological time series are quantified in terms of their magnitude, using measures such as the standard deviation (SD) and coefficient of variation (CV; Pincus and Goldberger, 1994; Jones et al., 2002; Rose et al., 2009). However, the complex dynamics of

physiological outputs are characterised by a number of properties that measures of the magnitude of variability cannot quantify; namely temporal irregularity, time irreversibility and long-range (fractal) correlations (Pincus, 1994; Goldberger et al., 2002). In other words, physiological outputs contain meaningful structural information, with the relationship between successive data points holding the key to understanding this structure (Grassberger, 1991; Lipsitz and Goldberger, 1992). Measures such as the SD do not account for data order and simply provide an index of the degree of deviation from a point in a distribution of scores (Bland and Altman, 1996; Slifkin and Newell, 1999), and are thus unable to provide any information on an output's temporal structure (Slifkin and Newell, 1999; Rose et al., 2009). As such, new statistical measures, based around the concepts of entropy and fractals, have been developed to provide information about the complexity and fractal-like properties exhibited by physiological processes. These measures characterise the moment-to-moment relationship between successive points in a data series (Slifin and Newell, 1999), providing information on the underlying dynamical state of the system (Sosnoff et al., 2009) and its deterministic and stochastic components (Slifkin and Newell, 1999).

Approximate entropy

Entropy, as embodied in the second law of thermodynamics, is a measure of disorder or randomness that tends towards a maximum in an isolated system (Schneider and Kay, 1994; Seely and Macklem, 2004). As it relates to dynamical systems, entropy can be thought of as the rate of information production (Eckmann and Ruelle, 1985; Richman and Moorman, 2000; Seely and Macklem, 2004) and can be used to quantify the apparent randomness and regularity, i.e. the complexity, of a system (Pincus, 1991; Seely and Macklem, 2004).

Approximate entropy (ApEn) derives from the Kolmogorov-Sinai entropy in an information theory sense, and was developed as a model-independent quantification of the regularity of sequences and time-series data, motivated by applications to relatively short, noisy data sets (Pincus 1991; Pincus, 1995; Pincus, 2006; Forrest et al., 2014). It provides an index of predictability of future values, i.e. long-range fractal correlations, in a time-series based on past events (Pincus, 1991). To understand ApEn and why it was developed, consider the following example, adapted from Pincus and Keefe (1992):

There are two time-series. Series one is 1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2, and so on. This time-series alternates between 1 and 2 in sequence. Series two is 1, 2, 2, 1, 2, 1, 2, 2, 1, 1, 2, 1, 2, 2, 1, 1, and so on. In this series, each term is either 1 or 2, but randomly chosen with the probability of ½ of either value. Traditional moment statistics, such as the mean and variance, and rank order statistics, such as the median, are unable to distinguish between the two series. However, series one is "perfectly regular"; knowing that one term is 1 allows one to predict with certainty that the next term will be 2. Series two, on the other hand, is randomly valued and irregular; knowing that one term is 1 gives no indication as to whether the next term will be 1 or 2. ApEn allows one to distinguish between such clearly different time-series and also allows the determination of subtler differences in regularity.

In order to compute ApEn, two parameters must be specified: a run length 'm', which is the length of the sequences to be compared, and a tolerance window 'r', which is the tolerance for accepting matches (Pincus, 1991; Richman and Moorman, 2000; Pincus, 2006). ApEn is approximately equal to the negative average natural logarithm of the conditional probability that two sequences of length N that are similar (within r) for m observations remain similar (within the same tolerance width r) for m + 1 observations (Pincus, 1991; Richman and Moorman, 2000; Pincus, 2006; Rose et al., 2009). The exact calculation of ApEn is given in detail in the General Methods (Chapter 3).

ApEn quantifies a continuum, ranging from totally ordered to completely random, assigning a non-negative number between 0 and 2 reflecting the complexity and regularity of the sequence (Seely and Macklem, 2004; Pincus, 2006). Low values, approaching 0, correspond to high system regularity and low complexity (i.e. sinusoidal behaviour); while high values, approaching 2, correspond to low system regularity and high complexity (i.e. white Gaussian noise; Figure 2.15; Pincus, 1991; Pincus, 2006; Rose et al., 2009; Sosnoff et al., 2009). Increases in ApEn reflect greater likelihood that values similar for m observations are not similar for m + 1 observations, and thus reflect an increase in a signal's time domain complexity (Pincus, 1991; Pincus, 1995).

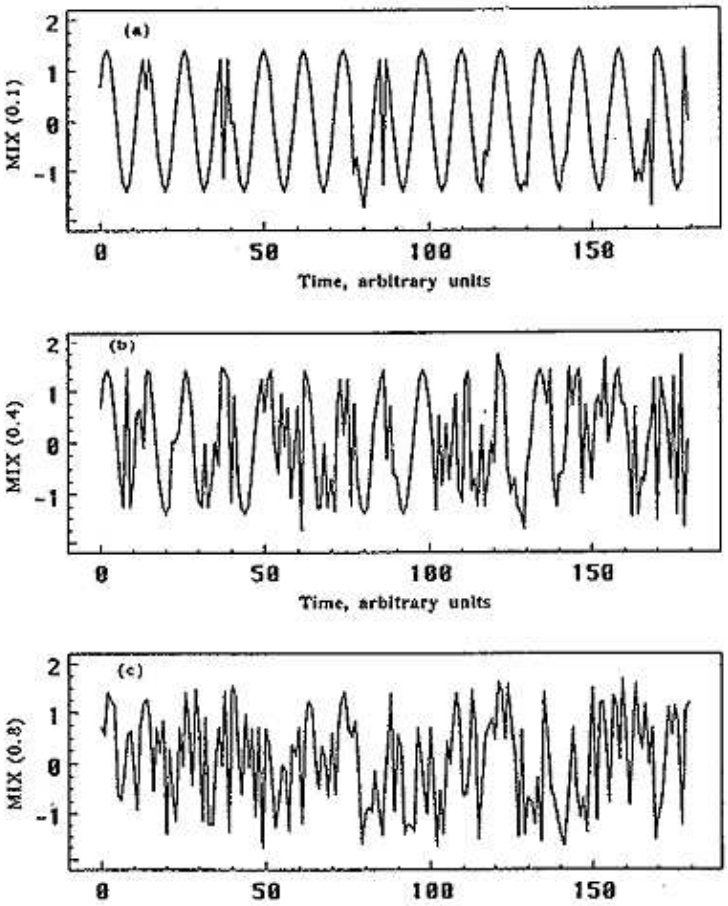

Figure 2.15. A regular trace, demonstrating near sinusoidal behaviour; ApEn = 0.436 (top panel). A less regular trace, still demonstrating some evidence of sinusoidal behaviour; ApEn = 1.455 (middle panel). An irregular trace, demonstrating random behaviour, ApEn = 1.901 (bottom panel). From Pincus and Goldberger, 1994.

ApEn has been used to characterise complexity in a wide range of physiological processes. It was first applied in the analysis of heart rate, the variations in which are exceedingly complex due to the large number of interacting factors, such as sympathetic and parasympathetic stimulation, hormones, temperature and physical activity, operating across a variety of time scales (Kaplan et al., 1991; Manor and Lipsitz, 2012). ApEn has been extensively applied in characterising the complexity of heart rate control and variability across gender, age groups and disease states (Pincus et al., 1991; Ryan et al., 1994). Such studies have, for example, found lower ApEn for males compared to females (0.78 ± 0.03 vs. 0.90 ± 0.03; Ryan et al., 1994); decreases in ApEn with increasing age from young (0.89 ± 0.05) to middle-aged (0.78 ± 0.03) to elderly subjects (0.66 ± 0.04; Ryan et al., 1994); and differences between severely ill neonates compared with healthy controls (0.80 ± 0.31 vs. 1.22 ± 0.12; Pincus et al., 1993).

ApEn has also been used to characterise changes and irregularities in hormonal secretion (Pincus and Keefe, 1992; Veldhuis et al., 2001), finding a decrease in the regularity of growth hormone release throughout puberty and to adulthood (Veldhuis et al., 1997); and changes in electroencephalogram dynamics (Bruhn et al., 2000; Ocak, 2009), finding differences in the electroencephalogram output between various sleep stages (Burioka et al., 2005). Importantly, ApEn has also been the preferred method to quantify motor-system regularity, as evaluated by torque time-series during isometric contractions (Slifkin and Newell, 1999; Vaillancourt and Newell, 2002; Vaillancourt and Newell, 2003; Deutsch and Newell, 2004; Sosnoff et al., 2007; Rose et al., 2009). With the right parameter settings, ApEn has also been demonstrated to be sensitive to distinguish effort levels corresponding to changes in force gradation strategies (Forrest et al., 2014).

There are many properties of ApEn that facilitate its use within clinical and experimental time-series analysis, such as those mentioned above. ApEn can be applied to relatively short series of 60 or more points with good confidence; it is robust/insensitive to outliers; it is nearly unaffected by noise of magnitude below a de facto specified filter level; and it is finite for stochastic, noisy deterministic data sets (Pincus and Keefe, 1992; Pincus, 1995; Veldhuis et al., 2001; Pincus, 2006). Furthermore, ApEn is able to detect subtle, subclinical changes undetected by more classical time-series measures, with these changes in ApEn having been mathematically shown to correspond to mechanistic inferences concerning coupling, feedback and subsystem autonomy in a variety of settings (Pincus, 2006); for example demonstrating changes in the complexity of postural tremor in Parkinson’s disease (Vaillancourt and Newell, 2000) and in heart rate with ageing (Figure 2.17; Lipsitz and Goldberger, 1992) in the absence of any change in the SD; distinguishing between force levels and gradation strategies (Forrest et al., 2014); and characterising changes in hormone secretion (Pincus and Keefe, 1992; Veldhuis et al., 2001).

Sample entropy

Although ApEn is one of the most popular metrics used to estimate complexity in physiological outputs (Aboy et al., 2007), it is not without shortcomings. Richman and Moorman (2000) have criticised ApEn, citing a bias due to the algorithm counting each sequence as matching itself. They claimed that this bias results in ApEn being heavily

dependent on the run length m, making it uniformly lower than expected for short runs, and resulting in it lacking relative consistency (Richman and Moorman, 2000; Lake et al., 2002; Chen et al., 2005). To reduce this bias, they developed a new statistic, sample entropy (SampEn), that does not count self-matches (Richman and Moorman, 2000). Self-matches were discounted from SampEn on the basis that entropy is the rate of information production (Eckmann and Ruelle, 1985) and that comparing data with themselves is meaningless (Richman and Moorman, 2000). In addition to eliminating self-matches, the SampEn algorithm is simpler than ApEn, is largely independent of run length and displays a relative consistency in circumstances where ApEn does not (Richman and Moorman, 2000; Richman et al., 2004; Chen et al., 2005).

Just as with ApEn, in order to compute SampEn a run length 'm' and a tolerance window 'r' must be specified (Richman and Moorman, 2000; Chen et al., 2005; Chen et al., 2009). SampEn is precisely the negative natural logarithm of the conditional probability that two sequences similar for m points remain similar at the next point, without allowing self-matches in calculating the probability (Richman and Moorman, 2000; Lake et al., 2002). The exact calculation of SampEn is given in the General Methods (Chapter 3). As with ApEn, SampEn quantifies a continuum from 0 to 2; with lower values (approaching 0) in a time-series indicating high system regularity and low complexity (i.e. greater self-similarity), and higher values (approaching 2) indicating low system regularity and high complexity (i.e. less self-similarity; Richman and Moorman, 2000; Richman et al., 2004; Ramdani et al., 2009).

SampEn has been used less widely than ApEn, though has still been applied to a range of physiological data, such as heart rate variability (Richman and Moorman, 2000; Lake et al., 2002), postural sway (Roerdink et al., 2006; Ramdani et al., 2009), neural respiratory signals (Chen et al., 2005) and electroencephalogram changes (Ramanand et al., 2004; Abásolo et al., 2006). It has also been applied to EMG recordings during various upper limb movements (Chen et al., 2009; Cashaback et al., 2013), though limited data on its application to torque time-series exists (Svendsen and Madeleine, 2010).

Detrended fluctuation analysis

Detrended fluctuation analysis (DFA) is a scaling analysis method that was originally conceived to investigate the long-range dependence in coding and non-coding DNA nucleotide sequences (Peng et al., 1994; Buldyrev et al., 1995; Stanley et al., 1999). Its wider utility was quickly recognised for determining the hidden fractal nature in physiological time-series (Kantelhardt et al., 2002) and it is now recognised as one of the most robust fractal fluctuation analysis methods (Taqqu et al., 1995). DFA permits the detection of long-range correlations embedded in non-stationary time-series and avoids the spurious detection of apparent long-range correlations that are an artefact of non-stationarity (Hu et al., 2001; Kantelhardt et al., 2002). Whereas entropy statistics such as ApEn and SampEn measure the complexity of a signal (Pincus, 1991; Richman and Moorman, 2000), DFA represents the long-range fractal correlation properties of a signal and relates to noise colour (Hu et al., 2001; Seely and Macklem, 2004).

The calculation of DFA involves several steps. Briefly, a moving window of size n is used to study how the fluctuation F(n) grows with n for the time-series (Stanley et al., 1999; Seely and Macklem, 2004). The relationship between F(n) and n can be graphed, with a fractal correlation present if the data is linear on a graph of log F(n) versus log (n) (Seely and Macklem, 2004). The slope of the line relating log F(n) to log (n) determines the scaling exponent (self-similarity parameter) α (Stanley et al., 1999; Hu et al., 2001; Seely and Macklem, 2004). Detailed description of the DFA calculation is given in the General Methods (Chapter 3). The scaling exponent, α, can vary from 0 (a process exhibiting anti-correlations) to 2 (a non-stationary process; Hardstone et al., 2012). Typically, physiological signals vary from 0.5 (white noise, random numbers) to 1.5 (Brownian noise, random walk; Seely and Macklem, 2004; Heffernan et al., 2008; Hardstone et al., 2012), with healthy signals yielding a scaling exponent close to 1, reflecting fractal scaling behaviour and pink (1/f) noise. Deviations either side of this are indicative of fractal collapse (Peng et al., 1995b; Seely and Macklem, 2004; Heffernan et al., 2008).

DFA was first applied to a physiological signal by Peng et al. (1995a), to quantify the fractal structure of heart rate. Since then, it has been widely applied to heart rate data from both healthy and pathological subjects (Mäkikallio et al., 1999; Castiglioni et al., 2011),

with older subjects, patients with heart disease and asymptomatic relatives of patients with dilated cardiomyopathy who have enlarged left ventricles all exhibiting a loss of fractal scaling (Iyengar et al., 1996; Viswanathan et al., 1997; Mahon et al., 2002). It has also been applied to investigate long-term correlations in neuronal discharge (Blesić et al., 1999; Blesić et al., 2003), gait (Hausdorff et al., 1997; Hausdorff, 2007) and breathing dynamics (Baldwin et al., 2004; Thamrin et al., 2009). Furthermore, DFA has been applied to torque time-series (Vaillancourt and Newell, 2003) and to fatiguing contractions (Vázquez et al., 2016).

2.7 – Applications of complexity and fractals

The concepts of complexity and fractals have been extensively applied in a variety of studies across many areas of physiology. The results of such studies have provided an interesting challenge to some previously well-held beliefs, as well as helping to characterise changes that occur in physiological systems as a result of both acute and chronic perturbations.

Complexity and fractals in homeostasis

Homeostasis has been a dominant principle in physiology for approaching a century (Goldberger, 1991). Coined by W.B. Cannon in 1929, the term homeostasis describes the observation that an organism reduces variability in its physiological processes in order to maintain a constant internal environment (Cannon, 1929; Goldberger, 2001; Que et al., 2001). It can be viewed as an evolutionary strategy that nature has selected and employed to keep the internal state of the body under control and within the operational range that allows cells to continue to function without change (West, 2010). Based on this principle, it would be expected that any physiological variable would return to its normal value after it has been perturbed, and to remain steady at that value until it is perturbed again (Goldberger, 1991).

The notion of the physiological steady state implied by homeostasis has been challenged in the last 25 years by the observation that many physiological processes are not predictably regular but are erratic and exhibit long-range fractal correlations (West,

2006). For example, normal healthy heart rate and heart rate variability show fluctuations that are not simply associated with regular sinus rhythm, but are reflective of fractal scaling (Figure 2.16; Goldberger, 1992; West, 2006). Studies on healthy resting subjects have demonstrated an irregular fractal pattern of heart rate variability (Yamamoto and Hughson, 1994; Yamamoto et al., 1995), that has a fractal dimension midway between that of a regular process and that of an uncorrelated, random process (West, 2006). It has been reported that this fractal component accounts for >70% of the total variance of heart rate variability (Yamamoto and Hughson, 1994).

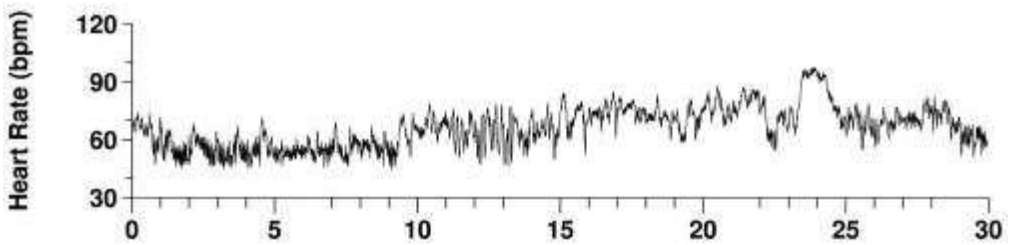

Figure 2.16. The heart rate trace from a healthy individual is far from homeostatic and is characterised by non-stationarity and irregular fluctuations. From Goldberger et al. 2002.

In addition to heart rate, many other previously assumed homeostatically controlled systems show fractal scaling. Normal, healthy blood pressure (Wagner and Persson, 1994; Wagner and Persson, 1995), ventilatory parameters (Bruce, 1996; Frey et al., 1998; Que et al., 2001) and gait (Hausdorff et al., 1995) also exhibit complexity and are characterised by non-linear and non-stationary fluctuations over time. The presence of such complexity and fractal scaling across a variety of systems and processes provides an interesting challenge to the classic theory of homeostasis. Indeed, with continuous irregular fluctuations appearing to characterise normal, healthy function and being associated with systems with the highest capacity to adapt to internal and external perturbations (Pincus and Goldberger, 1994; Goldberger, 1996; Lipsitz, 2004; Seely and Macklem, 2004; Peng et al., 2009), it seems that the term homeostasis may be inaccurate. Instead, homeokinesis, defined as “the ability of an organism functioning in a variable external environment to maintain a highly organised internal environment fluctuating within acceptable limits by dissipating energy in a far from equilibrium state,” may be more appropriate (Yates, 1982; Que et al., 2001). This term reflects the fact that variations in the internal environment are normal and that it is either the lack of or excessive variation that is abnormal (Que et al., 2001).

Complexity and fractals in ageing and disease

As highlighted above, the irregular fluctuations and variability seen across a wide range of physiological systems and processes under resting conditions are not noise; rather they are systematic and form a key component of system control (Lipsitz and Goldberger, 1992; Lipsitz, 2004). The complexity and fluctuations in physiological outputs are actually adaptive, allowing the organism to quickly respond to internal or external stimuli (Lipsitz and Goldberger, 1992; Lipsitz, 2004; Peng et al., 2009) and inhibit the emergence of periodic behaviours that narrow system responsiveness (Goldberger et al., 2002; Goldberger, 2006). Deviations from the "normal" complexity in an output, be it increased regularity or increased randomness, can be viewed as a sign of a loss of system control and result in reduced function of the system (Lipsitz, 2004; Peng et al., 2009; Manor and Lipsitz, 2012). This concept has been extensively applied in the contexts of ageing and disease states (Lipsitz and Goldberger, 1992; Goldberger et al., 2002; Lipsitz, 2004; Manor and Lipsitz, 2012).

Lipsitz and Goldberger (1992) first proposed the "loss of complexity" hypothesis, stating that the ageing process from adulthood to senescence can be characterised by a progressive loss of complexity within the dynamics of physiological outputs. This loss of complexity is manifest as either increased randomness or greater periodicity in a system's output, and can be characterised by a move away from 1/f or pink noise towards either white or Brownian noise (Vaillancourt and Newell, 2002; Lipsitz, 2004). Such losses of complexity are believed to derive from either a reduction in the number of individual structural components, the gradual deterioration of the underlying structural components, and alterations within the non-linear coupling and interaction between systems (Lipsitz, 2002; Vaillancourt and Newell, 2002; Lipsitz, 2004; Peng et al., 2009; Manor and Lipsitz, 2012). Using a variety of complexity and fractal analysis measures, ageing has been shown to be associated with a loss of complexity across a wide range of physiological systems and processes.

The study of alterations in complexity with ageing began with the examination of cardiovascular dynamics. The first study to investigate this was Kaplan et al. (1991), in which heart rate variability in young adults (21-35 years) and old adults (62-90 years) was compared. Using ApEn, they found that the older adults displayed reduced

complexity in electrocardiographic (R-R) interval and blood pressure variability for both normal spontaneous breathing and metronome breathing. Similarly, Pikkujämsä et al. (1999) found decreased complexity of R-R intervals, as measured using ApEn, with increasing age from young (<40 years; ApEn = 1.21± 0.14) to middle age (40-60 years; ApEn = 1.01 ± 0.16) to old age (>60 years; ApEn = 0.88 ± 0.16). It is now well-established that biological ageing from adulthood to senescence results in greater periodicity in cardiovascular dynamics (Figure 2.17), which is described as a loss of complexity and is associated with alterations in the multi-scale organisation of heart rate (Lipsitz, 1995; Iyengar et al., 1996; Beckers et al., 2006). This loss of complexity and breakdown in the fractal scaling of heart rate variability with age could be caused by decreasing autonomic modulation (Bigger et al., 1996; Beckers et al. 2006) or it could potentially relate to a reduction in the number of structural components in the sinus node cells in the heart (Wei and Gersh, 1987).

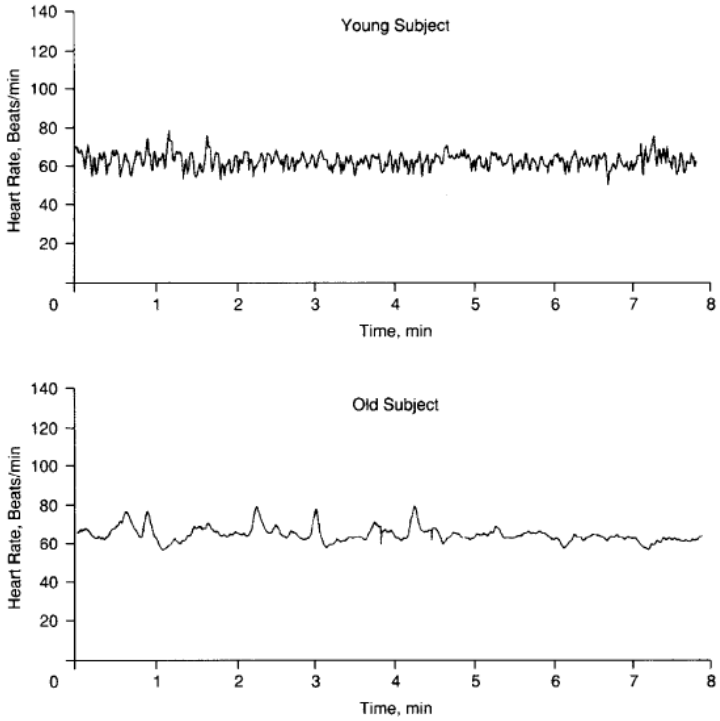

Figure 2.17. The means and standard deviations for the above heart rate traces from a young and old subject are nearly identical. The old subject shows greater periodicity, reflected in the lower ApEn value of 0.48, compared with 1.09 for the young subject. From Lipsitz and Goldberger, 1992.

Further studies have shown that ageing is associated with a loss of complexity and breakdown in fractal scaling in a variety of other physiological systems, including the respiratory and motor control systems. In terms of the respiratory system, older adults exhibit decreased complexity in a variety of respiratory measures, such as inter-breath

interval (Schumann et al., 2010; Bucaoto et al., 2011), with this loss particularly prevalent amongst males (Peng et al., 2002; Nemati et al., 2013). A loss of complexity in the respiratory system with age could be related to changes in the number of and mechanical properties of the alveoli (Thurlbeck and Angus, 1975; Vaillancourt and Newell, 2002) or could be due to factors such as decreased maximal expiratory flow and lung reserve volume (Babb, 1999). In terms of the motor control system, older adults exhibit decreased complexity in gait variables, such as stride interval and duration (Hausdorff et al., 1997; Hausdorff et al., 2001; Delignières and Torre, 2009), and in postural control (Costa et al., 2007; Duarte and Sternad, 2008; Manor et al., 2010).

The fractal scaling and complexity of physiological systems have also been demonstrated to decrease with various disease states. Certain cardiac diseases and disorders, such as heart murmur, atrial fibrillation (Vikman et al., 1999; Costa et al., 2002) and heart failure (Ho et al., 1997; Ho et al., 2011) are all associated with a loss of complexity. Furthermore, low heart rate complexity is predictive of suffering post-surgical complications, with greater complexity more likely to predict survival in the follow-up period (Mäkikallio et al., 1997; Mäkikallio et al., 1999; Ho et al., 2011). Decreases in complexity have also been observed within the temporal fluctuations of brain function in traumatic brain injury patients (Beharelle et al., 2012), with motor activity in Alzheimer's disease (Hu et al., 2009) and with stride-to-stride fluctuations (Herman et al., 2005), force tremor (Vaillancourt et al., 2001) and postural tremor (Vaillancourt and Newell, 2000) in Parkinson's disease.

The acknowledgement of a link between the complexity of physiological systems and their functionality has important implications, particularly in the design and implementation of interventions for the prevention and rehabilitation of functional loss with ageing and disease (Peng et al., 2009; Manor and Lipsitz, 2012). For example, as the degradation of complexity with ageing and disease is typically associated with a decoupling between the sub-components of a system (Peng et al., 2009), interventions with the greatest potential to restore healthy dynamics may need to have effects on multiple systems (Lipsitz, 2004; Manor and Lipsitz, 2012). One such intervention that has proven successful is exercise, with increases in the complexity of heart rate dynamics in older adults being observed following aerobic exercise training (Tulppo et al., 2001), resistance training (Millar et al., 2013) and Tai Chi (Lough et al., 2012).

Complexity and fractals in force production

One of the most common features of human movement is noise or variability (Hamilton et al., 2004; Stergiou and Decker, 2011); no matter how hard we try, we will never be able to replicate two actions identically. At rest, muscles are subject to physiological tremor in the 8-12 Hz range (Matthews and Muir, 1980; Lakie, 2010), and when contracting do not produce a steady or smooth force (Hamilton et al., 2004; Contessa et al., 2009; Sosnoff et al., 2009). The amount of variability during a contraction is intensity dependent, increasing in proportion to the mean force exerted (Galganski et al., 1993; Slifkin and Newell, 1999; Laidlaw et al., 2000; Jones et al., 2002). This variability is thought to be a function of multiple features of motor unit activity (Taylor et al., 2003). One cause is motoneuron action potential discharge rates rarely achieving tetanic rates (Enoka and Fuglevand, 2001), meaning the force exerted by a muscle will be subject to fluctuations due to the submaximal activation of motor units. Other causes of force fluctuations are the number of motor units recruited, changes in discharge rates and motor unit synchronisation (Slifkin and Newell, 1999; Yao et al., 2000; Taylor et al., 2003). It has also been proposed that noise in the motor command and common synaptic drive, which increase with increasing effort level, determine the magnitude of force variability (Jones et al., 2002; Farina and Negro, 2015).

In recent years, it has been recognised that the fluctuations seen in force output during contractions have an important structural component, are indicative of a flexible and adaptive force output, and represent a complex system with fractal scaling (Newell et al., 2003; Vaillancourt and Newell, 2003). The first study to examine the structural variability and complexity of force output, rather than simply the amount of variability, was that of Slifkin and Newell (1999), which measured ApEn for index finger flexion at contraction intensities between 5 and 95% MVC. In addition to the well-established increase in the amount of variability with increasing force, they observed an inverted-U shaped relationship between increasing force and complexity. Specifically, ApEn increased (indicating an increase in complexity) as force increased and reached a projected maximum at approximately 40% MVC, and then decreased with further increases in force (Figure 2.18). A similar inverted-U shaped relationship was observed for the power spectra, with power narrowly distributed and peaking at low frequencies for low and high forces, and becoming more broadband with a high proportion of power spreading to

higher frequencies for forces in the midrange. These results led the researchers to conclude that the peak in complexity at 40% MVC was the point of maximum information transfer. They reasoned that at this point, force could be modulated through either or both increasing the recruitment of motor units and increasing discharge rates, whereas below that point modulation would typically be through increased recruitment alone and above that point through increased discharge rates alone (Kukulka and Clamann, 1981; De Luca et al., 1982a; Bernardi et al., 1995).

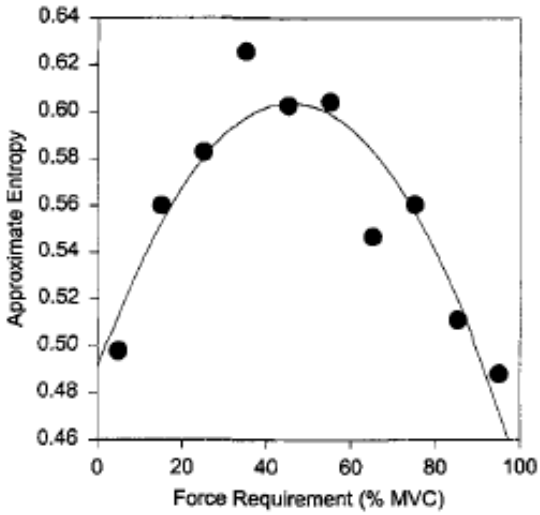

Figure 2.18. Inverted-U shaped relationship between force and ApEn, with a projected maximum at 40% MVC. From Slifkin and Newell, 1999.

Similar inverted-U shaped relationships have subsequently been found in several further studies (Slifkin and Newell, 2000; Hong et al., 2007; Svendsen and Madeleine, 2010). Slifkin and Newell (2000), using the same approach as their previous (1999) study, observed that the ApEn of index finger flexion increased as contraction intensity rose from 3 to 24% MVC, remained at approximately the same level for 48% MVC and declined at 60% MVC to a level similar to that obtained at 12% MVC. In a separate trial, they also observed a decrease in ApEn with contraction intensities from 55 to 85% MVC. Svendsen and Madeleine (2010) subsequently extended this inverted-U shaped relationship to the elbow flexors; finding that SampEn peaked between 40 and 50% MVC, and interestingly that SampEn was also greater in males than females.

Other studies have, however, not demonstrated such inverted-U shaped relationships. For example, Newell et al. (2003) found ApEn to be greater at 10% than 40% MVC for isometric index finger abduction; and Sosnoff et al. (2007), also using index finger

abduction, found ApEn to be higher at 5% than at 25% MVC. Unfortunately, in each of these studies, the objective was to either characterise how complexity is modulated with practice, visual feedback or increasing age, rather than to characterise how it varies with force, thus no attempt was made to test further force levels.

A recent study by Forrest et al. (2014), motivated by the frequent use of different signal acquisition and processing choices for ApEn in previous studies, found that differences in these had significant effects on the relationship between contraction intensity and complexity. For example, it was demonstrated that if 'r' was set to a fixed value accounting for the measured noise then ApEn increased with increasing contraction intensity; whereas, if 'r' was set to 0.1 of the signal SD ApEn decreased with increasing contraction intensity. Differences in the pattern of ApEn were also observed for different data sampling frequencies. Based on their findings, Forrest et al. recommended that for determination of torque ApEn, sampling frequencies in excess of 200 Hz be used and 'r' set to 0.1 of the signal standard deviation. The previous studies of Slifkin and Newell (1999; 2000) used a sampling frequency of 100 Hz and set 'r' to 0.2 of the signal SD. The results of Forrest et al. (2014) thus provide the first real challenge to the previously observed inverted-U shaped relationship.

It is clear from the studies mentioned above that complexity in force production is load dependent, though there is some disagreement on the exact nature and shape of the relationship. Further research is required to unequivocally characterise the load dependence of complexity during isometric force production. Currently, the evidence suggests that the relationship takes the form of an inverted-U, or at the very least that medium-intensity contractions are more complex than high-intensity and maximal contractions. A peak in complexity at approximately 40% MVC, as observed in several studies, makes some sense, as this represents a region where the motor system may engage in either modulation of recruitment or discharge rates to alter force, thus providing greater flexibility and adaptability in scaling force to a target (Slifkin and Newell, 2000). Reduced complexity at higher intensities also makes sense, as higher intensity contractions require a greater discharge rate (Bigland and Lippold, 1954; Moritz et al., 2005), which is more likely to result in a fused tetanus (Buller and Lewis, 1965) and a smooth force output.

To date, the inverted-U shaped relationship between contraction intensity and complexity has only been demonstrated in the first dorsal interosseous (Slifkin and Newell, 1999; Slifkin and Newell, 2000) and the elbow flexors (Svendsen and Madeleine, 2010). It could be argued that different muscles groups may have a peak in complexity at a different intensity or may exhibit a different shaped relationship entirely. This is due to that fact that gradation of muscle force, caused by the recruitment of motor units and discharge rates, is specific to each muscle (Enoka, 1997). For example, in the first dorsal interosseous recruitment of motor units occurs up to 40% MVC (De Luca et al., 1982a), in the adductor pollicis occurs up to 50% MVC (Kukulka and Clamann, 1981; Bernardi et al., 1995) and in the biceps brachii, deltoid and quadriceps occurs up to 80% MVC (Kukulka and Clamann, 1981; De Luca et al., 1982a; Bernardi et al., 1999).

Studies have also shown that EMG signals from contracting muscles exhibit fractal characteristics. Initial studies found that EMG signals demonstrated a fractal dimension, as measured using a box-counting algorithm, and that this estimated fractal dimension increased as EMG amplitude increased with increasing contraction intensity, indicating an output becoming more complex and less self-similar (Anmuth et al., 1994; Gitter and Czerniecki, 1995). The box-counting methods for determining the fractal dimension used in those initial studies, though, have been criticised by others (Talebinejad et al., 2009), who claim that box-counting algorithms applied to an EMG signal consistently saturate at around 1.5 and do not provide any useful information. However, subsequent studies have demonstrated that the fractal dimension of EMG is sensitive to perturbations such as neuromuscular fatigue (Mesin et al., 2009; Beretta-Piccoli et al., 2015), and have confirmed, using various measures of entropy, that EMG signals are non-linear deterministic signals with fractal properties that are significantly different from random noise (Kaufman et al., 2007; Sung et al., 2007; Cashaback et al., 2013).

In terms of a relationship between contraction intensity and complexity, Cashaback et al. (2013) hypothesised that greater contraction intensity would elicit greater EMG complexity, since higher-intensity contractions have the greatest discharge rates and recruitment. Using multiscale entropy as their measure of complexity, they evaluated isometric contractions of the biceps brachii at 40, 70 and 100% MVC, finding that complexity was higher at 70 than 40% MVC, but that neither condition was significantly different from 100% MVC. That the maximal contraction was not accompanied by

significantly greater complexity was explained by the fact that the rapid force loss during the contraction led to a rapid decrease in the EMG amplitude.

Interestingly, it has also been suggested that the complexity of the EMG signal changes over the course of a contraction. Ahmad and Chappell (2008), using moving ApEn to examine the complexity of EMG signals during wrist flexion and extension, noted a similar pattern of changing complexity throughout the contraction for both movements. Upon commencement of a contraction, ApEn was approximately 0.54; in the middle of the contraction, it rose to approximately 0.79; and at the end of the contraction, it fell again to approximately 0.46. T-tests revealed significant differences in ApEn between the start, middle and end of the contraction and also the relaxed state of the muscle. These changes in complexity across the course of a contraction could be reflective of the mechanisms of force modulation and could provide indirect evidence to confirm the hypothesis of Cashaback et al. (2013) that higher-intensity contractions are more complex. The increase in ApEn, and thus in complexity, from the beginning to the middle of the contraction could be reflective of increased recruitment and discharge rates until a target force is reached, while the subsequent decrease at the end of the contraction could be reflective of a progressive de-recruitment and decreased discharge rates as the muscle starts to relax.

Complexity and fractals in force production and ageing

Changes in the complexity of force production have been studied in the context of ageing. It has been observed that performance error decreases and complexity increases throughout childhood to adulthood (Deutsch and Newell, 2001; Deutsch and Newell, 2002). Deutsch and Newell (2004) studied the complexity of force production during a thumb and index finger pinch grip in three age groups: 6-year-old children, 10-year-old children and young college age adults; observing a significant effect of age, with ApEn becoming successively higher with increasing age. Similarly, Sosnoff et al. (2007) observed that ApEn increased as a function of age, from 6 to 8 to 10 years and to young adults. They also concluded that the increase in ApEn with age could not be entirely explained by the increase in muscular strength with age, with multiple developmental processes, including an increase in the dynamical degrees of freedom, contributing.

While ageing from childhood to adulthood is associated with positive gains in muscle function, performance and complexity (Lexell et al., 1992; Sosnoff et al., 2007), ageing from adulthood to senescence is associated with deleterious effects on the muscle itself and its function and performance (Evans and Hurley, 1995; Kent-Braun and Ng, 1999), and, as such, has been more extensively studied. Ageing from adulthood to senescence induces a broad range of performance decrements in the human motor system that affects both the quantity and quality of muscle (Frontera et al., 2000). Muscle cross-sectional area is reduced, particularly in males (Overend et al., 1992; Evans and Lexell, 1995; Kent-Braun and Ng, 1999); fibre type distribution changes, with a shift towards slower predominantly type I muscle fibres (Larsson et al., 1978; Larsson et al., 1979; Evans and Lexell, 1995); and the number of motor units within a muscle decreases (Brown, 1972; Galea, 1996). These changes combine to result in a decrease in force production and functionality (Larsson et al., 1979; Jubrias et al., 1997).

The negative effects of ageing on muscle also influence motor control, resulting in an increase in the amount of variability in a contraction, which could potentially decrease the quality of voluntary movements. Increases in the amount of variability, as measured by increases in the SD and CV, have been shown in older adults (>60 years) during isometric, concentric and eccentric contractions across a range of muscle groups and contraction intensities (Galganski et al., 1993; Laidlaw et al., 2000; Tracy and Enoka, 2002; Tracy et al., 2005). Such increases in variability have been found to result in decreased steadiness and performance in tasks of manual dexterity (Carmeli et al., 2003; Kornatz et al., 2005) and typically lead older adults to perform tasks more slowly in an attempt to eliminate errors and maintain balance (Schultz et al., 1992; Hortobagyi and DeVita, 1999). It has been shown, however, that these age-related increases in variability can be lessened, and motor control enhanced, by strength training (Keen et al., 1994; Laidlaw et al., 1999).

Not only has senescence been found to increase the amount of variability in motor control, but also the structure of variability, and thus complexity. Sturman et al. (2005) compared loaded postural tremor in young adults (aged 20-30) and three groups of old adults (aged 60-69, 70-79 and 80-94), finding ApEn to decrease as a function of age, with the oldest group displaying the greatest regularity, and with this decrease becoming more pronounced under increasing load. The increase in regularity with ageing was

accompanied by and linearly related to an increase in peak tremor-EMG coherence in the 1-8 Hz frequency band. As tremor-EMG coherence has been shown to provide a predictive measure of motor unit synchronisation (Halliday et al., 1999), the increase in the regularity of tremor with ageing was suggested to be due to enhanced motor unit synchronisation.

The first studies to investigate the effect of ageing on the complexity of isometric force output were by Vaillancourt and Newell (2003) and Vaillancourt et al. (2003). They demonstrated that during constant submaximal isometric index finger abductions at 5, 10, 20 and 40% MVC there was a progressive decrease in complexity from young adults (aged 20-24) to old adults (aged 60-69) and older-old adults (aged 75-90). This decrease in complexity was observed across numerous metrics, notably ApEn and DFA, and also spectral degrees of freedom and spectral slope analysis. Similar results were subsequently obtained by Sosnoff and Newell (2006; 2008), again for index finger abduction; with both studies indicating that older adults had greater periodicity in the production of submaximal force, indicating a loss of complexity with ageing. Decreases in complexity with ageing have also been observed, by Challis (2006), during maximal isometric contractions of the plantar flexors.

The cause of the decrease in the complexity of force production with ageing has yet to be identified, and could be related to any of the variety of processes that contribute to decreased muscular function with ageing. Challis (2006) suggested the loss of complexity could simply be a function of older adults having fewer motor units to co-ordinate (Galea, 1996). The results of other studies have, however, suggested the loss of complexity could be related to changes in the low-frequency portion of force and EMG outputs. For example, Vaillancourt and Newell (2003) found that the oldest group of adults in their study had a greater magnitude of power in the 0-4 Hz frequency band of force output across the range of forces tested; while Vaillancourt et al. (2003) found a progressive decrease in the relative power of 40 Hz EMG activity and a progressive increase in the relative power of 10 Hz EMG activity with increasing age. These findings suggest that alterations in the frequency structure of neural and motor outputs could be the cause of reduced complexity with ageing.

Interestingly, in contrast to the reduced complexity with ageing observed in isometric tasks, older adults have been found to demonstrate increased complexity compared to young adults for sine wave force tasks (Vaillancourt and Newell, 2003; Sosnoff and Newell, 2008). This provides support for Vaillancourt and Newell's (2002) "bi-directional theory of complexity", in which change is dependent on task dynamics. In tasks where the dynamic is a dimension of zero (fixed point; i.e. isometric), more complexity is required to maintain optimal output (Vaillancourt and Newell, 2002). For these tasks, there will be a decrease in complexity with ageing because, in order to realise the goal of no motion, additional degrees of freedom must be introduced by the neuromuscular system: something which older adults find difficult to accomplish (Vaillancourt and Newell, 2003). In contrast, in tasks where the dynamic is oscillatory, less complexity is required to closely track the oscillations and reduce error. The increase in complexity with ageing is reflective of greater fluctuations around the intrinsic dynamic (Vaillancourt and Newell, 2002) and is due to older adults having difficulty reducing the dimension of their output to a lower dimension than the resting state of the system (Vaillancourt and Newell, 2003; Sosnoff and Newell, 2008).

It has been demonstrated that the increased variability and decreased complexity observed in older adults can, to some extent, be reversed through strength training. Several studies have demonstrated reductions in force error and variability in older adults following high intensity strength training (Hortobágyi et al., 2001; Tracy et al., 2004). Subsequently, Keogh et al. (2007) observed that older males (aged 70 to 80) who underwent seven weeks of upper body strength training applied to just one upper limb not only decreased their force variability, but also increased the SampEn of force production in both the trained and untrained limb compared to a control group who underwent no training. Such an increase in SampEn could be related to an increase in strength with training, or could relate to changes in motor unit characteristics, such as innervation ratio. This contralateral effect, with changes in SampEn being observed in untrained limb, suggests that central mechanisms could be of greater importance to complexity.

Recent studies have also demonstrated that the complexity of EMG signals are affected by ageing. Arjunan et al. (2013) observed a significant decrease in the fractal dimension of EMG from the biceps brachii in old adults (61-69 years) compared to young adults (20-29 years). Arjunan and Kumar (2013) went on to study subjects in each decade of

life, from a group in their 20s to a group in their 60s. A gradual increase in the CV of biceps brachii force was observed for each decade until approximately 45 years, when the increase steepened. A gradual decrease in the fractal dimension of the EMG signal was also observed for each decade until approximately 48 years, when the decrease steepened. This decrease in complexity was observed for both submaximal and maximal contraction intensities and exhibited a strong correlation with the increase in muscle force CV. It has been suggested that this reduced EMG complexity with age may be reflective of the fact that older adults have a reduced number of motor units (Galea, 1996); a phenomenon caused by neuronal cell death and a loss of motoneurons, particularly those innervating fast motor units (Campbell et al., 1973).

2.8 – Complexity and fractals in neuromuscular fatigue

As previously mentioned, neuromuscular fatigue results in well-established performance decrements (i.e. reduced force generating capacity, reduced shortening velocity, reduced power output; Merton, 1954; Bigland-Ritchie et al., 1983a; Allen et al., 1995a; De Ruiter and De Haan, 2000; Jones et al., 2006), that are brought about by both central and peripheral perturbations (Søgaard et al., 2006; Burnley et al., 2012). A further, and often overlooked, effect of neuromuscular fatigue is on motor control and variability. Such an effect has implications for both functional and skilled activities (Singh et al., 2010), across a range of populations, from older adults to elite athletes.

To date, the majority of research on motor control and variability with neuromuscular fatigue has focused on the amount of variability during movements and contractions, as measured by the SD and CV. An effect of muscular action on force variability was first observed by Binet in 1920, who stated “tremor increases as a result of muscular contraction and becomes exaggerated under the influence of work.” Subsequent research has demonstrated increases in the amount of variability in force output during sustained fatiguing isometric contractions, typically conduced to the point of task failure, in numerous muscles (Figure 2.19; Bousfield, 1932; Furness et al., 1977; Gottlieb and Lippold, 1983; Löscher et al., 1996; Hunter and Enoka, 2001; Hunter et al., 2004; Hunter et al., 2005; Yoon et al., 2007; Singh et al., 2010). Such increases in the amount of variability serve to hinder the ability to exert a desired force, reducing the accuracy and

steadiness, and thus the quality, of a contraction (Missenard et al., 2009; Singh et al., 2010), and also impairs the ability to produce an intended movement trajectory (Harris and Wolpert, 1998). While the results of the studies mentioned above have proved useful, their methodologies can be criticised somewhat due to the fact that they investigated sustained contractions, while most tasks of daily life and in athletic/sporting events are intermittent rather than continuous. Nevertheless, increased variability still occurs during intermittent contractions; it simply takes longer for such changes to occur and to have significant effects (Contessa et al., 2009).

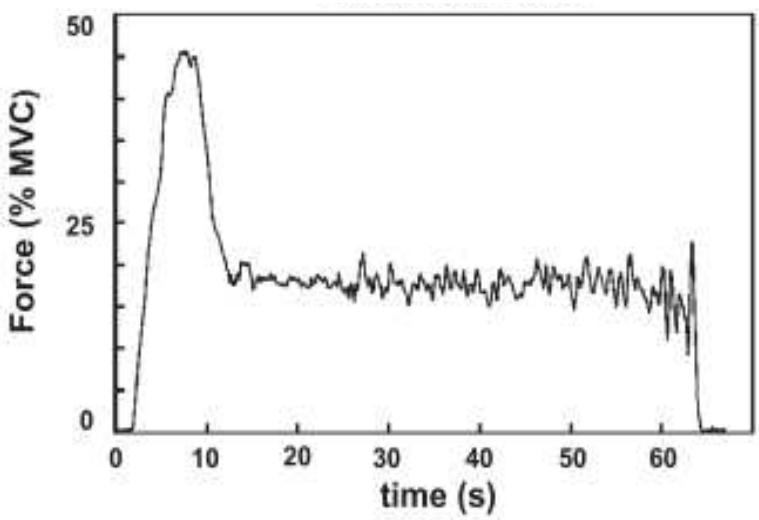

Figure 2.19. Increase in variability as a function of time during a steady sustained isometric contraction of the quadriceps. From Contessa et al. (2009).

The increase in the amount of variability in force output with neuromuscular fatigue has been suggested to reflect alterations in motor unit recruitment and discharge rate/timing as task failure approaches (Hunter and Enoka, 2003; Contessa et al., 2009). During neuromuscular fatigue, the relative difficulty of a submaximal task increases and a given force can only be maintained with an increased effort and motor command (Bigland-Ritchie, 1981; Missenard et al., 2008). In a non-fatigued state, the amount of variability in a contraction increases with, and is proportional to, contraction intensity (Slifkin and Newell, 1999; Jones et al., 2002). The increase in relative difficulty with neuromuscular fatigue means that the muscle is operating at operating at a higher percentage of its maximal output, which should lead to an increase in variability. This is partially due to the fact that more motor units need to be recruited to produce the force. As new motor units are recruited, they fire with lower firing rates and are unfused, meaning the individual twitch forces will increase variability (Contessa et al., 2009).

Several other mechanisms for the increased variability with neuromuscular fatigue have been proposed. Simulation studies have highlighted a causal relationship between motor unit firing rate variability and force variability (Jones et al., 2002; Moritz et al., 2005). The CV of motor unit firing rate variability has been speculated as a likely cause of increased force fluctuations (Laidlaw et al., 2000; Tracy et al., 2005), though there have been contrasting reports on how it changes with neuromuscular fatigue. Several studies have found increases in firing rate variability with fatiguing exercise (Enoka et al., 1989; Garland et al., 1994), while others have found no change (Galganski et al., 1993; Macefield et al., 2000). It has also been speculated that the increased muscle activation results in a shift in the frequency spectrum of force, from 0-3 Hz (Kouzaki et al., 2004) to 8-12 Hz (Singh et al., 2010). This shift to high frequencies is associated with the occurrence of increased tremor (Löscher et al., 1996; Singh et al., 2010).

It has recently been demonstrated that common drive, the synaptic input common to all motoneurons, increases with fatigue (Castronovo et al., 2015). This common synaptic input has been proposed to be a major determinant of force variability (Dideriksen et al., 2012; Farina et al., 2014) and has been demonstrated to increase with fatigue (Castronovo et al., 2015). A necessary consequence of a common synaptic input is motor unit synchronisation, the correlated discharge of motor unit action potentials (Milner-Brown and Lee, 1975; Semmler, 2002). This motor unit synchronisation must also necessarily increase with fatigue. A simulation study by Yao et al. (2000) demonstrated that motor unit synchronisation substantially increases the amplitude of force fluctuations (Yao et al., 2000), and several experimental studies have subsequently found increases in both motor unit synchronisation and force fluctuations with neuromuscular fatigue (Boonstra et al., 2008; Holtermann et al., 2009), suggesting a causal link between enhanced motor unit synchronisation and an increase in force variability.

Similar changes in the variability of EMG signals are also present during fatiguing contractions. Increases in EMG amplitude (Jørgensen et al., 1988; Hunter and Enoka, 2001) and in EMG variability (Enoka et al., 1989; Garland et al., 1994; Bilodeau et al., 2003; Hunter et al., 2005) have been observed during submaximal contractions. The increase in EMG amplitude can be attributed to the progressive recruitment of motor units necessary to maintain performance (Moritani et al., 1986), while the increase in variability may be to do with firing rates of the newly recruited motor units (Contessa et

al., 2009). It has also been demonstrated that neuromuscular fatigue results in a change in the frequency spectra of a muscle's EMG output, causing a compression of the spectra and a decrease in the median frequency (Mills, 1982; Bilodeau et al., 2003; Yoshino et al., 2004; Ravier et al., 2005; Cifrek et al., 2009). While the shape of the power spectrum in a fatigued muscle remains the same as in the unfatigued state, the spectral compression it undergoes results in greater power at lower frequencies. This shift can be interpreted as a scale factor; a behaviour typical of a fractal series (Figure 2.20; Ravier et al., 2005).

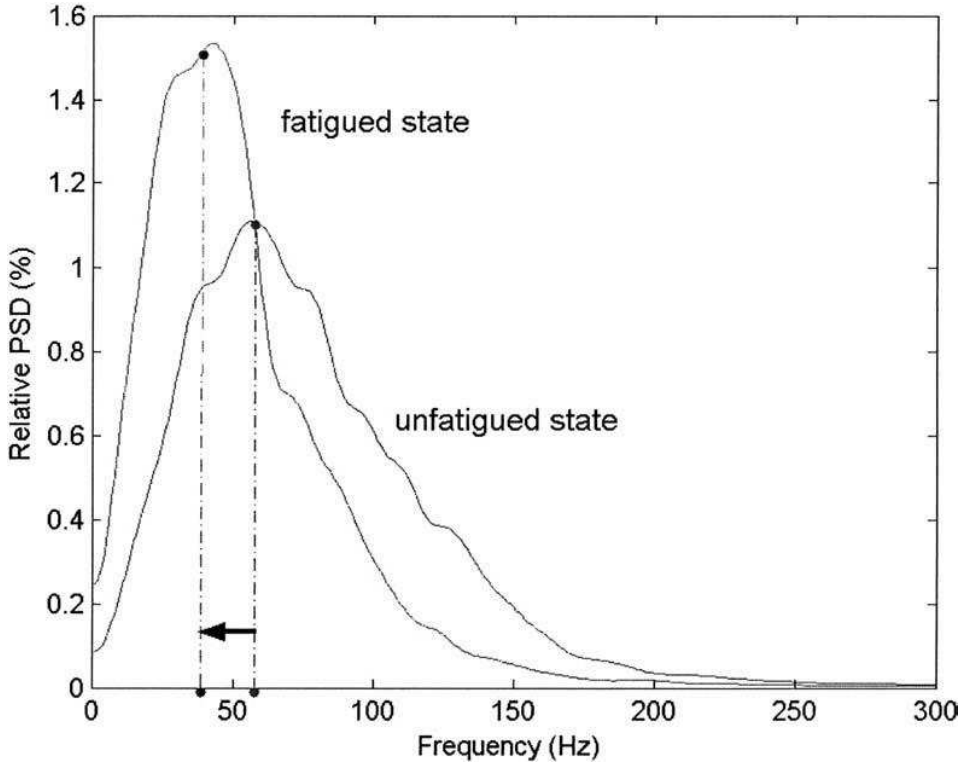

Figure 2.20. The frequency shift associated with fatigue can be interpreted as a scale factor, typical of fractal series. From Ravier et al. (2005).

Based on previous research, it is apparent that, in particular, the force output (Slifkin and Newell, 1999; Slifkin and Newell, 2000; Vaillancourt and Newell, 2003; Svendsen and Madeleine, 2010), and also the EMG output (Ahmad and Chappell, 2008; Cashaback et al., 2013), from contracting muscles exhibit structural variability and complexity typical of fractal scaling during non-fatiguing contractions. It is also clear that neuromuscular fatigue results in an increase in the amount of variability of both a muscle's force and EMG outputs (Enoka et al., 1989; Hunter et al., 2005; Contessa et al., 2009). However, prior to the commencement of this thesis, virtually no attention had been given to the structural variability and complexity of force and EMG outputs during neuromuscular fatigue.

The investigation of an effect of neuromuscular fatigue on the complexity of physiological systems and outputs is very much in its infancy. West et al. (2005) initially observed that the fractal dimension of the R-R interval decreased, indicating increased regularity, when going from rest to low-intensity exercise and then to heavy-intensity exercise. Lewis and Short (2007), using a more rigorous incremental exercise test, observed that the SampEn of R-R interval increased when going from rest to low-intensity exercise, and then progressively decreased as exercise intensity was increased until volitional fatigue, at which point the SampEn values were similar to or lower than those taken at rest. More recently, Millar et al. (2009) found that the SampEn of heart rate variability was significantly reduced following single and multiple Wingate tests and remained reduced throughout a two-hour recovery period; while Stewart et al. (2014) found that the ApEn of heart rate variability was significantly reduced following cycling to exhaustion at a power output equivalent to the gas exchange threshold, and that it remained lower over the course of a one-hour recovery period. Taken together, these decreases in the fractal dimension, ApEn and SampEn of cardiac dynamics indicate a loss of system complexity with neuromuscular fatigue, which would serve to decrease the adaptability of the individual in response to further perturbation.

Control entropy, a metric based on SampEn and proposed by its developers to better reflect changes in system complexity under dynamic exercise conditions (Bollt et al., 2009; McGregor and Bollt, 2012), has recently been applied to several other physiological outputs during fatiguing exercise. In application to cycling to exhaustion at maximal aerobic power, Bollt et al. (2009) observed a decrease in the control entropy of power output, which reached a nadir at exhaustion. McGregor et al. (2009) went on to demonstrate an increase in the control entropy of gait with increasing walking speed, and then a decrease with increasing running speed, with the values obtained at exhaustion being significantly lower than those obtained at rest. Similar results were also obtained by Bollt et al. (2009) for the control entropy of accelerometry during an incremental treadmill $\dot{V}O_{2max}$ test, with a decrease in control entropy evident from the point of transition from walking to running. These results, taken together with those on cardiac dynamics, all seem to indicate that neuromuscular fatigue results in a progressive decrease in the complexity of a wide variety of physiological outputs.

The first application of complexity measures to motor control with fatiguing exercise was by Cignetti et al. (2009), who examined fluctuations of limb movements in cross country skiers before and after skiing on a cross-country skiing treadmill at 90% $\dot{V}O_{2max}$ until exhaustion. At the end of the fatiguing protocol, participants exhibited significantly decreased maximal power for the arms and legs, and significantly greater standard deviations for the angular displacement of their arms and legs. Further analysis revealed significant increases in the mean Lyapunov exponent and correlation dimension, measures of fractal scaling, for the arm and leg angular displacements with neuromuscular fatigue. These results indicated that the fluctuations in the limb movements of the skiers when fresh displayed a chaotic behaviour, typical of fractal scaling, and reflected flexibility to adapt to perturbations; but that this behaviour was degraded by fatigue, which resulted in increased and more random fluctuations, making the movements more unstable and less adaptable. Though this study utilised neither force nor EMG traces and only investigated limb angular displacements, the results provided the first suggestion that neuromuscular fatigue does negatively influence the complexity of motor control.

The next study investigating complexity and motor control was by McGregor et al. (2011), who applied control entropy to investigate changes in postural control with neuromuscular fatigue. They used high-resolution accelerometers to measure centre of mass accelerations during single-legged stance before and after the performance of two Wingate tests. Compared to pre-test measures and to a control group performing no exercise, control entropy was significantly increased for centre of mass accelerations in the vertical and medio-lateral directions following the Wingate tests. This finding was in contrast to the authors' hypothesis of a decrease in control entropy, and was attributed to the fatigued muscles being ineffective at implementing commands and a subsequent need for greater exploration of solutions to the postural control problem.

Cortes et al. (2014) recently investigated the complexity of lower limb kinetics and kinematics before and after fatiguing exercise during a side-step cutting task. They observed that following a 45-minute fatiguing treadmill protocol, participants exhibited significantly decreased absolute force and decreases in the SD of knee moments. These decreases were accompanied by significant increases in the SampEn, and thus in the irregularity and complexity, of ground reaction forces in the vertical and medio-lateral directions, knee abduction angle and the moment about the knee during abduction. It was

concluded that exercise-induced fatigue does not simply decrease the amount of force capable of being generated, but that it also influences the ability of an individual to perform a smooth and controlled movement. It was also postulated that the loss of movement control could be reflective of an overall decline in coordination, which could have important implications for likelihood of injury.

Several recent studies have investigated the complexity of sEMG with neuromuscular fatigue. Using multiscale entropy, Cashaback et al. (2013) examined the complexity of sEMG of the biceps brachii during fatiguing elbow flexions at 40, 70 and 100% MVC. They observed that each contraction intensity resulted in a decrease in entropy near exhaustion, reflecting a decrease in sEMG complexity. It was therefore suggested that neuromuscular fatigue compromises muscle regulatory mechanisms, making the muscle less adaptable. The researchers postulated that this reduced complexity could arise from a combination of impaired peripheral factors and altered motor unit discharge rates and recruitment strategies, leading to a decrease in muscle action potential velocity and amplitude (Fuglevand et al., 1993b), though they stressed it is difficult to determine the exact mechanisms that influence sEMG complexity. Decreases in the complexity of vastus lateralis EMG, as measured by a decrease in the fractal dimension, have also been observed following sustained contractions at 60 and 70% MVC (Beretta-Piccoli et al., 2015 Boccia et al., 2015).

The increases in the complexity of motor control observed by Cignetti et al. (2009), McGregor et al. (2011) and Cortes et al. (2014) are in contrast to the decreases in complexity observed for cardiac dynamics with neuromuscular fatigue (Millar et al., 2009; Stewart et al., 2014), sEMG with neuromuscular fatigue (Cashaback et al., 2013; Beretta-Piccoli et al., 2015; Boccia et al., 2015) and for isometric force production with ageing (Vaillancourt and Newell, 2003; Vaillancourt et al., 2003; Challis, 2006). Such contrasting results provide support for Vaillancourt and Newell's (2002) bi-directional theory of complexity, and appear to indicate that changes in the direction of complexity are task and measure dependent. The results of Cignetti et al. (2009), McGregor et al. (2011) and Cortes et al. (2014) all indicate that the complexity of motor control is increased, that is, it becomes more random with neuromuscular fatigue. It must be noted, though, that all of their measures were indicative of whole body motion, rather than muscular output.

Further evidence that the neuromuscular system may become less complex and less adaptable with neuromuscular fatigue was obtained by Forestier and Nougier (1998), who examined adaptation to neuromuscular fatigue in a multi-joint skilled movement. Participants were required to throw a ball towards one of three targets in a no fatigue condition and a fatigue condition, in which they performed the task following completion of a series of 70% MVC contractions of the wrist flexors. The results indicated that throwing accuracy was significantly lower in the fatigue condition than the control condition, suggesting that neuromuscular fatigue significantly influenced movement organisation. More detailed analysis revealed that in the no fatigue condition, final hand velocity results from a proximal to distal chain and the summation of speed principle (Neal et al., 1991), but that in the fatigue condition there was no temporal delay between the elbow and hand peak velocities. This suggests that with neuromuscular fatigue movement rigidity increases, which could be reflective of low complexity and high periodicity.

The few studies that have investigated the complexity and fractal properties of motor control during neuromuscular fatigue have revealed perturbations arising from fatiguing protocols. Despite evidence of the potential importance of physiological complexity (Lipsitz and Goldberger, 1992; Lipsitz, 2004; Peng et al., 2009), there are currently no compelling models of complexity regulation for any system, nor have the causes and functional implications of reduced complexity been investigated in depth (Manor et al., 2010). Further systematic research is clearly required to properly characterise any potential relationship between neuromuscular fatigue and complexity. High complexity is thought to be adaptive, endowing a system with greater ability to adapt to perturbations and changes in task demand; while low complexity is associated with reduced functionality and decreased performance (Lipsitz and Goldberger, 1992; Lipstiz, 2002). Thus, any change towards less complexity with neuromuscular fatigue could have important functional implications, indicating that the neuromuscular system has become progressively less adaptable (Lipsitz and Goldberger, 1992; Lipsitz, 2004) and potentially more energetically expensive (Glenny, 2011; Seely and Macklem, 2012) as the contractions progress. Both of these factors may significantly increase the probability of task failure and may, in part, underpin exercise intolerance in a wide range of situations.

2.9 – Aims and hypotheses

The overall aim of this thesis is to investigate changes in the complexity and fractal scaling of isometric muscle torque output in response to neuromuscular fatigue. The specific aims of the five experimental studies are as follows:

1. To determine whether the temporal complexity and fractal scaling of isometric knee extensor torque and EMG outputs are perturbed by neuromuscular fatigue.

2. To determine whether changes in complexity and fractal scaling with neuromuscular fatigue differ above and below the critical torque (CT), and to establish the relationship between contraction intensity and complexity for fresh muscle across the full range of knee extensor joint torques.

3. To attempt to separate the effects of central fatigue, peripheral fatigue and afferent feedback, in order to investigate the mechanism(s) responsible for the reduced torque complexity seen with neuromuscular fatigue.

4. To investigate the effect of caffeine, a well-tolerated central nervous system stimulant, on the complexity and fractal scaling of knee extensor torque output during fatiguing contractions.

5. To investigate the effect of eccentric contractions on the complexity and fractal scaling of knee extensor torque output, and to compare the recovery kinetics with those following isometric contractions.

The hypotheses to be tested within the five experimental studies of this thesis are:

1. That knee extensor torque and EMG outputs will exhibit greater complexity (as measured by higher ApEn and SampEn and more fractal-like scaling (DFA $\alpha \sim 1.00$) during submaximal contractions.

That, compared to fresh muscle, neuromuscular fatigue will cause changes in the complexity of both torque and EMG outputs that are indicative of a reduction in complexity (as measured by decreased ApEn and SampEn).

That any reduction in the complexity of the torque and EMG outputs will be accompanied by a change in fractal scaling from pink (DFA $\alpha = 1.00$) to Brownian noise (DFA $\alpha = 1.50$).

2. That below the CT, fatigue development will be limited, a steady state in the temporal complexity of torque output will be attained and there will be no breakdown in the fractal scaling of torque output within 30 minutes.

   That above the CT, central and peripheral fatigue will develop, the temporal complexity of torque output will be reduced, and this reduction in temporal complexity (measured by decreased ApEn and SampEn) will be accompanied by a shift towards increasingly Brownian noise (DFA $\alpha = 1.50$).

3. That pre-existing central fatigue, induced by prior exercise of the contralateral limb, will decrease time to task failure and reduce the temporal complexity of torque output at the start of a subsequent bout of exercise (as measured by decreased ApEn and SampEn, and increased DFA α).

   That pre-existing peripheral fatigue, induced by circulatory occlusion, will decrease time to task failure and reduce the temporal complexity of torque output at the start of a subsequent bout of exercise.

   That enhanced afferent feedback, induced by prior exercise and circulatory occlusion, will decrease time to task failure and reduce the temporal complexity of torque output at the start of a subsequent bout of exercise.

4. That pre-exercise caffeine ingestion will increase time to task failure.

   That pre-exercise caffeine ingestion will attenuate/delay the loss of temporal torque complexity seen with neuromuscular fatigue (as measured by slower rates

of decrease in ApEn and SampEn), and attenuate/delay the shift towards Brownian noise (as measured by increased DFA α).

5. That eccentric exercise will reduce the temporal complexity of isometric torque (as measured by decreased ApEn and SampEn, and increased DFA α).

   That the temporal complexity of isometric torque will still be depressed 60 minutes after the cessation of eccentric exercise.

   That the reduction in temporal torque complexity seen following isometric exercise will have recovered 60 minutes after the cessation of fatiguing isometric exercise.

Chapter 3 – General methods

Health and safety

Full ethical approval of the experimental designs and procedures were obtained from the ethics committee of the University of Kent prior to the commencement of each experimental study (see Appendix). All procedures were performed in accordance with the Declaration of Helsinki (1964).

All experiments were conducted in a well-ventilated laboratory at 18-22 °C, at the University of Kent. Throughout all experimental testing, care and attention was taken to ensure that the laboratories and equipment were clean and safe for the assessment of, and use by, human participants. Any contaminated disposables were disposed of immediately after use for later incineration.

Participant recruitment, preparation and care

Participants for the experimental studies were recruited from among students and staff at the University of Kent. Approximately 10 participants were recruited for each study. No a priori power calculations were made for participant number, as no previous information on the complexity of isometric torque output with neuromuscular fatigue was available. This stance was maintained throughout the thesis. Prior to commencement of any experimental procedures, participants were given a full written and verbal explanation of the procedures, risks and benefits associated with participation and the commitment required for each study. Participants completed a health and physical activity questionnaire (see Appendix), to ensure adequate health to undertake high intensity exercise, and then signed an Informed Consent form. It was made clear to participants that they could withdraw their consent at any time, without having to give a reason for their withdrawal. It was also stressed that all data would be coded to ensure anonymity, and treated in the strictest confidence.

Prior to data collection, familiarisation trials were conducted to ensure participants were comfortable with the laboratory surroundings, the equipment and protocols used (particularly the electrical stimulation), and to minimise the learning effects associated

with repeated high intensity exercise bouts. Participants were instructed to arrive in the laboratory for testing in a well hydrated and rested state (having refrained from strenuous exercise in the preceding 24 hours), and to have refrained from consuming alcohol for 24 hours, and food or caffeine for three hours prior to each test. Adherence to these instructions was verbally verified prior to each test.

Descriptive data

Descriptive data and anthropometric measurements, including age, height, mass and a description of typical activity level, were recorded prior to the commencement of each experimental study. Height was measured to the nearest 0.01 m using a Harpenden Stadiometer (Holtain Ltd., Crymych, UK). Body mass was measured to the nearest 0.1 kg using regularly calibrated laboratory scales (Seca GmbH & Co., Hamburg, Germany). Both measurements were recorded with the participants standing unshod and wearing the attire with which they were to perform the experimentation.

Dynamometry

During all visits of each study, participants were seated in the chair of a Cybex isokinetic dynamometer (HUMAC Norm; CSMi, Massachusetts, USA), initialised and calibrated according to the manufacturer's instructions. Their right leg was attached to the lever arm of the dynamometer, with the seating position adjusted to ensure that the lateral epicondyle of the right femur was in line with the axis of rotation of the lever arm. Participants sat with relative hip and knee angles of 85° and 90°, respectively, with full extension being 0°. The lower leg was securely attached to the lever arm above the malleoli with a padded Velcro strap, while straps secured firmly across both shoulders and the waist prevented any extraneous movement and the use of the hip extensors during the isometric contractions. The seating position was recorded during the first visit of each study and replicated for each subsequent visit. The between day intraclass coefficient (ICC) of fresh maximal torque, as measured from the six experimental visits of Chapter 5, was 0.98; indicating very high reliability. This was higher than the 0.79 obtained for the knee extensors by Todd et al., (2004).

Electrical stimulation

An Ag/AgCl electrode (32 x 32 mm; Nessler Medizintechnik, Innsbruck, Austria) coated in conductive gel acted as the cathode, and was placed in the femoral triangle, over the femoral nerve of the right leg. The anode, a carbon rubber electrode with adhesive gel (100 x 50 mm; Phoenix Healthcare Products Ltd., Nottingham, UK), was placed lateral to the ischial tuberosity, on the posterior aspect on the leg. A constant-current, variable voltage stimulator (Digitimer DS7AH, Welwyn Garden City, UK) was then used to deliver single (200-µm pulses) and doublet stimuli (200-µm pulses, 10-ms interval) at 400 V. The precise location of the cathode was first established using a motor point pen (Compex, DJO Global, Guildford, UK), and determined based on the location giving the largest twitch and greatest peak-to-peak amplitude of the compound muscle action potential (M-wave) following single stimulation at 100 mA.

Following establishment of the cathode location, single stimuli were initiated starting at 100 mA, and the stimulator current increased in steps of 20 mA until there was no further increase in the measured twitch torque or M-wave. Participants then produced a maximal voluntary contraction (MVC) and subsequently reproduced 50% MVC, with a single pulse delivered during this contraction, in order to test whether there was any further increase in the M-wave. If there was a decrease in the M-wave, the current was increased again until the M-wave plateaued. Once a plateau in the M-wave was obtained, the current was increased to 130% of the plateau current to ensure supramaximality, and this intensity was used for stimulation during the subsequent tests. In all subsequent tests, doublet stimulation (two 200 µs pulses with 10 ms interpulse interval) was used.

Surface EMG

The EMG of the vastus lateralis and biceps femoris on the right leg were sampled using Ag/AgCl electrodes (32 x 32 mm; Nessler Medizintechnik, Innsbruck, Austria). Prior to attachment of the electrodes, the skin of the participants was shaved, abraded and then cleaned with an alcohol soaked cotton pad, in order to reduce impedance. Positioning of the electrodes was preceded by palpation of the muscle during a manually resisted contraction, to outline its length and belly. The electrodes were placed in a direction parallel to the alignment of the muscle fibres over the belly of the muscle. A reference

electrode was placed on prepared skin medial to the tibial tuberosity. The raw EMG signals were sampled at 1000 Hz, amplified (gain 1000; Biopac MP150, Biopac Systems Inc., California, USA) and band-pass filtered (10-500 Hz, Biopac MP150, Biopac Systems Inc., California, USA).

Contraction regime

The contraction regime of Bigland-Ritchie et al. (1986a) was used. This consisted of intermittent isometric contractions, alternating between 6 seconds of contraction and 4 seconds of rest (0.6 duty cycle), at a specified target torque (Figure 3.1). Such a regime was chosen in order to provide sufficient data for the quantification of complexity and fractal scaling (Lipsitz and Goldberger, 1992).

The dynamometer torque and target torque were presented on a display ~ 1m in front of the participant. Participants were verbally instructed to match their instantaneous torque with the target torque and were required to continue matching this torque for as much of the 6 second contraction as possible. A command of “push” was given to inform the participants to start the contractions, and a command of “stop” given to terminate the contractions. The tests were conducted to task failure, the point at which the participant failed to reach the target torque on three consecutive occasions, despite strong verbal encouragement. During the test, participants were required to perform an MVC (3 seconds long), accompanied by doublet stimulation of the femoral nerve during and at rest after the contraction, every sixth contraction (i.e. at the end of each minute). After the third missed contraction, participants were instructed to immediately produce an MVC, which was accompanied by peripheral nerve stimulation.

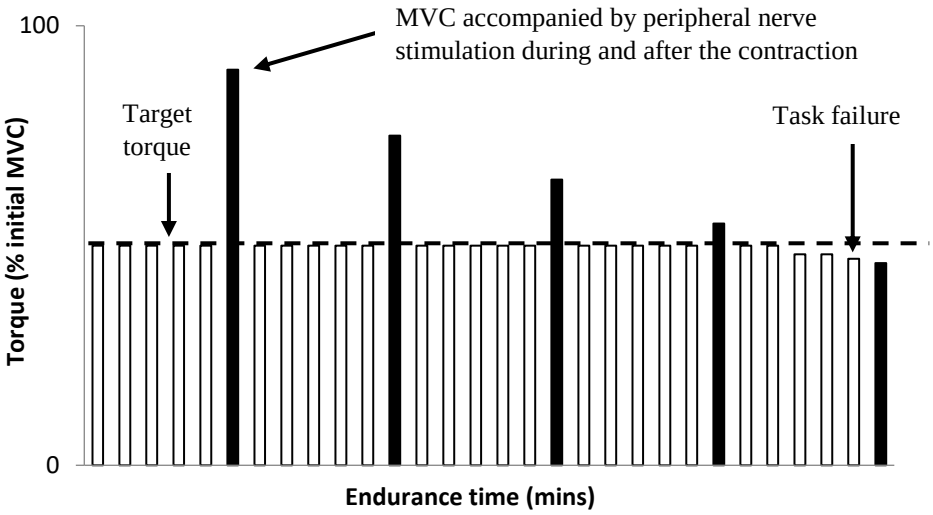

Figure 3.1. Contraction regime based on Bigland-Ritchie et al. (1986a).

Data acquisition and participant interface

Data were acquired from all peripheral devices through BNC cables connected to a Biopac MP150 (Biopac Systems Inc., California, USA) and CED Micro 1401-3 (Cambridge Electronic Design, Cambridge, UK) interfaced with a personal computer. All signals were sampled at 1000 Hz. The data were collected in Spike2 (Version 7; Cambridge Electronic Design, Cambridge, UK) A chart containing the instantaneous torque was projected onto a screen place in front of the participant. A scale consisting of a 1 mm thick purple line was superimposed on the torque chart and acted as a target, so that participants were able to match their instantaneous torque output (1 mm thick green line) to the target torque during the tests.

Torque and EMG

The mean and peak torques for each contraction in every test were determined. The mean torque was calculated based on the steadiest five seconds of each contraction, i.e. the five seconds with the least variation. The EMG output from the vastus lateralis for each contraction was filtered (10-500 Hz) and full-wave rectified with a gain of 1000. The average rectified EMG (arEMG) was then calculated and normalised by expressing the arEMG as a fraction of the arEMG obtained during an MVC from the fresh muscle preceding commencement of the tests.

Determination of task failure during submaximal tests

Due to the use of intermittent contractions, calculation of task failure during the submaximal tests was more problematic than defining failure during a sustained contraction. During the test itself, task failure was deemed to have occurred, as mentioned above, when the participant failed to reach the target torque on three consecutive occasions. The exact point of task failure was determined post-hoc. Due to the rise and fall of torque at the on- and off-set of each contraction, the mean torque for each successful contraction was inevitability less than the target torque. To establish the exact point of task failure the actual test torque had to be determined, and was defined as the mean torque recorded during the first five contractions of the test. Task failure was then deemed to have occurred when the mean torque recorded during three consecutive contractions was more than 5 N·m below the mean torque of the first five contractions, with the first of these contractions being considered the point of task failure (Figure 3.1).

Calculation of central and peripheral fatigue

Central and peripheral fatigue were calculated based on stimuli delivered during and after maximal contractions, performed at various points prior to, during and after the bouts of fatiguing contractions. The potentiated doublet torque, a measure of peripheral fatigue, was calculated as the peak torque attained following doublet stimuli at rest after a maximal contraction. Voluntary activation, a measure of central fatigue, was determined using the twitch interpolation technique (Belanger and McComas, 1981; Behm et al., 1996), and was calculated as:

$$Voluntary\ activation\ (\%) = 1 - \left(\frac{superimposed\ doublet}{potentiated\ doublet}\right) \times 100$$

Equation 1

where the superimposed doublet was that measured during the contraction of interest and the potentiated doublet was measured at rest 2 seconds after the contraction. The between day ICC of potentiated doublet torque, as measured from the six experimental visits of Chapter 5, was 0.97, indicating very high reliability; while the ICC for voluntary

activation was 0.70, indicating high reliability. These values were higher than those obtained by Todd et al. (2004; 0.87 and 0.60, respectively).

Quantification of variability

The amount of variability in the torque output of each contraction was examined using the standard deviation (SD), which provides a measure of the absolute amount of variability in a time-series, and the coefficient of variation (CV), which provides a measure of the SD of a time-series normalised to the mean of that time-series. The CV was calculated using the formula:

$$C_v = \left(\frac{\sigma}{\mu}\right) 100$$

Equation 2

where σ is the standard deviation and μ is the mean.

The between day ICC of SD, as measured from the contractions at 50% MVC in the discontinuous incremental tests performed in the six experimental visits of Chapter 5, was 0.91, while the ICC of CV was 0.74.

Quantification of complexity and fractal scaling

The temporal complexity of the torque (and in some cases EMG) outputs was examined using multiple time domain metrics derived from information theory. In information theory, a highly predictable, simple or regular output has low entropy because little information is conveyed. For example, the signal “HHHHH” would have low entropy in comparison to the signal “HELLO” since there is less predictability, and more information conveyed, in the letters of the second signal. Approximate entropy (ApEn) and sample entropy (SampEn) were used to determine the complexity of the torque outputs; while detrended fluctuation analysis (DFA) was used to estimate the noise colour and temporal fractal scaling of the torque outputs. All measures were taken from the steadiest five seconds of the relevant contraction; that is, the five seconds with the lowest SD.

Approximate entropy (ApEn)

ApEn, developed by Pincus (1991), provides a value between 0 and 2 reflecting the predictability of future values in a time-series based on previous values (Vaillancourt and Newell, 2003). Low values, approaching 0, are indicative of high regularity and low complexity; while high values, approaching 2, are indicative of low regularity and high complexity.

The ApEn statistic quantifies the negative natural logarithm of the conditional probability that a sequence of m data points is similar to other sequences of data points in the time series. Matching templates that remain similar (i.e. within the tolerance, r) are then counted, with the number of matches to the ith template of length m being designated B_i. The number of matches that remain similar for the $m+1^{th}$ point is then counted, with this number for the ith template being designated A_i. The conditional probability that the template including the $m+1^{th}$ data point matches, given the template length of m, is then calculated for each template match. The negative logarithm of the conditional probability is calculated for all templates and the results averaged (equation 3). If the data is highly ordered, then templates that are similar for m points are likely to also be similar for m+1 points. For such a data set, the conditional probability will therefore be close to 1, and the negative log and therefore the entropy will be close to zero. This will reflect low complexity and high predictability. Further details of the ApEn calculation are given by Pincus (1991) and in the appendix of Slifkin and Newell (1999).

$$ApEn\ (m, r, N) = \frac{1}{N-m} \sum_{i=1}^{N-m} log \frac{A_i}{B_i}$$

Equation 3

where: N is the number of data points in a time series; m is the length of the template; A_i is the number of matches to the ith template of length m+1 data points; and B_i is the number of matches of the ith template of length m data points.

The ApEn parameter settings used were m = 2 and r = 0.1 of the SD of the force. These parameters were chosen based on the use of similar parameters in previous studies (Slifkin and Newell, 1999; Vaillancourt and Newell, 2003) and based on the recommendations of

Pincus and Goldberger (1994) that r should be between 10 and 25% of the SD in order to produce good statistical validity, and of Forrest et al. (2014) that r should be 0.1 of the SD in order to distinguish between effort levels. The between day ICC of ApEn, as measured from the contractions at 50% MVC during discontinuous incremental tests performed in the six experimental visits of Chapter 5, was 0.80.

Sample entropy (SampEn)

The ApEn statistic has been criticised for its reliance on the matching of templates in a data series. To avoid the occurrence of ln(0), a template is allowed to match itself, meaning that each template occurs at least once, introducing a bias that has been shown to lead to inconsistent results from data series of similar lengths (Richman and Moorman, 2000). Sample entropy was therefore developed to correct this bias, by taking the logarithm after averaging (as shown in equation 4), and thus avoiding the introduction of self-matching. Further details of the SampEn calculation are given by Chen et al. (2005).

$$\mathrm{SampEn}(m, r, N) = -\log\left(\frac{\sum_{i=1}^{N-m} A_i}{\sum_{i=1}^{N-m} B_i}\right) = -\log\left(\frac{A}{B}\right)$$

Equation 4

As with ApEn, SampEn provides a value between 0 and 2, with low values being indicative of high regularity and low complexity, and high values being indicative of low regularity and high complexity. The SampEn parameter settings used were m = 2 and r = 0.1 of the SD of the force; the same parameters chosen for the calculation of ApEn. The between day ICC of SampEn, as measured from the contractions at 50% MVC from the discontinuous incremental tests performed in the six experimental visits of Chapter 5, was 0.75.

Detrended fluctuation analysis (DFA)

DFA, developed by Peng et al. (1994), relates to the colour of noise and detects long-range correlations in time-series, thus providing an indication of temporal fractal scaling. In the DFA algorithm, the time series is integrated, and the vertical characteristics scale of this integrated time series is measured. The integrated time series is then divided into

boxes of length n and a least-squares line is fitted, representing the trend in each box. The y co-ordinate of the straight-line segment of length n in the kth box is denoted by $y_n(k)$, and the integrated time series is detrended by subtracting the local trend in each box. For a given box size, n, the characteristic size of fluctuation for the integrated and detrended time series is given by:

$$F(n) = \sqrt{\frac{1}{N}\sum_{k=1}^{N}\left[y(k) - y_n(k)\right]^2}$$

Equation 5

This computation is then repeated over all time scales or box sizes (57 box sizes ranging from 1250 to 4 data points) to provide a relationship between box size and F(n). The slope of the log-log plot of n and F(n) determines the scaling parameter α. Further details of the DFA calculation are given by Stanley et al. (1999).

When $\alpha = 0.5$, this indicates that the time series is completely random, in the same way that white noise is completely random. For such a process, every value will be completely independent of the values of previous observations. When $\alpha \neq 0.5$, each observation is not completely independent and is correlated to some extent with the values of previous observations. When $0.5 < \alpha < 1$ power law correlations are present; when $\alpha > 1$, correlations exist but cease to be of power law form. 1/f or pink noise is indicated by $\alpha = 1$; Brownian noise is indicated by $\alpha = 1.5$. The between day ICC of DFA α, as measured from the contractions at 50% MVC during discontinuous incremental tests performed in the six experimental visits of Chapter 5, was 0.88.

Statistical analyses

Extraction of the relevant data from the raw files was performed in MATLAB (The MathWorks, Massachusetts, USA). Statistical analyses of data were then performed using the Statistical Package for Social Sciences (SPSS), with specific procedures detailed in each experimental chapter. Statistical significance was accepted at $P < 0.05$ level. Post-hoc power calculations were performed in G*Power (Heinrich-Heine Universität, Düsseldorf).

Chapter 4 – Study 1

Fatigue reduces the complexity of knee extensor torque fluctuations during maximal and submaximal intermittent isometric contractions

Introduction

The Review of Literature introduced the notion that physiological systems are characterised by an extraordinary inherent "complexity" (Chapter 2: 2.5 – Non-linear dynamics and complexity). This "complexity" refers to the fact that physiological systems, even under resting conditions, exhibit a great deal of intrinsic structural variability in their outputs (Goldberger et al., 1990; Lipsitz, 2002). Recent research has demonstrated that ageing and disease have the potential to perturb this structure, and reduce the complexity of physiological outputs, such as heart rate, respiration and gait (Pikkujämsä et al., 1999; Hausdorff et al., 1997; Peng et al., 2002). Such reductions in complexity are viewed as a loss of system control and have important implications for system adaptability (Lipsitz and Goldberger, 1992; Goldberger et al., 2002).

In neuromuscular physiology, it has been demonstrated that the complexity of torque output during isometric contractions is reduced with ageing (Vaillancourt & Newell, 2003; Vaillancourt et al., 2003; Sosnoff & Newell, 2008). It has also been demonstrated that the complexity of torque output is dependent on contraction intensity, with complexity (measured using approximate entropy [ApEn]) peaking at ~40% MVC and decreasing as torque requirements are increased (Slifkin and Newell, 1999). In terms of neuromuscular fatigue, an increase in the amount of variability in contractions has been frequently observed (Galganski et al., 1993; Slifkin and Newell, 2000; Contessa et al., 2009). Furthermore, prior exercise has been shown to increase signal complexity during single-leg stance and side-cutting movements (McGregor et al., 2011; Cortes et al., 2014). However, such movements represent the effect of fatigue on whole-body motion rather than muscular output per se. The effect of neuromuscular fatigue on the complexity of joint torque during isometric contractions is currently unknown.

The aim of the present study, therefore, was to determine whether the complexity of isometric knee extensor torque and EMG outputs are perturbed by neuromuscular fatigue,

and to test the following experimental hypotheses: 1) that for fresh muscle, knee extensor torque and EMG outputs would be more complex during submaximal contractions and exhibit more fractal-like (1/f; detrended fluctuation analysis [DFA] $\alpha = 1.00$) scaling; 2) that neuromuscular fatigue would result in a reduction in the complexity of the torque and EMG outputs from their fresh values during maximal and submaximal fatiguing isometric contractions; and 3) that this reduction in the complexity of the torque and EMG outputs would be accompanied by a change in fractal scaling from pink (DFA $\alpha = 1.00$) towards Brownian noise (DFA $\alpha = 1.50$).

Methods

Participants

Eleven healthy participants (10 male, 1 female; mean ± SD: age 25.0 ± 5.6 years; height 1.77 ± 0.05 m; body mass 78.6 ± 13.7 kg) provided written informed consent to participate in the study, which was approved by the ethics committee of the University of Kent, and which adhered to the Declaration of Helsinki. Participants were instructed to arrive at the laboratory rested (having performed no heavy exercise in the preceding 24 hours) and not to have consumed any food or caffeinated beverages in the three hours before arrival. Participants attended the laboratory at the same time of day (± 2 hours) during each visit.

Experimental design

Participants were required to visit the laboratory on three occasions over a two to three-week period, with a minimum of 48 hours between each visit. During their first visit, participants were familiarised with all testing equipment and procedures, and the settings for the dynamometer and stimulator were recorded. During their second visit, participants performed either a series of 30 intermittent maximal isometric contractions, interspersed with peripheral nerve stimulation (the “Maximal test”; see below) or a series of intermittent submaximal isometric contractions until task failure, interspersed with peripheral nerve stimulation (the “Submaximal test”; see below). The maximal and submaximal tests were presented in a randomised order. The methods used for the setup of the dynamometer, peripheral nerve stimulation and surface EMG are described in the General Methods (Chapter 3).

Protocol

All visits followed a similar pattern of data acquisition, beginning with the instrumentation of the participants and the (re-)establishment of the correct dynamometer seating position and supramaximal stimulation response. Participants then performed a series of brief (3 second) MVCs to establish their maximum torque. The contractions were separated by 60 seconds rest, and continued until three consecutive peak torques were within 5% of each other. Participants were given a countdown, followed by very strong verbal encouragement to maximise torque. The first MVC was used to establish the fresh maximal EMG signal, against which the subsequent EMG signals were normalised (Data analysis; see below). The second and third MVCs were performed with peripheral nerve stimulation; at ~1.5 seconds into the contraction, during a plateau in torque, a doublet was delivered and a further doublet delivered at rest 2 seconds after the contraction. The pulses superimposed on the contraction tested the maximality of the contraction and provided the fresh voluntary activation, while the pulses after the contraction established the fresh potentiated doublet response (see General Methods; Chapter 3). All subsequent contractions with peripheral nerve stimulation were conducted in this manner. Following the final MVC, participants rested for 10 minutes, and then performed either the maximal or submaximal test.

Maximal test

A five-minute all-out test (Figure 4.1), adapted from the test developed by Burnley (2009), was performed by all participants during their familiarisation visit and again during either their second or third visit to the laboratory. The test consisted of 30 intermittent MVCs, with a contraction regime of 6 seconds contraction and 4 seconds rest. Participants were given feedback on their previous MVCs and were encouraged to equal or exceed these values during the first 2-3 contractions. Participants were also informed to expect their torque to decrease by more than 50% during the test, but to still produce a maximal effort during each contraction despite this occurrence. During the test participants were very strongly encouraged to maximise and maintain their torque, but were not informed of the number of contractions remaining or the elapsed time. The test was ended after the 30th contraction was completed. During the test, peripheral nerve stimulation was delivered every sixth contraction (i.e. at the end of each minute).

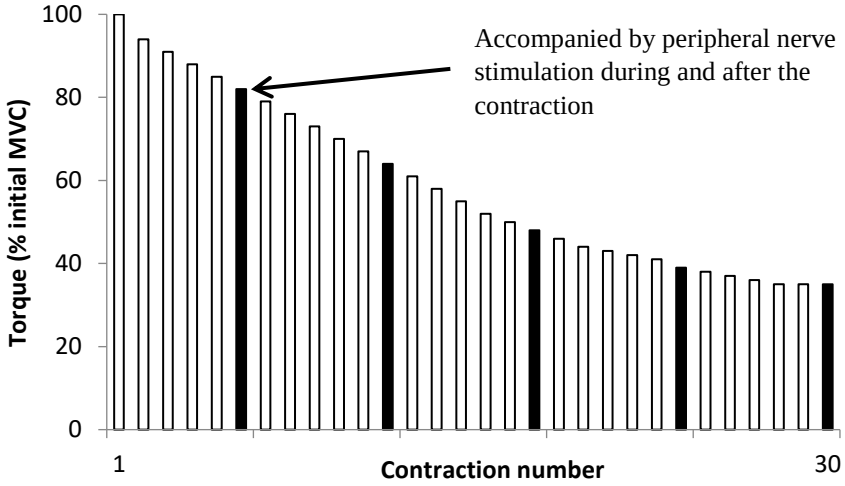

Figure 4.1. Contraction and stimulation protocol for the maximal test.

Submaximal test

A test at 40% MVC was performed by all participants during either their second or third visit to the laboratory. 40% MVC was determined from the highest instantaneous peak torque measured during the MVCs at the start of the visit. The same contraction regime as in the maximal test (6 seconds contraction, 4 seconds rest) was used. Participants were instructed to match their instantaneous torque with a target bar superimposed on the display in front of them and were required to continue matching this torque for as much of the 6 second contraction as possible. The test was conducted until task failure, the point at which participants failed to reach the target torque on three consecutive occasions, despite strong verbal encouragement. Participants were not informed of the elapsed time during the test, but were informed of each "missed" contraction. During the test, participants were required to perform an MVC, accompanied by peripheral nerve stimulation, every sixth contraction (i.e. at the end of each minute). After the third missed contraction, participants were instructed to immediately produce an MVC, which was accompanied by peripheral nerve stimulation. A graphical representation of the contraction regime is given in the General Methods (Chapter 3; Figure 3.1).

Data analysis

The data analysis focused on three specific areas: 1) basic measures of torque and EMG; 2) measures of central and peripheral fatigue; and 3) the variability and complexity of the torque and EMG outputs. All data were analysed using code written in MATLAB R2013a (The MathWorks, Massachusetts, USA).

Torque and EMG. The mean and peak torques, and vastus lateralis EMG, were calculated for each contraction, as described in the General Methods (Chapter 3). Due to the use of intermittent contractions, calculation of task failure was more problematic than defining failure during a sustained contraction. Details of how task failure was calculated are given in the General Methods (Chapter 3).

Central and peripheral fatigue. Measures of central and peripheral fatigue were calculated based on the peripheral nerve stimuli delivered during and after the MVCs performed pre-test, at the end of each minute during the fatiguing tests and at task failure. Further details are given in the General Methods (Chapter 3).

Variability and complexity. All measures of variability and complexity were calculated using the steadiest five seconds of each contraction. The amount of variability in the torque output of each contraction was measured using the standard deviation (SD) and coefficient of variation (CV). The temporal fluctuations were examined using multiple time domain analyses. Approximate entropy (ApEn) and sample entropy (SampEn) were used to determine the complexity of the torque and EMG outputs; while detrended fluctuation analysis (DFA) was used to estimate the temporal fractal scaling. Further details are given in the General Methods (Chapter 3).

Statistics

All data are presented as means ± SEM, and results were deemed statistically significant when $P < 0.05$. One-way ANOVAs with repeated measures were used to analyse the temporal profiles of MVC torque, arEMG, potentiated doublet torque and voluntary activation over the course of the tests. Contrasts between end-test values and all other time points were made using Bonferroni-adjusted 95% paired-samples confidence intervals. The variability and complexity of the torque and EMG outputs were analysed using averages from the first minute and final minute before task failure of each test. Two-way ANOVAs with repeated measures were used to test for differences between conditions and time points, and for a condition x time interaction. When main effects were observed Bonferroni-adjusted 95% confidence intervals were then used to determine specific differences.

Results

Preliminary measures

The peak instantaneous MVC torque recorded prior to the maximal test was 235.6 ± 15.5 N·m and the voluntary activation achieved was 89.8 ± 2.0%; while the peak instantaneous MVC torque recorded prior to the submaximal test was 237.7 ± 14.8 N·m and the voluntary activation achieved was 90.6 ± 1.8%. Neither of these values were significantly different (95% paired samples confidence intervals (CIs): peak MVC torque, –11.9, 16.2 N·m; voluntary activation, –1.4, 3.0%).

Torque and EMG

The mean data for peak and mean torque amongst all participants during each contraction for the maximal test are shown in Figure 4.2. Torque declined from a peak of ~96% MVC during the first contraction to an average of ~44% during the last three contractions. The mean data for the arEMG of the vastus lateralis for each contraction of the maximal test are shown in Figure 4.3. The arEMG, normalised to a fresh pre-test MVC, significantly decreased from 96.3 ± 2.5% (first contraction) to 73.8 ± 5.8% (last contraction; CIs –38.2, –9.7%).

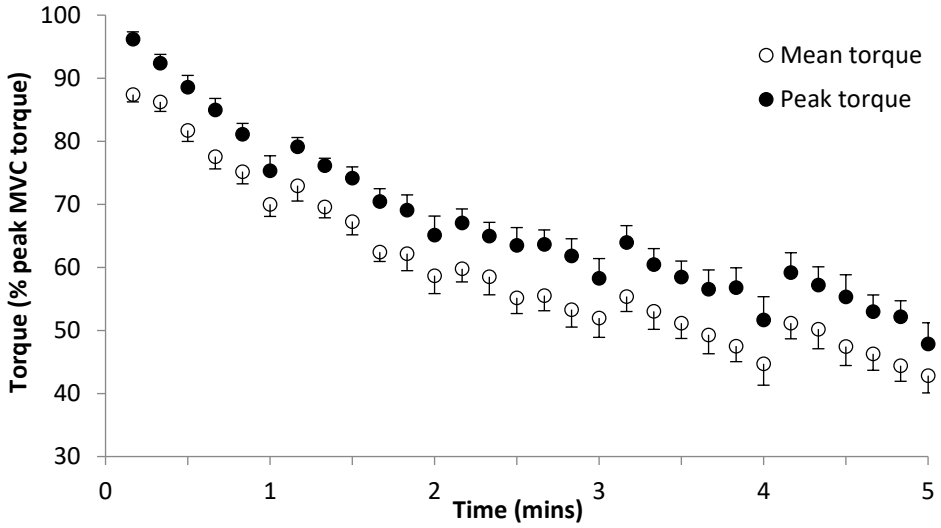

Figure 4.2. Group peak and mean torque (±SEM) for each contraction of the maximal test. Peak torque refers to the highest measured torque during each contraction; mean torque refers to the average torque calculated over the steadiest five seconds of the contraction. All contractions are normalised to a control MVC performed 10 minutes before the test commenced.

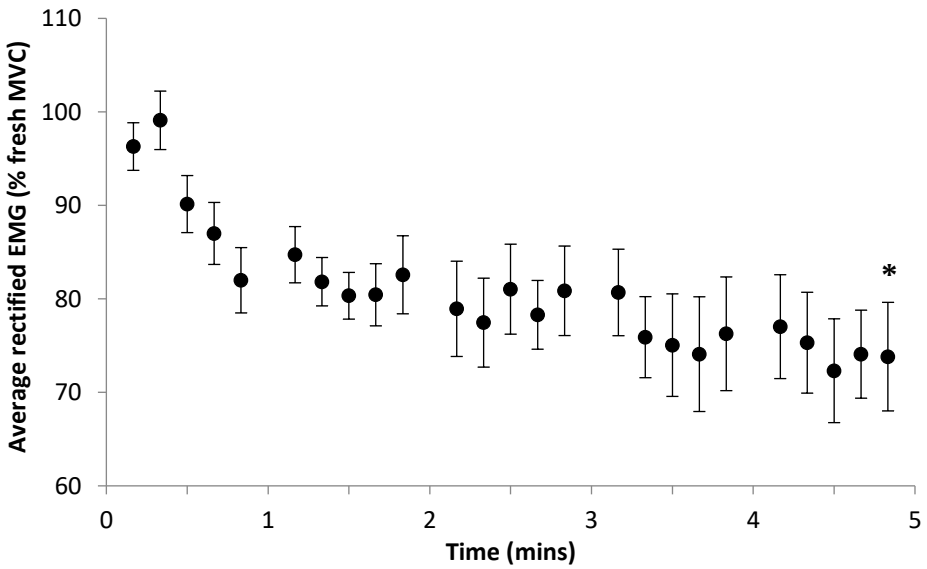

Figure 4.3. Group mean arEMG (±SEM) for each contraction of the maximal test. arEMG is normalised to a control MVC performed 10 minutes before the test commenced. * = Significantly different from the first contraction ($P < 0.05$).

The mean data for peak MVC torque and mean target torque during each common contraction and at task failure in all participants for the submaximal test are shown in Figure 4.4. The mean target torque, as calculated from the pre-test MVCs, was 95.1 ± 5.9 N·m, and task failure occurred when participants were not able to achieve this target torque, despite a maximal effort. The mean torque achieved during the MVC at task failure (87.4 ± 8.3 N·m) was not significantly different from the test target torque (CIs – 23.9, 8.4 N·m), indicating that participants required a maximal effort to achieve the target torque. The mean time to task failure was 17.3 ± 4.3 minutes, with a range of 8.2 to 58.0 minutes. The mean data for the arEMG of the vastus lateralis for each common contraction and task failure for the submaximal test are shown in Figure 4.5. The arEMG, normalised to a fresh pre-test MVC, significantly increased from 44.4 ± 5.2% (first contraction) to 68.5 ± 5.6% (last contraction; CIs 10.7, 37.5%).

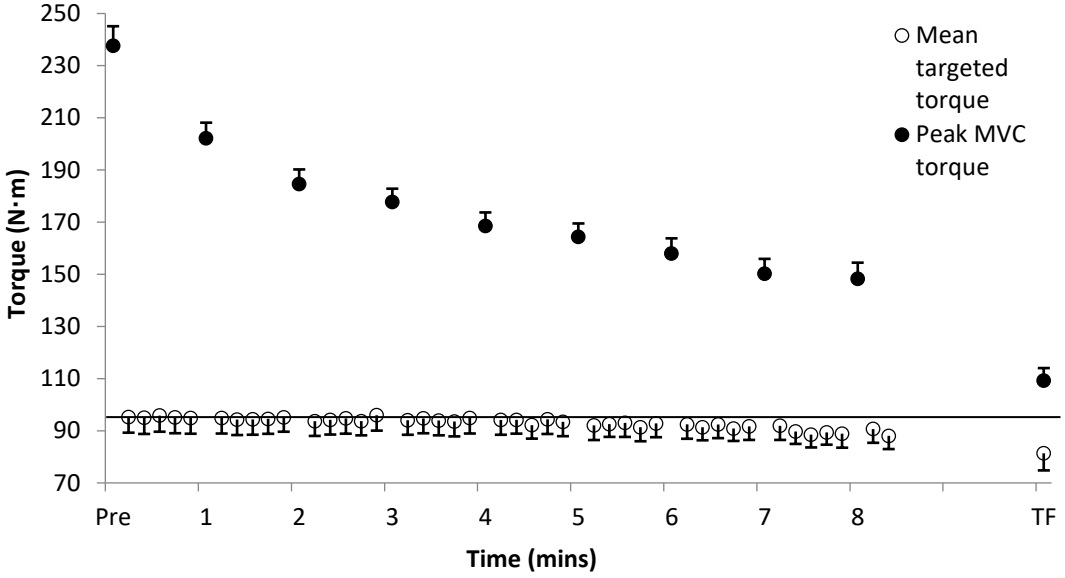

Figure 4.4. Group mean peak MVC torque and mean targeted torque (±SEM) for each common contraction and task failure during the submaximal test.

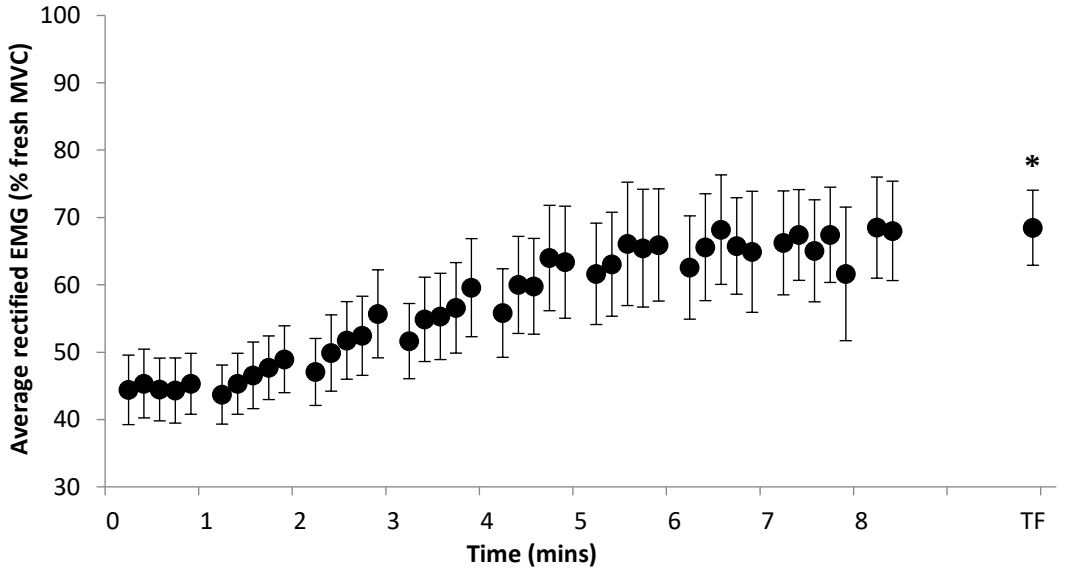

Figure 4.5. Group mean arEMG (±SEM) for each contraction of the submaximal test. arEMG is normalised to a control MVC performed 10 minutes before the test commenced. * = Significantly different from the first contraction ($P < 0.05$).

Peripheral and central fatigue

The potentiated doublet torque and voluntary activation decreased as both tests progressed, indicating the presence of peripheral and central fatigue, respectively. During the maximal test, the potentiated doublet torque decreased from 105.3 ± 7.1 N·m prior to commencement of the test to 62.4 ± 8.6 at the end of the test ($F_{5,10} = 69.98$, $P < 0.001$; Figure 4.6); while voluntary activation fell from 89.8 ± 2.0% prior to the commencement of the test to 68.6 ± 5.6% at the end of the test ($F_{5,10} = 3.74$, $P = 0.01$; Figure 4.7). During the submaximal test, the potentiated doublet torque decreased from 112.4 ± 7.9 N·m prior

to commencement of the test to 70.3 ± 10.0 N·m at task failure ($F_{9,10}$ = 18.65, P < 0.001; Figure 4.6); while voluntary activation fell from 90.6 ± 1.8% prior to the commencement of the test to 66.2 ± 4.7% at task failure ($F_{9,10}$ = 11.91, P = 0.004; Figure 4.7).

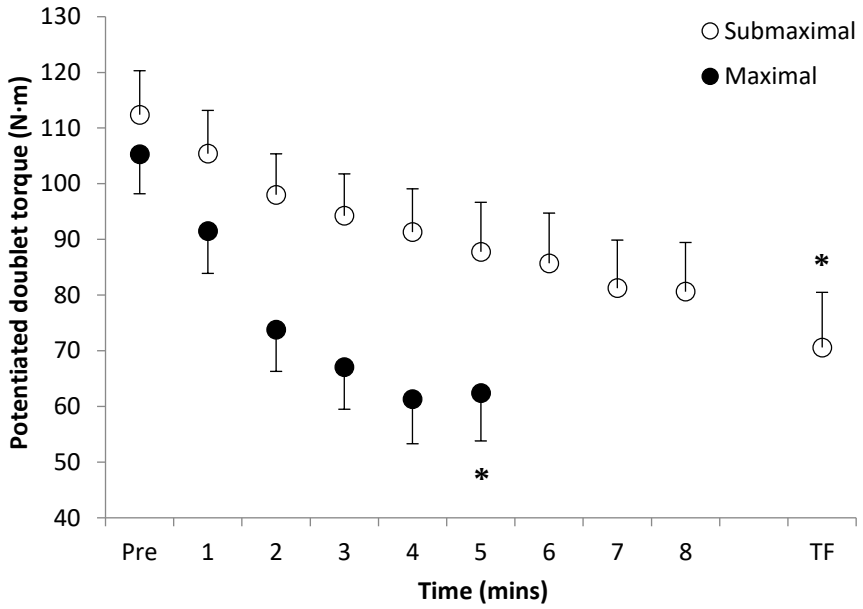

Figure 4.6. Group mean potentiated doublet torque (± SEM) for the maximal and submaximal tests. * = Significantly different from the pre-test value (P < 0.05).

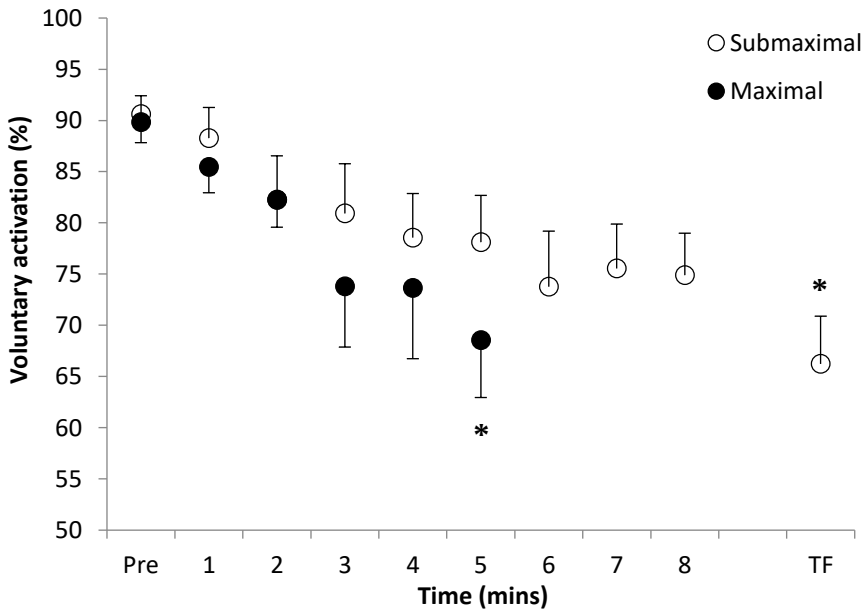

Figure 4.7. Group mean voluntary activation (± SEM) for the maximal and submaximal tests. * = Significantly different from the pre-test value (P < 0.05).

Variability and complexity

The metrics associated with the amount of variability (SD and CV) in the torque output are shown in Figure 4.8A AND B. The SD of the torque fluctuations was higher in the maximal test ($F_{1,10}$ = 14.59, P = 0.003) and increased over time during the submaximal test ($F_{1,10}$ = 42.55, P < 0.001). The CV significantly increased over time in both tests ($F_{1,10}$ = 95.7, P < 0.001). The SD was significantly higher at the start of the maximal test (8.4 ± 1.3 vs. 2.6 ± 0.3 N·m; CIs 2.6, 9.2 N·m). During the maximal test, the SD did not increase (CIs –0.4, 3.8 N·m), in contrast to the increased CV (CIs 2.4, 6.0%). During the submaximal test, there were significant increases in both the SD (CIs 2.9, 6.1 N·m) and CV (CIs 3.5, 7.2%).

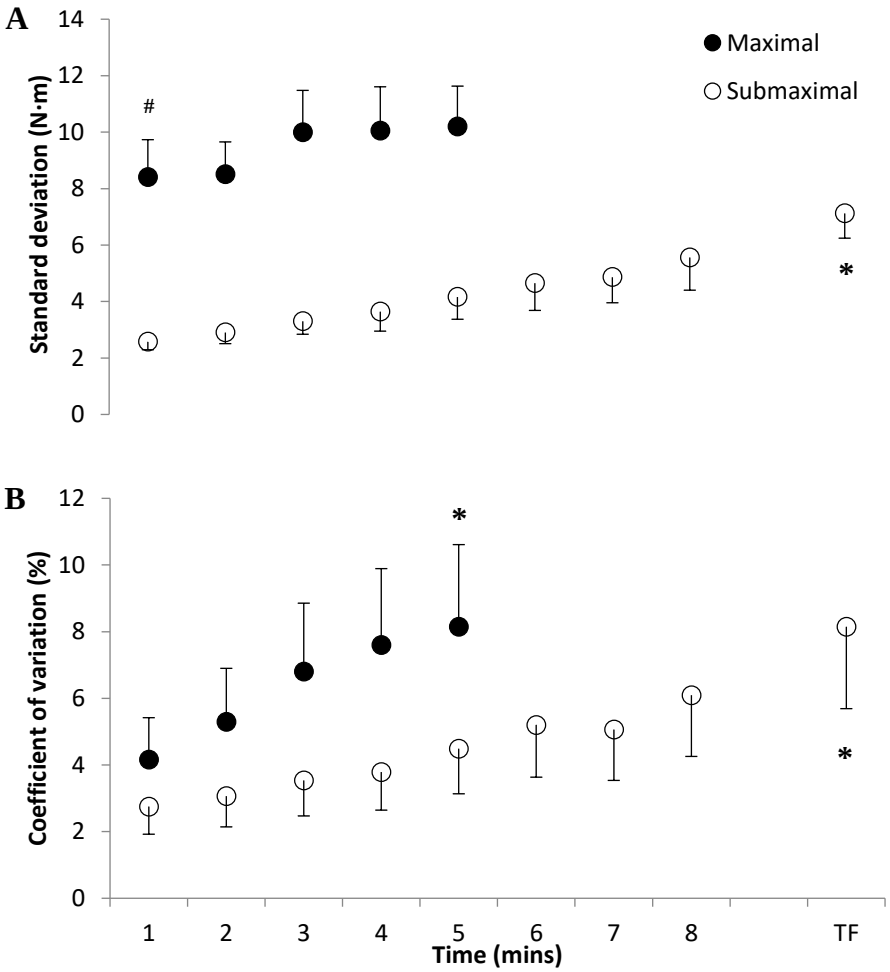

Figure 4.8. Mean standard deviation (± SEM) (A) and mean coefficient of variation (± SEM) (B) for each minute of the maximal and submaximal tests. * = Significantly different from first minute; # = Significantly different from submaximal value (P < 0.05).

In terms of the complexity of the torque output, maximal contractions were associated with smaller ApEn ($F_{1,10}$ = 34.79, P < 0.001) and SampEn values ($F_{1,10}$ = 33.50, P < 0.001) and a larger DFA α scaling exponent ($F_{1,10}$ = 37.22, P < 0.001) than submaximal contractions. For fresh muscle, there was significantly lower complexity in the torque output during the maximal test compared to the submaximal test, as evidenced by significantly lower ApEn (0.15 ± 0.02 vs. 0.65 ± 0.09; CIs –0.69, –0.30) and SampEn (0.14 ± 0.02 vs. 0.62 ± 0.09; CIs –0.68, –0.28); and a significantly higher DFA α scaling exponent (1.55 ± 0.03 vs. 1.35 ± 0.04; CIs 0.13, 0.27). Representative raw torque outputs from the initial contractions of the maximal and submaximal tests are shown in Figure 4.9A and B, respectively.

In both tests, complexity declined as a function of time; ApEn ($F_{1,10}$ = 73.86, P < 0.001) and SampEn were reduced ($F_{1,10}$ = 58.93, P < 0.001) and the DFA α scaling exponent increased ($F_{1,10}$ = 48.88, P < 0.001). In the maximal test, ApEn significantly decreased from 0.15 ± 0.02 (first minute) to 0.10 ± 0.02 (last minute; CIs –0.08, –0.01; Figure 4.10A), while SampEn significantly decreased from 0.14 ± 0.02 (first minute) to 0.10 ± 0.023 (last minute; CIs –0.08, –0.01; Figure 4.10B). The DFA α scaling exponent significantly increased from 1.55 ± 0.03 (first minute) to 1.63 ± 0.02 (last minute; CIs 0.04, 0.13; Figure 4.10C). Representative raw torque outputs from the beginning and end of the maximal test are shown in Figure 4.9A.

Over the course of the submaximal test, ApEn significantly decreased from 0.65 ± 0.09 (first minute) to 0.27 ± 0.04 (last minute; CIs –0.55, –0.28; Figure 4.10A) and SampEn significantly decreased from 0.62 ± 0.09 (first minute) to 0.22 ± 0.04 (last minute; CIs –0.54, –0.25; Figure 4.10B). The DFA α scaling exponent significantly increased from 1.35 ± 0.04 (first minute) to 1.55 ± 0.03 (last minute; CIs 0.12, 0.28; Figure 4.10C). Representative raw torque outputs from the beginning and end of the submaximal test are shown in Figure 4.9 B.

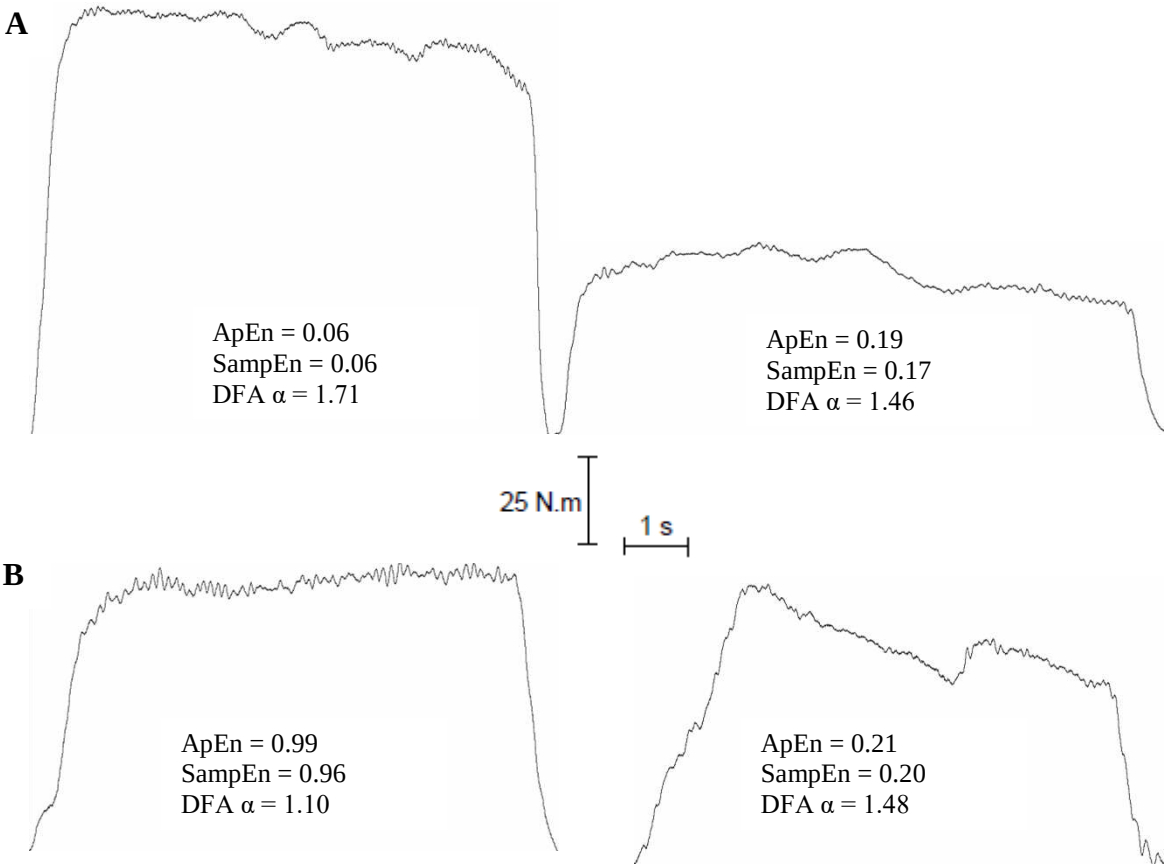

Figure 4.9. Raw torque outputs from representative contractions from the beginning (left) and end (right) of the maximal test (A) and the beginning (left) and end (right) of the submaximal test (B).

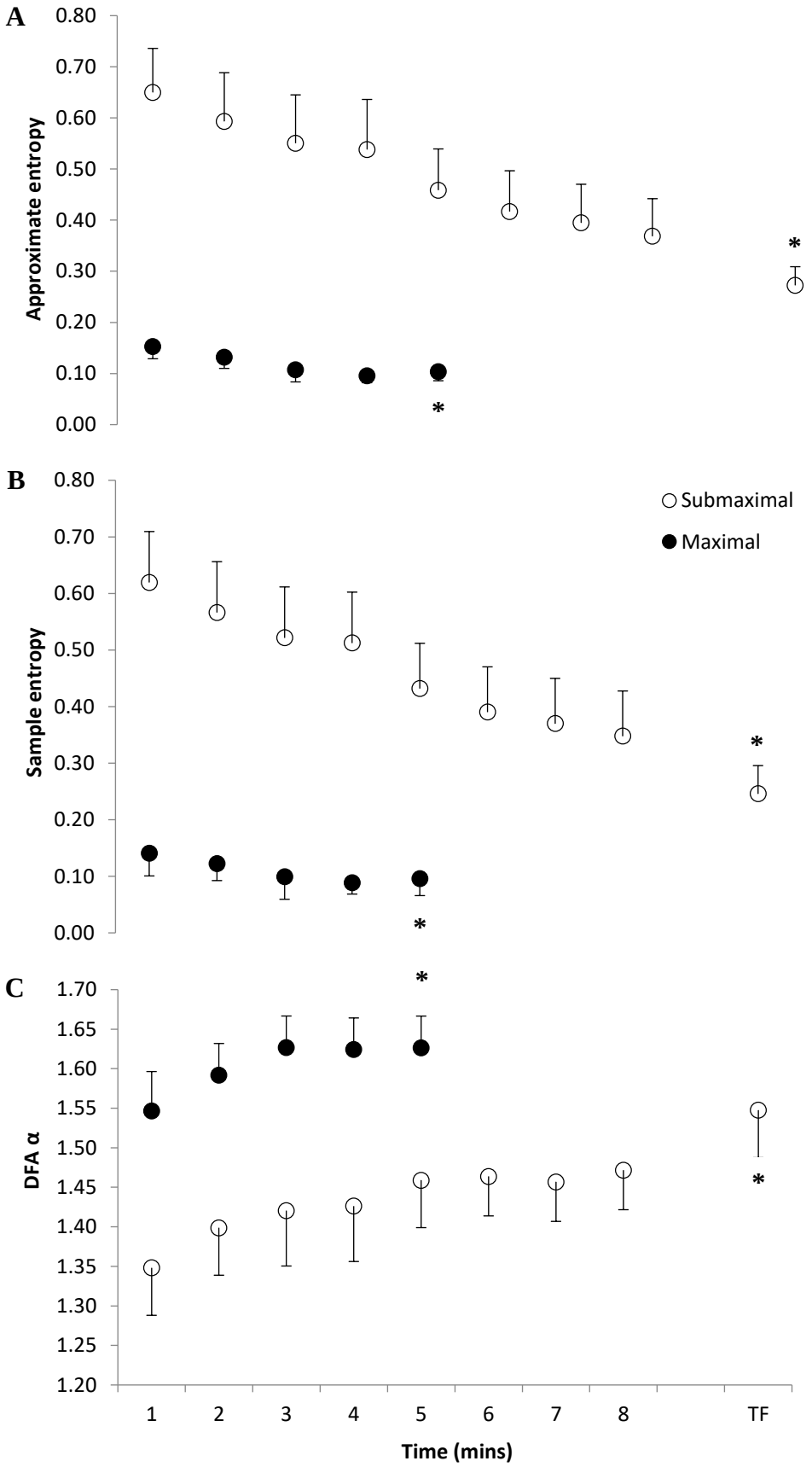

Figure 4.10. Changes in torque ApEn (A), SampEn (B) and DFA α scaling exponent (C) over the course of the maximal and submaximal tests. All values are the mean (± SEM) for all participants for each minute. * = Significantly different from value at one minute ($P < 0.05$).

In terms of the complexity of the vastus lateralis EMG output, maximal contractions were associated with smaller ApEn ($F_{1,10}$ = 22.93, P = 0.001) and SampEn ($F_{1,10}$ = 16.59, P = 0.002) values. For fresh muscle, there was lower complexity in the vastus lateralis EMG output during the maximal test, as evidenced by significantly lower ApEn (1.19 ± 0.04 vs. 1.38 ± 0.04; CIs –0.34, –0.03) and SampEn (1.19 ± 0.03 vs. 1.37 ± 0.05; CIs –0.34, –0.01).

In both tests, complexity changed as a function of time (SampEn, $F_{1,10}$ = 16.59, P = 0.002; and DFA, $F_{1,10}$ = 52.21, P < 0.001). During the maximal test SampEn significantly decreased (first minute 1.19 ± 0.03, last minute 1.05 ± 0.04; CIs –0.25, –0.04); and the DFA α scaling exponent significantly increased (first minute 0.32 ± 0.01, last minute 0.36 ± 0.01; CIs 0.07, 0.02). During the submaximal test, ApEn (first minute 1.38 ± 0.03, last minute 1.24 ± 0.05; CIs –0.24, –0.03) and SampEn (first minute 1.37 ± 0.05, last minute 1.21 ± 0.06; CIs –0.28, –0.03) both significantly decreased, and the DFA α scaling exponent significantly increased (first minute 0.32 ± 0.01, last minute 0.37 ± 0.01; CIs 0.07, 0.02).

Discussion

The aim of the present study was to investigate the complexity and fractal scaling of motor output during fatiguing contractions. The major novel finding was that neuromuscular fatigue was associated with reduced complexity in motor output (torque), regardless of whether the muscle was driven maximally or submaximally, during repeated intermittent isometric contractions. As the complexity of the torque output decreased during fatiguing contractions, the fluctuations became increasingly Brownian (DFA α = 1.50). The results also demonstrated that when the muscle was driven submaximally in the absence of fatigue, the fluctuations in torque were substantially more complex than when the muscle was driven maximally. Though these fluctuations were not consistently 1/f noise (DFA α = 1.00), the fresh torque signal nevertheless possessed long-range correlations, and this fractal-like scaling of torque broke down as fatigue progressed.

Complexity during "fresh" maximal and submaximal contractions

It has been suggested that an inverted-U shaped relationship exists between contraction intensity and the complexity of torque output, with complexity peaking at ~40% MVC (Slifkin and Newell, 1999; Svendsen and Madeleine, 2010). Such previous research informed the first hypothesis of the present study: that torque output during submaximal contractions would be more complex than during maximal contractions. The data obtained support this hypothesis, with ApEn and SampEn both being significantly greater during the initial contractions of the submaximal test, indicating a more complex output at 40% MVC (Figures 4.9 and 4.10). Furthermore, the DFA α scaling exponent was significantly lower during the initial contractions of the submaximal test (Figures 4.9 and 4.10). The greater complexity observed during fresh submaximal contractions is representative of a more adaptable torque output, compared to the more periodic output of maximal contractions. Slifkin and Newell (1999) reasoned that complexity peaked at ~40% MVC because, at least for muscle of the fingers, torque could be modulated by either increasing the recruitment of motor units or increasing discharge rates; whereas below that point modulation would typically occur through increased recruitment alone, and above that point through increased discharge rates alone. 40% MVC thus represented the point of maximum information transfer and system adaptability. However, whether the inverted-U shaped relationship holds for the knee extensors is unclear. Nevertheless, the present results confirm that submaximal contractions are more complex than maximal contractions for the knee extensors.

Submaximal contractions rarely achieve tetanic rates of discharge (Enoka and Fuglevand, 2001), meaning the force exerted by a muscle will exhibit fluctuations due to the submaximal activation of motor units (Taylor et al., 2003). As contraction intensity increases, a higher discharge rate is required (Bigland and Lippold, 1954; Moritz et al., 2005). Such higher discharge rates are more likely to result in an output approximating a fused tetanus (Buller and Lewis, 1965), which may result in a smoother, more periodic torque output with fewer fluctuations, similar to that observed during the maximal contractions (Figure 4.9). Interestingly, this greater complexity during fresh submaximal contractions was in contrast to the amount of variability, which was significantly lower for submaximal contractions (Figure 4.8). This reaffirms that variability metrics assess different properties of the signal to complexity and fractal scaling statistics; with the

former characterising the amplitude of torque fluctuations, and the latter quantifying their irregular temporal structure (Lipsitz and Goldberger, 1992).

Fatigue-induced reduction in torque complexity

During the submaximal test, MVC torque gradually declined (Figure 4.4), central and peripheral fatigue developed progressively (Figures 4.6 and 4.7) and the arEMG gradually increased (Figure 4.5). These responses are typical of exhaustive submaximal contractions, in which the neuromuscular system compensates for a loss of peripheral output by increasing central motor drive at the expense of a loss of maximal torque-generating capacity (Bigland-Ritchie et al., 1985; Bigland-Ritchie et al., 1986a; Burnley et al., 2012). More importantly, it was also observed that the complexity of submaximal torque output, measured using ApEn, SampEn and DFA α, progressively decreased as fatigue developed (Figure 4.10). Such reductions in complexity indicate a torque output that became smoother and more periodic as fatigue developed and as exhaustion approached. This decrease in complexity was in contrast to the amount of variability, which significantly increased with the progression of the test (Figure 4.8). Such an increase in variability is a consistent observation across previous work, and is attributable to various properties of motor unit recruitment and firing frequency (Hunter & Enoka, 2003; Missenard et al., 2008). The decrease in complexity as exhaustion approaches could be attributed to the fact that neuromuscular fatigue functionally reduces MVC torque, thus systematically increasing the relative demand of a submaximal task. Such an explanation is in line with the inverted-U shaped hypothesis (Slifkin and Newell, 1999), in which complexity decreases as the intensity of contractions increases from ~40% MVC to the maximum attainable torque. In this respect, as fatigue develops and the relative task demand increases, the increased torque variability increases targeting error, whilst changes in motor output (e.g. increased motor unit pool firing rate, but reduced muscle fibre force output) likely result in a greater structural regularity, manifest as reduced complexity.

The DFA α analysis demonstrated that the reduced complexity was also associated with a change in the fractal scaling of torque as fatigue developed (Figure 4.10). It was hypothesised that fresh submaximal contractions at 40% MVC would produce pink (1/f) noise, and that this output would become more Brownian as fatigue developed. The DFA α scaling exponent was ~1.35 for fresh muscle at 40% MVC and rose to ~1.55 at task

failure. Though the value obtained from fresh muscle represents an output containing long-range correlations, it cannot, in the strictest sense, be described as 1/f noise (α = 1.00); on the other hand, the value obtained at task failure clearly approximates Brownian noise (α = 1.50). Although a change from 1/f to Brownian noise was not observed, neuromuscular fatigue was, nonetheless, associated with a change in the fractal scaling of torque. Such a change in fractal scaling, and the loss of complexity, have not previously been assessed in the output of fatiguing muscle, but are a common characteristic of motor output in ageing (Vaillancourt and Newell, 2003; Vaillancourt et al., 2003; Challis, 2006; Sosnoff and Newell, 2008).

During the maximal test, MVC torque started to decline immediately (Figure 4.2), central and peripheral fatigue developed rapidly (Figures 4.6 and 4.7) and the arEMG progressively decreased (Figure 4.3). Despite the lower complexity of these contractions in comparison to the submaximal contractions, a reduction in complexity with the development of fatigue was still evident, as ApEn and SampEn decreased, and the DFA α scaling exponent increased with the progression of the test (Figure 4.10). These results again suggest that the torque output became smoother as the test progressed and as fatigue developed, although as with the submaximal contractions, the amount of variability increased (Figure 4.8). While the increased magnitude of variability in the submaximal test can be attributed to properties of motor unit recruitment and firing frequency (Hunter and Enoka, 2003; Contessa et al., 2009), the increase during the maximal test is likely due to an inability to maintain a stable plateau in torque during an MVC for 6 seconds, rather than being variance around a stable mean torque. This suggests that the losses of complexity in the submaximal and maximal tests have different causes; with the loss in the submaximal test potentially reflecting a loss of motor control in achieving a required torque, and the loss in the maximal test directly relating to the loss of torque generating capacity.

Though the present study is the first to demonstrate reduced complexity of torque output with neuromuscular fatigue, previous studies have investigated changes in complexity with fatigue for other physiological variables. Increases in the complexity of gait (McGregor et al., 2009), single-legged stance (McGregor et al., 2011) and ground reaction force (Cortes et al., 2014) have all been observed with neuromuscular fatigue, though these represent the effect of fatigue on whole-body motion rather than muscular

output. Of greater relevance to the present study, Cashaback et al. (2013) observed a decrease in the complexity of biceps brachii EMG output during continuous isometric contractions, as measured using multiscale entropy. The results obtained from the vastus lateralis EMG in the present study provide partial support for such a finding (see Fatigue induced changes in EMG complexity).

The present work extends the "loss of complexity" hypothesis in ageing (Lipsitz and Goldberger, 1992) to neuromuscular fatigue. This hypothesis states that healthy physiological systems produce inherently complex temporal outputs, which allow the system to explore a variety of control solutions, while the age-related loss of complexity reduces this exploratory freedom (Peng et al., 2009; Stergiou and Decker, 2011). The loss of complexity observed in the present study signifies a reduced adaptability of torque output and could indicate greater system isolation, wherein system components share less information and/or become less responsive to it (Pincus, 1994). Such a reduction in torque complexity, and motor output adaptability, significantly increases the probability of a mismatch between torque demand and its instantaneous output, thus increasing the probability of task failure. In this respect, task failure, which often occurs at apparently submaximal levels of muscle activity (Dideriksen et al., 2011) and with a significant torque "reserve" (Hunter et al., 2004; Klass et al., 2008), may be a failure of the neuromuscular system to adapt to increasing task demands, rather than a failure of neuromuscular capacity.

Physiological bases for changes in neuromuscular system behaviour

As with the exact mechanisms of neuromuscular fatigue, the mechanism(s) behind this fatigue-induced loss of complexity is, as yet, unclear. However, a loss of torque complexity must, by definition, be the result of mechanisms acting directly on the motor unit pool. The presence of both central and peripheral fatigue does not allow the identification of a mechanism, but it is possible that perturbations throughout the motor pathway could contribute to the lower torque complexity. Centrally, factors such as reductions in discharge rates (Bigland-Ritchie et al., 1983b; Garland et al., 1997), disfacilitation/inhibition of α-motoneurons (Garland, 1991; Macefield et al., 1991) or suboptimal descending drive (Gandevia et al., 1996; Smith et al., 2007) may be involved. Peripherally, factors such as metabolic changes affecting action potential velocity

(Fuglevand et al., 1993b) or metabolite-mediated derangements in excitation-contraction coupling (Allen et al., 2008a) may play a role.

Conclusions drawn from previous studies on variability and on changes in complexity with ageing may provide some insight into the causes of the reduced complexity observed in the present study. Firstly, Slifkin and Newell (1999) concluded that torque complexity is positively correlated with the amount of information transmitted. Such a conclusion, applied in the context of neuromuscular fatigue, would suggest that it is central factors, related to recruitment and discharge rates, which cause the reduction in complexity. In support of this, Jones et al. (2002) demonstrated that the signal-dependent noise observed during contractions is not a result of peripheral neuromuscular noise, and concluded that the mechanics of muscular contraction do not contribute to the variability of torque output. Secondly, Missenard et al. (2008) hypothesised that changes in variability with fatigue could arise from variability in the contractile machinery due to accumulation of metabolites, changes in motor command due to increased relative task difficulty, or variability of motoneuron firing and changes in inter-spike interval. And thirdly, Taylor et al. (2003) proposed that motor unit synchronisation is a likely contributor to the time and frequency domain features of torque output. It has since been postulated that the increased regularity of loaded postural tremor with ageing is due to enhanced motor unit synchronisation (Sturman et al., 2005), and has also been demonstrated that motor unit synchronisation is enhanced following fatiguing exercise and is associated with an increase in the magnitude of force fluctuations (Yao et al., 2000; Boonstra et al., 2008; Holtermann et al., 2009; Castronovo et al., 2015). Whatever the cause of the reduction in complexity and breakdown in fractal scaling of torque output with neuromuscular fatigue, there is likely to be some degree of task dependence, due to the different fatiguing mechanisms acting and predominating during maximal and submaximal contractions.

Fatigue-induced changes in EMG complexity

The data obtained from the vastus lateralis EMG provides partial support for the three experimental hypotheses and the findings from the torque output. In line with the first hypothesis, maximal contractions were less complex than submaximal contractions, with both ApEn and SampEn being significantly greater during the submaximal test. This is in contrast to previous findings, which seem to indicate greater complexity with greater EMG amplitude and contraction intensity (Cashaback et al., 2013; Beretta-Piccoli et al.,

2015). The relatively high ApEn and SampEn values at the start of both tests indicate a complex output; while the very low DFA α scaling exponents ($\alpha < 0.50$) indicate an output that exhibits anti-correlations and is akin to white noise (Hardstone et al., 2012).

In support of the second hypothesis, it was observed that complexity was reduced with neuromuscular fatigue, as evidenced by decreased ApEn and SampEn values. Such a finding is in line with that of Cashaback et al. (2013), who observed decreased multiscale entropy for the EMG output of the biceps brachii during fatiguing contractions at 40, 70 and 100% MVC. Furthermore, the fractal dimension of vastus lateralis EMG has been demonstrated to decrease during sustained contractions at 60% (Beretta-Piccoli et al., 2015) and 70% MVC (Boccia et al., 2015); a change indicative of the signal becoming more periodic (Mesin et al., 2009). Previously the ApEn of the discharge rate of single motor units has been shown to linearly increase with increases in motor unit discharge rate (Vaillancourt et al., 2002; Vaillancourt et al., 2003). As motor unit discharge rate typically decreases with neuromuscular fatigue (Bigland-Ritchie et al., 1983b; Kuchinad et al., 2004), this could explain the observed decrease in EMG complexity in the present and previous studies. The EMG complexity data does not provide support for the third hypothesis, relating to changes in fractal scaling. The increases in the DFA α scaling exponent in both conditions cannot be viewed as a change in fractal scaling, as the low values indicate no fractal scaling and long-range correlations were present to begin with, and despite the increases with the progression of the tests, the values at task end/failure were still anti-correlated and indicative of white noise (DFA $\alpha < 0.50$). This discrepancy between the entropy statistics and DFA α could be a reflection of the relative difficulty in analysing the complexity of EMG in comparison to torque. It may be the intramuscular EMG or recordings from individual motor units may be more suitable to characterise the complexity of EMG output.

Conclusion

In summary, the performance of fatiguing maximal and submaximal intermittent isometric contractions of the knee extensors resulted in a progressive decrease in voluntary torque that was accompanied by significant reductions in the structural complexity of torque output. These reductions in the temporal fluctuations of the torque output provide the first evidence that neuromuscular fatigue reduces the complexity of

motor output and shifts it from noise containing fractal characteristics to Brownian noise. This evidence adds to the small, but growing body of literature indicating that neuromuscular fatigue perturbs the complexity of physiological systems and their outputs. The reduced complexity observed suggests that the impact of neuromuscular fatigue is not limited to a decrease in force generating capacity, but that it may also affect the adaptability of the neuromuscular system to external perturbations.

Chapter 5 – Study 2

Fatigue-induced loss of knee extensor torque complexity during isometric contractions occurs exclusively above the critical torque

Introduction

The first study of this thesis (Chapter 4) demonstrated that fresh submaximal contractions exhibit greater complexity than maximal contractions and, importantly, that neuromuscular fatigue results in a reduction in the complexity of torque output, which is accompanied by a shift towards increasingly Brownian noise (DFA $\alpha = 1.50$), during repeated isometric knee extensions. The results presented within Chapter 4 indicated that this reduction in complexity and breakdown in fractal scaling occurred for both maximal and submaximal contractions. However, only a single submaximal intensity was investigated, thus the exact nature of the relationship between contraction intensity and complexity, and the intensity dependence of changes in the complexity and fractal scaling of torque output with neuromuscular fatigue are still unknown. This latter point is of particular importance, given that it is well known that the mechanisms of neuromuscular fatigue are intensity dependent (Place et al., 2009).

It has long been established that the relationship between power or torque and the time to task failure is hyperbolic (Monod and Scherrer, 1965; Poole et al., 1988; Burnley et al., 2012). The asymptote of this relationship, the critical power (CP) or critical torque (CT), was introduced in the Review of Literature (Chapter 2: 2.4 – Critical Power), and was originally defined as the maximum rate of work that a muscle or muscle group can keep up for a very long time without fatigue (Monod and Scherrer, 1965). Based on such a definition, contractions performed below the CT should, theoretically, be fatigueless and task failure should not occur (Monod and Scherrer, 1965; Moritani et al., 1981), while contractions performed above the CT will inexorably lead to task failure, with this occurring sooner the further the exercise intensity is above the CT (Jones et al., 2008; Burnley, 2009).

It is now, however, recognised that the CT does not represent a threshold for fatiguing and fatigueless contractions, but rather demarcates an intensity above which there is a

significant and progressive loss of muscle metabolic homeostasis (Jones et al., 2008; Burnley et al., 2010; Vanhatalo et al., 2010) and above which a steady state cannot be attained (Poole et al., 1988; Jones et al., 2008), and represents a critical threshold for the development of peripheral fatigue (Burnley et al., 2012). Given these distinct physiological responses above and below the CT, it is reasonable to suggest that changes in the complexity and fractal scaling of torque may also depend on whether contractions are performed above or below the CT.

The purpose of the present study was, therefore, to investigate changes in the complexity of torque output in relation to the CT during intermittent isometric contractions of the knee extensors. An additional aim was to characterise the shape of the relationship between contraction intensity and complexity across the whole range of knee extensor joint torques when fresh. The specific experimental hypotheses tested were: 1) that below the CT, fatigue development would be limited; 2) that below the CT, neither complexity nor fractal scaling would not change; 3) that above the CT, central and peripheral fatigue would develop; and 4) that above the CT, complexity would be progressively reduced, as measured by decreased approximate entropy (ApEn) and sample entropy (SampEn), and increased detrended fluctuation analysis (DFA) α.

Methods

Participants

Nine healthy participants (5 male, 4 female; mean ± SD: age 25.3 ± 5.8 years; height 1.74 ± 0.10 m; body mass 69.2 ± 10.4 kg) provided written informed consent to participate in the study, which was approved by the ethics committee of the University of Kent, and which adhered to the Declaration of Helsinki. Participants were instructed to arrive at the laboratory rested (having performed no heavy exercise in the preceding 24 hours) and not to have consumed any food or caffeinated beverages in the three hours before arrival. Participants attended the laboratory at the same time of day (± 2 hours) during each visit.

Experimental design

Participants were required to visit the laboratory on seven occasions over a four to six-week period, with a minimum of 48 hours between visits. During their first visit, participants were familiarised with all testing equipment and procedures, and the settings

for the dynamometer and stimulator were recorded. During visits two to five, participants performed a series of intermittent isometric contractions to task failure ("Severe trials"; see below). From these four tests, the CT was calculated, and participants subsequently performed two further tests, during visits six and seven, at 50 and 90% of the calculated CT (50%CT and 90%CT, respectively; "Sub-CT trials"; see below) for 30 minutes or until task failure, whichever occurred sooner. The severe trials and the sub-CT trials were each presented in a randomised order. The methods used for the setup of the dynamometer, femoral nerve stimulation and surface EMG are described in the General Methods (Chapter 3).

Protocol

All visits followed a similar pattern of data acquisition, beginning with the instrumentation of the participants and the (re-)establishment of the correct dynamometer seating position and supramaximal stimulation response. Participants then performed a series of brief (3 second) MVCs to establish their maximum torque. These contractions were separated by 60 seconds rest, and continued until three consecutive peak torques were within 5% of each other. Participants were given a countdown, followed by very strong verbal encouragement to maximise torque. The first MVC was used to establish the fresh maximal EMG signal, against which the subsequent EMG signals were normalised (Data analysis; see below). The second and third MVCs were performed with peripheral nerve stimulation. In all instances where MVCs were performed with stimuli, the stimuli were delivered ~1.5 seconds into the contraction to coincide with maximal torque, and 2 seconds after the contraction to provide a resting potentiated doublet (see General Methods; Chapter 3).

Following the establishment of maximal torque, participants performed a discontinuous incremental test, in order to establish the baseline complexity across the full range of knee extensor joint torques under 'fresh' conditions. This test consisted of targeted contractions at intensities between 10 and 100% MVC, in 10% increments (Figure 5.1), with the target intensities calculated based on the highest instantaneous torque from the pre-test MVCs in visit two. The contractions were 6 seconds long and separated by 20 seconds rest. Participants were instructed to match their instantaneous torque output with a target torque superimposed on a screen in front of them, and to continue matching the target for as much of the 6 second contraction as possible. After the discontinuous incremental test,

participants rested for 10 minutes, and then performed either one of the severe or sub-CT trials (see below).

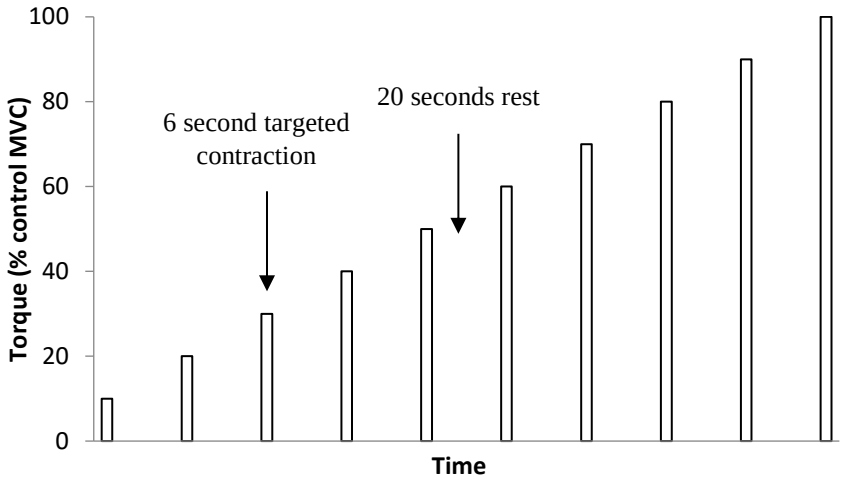

Figure 5.1. Contraction regime for the discontinuous incremental test.

Severe trials (performed above CT)

During visit two (the first of the severe trials), the highest instantaneous pre-test measure of voluntary torque was recorded as the peak MVC torque, and the target torques for the submaximal contractions in visits two to five were calculated from this value. The submaximal contractions (Figure 5.2) were performed using a duty cycle of 0.6, with contractions held for 6 seconds, followed by 4 seconds rest. The target for the submaximal contractions in visit two was set at 50% of the peak torque measured in the pre-test MVCs. Participants were instructed to match their instantaneous torque with a target bar superimposed on the display in front of them and were required to continue matching this torque for as much of the 6 second contraction as possible. The test was conducted until task failure, the point at which participants failed to reach the target torque on three consecutive occasions, despite strong verbal encouragement. Participants were not informed of the elapsed time during the test, but were informed of each “missed” contraction. After the third missed contraction, participants were instructed to immediately produce an MVC, which was accompanied by peripheral nerve stimulation.

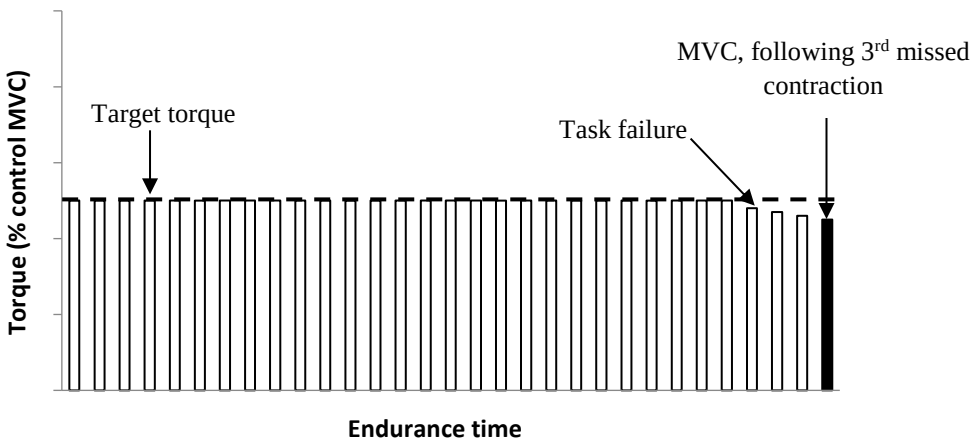

Figure 5.2. Contraction regime for the severe and sub-CT trials. The severe trials were terminated when three consecutive contractions were below the target torque, with the first of these being considered task failure. The sub-CT trials were terminated after 30 minutes or task failure, whichever occurred sooner.

The duration of the initial severe trial at 50% MVC was used to determine the percentage of MVC used in subsequent trials, which were performed in an identical manner. The objective of these tests was to yield trial durations of between two and fifteen minutes, which have been recommended for the assessment of CT (Hill, 1993). The subsequent trials were performed in a randomised order. Visits two to five were used to determine the CT; individual trials were identified as severe 1 (S1) to severe 4 (S4), with S1 being the lowest and S4 being the highest torque.

Sub-CT trials

The final two visits were performed at target torques of 50 and 90% of the calculated CT (identified as 50%CT and 90%CT). These trials were conducted in the same manner as the severe trials (Figure 5.2), requiring the participants to perform intermittent contractions (6 seconds contraction, 4 seconds rest) at a target torque. In these trials, the contractions continued for 30 minutes or until task failure, whichever occurred sooner. Immediately after completion of the trial or task failure, participants were instructed to perform an MVC, which was accompanied by peripheral nerve stimulation. The two sub-CT trials were performed in a randomised order.

Data analysis

The data analysis focused on four specific areas: 1) basic measures of torque and EMG; 2) calculation of the CT; 3) measures of central and peripheral fatigue; and 4) the

variability and complexity of the torque output. All data were analysed using code written in MATLAB R2013a (The MathWorks, Massachusetts, USA).

Torque and EMG. The mean and peak torques, and vastus lateralis EMG, were calculated as described in the General Methods (Chapter 3). The torque impulse (the integral of torque and time) was also calculated for each contraction.

Task failure and the CT. Due to the use of intermittent contractions, calculation of task failure was more problematic than defining failure during a sustained contraction. Details of the calculation of task failure are given in the General Methods (Chapter 3).

Visits two to five were used to determine the CT. To do this, the total torque impulse produced until task failure and the total contraction time during each individual trial were calculated. The torque impulse was then plotted against the contraction time (Figure 5.3). The parameters of the torque-duration relationship were then estimated using linear regression of the torque impulse vs. contraction time (Burnley, 2009; Burnley et al., 2012):

$$Torque\ impulse = W' + CT\ \cdot t$$

where W' represents the curvature constant parameter and t is the time to task failure.

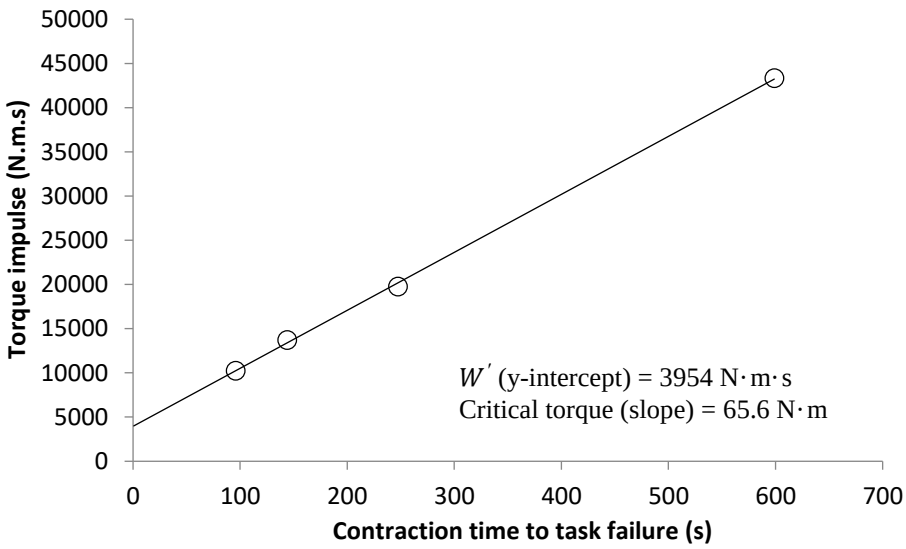

Figure 5.3. Torque impulse plotted against contraction time to task failure in the four trials performed above the CT from a representative participant.

Central and peripheral fatigue. Measures of central and peripheral fatigue were calculated based on the peripheral nerve stimuli delivered during and after the pre-test and task end/failure MVCs. Further details are given in the General Methods (Chapter 3). Additionally, muscle contractility was assessed for each peripherally derived potentiated doublet as contraction time to peak torque (TPT), measured as the time from delivery of the stimulus to the highest torque response; and one-half relaxation time ($RT_{0.5}$), measured as the half time from the peak torque to the recovery of baseline torque.

Variability and complexity. All measures of variability and complexity were calculated using the steadiest five seconds of each contraction. The amount of variability in the torque output of each contraction was measured using the standard deviation (SD) and coefficient of variation (CV). The temporal structure of variability was examined using multiple time domain analyses. Approximate entropy (ApEn) and sample entropy (SampEn) were used to determine the complexity of the torque output; while detrended fluctuation analysis (DFA) was used to estimate the temporal fractal scaling. Further details are given in the General Methods (Chapter 3).

Statistics

All data are presented as means ± SEM unless otherwise stated, and results were deemed statistically significant when $P < 0.05$. Two-way ANOVAs with repeated measures were used to test for differences between conditions and time points, and for a condition x time interaction for torque, arEMG, potentiated doublet torque, voluntary activation, measures of variability and measures of complexity. The variability and complexity measures were analysed using averages from the first minute and the final minute before task end/failure. The rates of change in all parameters were analysed using one-way ANOVAs with repeated measures. When main effects were observed, Student's paired samples t-tests and Bonferroni-adjusted 95% confidence intervals were then used to determine specific differences.

Results

Preliminary measures and the CT

The peak instantaneous MVC torque recorded during a pre-test MVC in visit two was 198.1 ± 17.2 N·m. This was used to set the target torques for the four tests performed above CT, which ranged from 78.7 ± 6.3 to 112.7 ± 9.0 N·m, or 40.8 ± 2.6 to 57.8 ± 2.5% MVC (Table 5.1). The CT was calculated to be 57.5 ± 4.7 N·m, which was equivalent to 29.7 ± 1.7% MVC, and the W' was 3,637 ± 537 N·m·s. The 95% CI for the estimation of CT was 11.8 ± 2.3 N·m. The two trials below CT (50%CT and 90%CT) were performed at 28.7 ± 2.3 and 51.7 ± 4.2 N·m, or 14.9 ± 0.9 and 26.7 ± 1.6% MVC, respectively (Table 5.1).

Fresh complexity/contraction intensity relationship

The relationships between contraction intensity and ApEn, SampEn and DFA α for fresh muscle are shown in Figure 5.4A, B and C, respectively. Complexity was found to decrease with increasing contraction intensity. ApEn and SampEn were both highest at 10% MVC (1.15 ± 0.04 and 1.14 ± 0.04, respectively) and decreased with increasing torque requirements, reaching nadirs at 100% MVC (0.26 ± 0.04 and 0.24 ± 0.04, respectively). The opposite relationship was observed for DFA α, which was lowest at 10% MVC (1.23 ± 0.02) and became increasingly Brownian as the torque requirement increased, peaking at 100% MVC (1.51 ± 0.02). The decrease in complexity with increasing contraction intensity was in contrast to the amount of variability, as measured by the SD, which increased in proportion to torque requirements from 10 to 100% MVC (0.80 ± 0.04 to 5.46 ± 0.67 N·m).

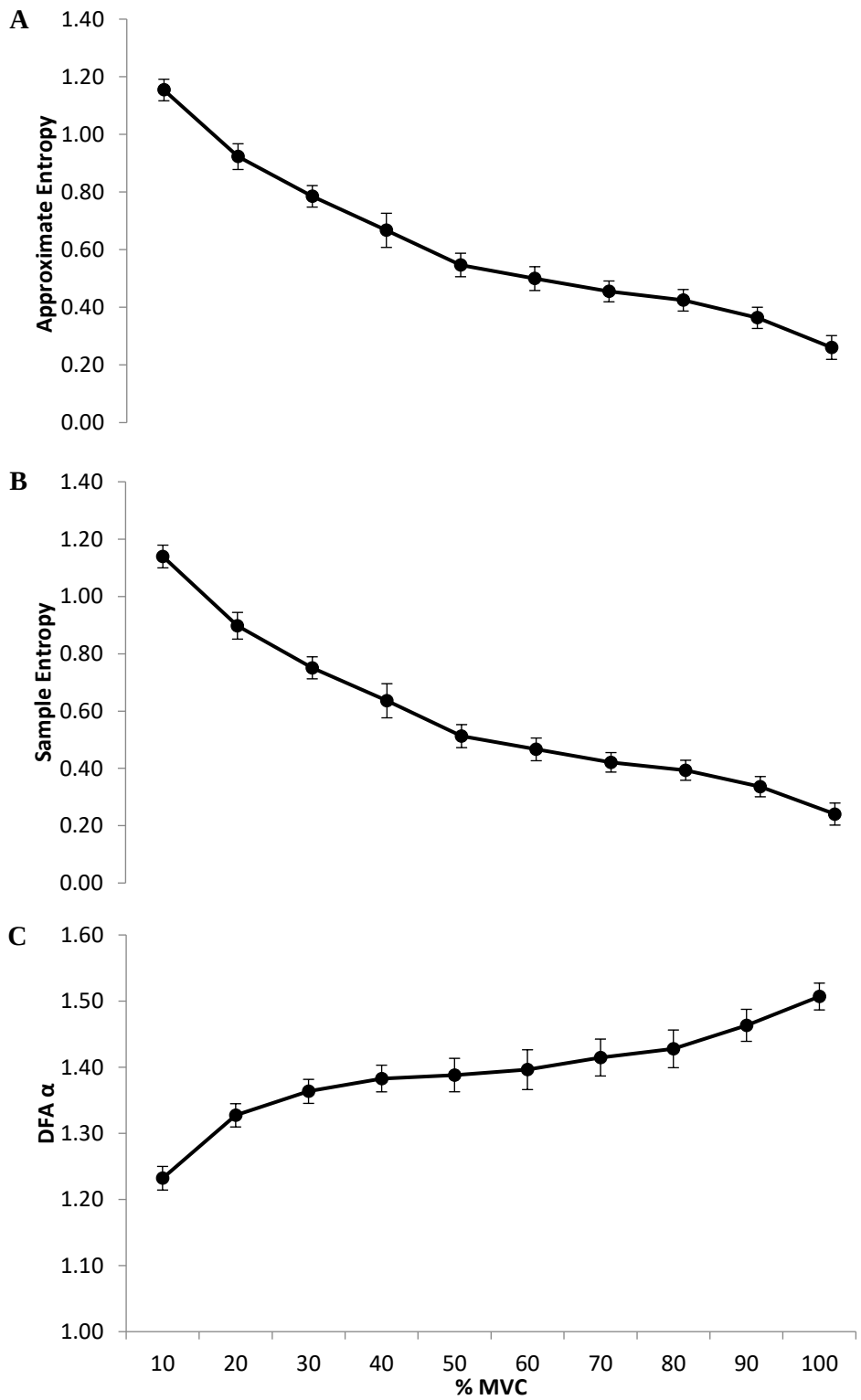

Figure 5.4. The relationship between contraction intensity and ApEn (A), SampEn, (B) and DFA α (C) for fresh muscle. All values are means (± SEM).

Torque and EMG

For the trials above the CT, task failure occurred when participants were no longer able to achieve the target torque, despite a maximal effort. All trials above the CT resulted in significant decreases in MVC torque ($F_{1,8} = 62.17$, $P < 0.001$), with the mean MVC torque at task failure being not significantly different from (S1-S3) or significantly lower than (S4) the torque produced during the submaximal contractions (Table 5.1). In contrast, all participants completed 30 minutes of contractions in both trials below the CT. At the end of these trials, the mean MVC torque was still significantly greater than the submaximal torque requirements (95% paired sampled confidence intervals (CIs): 90%CT, 52.6, 168.2 N·m; 50%CT, 92.2, 211.3 N·m; Table 5.1), indicating that contractions performed below the CT ended with a substantial reserve in maximal torque.

The arEMG responses to contractions above and below the CT are presented in Figure 5.5. The mean arEMG in the first minute, normalised to a fresh pre-test MVC, increased with increasing torque requirements from 50%CT to S4 ($F_{5,8} = 71.60$, $P < 0.001$; Table 5.1). The arEMG amplitude increased over time in all of the trials above the CT, reaching ~61-77% of the pre-test MVC value at task failure ($F_{1,8} = 14.33$, $P = 0.005$; Table 5.1). Contractions below the CT resulted in only modest increases in arEMG as the trials progressed, with the values at task end not significantly different from those at the start (CIs 90%CT, –4.7, 19.0%; 50%CT, –0.6, 2.9%; Table 5.1).

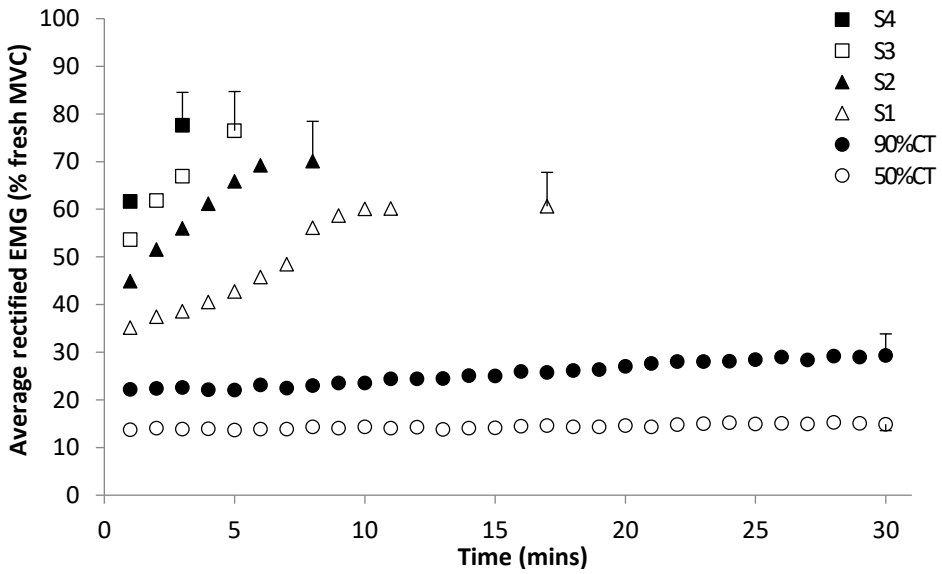

Figure 5.5. Group mean arEMG for each minute of the severe and sub-CT trials. arEMG is normalised to a control MVC performed before each trial commenced. For clarity, errors bars (± SEM) are only given at task end/failure.

Peripheral and central fatigue

All the trials above the CT resulted in significant reductions in potentiated doublet torque ($F_{1,7}$ = 34.34, P = 0.001; Table 5.1), indicating the presence of peripheral fatigue. The values attained at task failure were not significantly different between trials (CIs S1 vs. S2, –19.2, 20.5 N·m; S1 vs. S3, –10.9, 12.4 N·m; S1 vs. S4, –11.9, 17.2 N·m; Table 5.1). Voluntary activation significantly declined during all trials above the CT ($F_{1,7}$ = 192.21, P < 0.001; Table 5.1), indicating the presence of central fatigue. In terms of muscle contractility, neither TPT ($F_{1,7}$ = 2.84, P = 0.152) nor $RT_{0.5}$ ($F_{1,7}$ = 0.337, P = 0.620) were affected by contractions above the CT. Contractions below the CT also resulted in a significant reduction in potentiated doublet torque, though this reduction was modest (Table 5.1), with the values at task end being significantly higher than those at task failure above the CT for 90%CT (CIs S1 vs. 90%CT, 6.0, 39.6 N·m), but not for 50%CT (CIs –46.6. 0.5 N·m). Voluntary activation significantly decreased in 90%CT (CIs –9.4, –1.7%), though this change was more modest than above the CT (Table 5.1). There was no change in voluntary activation in 50%CT (CIs –5.9, 3.5%).

Variability and complexity

All trials above the CT resulted in a significant increase in the amount of variability, as measured by the SD ($F_{1,8}$ = 110.15, P < 0.001; Table 5.2) and CV ($F_{1,8}$ = 136.96, P < 0.001; Table 5.2). The values attained at task failure for the SD were not significantly different between trials. The trials below the CT resulted in no change in the amount of variability (CIs SD: 90%CT, –0.1, 0.7 N·m; 50%CT, –0.1, 0.5 N·m; CV: 90%CT, –0.2, 1.5%; 50%CT, –0.1, 2.0%), and the values at task end were significantly lower than those at task failure in the trials above the CT (Table 5.2).

The ApEn, SampEn and DFA α responses to contractions above and below the CT are presented in Figure 5.6A, B and C, respectively. Complexity at the beginning of the trials decreased with increasing torque requirements from 50%CT to S4 (ApEn, F = 35.54, P < 0.001; SampEn, F = 30.58, P < 0.001; DFA α, F = 38.97, P < 0.001; Table 5.2). All trials above the CT resulted in decreases in complexity, as measured by ApEn ($F_{1,8}$ = 192.30, P < 0.001), SampEn ($F_{1,8}$ = 172.55, P <0.001) and DFA α ($F_{1,8}$ = 46.28, P < 0.001; Table 5.2). ApEn and SampEn significantly decreased with the progression of each trial, with the values at task failure being common, despite starting at different values (Table 5.2). DFA α significantly increased, becoming more Brownian, as each trial

progressed, with the values at task failure again being common, despite starting at different values (Table 5.2). Representative contractions from the beginning and task failure of an above CT trial are shown in Figure 5.7A and B, respectively.

In contrast, the trials below the CT resulted in no significant change in complexity after 30 minutes of contractions. ApEn (CIs 90%CT, –0.23, 0.10; 50%CT, –0.29, 0.05) and SampEn (CIs 90%CT, –0.22, 0.24; 50%CT, –0.05, 0.32) showed no change, and the values at task end were significantly greater than those at task failure above the CT (Table 5.2). DFA α showed no change (CIs 90%CT, –0.12, 0.04; 50%CT, –0.06, 0.11), and the values at task end remained significantly lower than at task failure above the CT (Table 5.2). Representative contractions from the beginning and task end of a below CT trial are shown in Figure 5.7A and B, respectively.

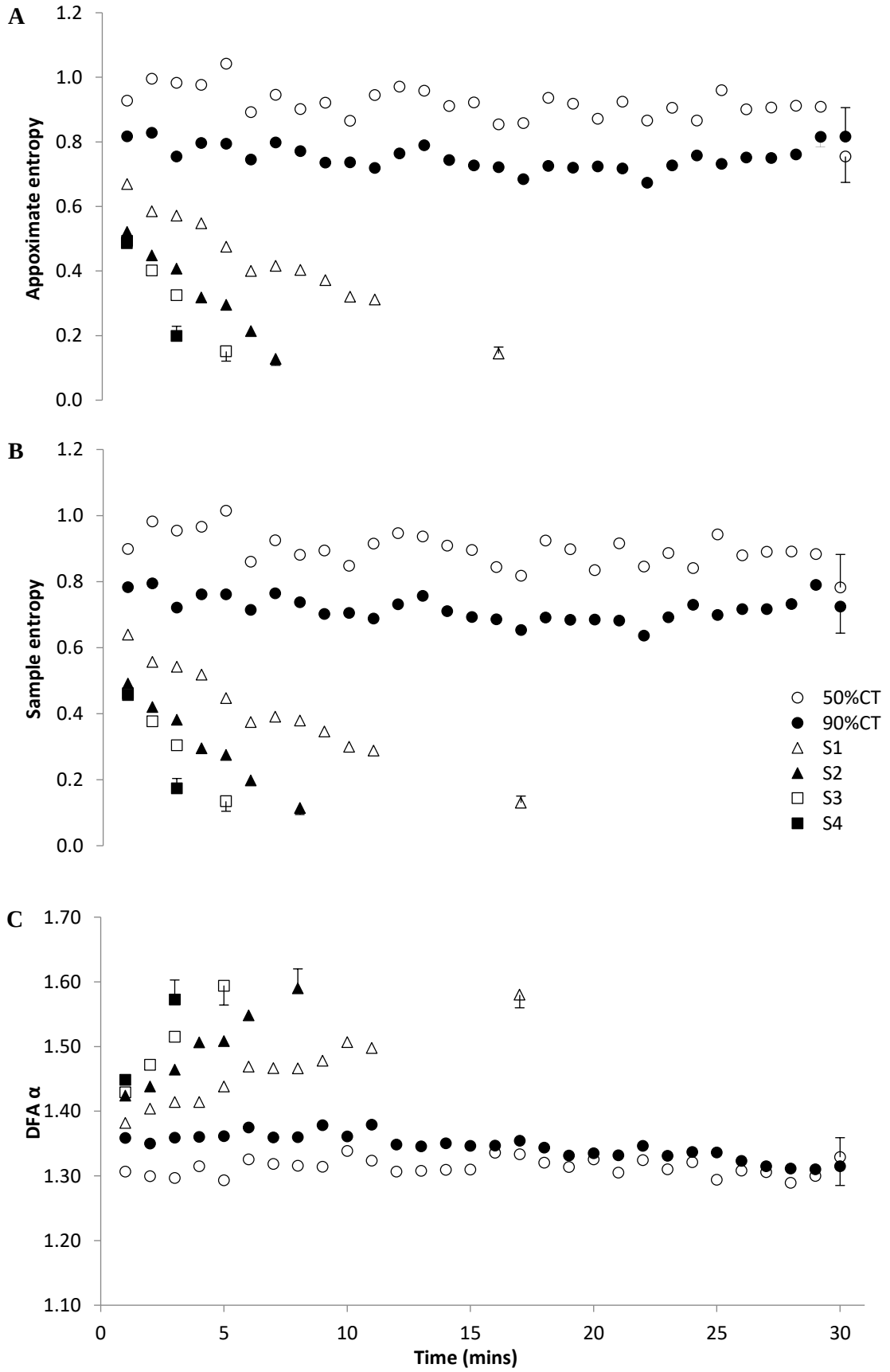

Figure 5.6. Changes in torque ApEn (A), SampEn (B) and DFA α (C) over the course of the severe and sub-CT trials. All values are the mean for all participants for each minute. For clarity, errors bars (± SEM) are only given at task end/failure.

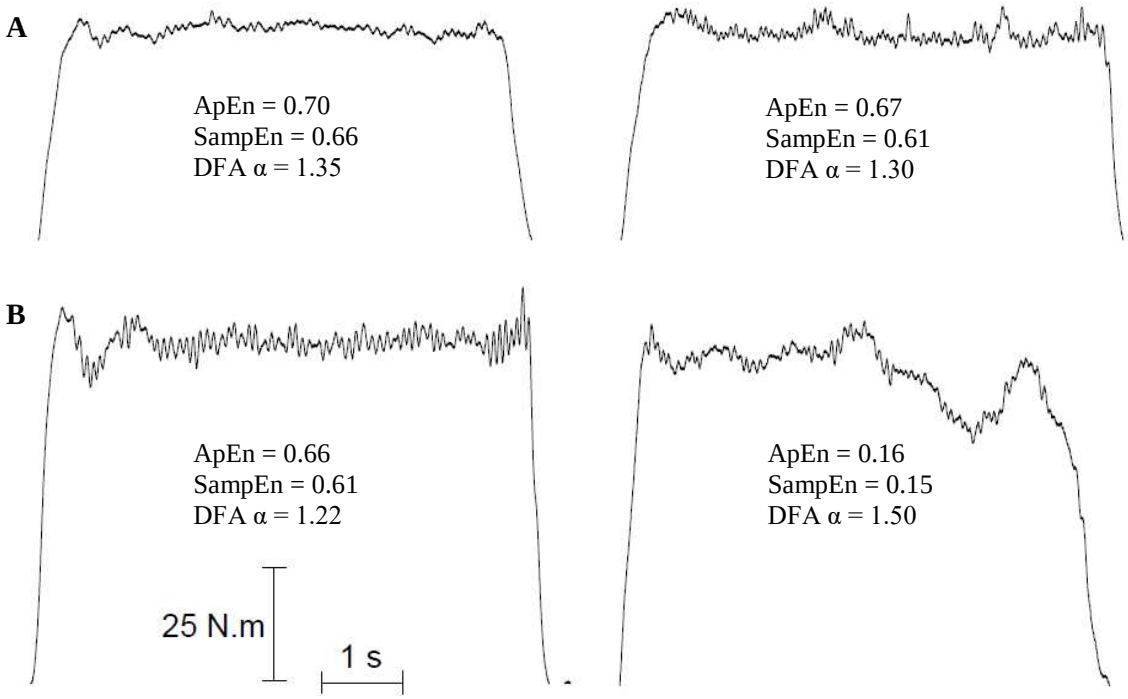

Figure 5.7. Representative contractions from the beginning (left) and task end (right) of a trial at 90%CT (A), and the beginning (left) and task failure (right) of an S1 trial (B).

Rates of fatigue development

The rates of decrease in MVC torque, potentiated doublet torque and voluntary activation all increased with increasing torque requirements from 50%CT to S4 and were significantly greater above compared to below the CT (MVC, $F_{5,8} = 34.41$, $P < 0.001$; potentiated doublet, $F_{5,7} = 16.68$, $P = 0.002$; voluntary activation, $F_{5,7} = 17.71$, $P = 0.001$; Figure 5.8; Table 5.1). The rate of increase in arEMG increased with increasing torque requirements and was significantly greater above compared to below the CT ($F_{5,8} = 8.63$, $P = 0.008$; Table 5.1).

The rates of decrease in ApEn and SampEn increased with increasing torque requirements from 90%CT to S4 and were significantly greater above compared to below the CT (ApEn, $F_{5,8} = 34.94$, $P < 0.001$; SampEn, $F_{5,8} = 34.22$, $P < 0.001$; Figure 5.8; Table 5.2). The rates of change below the CT were an order of magnitude smaller than above the CT. The rate of increase in DFA α (Figure 5.8) increased with increasing torque requirements from 90%CT to S4 and was significantly greater above compared to below the CT ($F_{5,8} = 14.52$, $P = 0.001$; Table 5.2). The rate of change below the CT was an order of magnitude smaller than above the CT.

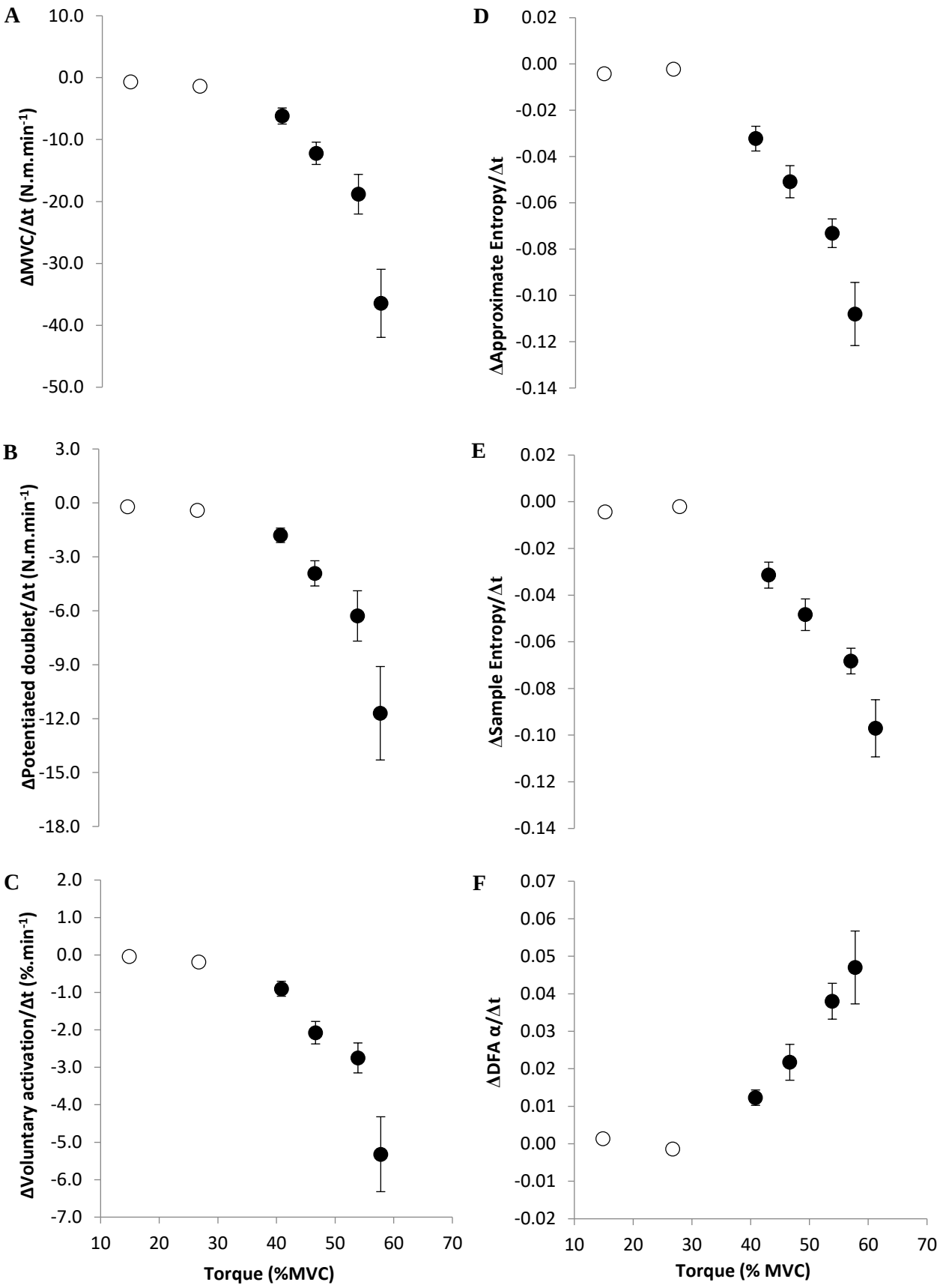

Figure 5.8. Mean (±SEM) rate of change (Δ) in MVC torque (A), potentiated doublet torque (B), voluntary activation (C), ApEn (D), SampEn (E) and DFA α (F). Open circles represent contractions below CT; closed circles represent contractions above CT.

Table 5.1. Voluntary torque, potentiated doublet, voluntary activation and EMG responses to contractions below (50%CT and 90%CT) and above (S1-S4) the critical torque.

Parameter	50%CT	90%CT	Severe 1	Severe 2	Severe 3	Severe 4
Mean test torque, N·m	28.7 ± 2.3	51.7 ± 4.2	78.7 ± 6.3	89.7 ± 6.8	104.0 ± 8.4	112.7 ± 9.0
Mean test torque, [a] % MVC	14.9 ± 0.9	26.7 ± 1.6	40.8 ± 2.6	46.7 ± 3.2	53.9 ± 3.4	57.8 ± 2.5
Time to task end/failure, min	30.0 ± 0.0	30.0 ± 0.0	17.5 ± 1.3	8.1 ± 0.7	4.8 ± 0.5	2.9 ± 0.3
Global fatigue						
Pre-exercise MVC, N·m	226.2 ± 19.7	223.8 ± 21.8	199.9 ± 18.6	198.1 ± 17.2	200.6 ± 15.5	209.6 ± 19.2
Peak MVC at task end/failure, N·m	205.6 ± 18.6*,†	182.2 ± 19.5*,†	101.9 ± 8.2*	110.7 ± 9.7*	119.4 ± 10.2*	112.7 ± 8.0*
Mean MVC at task end/failure, N·m	180.5 ± 18.3$^{\#}$	162.1 ± 17.8$^{\#}$	77.7 ± 5.7	87.4 ± 8.1	101.0 ± 8.5	95.1 ± 7.6$^{\#}$
ΔMVC/Δt, N·m·min^{-1}	–0.7 ± 0.1†	–1.4 ± 0.3†	–6.2 ± 1.3	–12.2 ± 1.8†	–18.5 ± 3.2†	–36.4 ± 5.5†
Peripheral fatigue						
Pre-exercise doublet, N·m	93.1 ± 7.9	98.6 ± 8.5	95.1 ± 7.9	94.4 ± 8.7	92.3 ± 8.5	91.9 ± 7.7
Doublet at task end/failure, N·m	86.6 ± 7.7*	86.3 ± 7.6*,†	63.5 ± 4.9*	63.8 ± 8.1*	62.8 ± 5.0*	60.9 ± 6.3*
% Change at task end/failure	7.1 ± 1.3	12.5 ± 2.3	32.2 ± 3.8	32.6 ± 4.9	29.8 ± 5.2	32.7 ± 5.5
Δdoublet/Δt, N·m·min^{-1}	–0.2 ± 0.04†	–0.4 ± 0.1†	–1.8 ± 0.4	–3.9 ± 0.7†	–6.1 ± 1.3†	–11.6 ± 2.5†
Central fatigue						
Pre-exercise VA, %	92.4 ± 0.5	93.6 ± 0.7	91.3 ± 0.9	91.5 ± 1.0	92.0 ± 1.3	92.4 ± 1.1
VA at task end/failure, %	91.2 ± 1.6†	88.0 ± 1.3*	75.0 ± 3.2*	76.1 ± 1.1*	80.0 ± 1.7*	76.9 ± 3.7*
% Change at task end/failure	0.7 ± 0.7	6.0 ± 1.1	17.7 ± 3.6	16.7 ± 1.8	13.0 ± 1.5	16.7 ± 3.8
ΔVA/Δt, %/min	–0.04 ± 0.04†	–0.2 ± 0.04	–0.9 ± 0.2	–2.1 ± 0.3†	–2.7 ± 0.4†	–5.3 ± 1.0†
Surface EMG						
arEMG at task beginning, % MVC	13.8 ± 1.1	22.2 ± 2.3	35.2 ± 3.1	44.9 ± 4.9	53.7 ± 4.9	61.7 ± 4.3
arEMG at task end/failure, % MVC	14.9 ± 1.3	29.4 ± 4.4	62.4 ± 7.1*	70.1 ± 8.4*	76.5 ± 8.2*	77.6 ± 7.0*
ΔarEMG/Δt, % MVC/min	0.04 ± 0.02†	0.2 ± 0.1†	1.6 ± 0.4	3.5 ± 0.9	4.9 ± 1.5	5.4 ± 1.7

Values are means ± SEM. EMG, electromyogram; 90%CT and 50%CT, 10 and 50% below the critical torque, respectively; MVC, maximal voluntary contraction; Δ, change; t, time; VA, voluntary activation; arEMG, average rectified EMG of the vastus lateralis;. [a] Mean test torque is expressed as a percentage of the peak torque measured during the MVCs in visit 2. Symbols indicate a statistically significant difference compared to the following: $^{\#}$mean test torque, *pre-exercise value/value at task beginning, †Severe 1.

Table 5.2. Variability, complexity and fractal scaling responses to contractions below (50%CT and 90%CT) and above (S1-S4) the critical torque.

Parameter	50%CT	90%CT	Severe 1	Severe 2	Severe 3	Severe 4
SD						
SD at task beginning, N·m	0.9 ± 0.1	1.3 ± 0.1	2.0 ± 0.1	2.4 ± 0.2	2.8 ± 0.3	3.2 ± 0.3
SD at task end/failure, N·m	1.1 ± 0.2†	1.7 ± 0.2†	7.6 ± 0.4*	8.4 ± 0.8*	8.5 ± 1.4*	7.1 ± 0.8*
ΔSD/Δt, N·m·min^{-1}	0.007 ± 0.003†	0.01 ± 0.003†	0.3 ± 0.03	0.8 ± 0.1	1.4 ± 0.4	1.6 ± 0.4
CV						
CV at task beginning, %	3.0 ± 0.2	3.0 ± 0.1	3.0 ± 0.1	3.0 ± 0.2	3.0 ± 0.3	3.0 ± 0.3
CV at task end/failure, %	4.0 ± 0.4†	3.0 ± 0.2†	11.0 ± 1.0*	11.0 ± 1.0*	9.0 ± 1.0*	7.0 ± 0.1*
ΔCV/Δt, %/min	0.03 ± 0.01†	0.02 ± 0.006†	0.5 ± 0.04	1.1 ± 0.2	1.6 ± 0.4	1.8 ± 0.5
ApEn						
ApEn at task beginning	0.93 ± 0.07	0.82 ± 0.03	0.67 ± 0.06	0.52 ± 0.05	0.49 ± 0.05	0.49 ± 0.05
ApEn at task end/failure	0.80 ± 0.07†	0.75 ± 0.06†	0.14 ± 0.01*	0.13 ± 0.02*	0.15 ± 0.04*	0.20 ± 0.03*
ΔApEn/Δt	–0.004 ± 0.002†	–0.002 ± 0.002†	–0.03 ± 0.01	–0.05 ± 0.01	–0.07 ± 0.01†	–0.11 ± 0.01†
SampEn						
SampEn at task beginning	0.90 ± 0.07	0.78 ± 0.03	0.64 ± 0.06	0.49 ± 0. †5	0.46 ± 0.05	0.46 ± 0.05
SampEn at task end/failure	0.77 ± 0.07†	0.72 ± 0.07†	0.13 ± 0.01*	0.12 ± 0.02*	0.14 ± 0.02*	0.20 ± 0.04*
ΔSampEn/Δt	–0.004 ± 0.002†	–0.002 ± 0.002†	–0.03 ± 0.01	–0.05 ± 0.01	–0.07 ± 001†	–0.10 ± 0.01†
DFA α						
DFA α at task beginning	1.31 ± 0.02	1.36 ± 0.01	1.38 ± 0.03	1.42 ± 0.03	1.43 ± 0.03	1.45 ± 0.03
DFA α at task end/failure	1.34 ± 0.03†	1.32 ± 0.03†	1.58 ± 0.01*	1.59 ± 0.02*	1.59 ± 0.02*	1.57 ± 0.03*
ΔDFA α /Δt	0.001 ± 0.001†	–0.001 ± 0.001†	0.01 ± 0.002	0.02 ± 0.005	0.04 ± 0.01†	0.05 ± 0.01†

Values are means ± SEM. 90%CT and 50%CT, 10 and 50% below the critical torque, respectively; Δ, change; t, time; SD, standard deviation; CV, coefficient of variation; ApEn, approximate entropy; SampEn, sample entropy; DFA α, detrended fluctuation analysis. Letters indicate a statistically significant difference compared to the following: *pre-exercise value/value at task beginning, †Severe 1.

Discussion

The present study sought to determine the intensity dependence of changes in torque complexity with neuromuscular fatigue, by investigating contractions performed above and below the CT. The major novel finding of the present study was that the complexity of knee extensor torque output was reduced only during contractions performed above the CT. Contractions performed above the CT were associated with the development of significant central and peripheral fatigue, accompanied by reduced complexity and increasingly Brownian (DFA $\alpha = 1.50$) fluctuations in torque output. At task failure above the CT, common values for torque complexity, central fatigue and peripheral fatigue were observed regardless of the torque requirement of the task. Contrastingly, contractions performed below the CT resulted in no change in the complexity or fractal scaling of torque output. Finally, it was observed that the complexity of fresh knee extensor torque was highest at 10% MVC and decreased with increasing torque requirements. These results provide new evidence that complexity and fractal scaling are sensitive to both increased torque demands and the development of neuromuscular fatigue during high-intensity voluntary contractions.

Fresh complexity/contraction intensity relationship

Many previous studies have demonstrated that torque variability is proportional to the mean torque exerted during isometric contractions (Schmidt et al., 1979; Galganski et al., 1993; Jones et al., 2002). Few studies, however, have examined the relationship between complexity and contraction intensity, with the present study being the first to do so for the knee extensors over the whole range of joint torques. Initial studies on the contraction intensity/complexity relationship, conducted on the index finger and elbow flexors, demonstrated an inverted-U shaped relationship, with complexity peaking at ~40% MVC (Slifkin and Newell, 1999; Svendsen and Madeleine, 2010). This peak at ~40% MVC was thought to represent the point of maximum information transfer and adaptability; where force could be modulated through a combination of motor unit recruitment and rate coding. The results of the present study, in contrast, indicate that for the knee extensors complexity is highest at 10% MVC and decreases, with torque becoming increasingly Brownian (DFA $\alpha = 1.50$), as contraction intensity rises to the maximum attainable torque (Figure 5.4).

Rather than contradicting previous findings, the present results may simply reflect the different torque modulation strategies utilised by different muscle groups. For example, it has been shown that for the index finger flexors, recruitment occurs up to ~40% MVC (De Luca et al., 1982a); while in larger muscles, such as the quadriceps, recruitment is dominant up to ~80% MVC (Kukulka and Clamann, 1981; De Luca et al., 1996). It may be that increased recruitment is responsible for the changes in complexity through much of the range of torque requirements, with a switch to rate coding and increased discharge rates occurring only for the highest torque demands. Thus, a combination of the activation of a large proportion of the motor unit pool and increased discharge rates at high contractile intensities leads to torque output approximating a fused tetanus (Buller and Lewis, 1965). Therefore, complexity systematically decreased as the torque requirement increased, reaching its lowest point when recruitment and discharge rates reached their peaks. Alternatively, the decreasing complexity with increasing contraction intensity could relate to common drive; that is, the common synaptic input to motoneurons (De Luca and Erim, 1994). This common drive (discussed further below) has been proposed to be the main determinant of torque variability (Negro et al., 2009; Farina et al., 2014) and has been demonstrated to increase with increasing contractile intensity (Castronovo et al., 2015; Farina and Negro, 2015). It must be noted, though, that direct measurement of motor unit discharge rates would be required to firmly establish either of these proposed relationships.

Complexity and neuromuscular fatigue below and above the critical torque

Although the CP/CT was originally proposed to distinguish fatiguing and fatigueless contractions (Monod and Scherrer, 1965), it is now known that fatigue occurs below the CT, but at a disproportionately slower rate than above CT (Burnley et al., 2012). The present results support this, with decreases in MVC torque occurring more than four times faster in the S1 compared to 90%CT condition, and with the rate of fatigue, in all its forms, increasing as torque demands increased above the CT (Table 5.1). The dominant mechanism of fatigue above the CT is believed to be metabolite-induced peripheral fatigue (Burnley, 2009; Burnley et al., 2012), on the basis that progressive phosphocreatine depletion and inorganic phosphate and proton accumulation only occur above the CT (Jones et al., 2008; Burnley et al., 2010). The loss of muscle force generating capacity in vivo results in additional motor unit recruitment to sustain the demands of the task (Adam and De Luca, 2003), which is indirectly reflected by an

increase in arEMG amplitude (Figure 5.5; Table 5.1). Such metabolic and neuromuscular responses drive the non-steady state increases in muscle and pulmonary $\dot{V}O_2$ observed above the CT (Poole et al., 1988; Saugen and Vøllestad, 1996). Consequently, neuromuscular fatigue above the CT leads to a progressive decrease in muscular efficiency (Grassi et al., 2015).

The present study has demonstrated, for the first time, that the fatigue-induced loss of torque complexity occurs exclusively above the CT (at least for tasks lasting 30 minutes or less). Specifically, significant decreases in ApEn and SampEn (indicating increased signal regularity) and an increase in DFA α (indicating a move towards more Brownian noise) were only observed when the CT was exceeded and did not change during 30 minutes of contractions below the CT (Figure 5.6; Table 5.2). Such changes above the CT indicate a progressive reduction in the complexity of the torque output as fatigue developed; with torque output becoming progressively smoother, with smaller fluctuations as task failure approached (Figure 5.7). Such a low complexity, smooth output indicates a loss of neuromuscular system adaptability (Lipsitz and Goldberger, 1992) and a narrowing of system responsiveness (Goldberger et al., 2002). It is not clear why complexity and the CT are linked, though an inverse relationship between the metabolic rate of a system and its output complexity has been hypothesised (Seely and Macklem, 2012). The present results support this hypothesis, since it is only above the CP that muscle $\dot{V}O_2$ rises inexorably as a function of time, and it is now known that complexity only falls as a function of time above the CT. This raises the possibility that the metabolic, neuromuscular and respiratory changes observed above the CP/CT are all fundamentally linked to the observed loss of complexity.

Although there was no significant change in complexity during contractions below the CT, modest reductions were evident (Table 5.2). It is possible that the relatively low sample size and high number of comparisons resulted in insufficient power to detect whether these differences were, in fact, significant. However, post-hoc power calculations revealed that the statistical powers for ApEn, SampEn and DFA α for CT10 were 0.05, 0.04 and 0.08, respectively, and for CT50 were 0.19, 0.20 and 0.04, respectively. Despite the lack of significant change in complexity below the CT, modest, but significant, changes in global, central and peripheral fatigue were, nevertheless, observed (Table 5.1). It must be noted, though, that the changes in global, central and peripheral fatigue were

not of the same magnitude as those above CT, and the values at task end tended to be significantly greater than at task failure above the CT. This may indicate that complexity and fractal scaling are dissociated from central and peripheral fatigue below the CT, and that fatigue mechanisms particular to contractions above the CT are responsible for the loss of complexity and change in fractal scaling observed. Specifically, the non-steady state responses seen above the CT are associated with peripheral fatigue that is, at least partly, of metabolic origin (Allen et al., 2008), while the metabolic changes seen below the CT are likely too small to affect the output of the system to a significant extent (Jones et al., 2008). Thus, neuromuscular adaptability, reflected by its output complexity (Vaillancourt and Newell, 2003; Peng et al., 2009) is not significantly perturbed and contractions continue with relative ease. These observations advance previous findings on both neuromuscular fatigue (Burnley et al., 2012) and torque complexity (Chapter 4); demonstrating that metrics derived from non-linear dynamics can be used to identify changes in neuromuscular system behaviour coincident with the CT.

Physiological bases for changes in neuromuscular system behaviour

The present study has demonstrated that knee extensor torque complexity can be reduced by increasing neuromuscular system demands (i.e. by increasing the torque demands of a task; Figure 5.4) or by reducing torque generating capacity (i.e. by fatiguing the muscle; Figure 5.6). During fatiguing submaximal contractions, the neuromuscular system must maintain torque output in the face of reduced muscle fibre twitch force (Allen et al., 2008a) and motoneuron excitability (McNeil et al., 2011) by increasing central drive and, therefore, motor unit recruitment and rate coding (Adam and De Luca, 2003; Dideriksen et al., 2011). As fatiguing contractions progress, a greater pool of fibres is engaged in the task, with each contributing progressively less to the overall output, likely resulting in the loss of temporal complexity observed. Previous studies have indicated a slowing of TPT and $RT_{0.5}$ in response to doublet stimulation (Cady et al., 1989b; Jones et al., 2006). Such responses could also contribute to a smoothing of torque output, though were not observed in the present study. The use of doublet stimulation is preferential for the assessment of central and peripheral fatigue (Place et al., 2007), though can prove problematic for the assessment of contractility, due to the greater discomfort caused and variable response during recovery from stimulation. As such, a single stimulus is the preferred method for assessing contractility (Bellemare et al., 1983; Alway et al., 1987; O'Leary et al., 1997). Nevertheless, that the fatigue-induced loss of complexity occurred

solely above the CT (at least for tasks lasting 30 minutes or less) is significant, as it suggests that only metabolically mediated peripheral fatigue is capable of creating the chain of events leading to that loss. These events include both peripheral alterations and the central adjustments required to counter them and to continue exercise.

One of the central adjustments that may be critical to the fatigue-induced loss of complexity is the common synaptic input to motoneurons and the modulation of motor unit discharge rates (i.e. common drive; De Luca and Erim, 1994; Farina and Negro, 2015). A necessary consequence of a common synaptic input to all motoneurons is the correlated discharge of action potentials, known as motor unit synchronisation (Farina and Negro, 2015). It has recently been demonstrated that there is an increase in common synaptic input, and thus in motor unit synchronisation, when the net excitatory input to motoneurons increases, whether this is due to an increase in contraction intensity or to the progression of fatigue and the necessary recruitment of a greater proportion of the motor unit pool (Castronovo et al. 2015). Crucially, both increased contractile intensity and neuromuscular fatigue decrease torque complexity (Figure 5.4; Figure 5.6; Table 5.2). Moreover, common synaptic input has been proposed as the main determinant of force variability in a contraction (Farina et al., 2014). Motor unit synchronisation has been suggested as a likely contributor to the time and frequency domains of torque output (Taylor et al., 2003), increased motor unit synchronisation has been postulated to be a cause of the increased regularity of loaded postural tremor with ageing (Sturman et al., 2005) and excessive levels of motor unit synchronisation are thought to disrupt motor performance (Yao et al., 2000; Holtermann et al., 2009). It may also be the case that fatigue processes may influence the temporal complexity of common synaptic input, as well as neural drive to the muscle (Farina et al., 2014), though the EMG measurements made in the study (using a single set of bipolar surface electrodes) do not allow us to address this hypothesis.

As previously observed (Burnley et al., 2012), peripheral fatigue developed more than four times faster above than below the CT and developed at an increasingly faster rate as the torque requirements increased above the CT (Figure 5.8; Table 5.1). At task failure, though, the potentiated doublet had declined to similar levels, regardless of the intensity of contractions and the rate of change (Table 5.1). This is consistent with previous data indicating a consistent level of metabolic disturbance and/or peripheral fatigue at task

failure (Amann et al., 2007; Vanhatalo et al., 2010; Burnley et al., 2012). A significant novel finding of the present study was that the values of ApEn, SampEn and DFA α at task failure were common for each of the trials above the CT, regardless of their starting values and rates of change during the trials (Table 5.2). This indicates that task failure is characterised not only by consistent levels of metabolic disturbance and peripheral fatigue, but also by consistently low levels of torque complexity.

The significance of low complexity outputs is thought to be related to system adaptability (Peng et al., 2009). High levels of complexity reflect a system able to rapidly adapt to sudden increases in demand (in this case, contractions above the CT; Lipsitz, 2002), because fluctuations occur at multiple frequencies and are correlated across multiple timescales (Lipsitz and Goldberger, 1992; Lipsitz, 2002; Sosnoff et al., 2004). As complexity falls, the system is increasingly unable to match the required output (Sosnoff et al., 2004), leading to diminished motor control. Consequently, torque output fluctuates with increased amplitude at lower frequencies (Lipsitz, 2002; Peng et al., 2009). This response is commonly observed in ageing (Sosnoff et al., 2004; Sturman et al., 2005), and the present study extends it to neuromuscular fatigue. At task failure above the CT, torque complexity reaches consistently low values that may be so low as to compromise motor control and thus limit task performance, in agreement with the purported functional importance of physiological complexity (Lipsitz, 2002; Peng et al., 2009). Furthermore, the loss of torque complexity may provide a sensitive, non-invasive surrogate index of the fatigue process and exercise capacity during free-running conditions in vivo, especially when the use of maximal contractions and/or electrical stimulation is impractical and undesirable. However, further research is warranted, firstly, to understand the mechanistic basis of complexity and how it relates to fatigue, and secondly, to calculate complexity statistics during exercise and not just post-hoc.

Conclusion

In summary, this study has demonstrated that the complexity of fresh knee extensor torque decreases with increasing contraction intensity and crucially, that a fatigue-induced loss of complexity occurs exclusively during contractions performed above the CT (at least for tasks lasting 30 minutes or less). During 30 minutes of contractions below the CT there were modest neuromuscular adjustments, but complexity, measured using ApEn, SampEn and DFA α, did not change. In contrast, above the CT substantial

neuromuscular fatigue developed and complexity progressively decreased until task failure. The complexity of torque output reached similarly low levels at task failure during all trials above the CT, implying a similar loss of neuromuscular system adaptability that may contribute to exercise intolerance. That the fatigue-induced loss of complexity occurred exclusively above the CT suggests that metabolite-induced peripheral fatigue is an essential part of this process. Compensatory adjustments to central drive in the face of developing peripheral fatigue are also likely to contribute to the loss of complexity during intermittent knee extensor contractions above the CT.

Chapter 6 – Study 3

The effects of central fatigue, peripheral fatigue and afferent feedback on isometric knee extensor torque complexity

Introduction

The first two studies of this thesis (Chapter 4 and 5) have demonstrated that neuromuscular fatigue significantly reduces the complexity of torque output, shifting it towards increasingly Brownian noise (DFA $\alpha = 1.50$), during repeated isometric knee extensions performed above the critical torque. The results presented indicated that this reduced complexity was associated with metabolically mediated peripheral fatigue, and the central adjustments required to counter this. It has not, however, been possible to discern whether the changes in complexity with neuromuscular fatigue occur as a result of central or peripheral mechanisms. The purpose of the present study was, therefore, to attempt to investigate central and peripheral mechanisms, in isolation, in order to determine the origin of the neuromuscular fatigue-induced reduction in complexity.

As peripheral fatigue is the dominant fatigue mechanism above critical torque, and torque complexity has been demonstrated to only decrease during contractions performed above the critical torque (at least for tasks lasting 30 minutes or less; Chapter 5), this supports the notion that only metabolically-mediated peripheral fatigue is capable of inducing a loss of torque complexity. Torque complexity and peripheral fatigue are further linked by the common values attained for both at task failure, regardless of contraction intensity (Chapter 5). If the loss of torque complexity is, indeed, mechanistically linked to peripheral fatigue, then enhanced peripheral fatigue at the start of an exercise bout would be expected to be accompanied by reduced torque complexity, in comparison to fresh muscle. Enhanced peripheral fatigue has often been investigated through the use of circulatory occlusion, which has been repeatedly demonstrated to decrease isometric endurance time (Edwards et al., 1971; Harris et al., 1975; Greenhaff et al., 1993). This is largely attributed to greater accumulation of metabolites associated with peripheral fatigue, such as hydrogen ions and inorganic phosphate (Sjøgaard et al., 1988; Lanza et al., 2006; Sugaya et al., 2011); the combination of which has recently been suggested to have an additive effect on force depression (Nelson and Fitts, 2014). It has further been

demonstrated that recovery of force is prevented (Bigland-Ritchie et al., 1986b; Gandevia et al., 1996) and that the muscle metabolic milieu does not recover (Quistorff et al., 1993; Lanza et al., 2006) when the muscle is held ischaemic following completion of exercise.

The loss of torque complexity previously demonstrated was also associated with central adjustments, such as decreased voluntary activation, and could potentially relate to common drive (Chapter 5); the synaptic input common to all motoneurons. If the loss of torque complexity is mechanistically linked to such central adjustments, increased central fatigue at the start of an exercise bout would be expected to be accompanied by reduced torque complexity, in comparison to fresh muscle. Several studies have observed that neuromuscular fatigue in the muscles of one limb has a small "cross-over" effect to the homologous unexercised muscles of the contralateral limb (Zijdewind et al., 1998; Todd et al., 2003; Rattey et al., 2006). While the fatigue experienced in the exercised limb can be attributed to both central and peripheral mechanisms, the fatigue in the unexercised contralateral limb appears to be solely due to central mechanisms, with participants displaying reduced voluntary activation but no change in twitch or M-wave properties (Rattey et al., 2006; Martin and Rattey, 2007). Interestingly, effects of unilateral exercise on tremor complexity in unexercised contralateral limbs have been observed. Morrison and Sosnoff (2010), using a wrist flexion/extension fatiguing protocol, found a significant increase in the approximate entropy (ApEn) of finger tremor in both the exercised and unexercised limbs. While this study focused on kinematic (movement) rather than kinetic (torque) measures, it indicates a potential effect of fatigue on complexity in unexercised contralateral limbs.

It has recently been proposed that central and peripheral fatigue mechanisms interact (Amann and Dempsey, 2008; Amann, 2011). According to this hypothesis, metabosensitive group III and IV muscle afferents within working muscle relate exercise-induced metabolic perturbations associated with peripheral fatigue to the central nervous system (Kaufman et al., 2002), which then adjusts central motor drive based on this inhibitory feedback (Amann and Dempsey, 2008). This feedback loop serves to limit voluntary drive (i.e. increases central fatigue) and restricts the development of further peripheral fatigue (Amann et al., 2006; Amann et al., 2013). Given that this hypothesis proposes that afferent feedback acts to increase central fatigue in order to restrict the development of peripheral fatigue, and consistent values for both peripheral fatigue and

complexity have been observed at task failure (Chapter 5), such an interaction of peripheral and central processes could be responsible for the fatigue-induced loss of torque complexity.

The purpose of the present study was to investigate the cause of the fatigue-induced loss of torque complexity previously observed, through attempting to separate the effects of central fatigue, peripheral fatigue and afferent feedback. The specific experimental hypotheses tested were: 1) that pre-existing peripheral fatigue, induced by circulatory occlusion, would decrease time to task failure and reduce torque complexity (measured by decreased ApEn and sample entropy [SampEn], and increased detrended fluctuation analysis [DFA] α) at the start of an exercise bout; 2) that pre-existing central fatigue, induced by prior exercise of the contralateral limb, would decrease time to task failure and reduce torque complexity at the start of an exercise bout; and 3) that enhanced afferent feedback, induced by prior exercise and occlusion of the contralateral limb, would decrease time to task failure and reduce torque complexity at the start of an exercise bout.

Methods

Participants

Nine healthy participants (5 male, 4 female; mean ± SD: age 23.9 ± 5.7 years; height 1.74 ± 0.09 m; body mass 66.0 ± 12.4 kg) provided written informed consent to participate in the study, which was approved by the ethics committee of the University of Kent, and which adhered to the Declaration of Helsinki. Participants were instructed to arrive at the laboratory in a rested state (having performed no strenuous exercise in the preceding 24 hours) and to have consumed neither any food nor caffeinated beverages in the three hours before arrival. Participants attended the laboratory at the same time of day (± 2 hours) during each visit.

Experimental design

Participants were required to visit the laboratory on five occasions over a three to five-week period, with a minimum of 48 hours between each visit. During their first visit, participants were familiarised with all testing equipment and procedures, and the settings for the dynamometer and stimulator were recorded. During the next four visits, participants performed a series of intermittent isometric contractions to task failure,

during which overall fatigue, central fatigue, peripheral fatigue and afferent feedback were manipulated ("Experimental trials"; see below). The methods used for the setup of the dynamometer, femoral nerve stimulation and surface EMG are described in the General Methods (Chapter 3). The present study involved contractions performed on both legs; therefore, the dynamometer, femoral nerve stimulation and surface EMG were also setup for the left leg.

Protocol

All visits followed a similar pattern of data acquisition, beginning with the instrumentation of the participants and the (re-)establishment of the correct dynamometer seating position and supramaximal stimulation response. Participants then performed a series of brief (3 second) MVCs to establish the maximum torque of their right leg. These contractions were separated by 60 seconds rest, and continued until three consecutive peak torques were within 5% of each other. Participants were given a countdown, followed by very strong verbal encouragement to maximise torque. The first MVC was used to establish the fresh maximal EMG signal, against which the subsequent EMG signals were normalised (Data analysis; see below). The second and third MVCs were performed with peripheral nerve stimulation. In all instances where MVCs were performed with stimuli, the stimuli were delivered ~1.5 seconds into the contraction to coincide with maximal torque, and 2 seconds after the contraction to provide a resting potentiated doublet (see General Methods; Chapter 3). In the visits involving contractions performed on both legs, after 10 minutes rest participants repeated this process for the left leg. Following the establishment of maximal torque, participants rested for a further 10 minutes and then performed one of the experimental trials (see below).

Experimental trials

The four experimental trials were termed: 1) pre-existing neuromuscular fatigue (NF); 2) pre-existing central fatigue (CF); 3) pre-existing peripheral fatigue (PF); and 4) enhanced afferent feedback (AF). All four trials consisted of two bouts of exercise. NF involved exercising the right leg to task failure, followed by three minutes rest, and then exercising the right leg to task failure again. CF involved exercising the left leg to task failure and then immediately switching to the right leg and exercising to task failure. PF involved exercising the right leg to task failure, then resting for three minutes with the right leg occluded, and then exercising the right leg to task failure again with the occlusion

released. AF involved exercising the left leg to task failure, then occluding the left leg, and immediately switching to the right leg and exercising to task failure. During this trial, the occlusion of the left leg was removed after six minutes of contractions or at task failure, whichever occurred sooner. Occlusion in the CF and AF trials was accomplished by inflating a sphygmomanometer cuff around the top of the thigh, using a standard, double-bladder, adult thigh cuff, rapidly inflated to a pressure of 200 mmHg using compressed air (AG101, D.E. Hokanson Inc., Washington, USA). The trials were conducted in a randomised order.

All exercise bouts were conducted in an identical manner, with a target torque of 50% MVC, based on the highest instantaneous torque recorded during the pre-test MVCs in the first experimental session, and with a duty cycle of 0.6 (6 seconds contraction, 4 seconds rest). At the end of each minute (i.e. every sixth contraction), participants performed an MVC, accompanied by peripheral nerve stimulation. Further details and a graphical representation of the contraction protocol can be found in the General Methods (Chapter 3; Figure 3.1). Each exercise bout was performed until task failure, the point at which participants failed to reach the target torque on three consecutive occasions, despite strong verbal encouragement. Participants were not informed of the elapsed time during the bouts, but were informed of each “missed” contraction. After the third missed contraction, participants were instructed to immediately produce an MVC, which was accompanied by peripheral nerve stimulation. Following this MVC at the end of the first exercise bout, participants either rested for three minutes and exercised the same leg again (NF and PF) or switched to exercising their other leg (CF and AF). The switch from the left to right leg in the CF and AF conditions took approximately 50 seconds, and the second exercise bout was commenced 60 seconds after completion of the first bout. Immediately prior to the commencement of the second exercise bout, participants performed an MVC, accompanied by peripheral nerve stimulation, of the leg to be exercised in the second bout. The second exercise bout was then performed in an identical manner to the first.

Data analysis

The data analysis focused on three specific areas: 1) basic measures of torque and EMG; 2) measures of central and peripheral fatigue; and 3) the variability and complexity of the

torque output. All data were analysed using code written in MATLAB R2013a (The MathWorks, Massachusetts, USA).

Torque and EMG: The mean and peak torques, and vastus lateralis EMG, were calculated as described in the General Methods (Chapter 3). Due to the use of intermittent contractions, calculation of task failure was more problematic than defining failure during a sustained contraction. Details of how task failure was calculated are given in the General Methods (Chapter 3).

Central and peripheral fatigue. Measures of central and peripheral fatigue were calculated based on the stimuli delivered during and after the MVCs performed pre-test, during the exercise bouts and at task failure. Further details are given in the General Methods (Chapter 3). Muscle contractility was assessed for each peripherally derived resting twitch as contraction time to peak torque (TPT) and one-half relaxation time ($RT_{0.5}$). Further details of the calculation of these are given in the Methods section of Chapter 5.

Variability and complexity: All measures of variability and complexity were calculated using the steadiest five seconds of each contraction. The amount of variability in the torque output of each contraction was measured using the standard deviation (SD) and coefficient of variation (CV). The temporal structure of variability was examined using multiple time domain analyses. Approximate entropy (ApEn) and sample entropy (SampEn) were used to determine the complexity of the torque output; while detrended fluctuation analysis (DFA) was used to estimate the temporal fractal scaling. Further details are given in the General Methods (Chapter 3).

Statistics

All data are presented as means ± SEM unless otherwise stated, and results were deemed statistically significant when $P < 0.05$. The first exercise bout of the NF condition (NF1) acted as a control, against which the second exercise bouts of the NF, CF, PF and AF conditions (NF2, CF2, PF2, AF2) were compared. The first exercise bouts of CF, PF and AF were used purely to induce pre-existing fatigue in the right leg, and were not considered for analysis. Two-way ANOVAs with repeated measures were used to test for differences between conditions, time points and a condition x time interaction for MVC

torque, arEMG, potentiated doublet torque, voluntary activation, measures of variability and measures of complexity. The variability and complexity measures were analysed using averages from the first minute and the final minute before task failure. The rates of change in all parameters were analysed using one-way ANOVAs with repeated measures. When main effects were observed, Bonferroni-adjusted 95% confidence intervals were then used to determine specific differences.

Results

Preliminary measures

The peak instantaneous MVC torque for the right leg recorded during the first experimental visit was 213.2 ± 21.1 N·m. This value was used to set the target torque for all the experimental bouts performed on the right leg, which was 106.6 ± 10.5 N·m.

Time to task failure and MVC torque

Time to task failure in NF1 (i.e. the control condition) was 4.7 ± 0.9 mins. There was a significant effect of condition on the time to task failure ($F_{4,8}$ = 17.52, P < 0.001). Time to task failure was significantly shorter in NF2 and PF2 compared to NF1 (95% paired samples confidence intervals (CIs): NF1 vs. NF2, –5.0, –0.4 minutes; NF1 vs. PF2, –6.8, –1.4 minutes; Table 6.1). Time to task failure was not significantly different in CF2 and AF2, but there was a tendency for it be reduced compared to NF1 (by ~1 minute; CIs NF1 vs. CF2, –1.1, 2.8 minutes; NF1 vs. AF2, –0.4, 2.0 minutes; Table 6.1).

The MVC torques prior to the start of each condition and at task failure are presented in Figure 6.1. Task failure occurred when participants were no longer able to achieve the target torque, despite a maximal effort. All conditions resulted in significant decreases in MVC torque ($F_{1,8}$ = 25.66, P = 0.001; Table 6.1), except for PF2 (CIs –26.6, 28.6 N·m), in which neither the pre- nor post-test MVC torques were significantly different from the target torque. At task failure, neither the peak, nor the mean MVC torques in any condition were significantly different from the torque produced during the submaximal contractions (Table 6.1). MVC torque was significantly lower at the start of the second bout of exercise compared to NF1 for all conditions ($F_{4,8}$ = 21.99, P < 0.001; Table 6.1), except for CF2 (CIs –8.3, 65.3 N·m). Significant recovery was observed at the start of NF2 (CIs 8.0, 75.5 N·m), but not PF2 (CIs –8.4, 38.4 N·m).

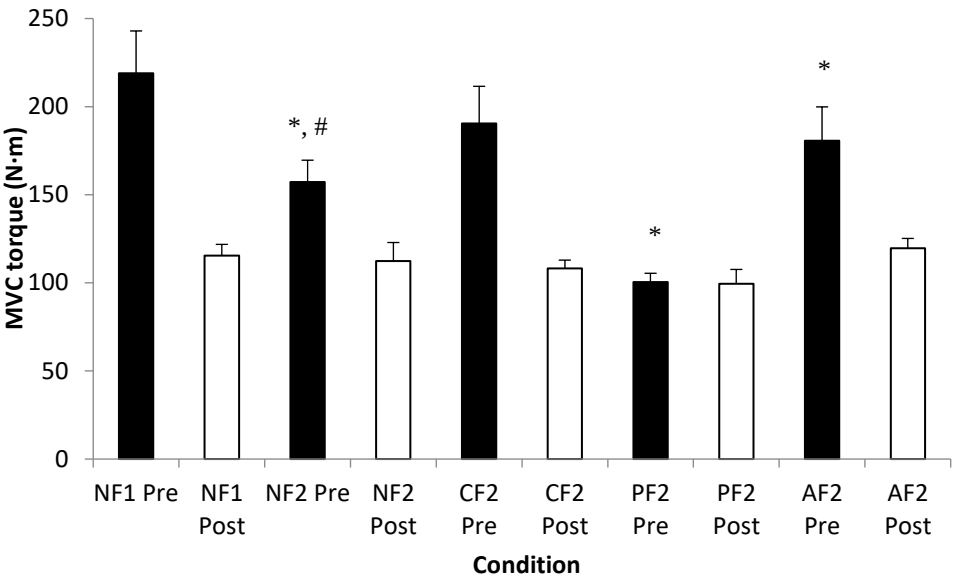

Figure 6.1. MVC torques prior to the start of each condition and at task failure. All values are means (± SEM). * indicates significantly different from NF1 Pre; # indicates significantly different from NF1 Post. There were significant pre to post differences for each condition, with the exception of PF.

Peripheral and central fatigue

The potentiated doublet torques prior to the start of each condition and at task failure are presented in Figure 6.2. All conditions resulted in significant reductions in potentiated doublet torque ($F_{1,8}$ = 47.22, P < 0.001; Table 6.1), indicating the presence of peripheral fatigue. There were also significant effects of condition ($F_{4,8}$ = 5.27, P = 0.003) and a condition x time interaction ($F_{4,8}$ = 8.92, P = 0.004). Potentiated doublet torque was significantly lower at the start of the second bout of exercise compared to NF1 for all conditions, except for AF2 (CIs –12.3, 26.7 N·m; Table 6.1). The values attained at task failure were not significantly different between the conditions (CIs NF1 vs. NF2, –8.9, 7.1 N·m; NF1 vs. CF2, –17.1, 11.4 N·m; NF1 vs. PF2, –17.0, 10.8 N·m; NF1 vs. AF2, –18.9, 6.9 N·m; Table 6.1). Significant recovery was observed at the start of NF2 (CIs 9.1, 39.9 N·m), but not PF2 (CIs –30.9, 5.1 N·m).

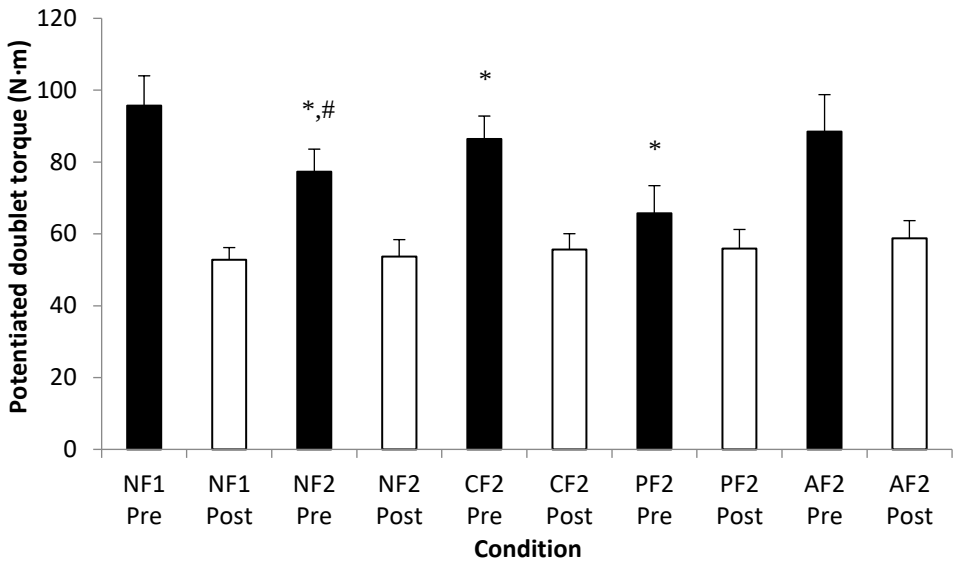

Figure 6.2. Potentiated doublet torques prior to the start of each condition and at task failure. All values are means (± SEM). * indicates significantly different from NF1 Pre; # indicates significantly different from NF1 Post. There were significant pre to post differences for each condition.

The voluntary activation values prior to the start of each condition and at task failure are presented in Figure 6.3. There was an effect of time on voluntary activation ($F_{1,8}$ = 30.49, P = 0.001; Table 6.1), with significant reductions across NF2 (CIs –22.2, –2.4%) and CF2 (CIs –35.0, –9.2%), indicating the presence of central fatigue. Reduced voluntary activation was also evident across NF1 and AF2, though greater variability in those conditions meant the changes did not reach significance (CIs NF1, –1.4, 41.0%; AF2, –5.6, 34.2%). There were also significant effects of condition ($F_{4,8}$ = 3.53, P = 0.046) and a condition x time interaction ($F_{4,8}$ = 4.45, P = 0.022). Voluntary activation was significantly lower at the start of the second bout of exercise compared to NF1 for NF2 (CIs –23.0, –2.9%) and PF2 (CIs –28.4, –8.5%; Table 6.1). The values attained at task failure were not significantly different between the conditions (CIs NF1 vs. NF2, –6.0, 16.8%; NF1 vs. CF2, –12.4, 24.1%; NF1 vs. PF2, –25.9, 23.4%; NF1 vs. AF2, –22.9, 17.8%; Table 6.1). No recovery was observed at the start of either NF2 (CIs –20.4, 6.7%) or PF2 (CIs –23.0, 20.2%).

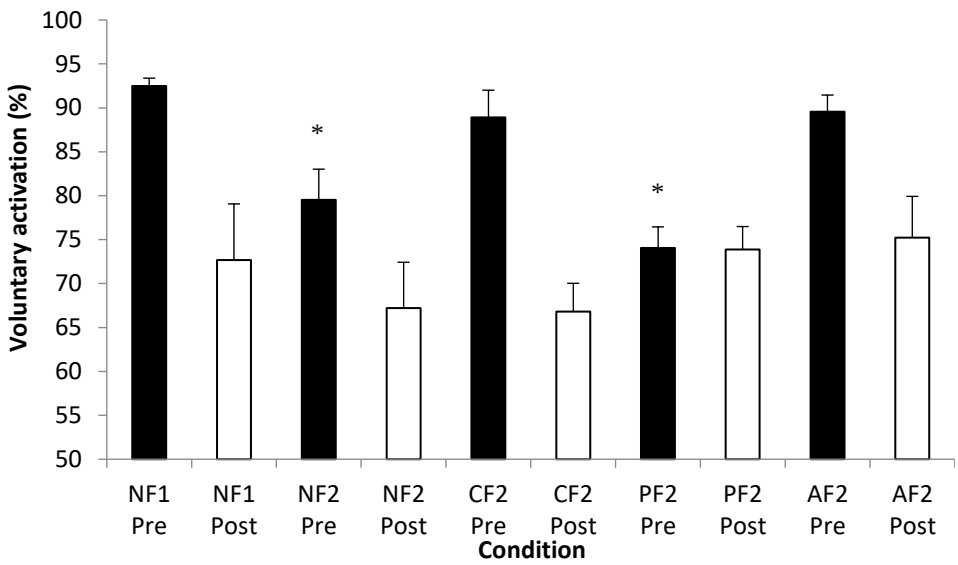

Figure 6.3. Voluntary activation prior to the start of each condition and at task failure. All values are means (± SEM). * indicates significantly different from NF1 Pre. There were significant pre to post differences for NF2 and CF2.

Variability and complexity

There was a significant effect of time on the amount of variability, as measured by the SD ($F_{1,8}$ = 24.11, P < 0.001) and CV ($F_{1,8}$ = 60.23, P < 0.001). The SD significantly increased over time in NF1 (CIs 2.8, 8.3 N·m) and AF2 (CIs 1.6, 3.9 N·m; Table 6.2), and had a tendency to increase, though not significantly, in NF2 (CIs –8.8, 0.6 N·m) and CF2 (CIs –9.7, 0.7 N·m). The CV significantly increased in all conditions, except for PF2 (CIs –0.02, 0.01%; Table 6.2). There were also significant condition x time interactions for both the SD ($F_{4,8}$ = 5.62, P = 0.002) and CV ($F_{4,8}$ = 7.74, P = 0.004). The amount of variability was significantly greater at the start of PF2 compared to NF1 (CIs SD, 0.3, 5.3 N·m; CV, 0.007, 0.06%; Table 6.2). The values attained at task failure were not significantly different for either the SD or CV (Table 6.2).

The ApEn and SampEn responses from the first minute and at task failure in each condition are presented in Figure 6.4A and B, respectively. ApEn ($F_{1,8}$ = 39.66, P < 0.001) and SampEn ($F_{1,8}$ = 39.22, P < 0.001) decreased with time in all conditions, except for PF2 (CIs ApEn, –0.02, 0.05; SampEn, –0.02, 0.04). There were also significant effects of condition (ApEn, $F_{4,8}$ = 4.70, P = 0.016; SampEn, $F_{4,8}$ = 4.86, P = 0.014) and a condition x time interaction (ApEn, $F_{4,8}$ = 14.97, P < 0.001; SampEn, $F_{4,8}$ = 14.35, P < 0.001). ApEn (CIs –0.5, –0.2) and SampEn (CIs –0.4, –0.2) were significantly lower at the start of PF2 compared to NF1, and had a tendency to be lower at the start of NF2 and AF2,

though these differences did not reach statistical significance (CIs NF2, ApEn, –0.06, 0.3; SampEn, –0.06, 2.7; AF2, ApEn, –0.08, 0.3; SampEn, –0.08, 0.3; Table 6.2). There were no significant differences at task failure between conditions for either ApEn (CIs NF1 vs. NF2, –0.09, 0.02; NF1 vs. CF2, –0.1, 0.1; NF1 vs. PF2, –0.08, 0.07; NF1 vs. AF2, –0.1, 0.07) or SampEn (CIs NF1 vs. NF2, –0.09, 0.02; NF1 vs. CF2, –0.1, 0.09; NF1 vs. PF2, –0.06, 0.06; NF1 vs. AF2, –0.1, 0.07; Table 6.2). Significant recovery was observed at the start of NF2 (CIs ApEn, 0.04, 0.4; SampEn, 0.04, 0.4), but not PF2 (CIs ApEn, –0.1, 0.07; SampEn, –0.09, 0.06).

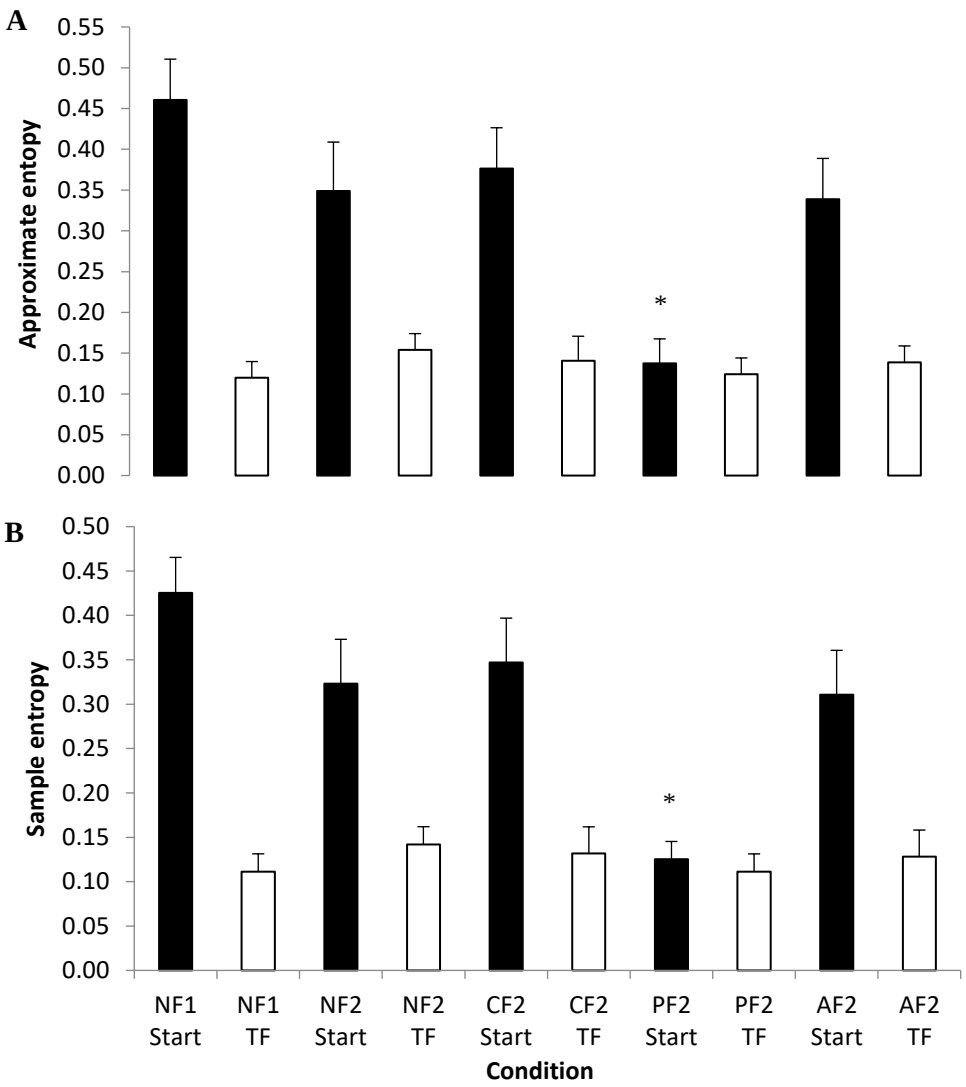

Figure 6.4. Torque ApEn (A) and SampEn (B) at the start and task failure in each condition. All values are means (± SEM). * indicates significantly different from NF1 Pre; # indicates significantly different from NF1 TF. There were significant start to TF differences for each condition, except for PF2.

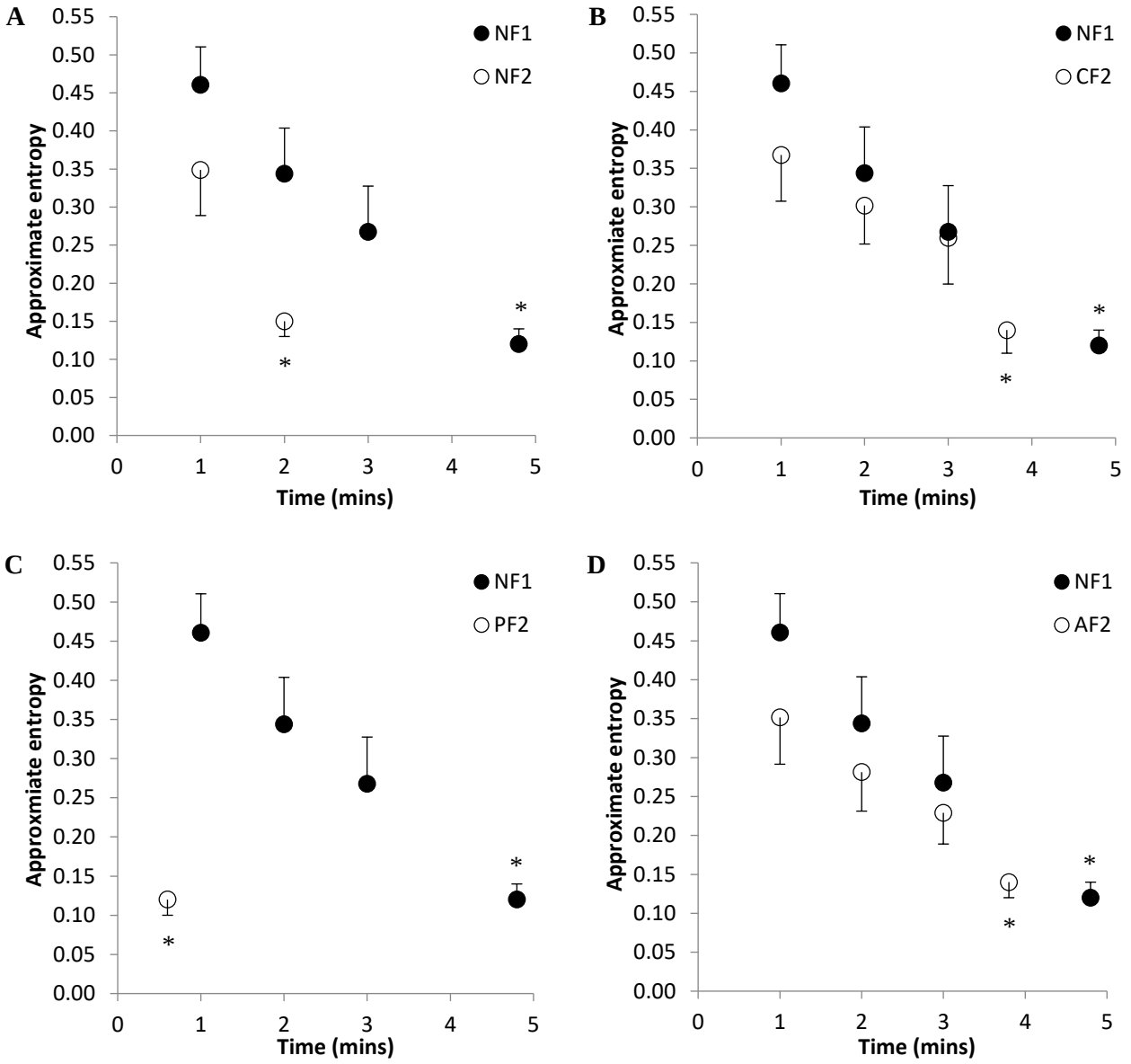

Figure 6.5. Changes in torque ApEn over time in NF1 and NF2 (A), NF1 and CF2 (B), NF1 and PF2 (C) and NF1 and AF2 (D). All values are means (± SEM). The last point represents task failure, while the penultimate point represents the last common minute. All values are means (± SEM). * indicates significantly different from NF1 at 1 minute.

The DFA α responses from the first minute and at task failure in each condition are presented in Figure 6.6. DFA α significantly increased with time in all conditions ($F_{1,8}$ = 31.49, P < 0.001), except for PF2 (CIs –0.03, 0.03). There was also a significant condition x time interaction ($F_{4,8}$ = 18.45, P < 0.001). DFA α was significantly greater at the start of PF2 compared to NF1 (CIs 0.03, 0.3), and had a tendency to be increased at the start of NF2 and AF2, though the differences did not reach significance (CIs NF2 –0.2, 0.004; AF2 –0.2, 0.2). There were no significant differences between the values attained at task failure between the different conditions (CIs NF1 vs. NF2, –0.03, 0.09; NF1 vs. CF2, –

0.09, 0.06; NF1 vs. PF2, –0.08, 0.2; NF1 vs. AF2, –0.1, 0.08; Table 6.2). Significant recovery was observed at the start of NF2 (CIs 0.03, 0.2), but not PF2 (CIs –0.08, 0.2).

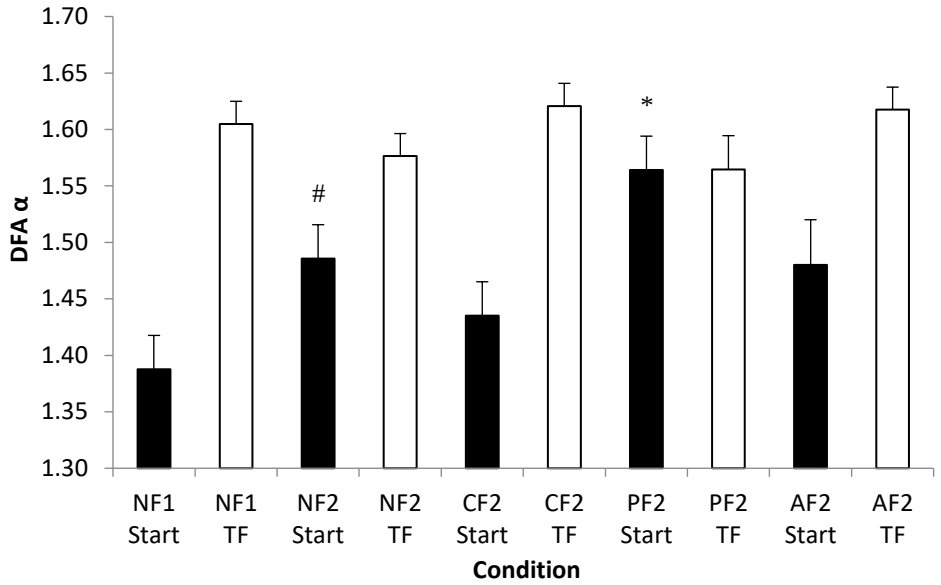

Figure 6.6. Changes in torque DFA α at the start and at task failure in each condition. All values are means (± SEM). * indicates significantly different from NF1 Pre; # indicates significantly different from NF1 Post. There were significant start to TF differences for each condition, with the exception of PF.

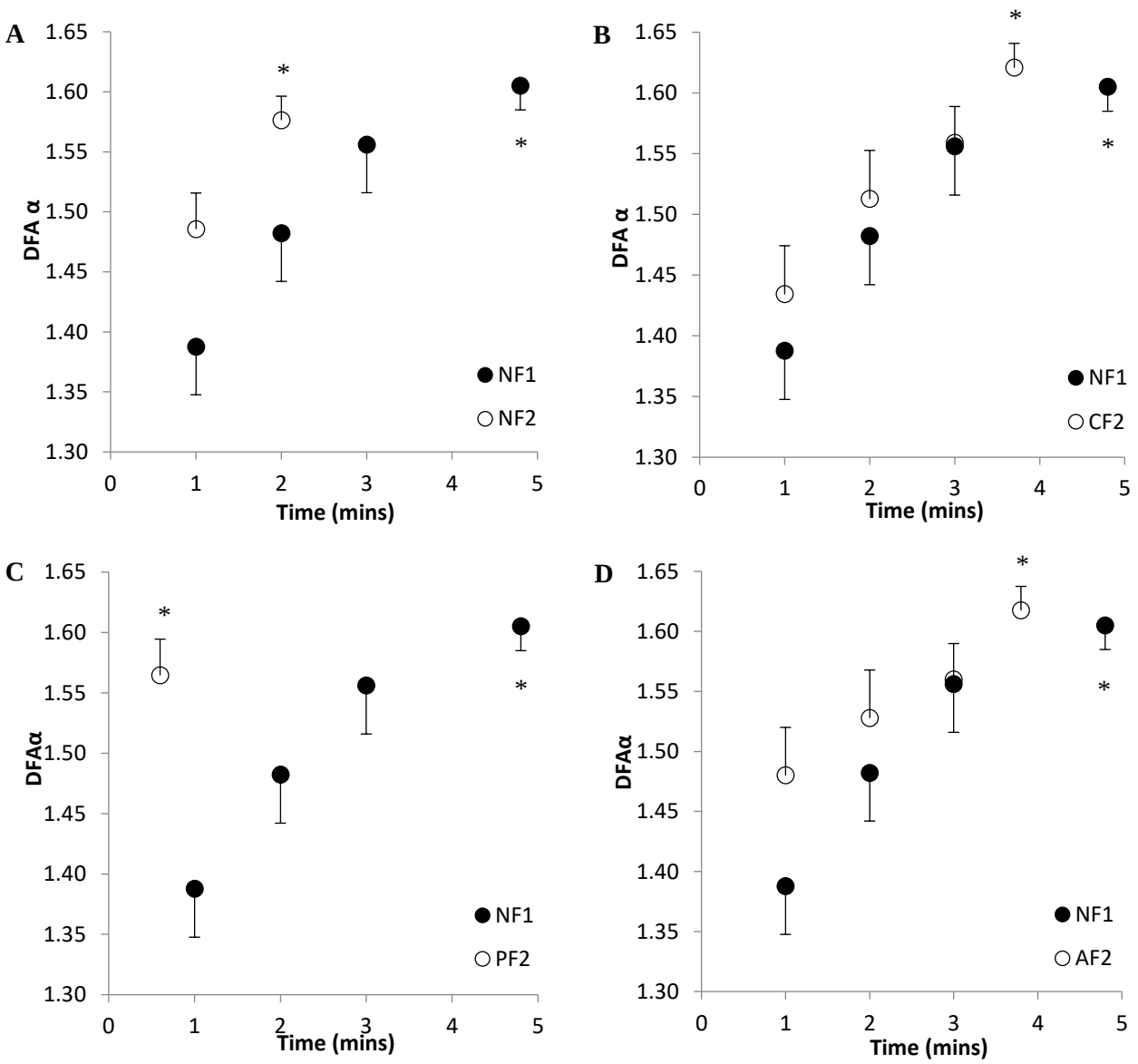

Figure 6.7. Changes in torque DFA α over time in NF1 and NF2 (A), NF1 and CF2 (B), NF1 and PF2 (C) and NF1 and AF2 (D). The last point represents task failure, while the penultimate point represents the last common minute. All values are means (± SEM). * indicates significantly different from NF1 at 1 minute.

Table 6.1. Voluntary torque, potentiated doublet, voluntary activation and EMG responses during contractions in the first exercise bout of NF and the second exercise bouts of NF, CF, PF and AF.

Parameter	NF1	NF2	CF2	PF2	AF2
Mean test torque, N·m	106.6 ± 10.5				
Time to task failure, min	4.7 ± 0.9	2.0 ± 0.3$^{\#}$	3.8 ± 0.8	0.6 ± 0.1$^{\#}$	3.9 ± 0.6
Global fatigue					
Pre-exercise MVC, N·m	218.9 ± 24.0	157.2 ± 12.4$^{\#,\dagger}$	190.4 ± 22.9	100.4 ± 5.0$^{\#}$	180.6 ± 19.2$^{\#}$
Peak MVC at task failure, N·m	115.4 ± 6.4*	112.4 ± 10.5*	108.1 ± 4.8*	99.4 ± 8.2	119.6 ± 5.6*
Mean MVC at task failure, N·m	97.9 ± 7.7*	93.2 ± 8.4*	97.3 ± 6.4*	85.1 ± 7.7	100.3 ± 4.4*
ΔMVC/Δt, N·m·min^{-1}	–31.6 ± 8.2	–32.9 ± 10.2	–27.7 ± 9.0	–25.2 ± 28.5	–22.7 ± 8.5
Peripheral fatigue					
Pre-exercise doublet, N·m	95.7 ± 8.3	77.3 ± 6.3$^{\#,\dagger}$	86.4 ± 7.8$^{\#}$	65.7 ± 5.5$^{\#}$	88.5 ± 10.3
Doublet at task failure, N·m	52.8 ± 3.4*	53.7 ± 4.7$^{*,\#}$	55.7 ± 4.4$^{*,\#}$	55.9 ± 5.3$^{*,\#}$	58.8 ± 4.9$^{*,\#}$
% Change at task failure	43.4 ± 3.9	30.7 ± 2.4	34.6 ± 3.8	15.2 ± 4.1	31.3 ± 5.2
Δdoublet/Δt, N·m·min^{-1}	–12.3 ± 3.3	–14.4 ± 4.2	–9.5 ± 2.2	–29.6 ± 11.9	–9.9 ± 3.4
Central fatigue					
Pre-exercise VA, %	92.5 ± 0.9	79.5 ± 3.5$^{\#}$	88.9 ± 3.1	74.1 ± 2.4$^{\#}$	89.6 ± 1.9
VA at task failure, %	72.7 ± 6.4	67.2 ± 5.2$^{*,\#}$	66.8 ± 3.2$^{*,\#}$	73.9 ± 2.6$^{\#}$	75.2 ± 4.7$^{\#}$
% Change at task failure	21.6 ± 6.7	16.2 ± 4.3	24.5 ± 3.6	0.2 ± 3.5	15.5 ± 6.0
ΔVA/Δt, %/min	–5.2 ± 2.2	–6.9 ± 2.0	–6.5 ± 1.2	7.2 ± 9.3	–4.0 ± 1.5
Surface EMG					
arEMG at task beginning, % MVC	55.6 ± 2.3	70.1 ± 4.9	56.3 ± 3.4	68.7 ± 4.6	61.0 ± 3.0
arEMG at task failure, % MVC	75.7 ± 6.8*	74.1 ± 5.6	71.5 ± 8.8	69.1 ± 4.7	77.2 ± 7.9
ΔarEMG/Δt, % MVC/min	5.6 ± 1.1	3.9 ± 1.8	3.9 ± 1.6	0.5 ± 0.4	4.6 ± 1.7

Values are means ± SEM. NF1 is the first exercise bout in the neuromuscular fatigue condition; NF2, CF2, PF2 and AF2 are the second exercise bouts in the neuromuscular fatigue, central fatigue, peripheral fatigue and afferent feedback conditions; MVC, maximal voluntary contraction; VA, voluntary activation; EMG, electromyogram; arEMG, average rectified EMG of the vastus lateralis; Δ, change; t, time. Letters indicate a statistically significant difference compared to the following: *pre-exercise value/value at task beginning, $^{\#}$NF1, †NF1 at task failure (only applicable for NF2 and PF2).

Table 6.2. Variability, complexity and fractal scaling responses during contractions in the first exercise bout of NF and the second exercise bouts of NF, CF, PF and AF.

Parameter	NF1	NF2	CF2	PF2	AF2
SD					
SD at task beginning, N·m	3.1 ± 0.3	3.6 ± 0.5	3.4 ± 0.6	5.9 ± 1.0$^{\#}$	4.4 ± 0.9
SD at task failure, N·m	8.6 ± 1.1*	7.6 ± 1.6	8.4 ± 2.0	6.3 ± 0.9	7.2 ± 1.0*
ΔSD/Δt, N·m·min^{-1}	1.6 ± 0.4	2.3 ± 0.6	1.4 ± 0.5	0.1 ± 0.4	0.9 ± 0.2
CV					
CV at task beginning, %	2.8 ± 0.1	3.4 ± 0.4	3.6 ± 0.4	6.1 ± 0.7$^{\#}$	4.1 ± 0.7
CV at task failure, %	8.8 ± 0.5*	7.7 ± 1.0*	8.4 ± 1.2*	6.8 ± 0.8	7.3 ± 0.8*
ΔCV/Δt, %/min	1.6 ± 0.3	2.4 ± 0.4	1.4 ± 0.3	0.6 ± 0.4	0.9 ± 0.1
ApEn					
ApEn at task beginning	0.46 ± 0.05	0.35 ± 0.06†	0.38 ± 0.05	0.14 ± 0.03$^{\#}$	0.34 ± 0.05
ApEn at task failure	0.12 ± 0.02*	0.15 ± 0.02*	0.14 ± 0.03*	0.12 ± 0.02	0.14 ± 0.02*
ΔApEn/Δt	–0.09 ± 0.01	–0.10 ± 0.02	–0.07 ± 0.01	–0.01 ± 0.01	–0.05 ± 0.01
SampEn					
SampEn at task beginning	0.43 ± 0.04	0.32 ± 0.05†	0.35 ± 0.05	0.13 ± 0.02$^{\#}$	0.31 ± 0.05
SampEn at task failure	0.11 ± 0.02*	0.14 ± 0.02*	0.13 ± 0.03*	0.11 ± 0.02	0.13 ± 0.02*
ΔSampEn/Δt	–0.08 ± 0.01	–0.09 ± 0.02	–0.06 ± 0.01	–0.01 ± 0.01	–0.05 ± 0.01
DFA α					
DFA α at task beginning	1.39 ± 0.03	1.49 ± 0.03†	1.44 ± 0.03	1.56 ± 0.03$^{\#}$	1.48 ± 0.04
DFA α at task failure	1.60 ± 0.02*	1.58 ± 0.02*	1.62 ± 0.02*	1.56 ± 0.03	1.62 ± 0.02*
ΔDFA α /Δt	0.06 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.002 ± 0.01	0.04 ± 0.01

Values are means ± SEM. NF1 is the first exercise bout in the neuromuscular fatigue condition; NF2, CF2, PF2 and AF2 are the second exercise bouts in the neuromuscular fatigue, central fatigue, peripheral fatigue and afferent feedback conditions; SD, standard deviation; CV, coefficient of variation; ApEn, approximate entropy; SampEn, sample entropy; DFA α, detrended fluctuation analysis; Δ, change; t, time; Letters indicate a statistically significant different compared to the following: *pre-exercise value/value at task beginning, $^{\#}$NF1, †NF1 at task failure (only applicable for NF2 and PF2).

Discussion

The present study sought to gain further insight into the causes of the reduced torque complexity previously observed with neuromuscular fatigue by varying the state of the knee extensor muscle group at the start of exercise, through interventions designed to increase global fatigue, central fatigue, peripheral fatigue and afferent feedback. The major novel finding was that pre-existing fatigue had a significant effect on torque complexity and time to task failure, but that complexity still declined to reach similarly Brownian (DFA $\alpha > 1.50$) values at task failure in all conditions, despite the different task demands. The consistent values of complexity at task failure were accompanied by similarly low and common values for potentiated doublet torque and voluntary activation. It was additionally observed that exercise of one limb results in no "cross-over" of central fatigue to the unexercised contralateral limb, but that it can, nevertheless, exert a fatiguing effect. These results provide further evidence of a link between changes in torque complexity with metabolically mediated peripheral fatigue and the compensatory central adjustments required to continue exercise, and suggest that the loss of complexity is not caused exclusively by central or peripheral fatigue, rather that it reflects changes in relative task demands and is an integrative response to fatigue.

Complexity and neuromuscular fatigue in pre-fatigued muscle

At the start of NF2, which sought to manipulate global fatigue, significant decrements in MVC torque, potentiated doublet torque and voluntary activation were evident (Figures 6.1, 6.2 and 6.3, respectively; Table 6.1) compared with fresh muscle (i.e. NF1 pre). These observations indicate that neuromuscular function remained compromised for the subsequent exercise bout; a fact confirmed by the significantly shorter time to task failure in NF2 compared to fresh muscle (Table 6.1). The complexity values at the start of NF2 were not significantly different from fresh muscle, though were, nonetheless, blunted; as measured by lower ApEn and SampEn, and greater DFA α (Table 6.2). It is possible that the relatively low sample size and high number of comparisons resulted in insufficient power to detect whether these differences were, in fact, significant. Post-hoc power calculations revealed that the powers of the test for ApEn, SampEn and for DFA α were 0.53, 0.45 and 0.48, respectively. The first two studies of this thesis have demonstrated that torque complexity can be systematically reduced by both increasing the absolute demand of a task (i.e. by increasing torque requirements) or by increasing the relative

demand of a task (i.e. by fatiguing the muscle). This suggests that complexity can act as a sensitive surrogate measure of the fatigue process, with lower values indicating increased relative task demands and decreased exercise tolerance. Further support for this contention comes from the observation that this reduced complexity at the start of NF2, though not significant, was accompanied by both significantly decreased MVC torque (indicating increased relative task demands) and time to task failure (indicating decreased exercise tolerance; Table 6.1). This indicates that decreases in complexity reflect an integrated response to neuromuscular fatigue and act as indicators of system function in the presence of fatigue.

At the start of PF2, which sought to manipulate peripheral fatigue, there were significant decrements in MVC torque, potentiated doublet torque and voluntary activation (Figures 6.1, 6.2 and 6.3 respectively; Table 6.1) compared to when fresh. None of these values were significantly different from task failure in NF1, indicating that circulatory occlusion prevented recovery; a finding in line with previous research (Bigland-Ritchie et al., 1986b; Woods et al., 1987; Quistorff et al., 1993). As such, time to task failure was significantly lower than when fresh, with participants unable to complete even one minute of contractions (Table 6.1). Complexity at the start of PF2 was also no different than at task failure in NF1 (Figures 6.4 and 6.6; Table 6.2), indicating that circulatory occlusion prevented its recovery. Circulatory occlusion holds the muscle ischaemic, preventing the recovery of the muscle metabolic milieu and high energy phosphates (Yoshida and Watari, 1997; Lanza et al., 2006), thus it is likely that the failure of ApEn and DFA α to demonstrate any recovery was mediated, at least partially, by this maintained peripheral fatigue. However, the failure of voluntary activation to demonstrate any recovery means that an effect of central fatigue on complexity cannot be excluded. Such a failure of voluntary activation to recover is predicted by the theory of Amann and Dempsey (2008), in which central motor drive is adjusted based on feedback of metabolic perturbations by metabosensitive group III and IV afferents. Based on this theory, the ischaemia induced prevention of recovery of the muscle metabolic milieu was related back to the central nervous system, which then decreased voluntary activation in order to restrict the development of further peripheral fatigue.

At the start of CF2 and AF2, which sought to manipulate central fatigue and afferent feedback, and involved exercise of the left leg then the right leg, there were no significant

differences in voluntary activation compared to when fresh (Figure 6.3; Table 6.1); indicating no "cross-over" of central fatigue to the unexercised, contralateral limb. While several previous studies have observed significant central fatigue and reductions in central motor drive in unexercised, contralateral limbs (Rattey et al., 2006; Martin and Rattey, 2007), this finding is equivocal, with other studies demonstrating no such cross-over, and fatigue being specific to the exercised muscles (Elmer et al., 2013; Kennedy et al., 2015). Prior exercise of the left leg, though, was not without a fatiguing effect on the right leg. Potentiated doublet torque was significantly reduced at the start of CF2 (Figure 6.2; Table 6.1) and MVC torque significantly reduced at the start of AF2 (Figure 6.1; Table 6.1). Such differences suggest that exercise of one limb does exert some fatiguing effect on the contralateral limb, and has the potential to influence performance in a subsequent exercise bout. The observation of an effect of exercise of one limb on the MVC of the unexercised limb is not new (Martin and Rattey, 2007; Halperin et al., 2014), though a "cross-over" of peripheral fatigue is a novel and curious observation. No previous studies on unilateral exercise have demonstrated a cross-over of peripheral fatigue. However, studies by Nordsborg et al. (2003) and Johnson et al. (2014) have found that severe upper body exercise increased markers associated with peripheral fatigue at the onset of a subsequent bout of cycling, and also decreased exercise tolerance. These studies, combined with the present observations, suggest that there is the potential for blood mediated migration of fatiguing metabolic by-products between exercised and unexercised muscle (Halperin et al., 2015).

Prior exercise of the left leg had no significant effect on complexity, though as with the start of NF2 there was a tendency for complexity to be reduced at the start of AF2 (Figures 6.4 and 6.6; Table 6.2). Post-hoc power calculations revealed powers for ApEn, SampEn and DFA α of 0.79, 0.81 and 0.70, respectively. Previous studies have observed increases in tremor amplitude and in tremor ApEn of an unexercised limb following fatiguing contractions performed by the opposite limb (Morrison et al., 2005; Morrison and Sosnoff, 2010; Kavanagh et al., 2013). Although such measures reflect movement rather than torque, they provide support for the contention of a small effect of fatiguing exercise of one limb on the complexity of torque in the unexercised limb. Such a finding would suggest the involvement of central processes in torque complexity and fractal scaling. Though no significant central fatigue was observed at the start of either CF2 or AF2 this

does not rule out potential effects of central mechanisms, such as increased common drive.

Physiological bases for changes in neuromuscular system behaviour

Increases in both absolute and relative task demands are accompanied by a decrease in torque complexity (Chapters 4 and 5). Such increasing demands have also been associated with common drive, the common synaptic input to motoneurons (Castronovo et al., 2015; Farina and Negro, 2015). It has been proposed that the main determinant of torque variability is the total common synaptic input to motoneurons (Dideriksen et al., 2012; Farina et al., 2014), and a recent study by Castronovo et al. (2015) has demonstrated that the relative strength of this common synaptic input increases with both increased torque requirements and increased fatigue. Motor unit synchronisation is a necessary consequence of common synaptic input (Farina and Negro, 2015), and must therefore also increase with both increasing torque requirements and fatigue. Increased motor unit synchronisation has been suggested to disrupt motor performance (Yao et al., 2000), has been postulated to be the cause of decreased complexity of postural tremor with ageing (Sturman et al., 2005) and has been linked to decreases in the fractal dimension (indicating greater regularity) of surface EMG with fatigue (Mesin et al., 2009; Beretta-Piccoli et al., 2015). It is therefore possible that the increased fatigue at the start of the second exercise bouts was accompanied by increased common drive and motor unit synchronisation, with this being responsible for the reduced complexity observed. Further to this, motor unit synchronisation has been described as “phase-locking” (De Luca et al., 1982b), which could relate to “mode-locking,” a phenomenon associated with a pathological loss of complexity and functional responsiveness in ageing and disease, in which outputs oscillate at a single dominant frequency (Peng et al., 1998; Peng et al., 2009).

The previous study (Chapter 5) observed that task failure was characterised by similarly low values of ApEn, SampEn, DFA α and potentiated doublet torque across all of the fatiguing conditions, suggesting links between complexity, fractal scaling, peripheral fatigue and exercise tolerance. The same was observed in the present study (Table 6.1; Table 6.2). This could be reflective of the “sensory tolerance limit” proposed by Amann (2011) actually being an emergent property of a fatiguing neuromuscular system. The sensory tolerance limit proposes that metabolic perturbations (i.e. peripheral fatigue) are

related back to the central nervous system, which then adjusts central motor drive in order to restrict the development of peripheral fatigue beyond a certain limit (Amann et al., 2006). However, the consistent values of complexity at task failure observed in the present and previous (Chapter 5) studies could indicate that it is, in fact, the loss of complexity which is restricted. Whether low complexity plays a direct role in precipitating task failure is not presently clear; though in accordance with the purported functional importance of physiological complexity, high values endow a system with the ability to rapidly adapt to changes in demand, while low values compromise motor control and limit task performance (Sosnoff et al., 2004; Peng et al., 2009). Such a restriction in the loss of complexity would be an adaptive response, serving to maintain some degree of adaptability in motor control and exploratory freedom in the face of compromised neuromuscular system function.

Conclusion

In summary, this study has demonstrated that pre-existing fatigue influences the complexity and fractal scaling of knee extensor torque complexity at the start of an exercise bout, but still results in consistent values at task failure regardless of the time taken to reach this point. Whilst the results provide support for the contention that peripheral fatigue is at least partially responsible for the changes in complexity and fractal scaling seen with fatigue, it is possible that such changes are, in fact, brought about by both peripheral and central mechanisms, and reflect the integrity of the neuromuscular system as a whole. Complexity and fractal scaling may, therefore, not act as measures of fatigue, but instead as indices of system functionality. This latter interpretation of complexity as a measure of system functionality allows it to be applied to other perturbations, and is in line with the loss of complexity previously demonstrated with ageing. Low values of complexity and high values of fractal scaling would thus represent a system with reduced functionality, predisposing it to task failure and reducing its adaptability.

Chapter 7 – Study 4

Fatigue-induced loss of knee extensor torque complexity is slowed by caffeine ingestion

Introduction

The first three studies of this thesis (Chapters 4, 5 and 6) have demonstrated that the development of neuromuscular fatigue is accompanied by a significant reduction in the complexity of torque output, shifting it towards increasingly Brownian noise (DFA α = 1.50), during intermittent isometric knee extension contractions. A failure to successfully uncouple central and peripheral fatigue, which develop alongside this reduced complexity, means that the exact mechanism(s) behind the loss of complexity remains obscure. The purpose of the present study was to investigate the effect of the ingestion of caffeine, an ergogenic aid thought to act primarily through central mechanisms, on torque complexity during neuromuscular fatigue. If central processes are mechanistically linked to torque complexity and the fatigue-induced loss previously observed, it would be expected that such a centrally acting ergogenic aid should exert a positive effect on torque complexity, potentially increasing baseline complexity or acting to delay/attenuate the fatigue-induced reduction.

Caffeine (1,3,7-trimethylxanthine) is one of the most widely consumed drugs in the world (Williams et al., 1987; Fredholm et al., 1999). It has long been recognised as a potent ergogenic aid, with studies as far back as the first decade of the 20th century demonstrating improved exercise capacity following caffeine ingestion (Rivers and Webber, 1907). Since that time, numerous studies have demonstrated that pre-exercise caffeine ingestion enhances performance during prolonged (> 1 hour) cycling and running (Costill et al., 1977; Spriet et al., 1992; Graham, 2001). Such is the effectiveness of caffeine at improving performance, various sporting governing bodies have banned or controlled its use over the last 40 years (Graham, 2001).

As well as enhancing performance in events of a predominantly whole-body nature, caffeine has also been shown to have a clear effect on neuromuscular performance (Warren et al., 2010). Evidence suggests that caffeine can potentiate twitch and tetanic

tensions (Lopes et al., 1983; Tarnopolsky and Cupido, 2000), increase MVC torque (Kalmar and Cafarelli, 1999; Park et al., 2008) and increase spinal excitability (Walton et al., 2003; Kalmar and Cafarelli, 2004b). More pertinently, caffeine has been repeatedly demonstrated to increase muscular endurance, with a recent meta-analysis finding a significant positive effect size of 0.28, which equated to an increase in time to task failure of ~18% (Warren et al., 2010). Studies conducted on the knee extensors have found single-leg isometric time to exhaustion to increase by as much as 25% (Kalmar and Cafarelli, 1999; Plaskett and Cafarelli, 2001; Meyers and Cafarelli, 2005).

It is thought that caffeine's primary ergogenic effect is to enhance central drive, through an antagonistic effect on adenosine receptors (Fredholm et al., 1999). Adenosine preferentially inhibits the release of excitatory neurotransmitters (Fredholm et al., 1999), and thus has the potential to alter α-motoneuron excitability and decrease the firing of central neurons. Caffeine's inhibitory effect on adenosine receptors should prevent inhibition or disfacilitation of α-motoneurons in a variety of ways, possibly by promoting dopaminergic transmission (Gandevia, 2001) or increasing spinal excitability (Walton et al., 2003), thus offsetting task failure (Kalmar and Cafarelli, 2004a). Though other mechanisms, such as a direct effect on skeletal muscle and calcium (Ca^{2+}) (Tarnopolsky and Cupido, 2000), an antinociceptive effect (Plaskett and Cafarelli, 2001) and an effect on cortical substrates and perceived exertion (de Morree et al., 2014), cannot be ruled out, there is considerable evidence to suggest that caffeine acts on the central nervous system and, indeed, it has been described as a valuable tool in the study of central fatigue (Kalmar and Cafarelli, 2004a).

The aim of the present study was to determine whether caffeine, a well-tolerated central nervous system stimulant and ergogenic aid, influences torque complexity during neuromuscular fatigue. The specific experimental hypotheses tested were: 1) that pre-exercise caffeine ingestion will increase time to task failure; 2) that pre-exercise caffeine ingestion will attenuate/delay the reduction in knee extensor torque complexity seen with neuromuscular fatigue (as measured by slower rates of decrease in approximate entropy [ApEn] and sample entropy [SampEn]); and 3) that pre-exercise caffeine ingestion will attenuate/delay the change in knee extensor torque fractal scaling seen with neuromuscular fatigue (as measured by a slower rate of increase in detrended fluctuation analysis [DFA] α).

Methods

Participants

Ten healthy participants (6 male, 4 female; mean ± SD: age 25.6 ± 6.1 years; height 1.75 ± 0.09 m; body mass 65.1 ± 7.7 kg) provided written informed consent to participate in the study, which was approved by the ethics committee of the University of Kent, and which adhered to the Declaration of Helsinki. All participants were non-smokers, as nicotine is known to alter the rate of caffeine degradation (Parson and Neims, 1978). Participants were instructed to arrive at the laboratory in a rested state (having performed no strenuous exercise in the preceding 24 hours) and to have consumed neither any caffeine nor alcohol in the preceding 24 hours. Participants attended the laboratory at the same time of day (± 2 hours) during each visit.

Experimental design

Participants were required to visit the laboratory on three occasions over a two to three-week period, with a minimum of 72 hours between each visit. During their first visit, participants were familiarised with all testing equipment and procedures, and the settings for the dynamometer and stimulator were recorded. During the next two visits, a double-blind, repeated measures, randomised design was used to assess the effect of caffeine on torque fluctuations during fatiguing intermittent isometric knee extension contractions. The methods used for the setup of the dynamometer, femoral nerve stimulation and surface EMG are described in the General Methods (Chapter 3).

Protocol

All visits followed a similar pattern of data acquisition, beginning with the instrumentation of the participants and the (re-)establishment of the correct dynamometer seating position and supramaximal stimulation response. Participants then performed a series of brief (3 second) MVCs to establish the maximum torque of their right leg. These contractions were separated by 60 seconds rest, and continued until three consecutive peak torques were within 5% of each other. Participants were given a countdown, followed by very strong verbal encouragement to maximise torque. The first MVC was used to establish the fresh maximal EMG signal, against which the subsequent EMG signals were normalised (Data analysis; see below). The second and third MVCs were performed with peripheral nerve stimulation. In all instances where MVCs were

performed with stimuli, the stimuli were delivered 1.5 seconds into the contraction to coincide with maximal torque, and 2 seconds after the contraction to provide a resting potentiated doublet (see General Methods; Chapter 3).

Following the establishment of maximum torque, participants ingested gelatin capsules containing either 6 $mg \cdot kg^{-1}$ caffeine (Myprotein, Cheshire, UK) or the same amount of all-purpose flour (placebo). A person not involved in the study prepared and arranged the order of the capsules, with this double-blind design not being broken until all the trials were complete. After ingestion of the capsules, participants rested in the laboratory, and repeated the process of establishing maximum torque after 1 hour, when plasma caffeine levels peak (Graham and Spriet, 1995). After a further 10 minutes of rest, participants then performed the experimental trial (see below).

Experimental trials

As in the previous studies of this thesis, participants performed intermittent isometric knee extension contractions until task failure. The target torque was 50% MVC, and was based on the highest instantaneous torque recorded during the pre-capsule ingestion MVCs in the first experimental session. The duty cycle for the contractions was 0.6; with contractions lasting 6 seconds and being followed by 4 seconds rest. At the end of each minute (i.e. every sixth contraction), participants performed an MVC, accompanied by peripheral nerve stimulation. The contractions were performed until task failure, the point at which the participant failed to reach the target torque on three consecutive occasions, despite strong verbal encouragement. Participants were not informed of the elapsed time during the trials, but were informed of each “missed” contraction. After the third missed contraction, participants were instructed to immediately produce an MVC, which was accompanied by peripheral nerve stimulation. Further details and a graphical representation of the contraction protocol can be found in the General Methods (Chapter 3, Figure 3.1).

Additional measures

Measures of heart rate were taken prior to capsule ingestion, 1 hour after capsule ingestion and at the end of each minute of the fatiguing trials, using a heart rate monitor (BHIP 9, BHIP Ltd., Norfolk, UK). Ratings of perceived exertion (RPE) were taken using the Borg scale (Borg, 1970) at the end of each minute of the fatiguing trials.

Data analysis

The data analysis focused on three specific areas: 1) basic measures of torque and EMG; 2) measures of central and peripheral fatigue; and 3) the variability and complexity of the torque output. All data were analysed using code written in MATLAB R2013a (The MathWorks, Massachusetts, USA).

Torque and EMG: The mean and peak torques, and vastus lateralis EMG, were calculated as described in the General Methods (Chapter 3). Due to the use of intermittent contractions, calculation of task failure was more problematic than defining failure during a sustained contraction. Details of the exact calculation of task failure are given in the General Methods (Chapter 3). The vastus lateralis arEMG was normalised to the post-capsule ingestion MVC.

Central and peripheral fatigue: Measures of central and peripheral fatigue were calculated based on the stimuli delivered during and after the MVCs performed pre-test, during the fatiguing contractions and at task failure. Further details are given in the General Methods (Chapter 3). Muscle contractility was assessed for each peripherally derived resting twitch as contraction time to peak torque (TPT) and one-half relaxation time ($RT_{0.5}$). Further details are given in the Methods section of Chapter 5.

Variability and complexity: All measures of variability and complexity were calculated using the steadiest five seconds of each contraction. The amount of variability in the torque output of each contraction was measured using the standard deviation (SD) and coefficient of variation (CV). The temporal structure of variability was examined using multiple time domain analyses. ApEn and SampEn were used to determine the complexity of the torque output; while DFA was used to estimate the temporal fractal scaling. Further details are given in the General Methods (Chapter 3).

Statistics

All data are presented as means ± SEM unless otherwise stated, and results were deemed statistically significant when $P < 0.05$. Two-way ANOVAs with repeated measures were used to test for differences between conditions, time points and a condition x time interaction for MVC torque, arEMG, potentiated doublet torque, voluntary activation, measures of variability and measures of complexity. The variability and complexity

measures were analysed using averages from the first minute and final minute before task failure. When main effects were observed, Student's paired samples t-tests and Bonferroni-adjusted 95% confidence intervals were then used to determine specific differences. The rates of change in all parameters were analysed using Student's paired samples t-tests.

Results

Preliminary measures

The contractile properties of the knee extensors, electrical activity of the vastus lateralis and heart rate responses in the two conditions are shown in Table 7.1 These data were obtained immediately before and 1h after capsule ingestion and were intended to assess the effects of caffeine in a rested state. Caffeine ingestion resulted in a significant increase in MVC torque of 5.2 ± 1.9 N·m (or 2.6 ± 1.0%; 95% paired samples confidence intervals (CIs): 1.0, 9.5 N·m); and a significant increase in EMG_{max} (CIs 0.004, 0.5 mV). Placebo ingestion resulted in no significant change in any of the variables.

Table 7.1. Effects of caffeine and placebo ingestion on control measurements

	Placebo		Caffeine	
Parameter	Pre	Post	Pre	Post
MVC, N·m	205.6 ± 16.2	202.5 ± 16.6	204.1 ± 14.6	209.3 ± 14.9*
Doublet, N·m	99.0 ± 9.6	94.4 ± 8.8	94.5 ± 8.6	91.6 ± 7.7
VA, %	92.1 ± 0.8	91.6 ± 0.4	92.0 ± 0.9	92.4 ± 0.1
EMG_{max}, mV	0.26 ± 0.03	0.26 ± 0.03	0.27 ± 0.03	0.30 ± 0.03*
HR, bpm	79 ± 4	72 ± 3	79 ± 5	71 ± 4

Values are means ± SEM. MVC, maximal voluntary contractions; VA, voluntary activation; EMG_{max}, maximal electrical activity of the vastus lateralis; mV, millivolts; HR, heart rate; bpm, beats per minute. * = significantly different from Pre.

Time to task failure, torque and EMG

Caffeine ingestion significantly increased endurance time by 2.4 ± 0.9 mins (CIs 4.3, 5.0 mins; Table 7.2) or 28.4 ± 5.2%. Task failure occurred when participants were no longer able to achieve the target torque (102.3 ± 8.2 N·m), despite a maximal effort. Both conditions resulted in significant decreases in MVC torque ($F_{1,9}$ = 65.80, $P < 0.001$; Figure 7.1; Table 7.2), with neither the peak nor the mean MVC torque at task failure being significantly different from the torque produced during the submaximal

contractions (Table 7.2). At task failure, there was no significant difference between the conditions for the peak MVC torque (CIs –16.2, 6.4 N·m). However, at the time point in the caffeine condition equivalent to task failure in the placebo condition (henceforth referred to as isotime), MVC torque was significantly higher (133.9 ± 7.8 vs. 105.8 ± 6.7 N·m; CIs 13.8, 42.5 N·m; Figure 7.1), indicating participants still had a significant reserve of maximal torque. Furthermore, the rate of decrease in MVC torque was significantly attenuated in the caffeine condition (CIs –1.3, –7.5 N·m·min^{-1}; Table 7.2).

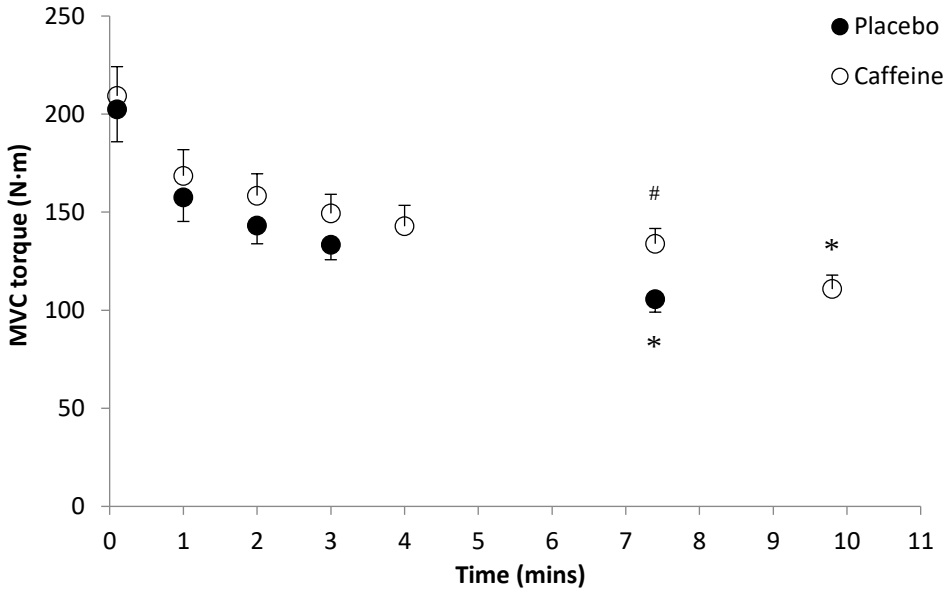

Figure 7.1. Group peak MVC torque (± SEM) for each common minute, isotime and task failure in each condition. The last point of each condition represents task failure. The penultimate point in the caffeine condition represents isotime, the equivalent of task failure in the placebo condition. * = Significantly different from fresh. # = Significantly different from task failure in the placebo condition.

The mean arEMG, normalised to a fresh post-capsule MVC, increased over time in both conditions, reaching 73.1 ± 5.1% in the placebo condition and 78.8 ± 7.5% in the caffeine condition at task failure ($F_{1,9}$ = 9.79, P = 0.012; Figure 7.2; Table 7.2). There were no significant differences between the conditions during the first minute of contractions, at task failure or at isotime; nor was there a significant difference in the rate of increase in arEMG (Table 7.2).

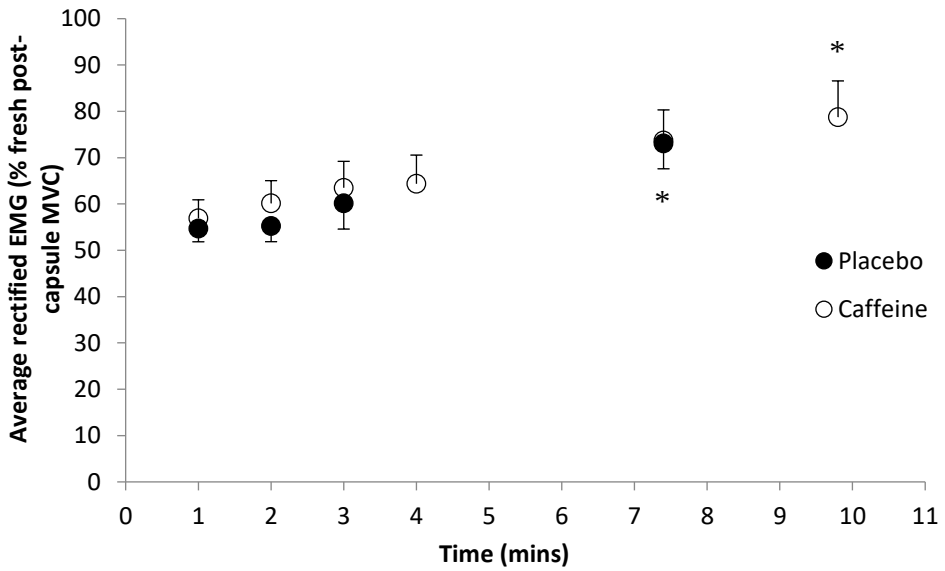

Figure 7.2. Group mean arEMG (± SEM) for each common minute, isotime and task failure in each condition. The last point of each condition represents task failure. The penultimate point in the caffeine condition represents isotime, the equivalent of task failure in the placebo condition. * = Significantly different from fresh.

Peripheral and central fatigue

Both conditions resulted in significant reductions in potentiated doublet torque ($F_{1,8}$ = 27.67, P = 0.001; Figure 7.3; Table 7.2), indicating the presence of peripheral fatigue. The values attained at task failure were not significantly different between the conditions (CIs –6.0, 15.8 N·m), nor was the value attained at task failure in the placebo condition different from the value at isotime in the caffeine condition (CIs –7.8, 13.8 N·m). The rate of decrease in potentiated doublet torque was, however, significantly attenuated in the caffeine condition (CIs –3.3, –0.07 $N \cdot m \cdot min^{-1}$). Voluntary activation significantly declined in both conditions ($F_{1,8}$ = 24.0, P = 0.001; Figure 7.4; Table 7.2), indicating the presence of central fatigue. The values attained at task failure were not significantly different between the conditions (CIs –20.5, 4.6%), nor was the value attained at task failure in the placebo condition different from the value at isotime in the caffeine condition (CIs –26.1, 2.1%). There was, however, a tendency for voluntary activation to be higher (~12%) at isotime in the caffeine condition (Figure 7.4). The rate of decrease in voluntary activation was significantly attenuated in the caffeine condition (CIs –4.0, –0.05 %/min). In terms of muscle contractility, neither TPT nor $RT_{0.5}$ were affected by either fatigue (TPT, $F_{1,8}$ = 1.8, P = 0.84; $RT_{0.5}$, $F_{1,8}$ = 2.7, P = 0.14) or condition (TPT, $F_{1,8}$ = 3.5, P = 0.10; $RT_{0.5}$, $F_{1,8}$ = 4.4, P = 0.07).

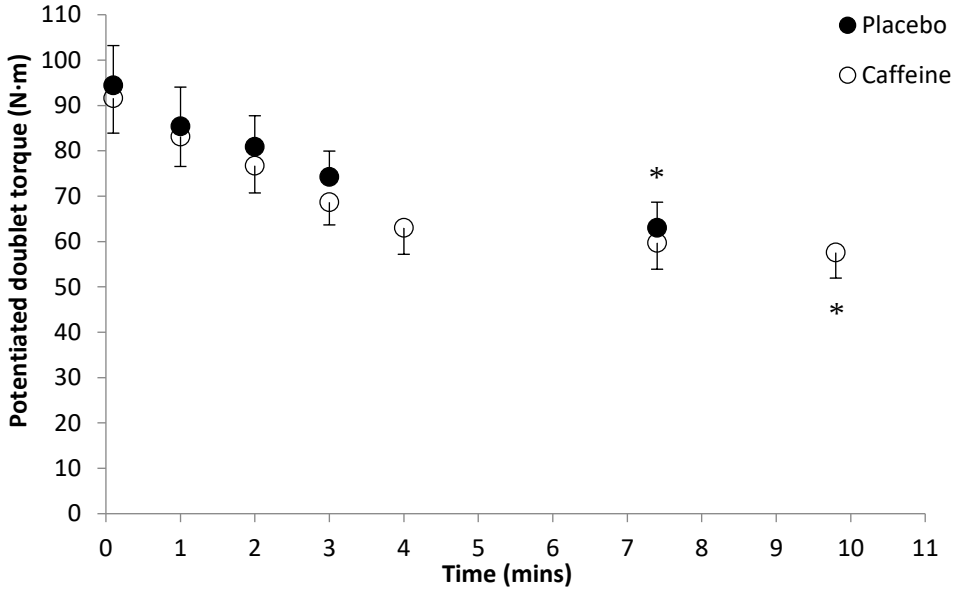

Figure 7.3. Group mean potentiated doublet torque (± SEM) for each common minute, isotime and task failure in each condition. The last point of each condition represents task failure. The penultimate point in the caffeine condition represents isotime, the equivalent of task failure in the placebo condition. * = Significantly different from fresh.

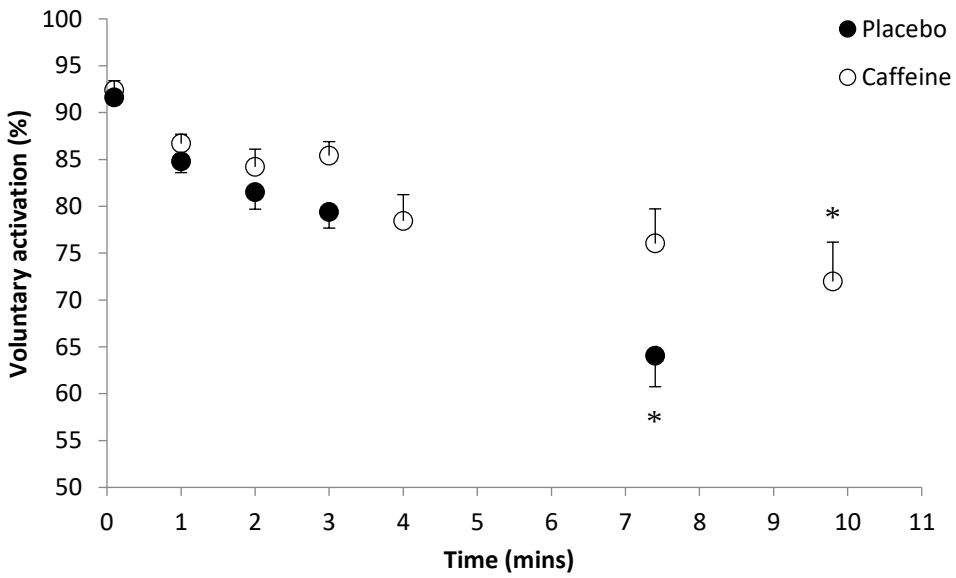

Figure 7.4. Group mean voluntary activation (± SEM) for each common minute, isotime and task failure in each condition. The last point of each condition represents task failure. The penultimate point in the caffeine condition represents isotime, the equivalent of task failure in the placebo condition. * = Significantly different from fresh.

Variability and complexity

Both conditions resulted in a significant increase in the amount of variability, as measured by the SD ($F_{1,9}$ = 58.21, P = 0.004; Table 7.3) and CV ($F_{1,9}$ = 65.87, P = 0.001; Table 7.3). The values attained at task failure for the SD (CIs –1.8, 2.6 N·m) and CV (CIs –1.9, 3.0%) were not significantly different between the conditions. The CV was significantly lower at isotime in the caffeine condition compared with task failure in the placebo

condition (CIs –5.8, –0.3%). The rates of increase in SD (0.8 ± 0.2 vs. 1.0 ± 0.3 $N \cdot m \cdot min^{-1}$; CIs 0.002, 0.6 $N \cdot m \cdot min^{-1}$) and CV (0.8 ± 0.2 vs. 1.1 ± 0.2 %/min; CIs 0.01, 0.6 %/min) were significantly lower in the caffeine condition (Table 7.3).

The ApEn, SampEn and DFA α responses to the fatiguing contractions in both conditions are presented in Figure 7.5A, B and C, respectively. There was no difference between conditions in terms of complexity values at the start of the trials (CIs ApEn, –0.08, 0.06; SampEn –0.07, 0.06; DFA α –0.05, 0.04). Complexity decreased with fatigue in both conditions, as measured by ApEn ($F_{1,9}$ = 64.75, P < 0.001), SampEn ($F_{1,9}$ = 61.78, P < 0.001) and DFA α ($F_{1,9}$ = 35.12, P < 0.001; Table 7.3). ApEn and SampEn significantly decreased as the trials progressed (Figure 7.5A and B), with the values at task failure being not significantly different between the conditions (CIs ApEn –0.1, 0.05; SampEn –0.09, 0.05; Table 7.3). At isotime in the caffeine condition, ApEn remained significantly higher than at task failure in the placebo condition (CIs 0.005, 0.3). SampEn had a tendency to be higher at isotime in the caffeine condition, though the difference did attain significance (0.25 vs. 0.12; CIs –0.3, 0.006). The rates of decrease in ApEn (CIs –0.004, –0.03) and SampEn (CIs –0.002, –0.03) were significantly lower in the caffeine condition. DFA α significantly increased, becoming more Brownian, as the trials progressed (Figure 7.5C), with the values at task failure being not significantly different between the conditions (CIs –0.08, 0.09; Table 7.3). At isotime in the caffeine condition, DFA α had a tendency to be lower than at task failure in the placebo condition, though as with SampEn, this difference did not attain significance (1.54 vs. 1.60; CIs –0.008, 0.1). The rate of change of DFA α was not significantly different between the conditions (CIs –0.003, 0.03); though given the values from the first minute and task failure did not differ between the conditions (Table 7.3) and time to task failure was extended with caffeine, this suggests that the rate of increase in DFA α was, in fact, slower following caffeine ingestion.

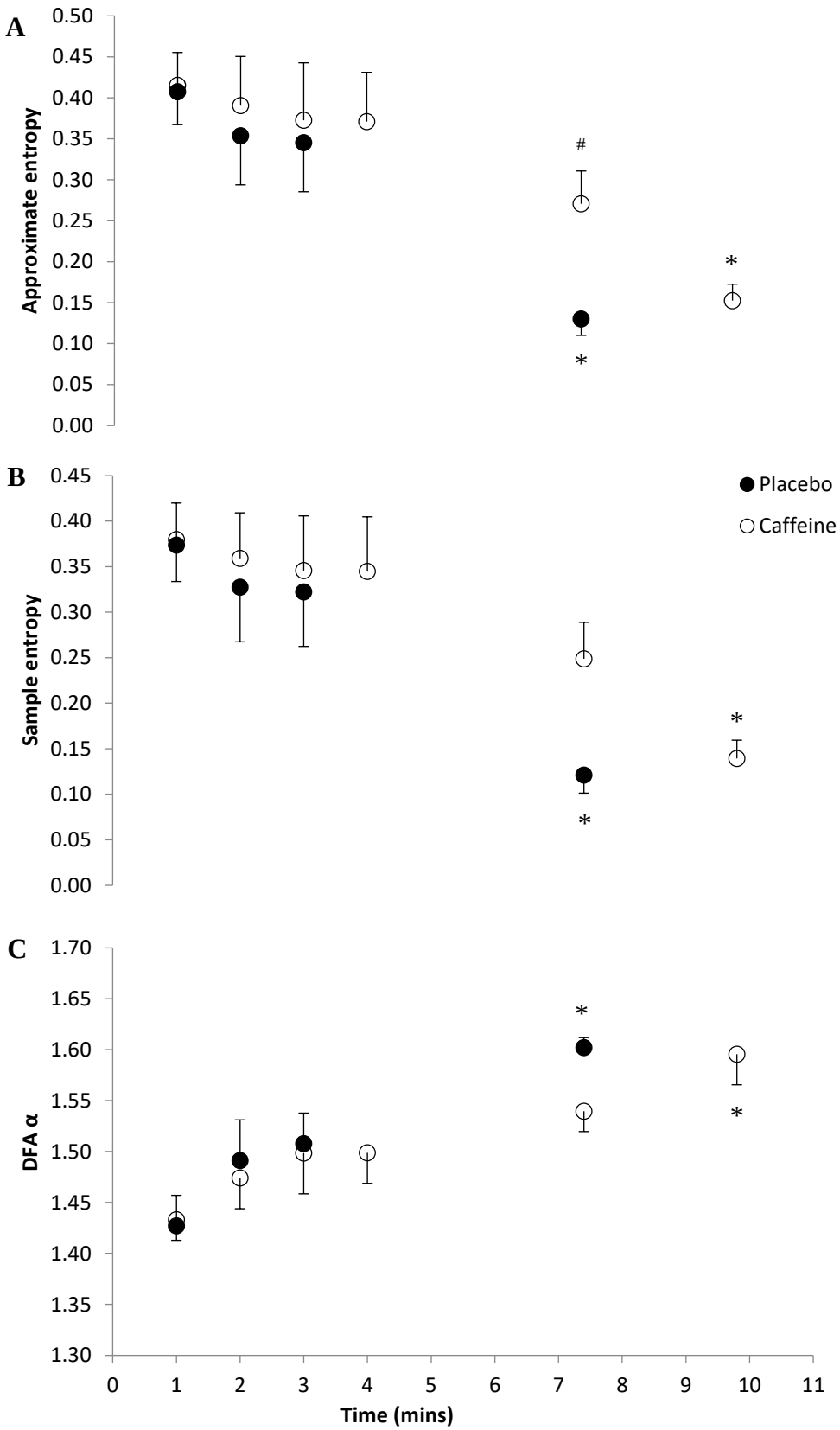

Figure 7.5. Group mean (± SEM) changes in torque ApEn (A), SampEn (B) and DFA α (C) for each common minute, isotime and task failure in each condition. The last point of each condition represents task failure. The penultimate point in the caffeine condition represents isotime, the equivalent of task failure in the placebo condition. * = Significantly different from fresh. # = Significantly different from task failure in the placebo condition.

Heart rate and perceived exertion

Heart rate significantly increased in both conditions as the trials progressed ($F_{1,9} = 126.65$, $P < 0.001$; Table 7.2). There were no significant differences in heart rate between the conditions at task failure (CIs –15.5, 9.7 bpm) or at task failure in the placebo condition and isotime in the caffeine condition (CIs –7.5, 20.2 bpm; Table 7.2). The rate of increase in heart rate was significantly lower in the caffeine condition (CIs –0.8, –3.4 bpm). Perceived exertion increased over time in both the placebo (CIs 5.4, 8.2) and caffeine conditions (CIs 5.8, 9.3; Table 7.2) Perceived exertion was significantly lower at the end of the first minute of the caffeine condition (CIs –1.8, –0.2) and also at isotime in the caffeine condition compared to task failure in the placebo condition (CIs –3.9, –1.2). The rate of increase in perceived exertion was significantly lower in the caffeine condition (CIs –0.3, –0.02).

Table 7.2. Changes in voluntary torque, potentiated doublet torque, voluntary activation, EMG, heart rate and RPE responses during contractions in the placebo and caffeine trials.

Parameter	Placebo	Caffeine
Time to task failure, min	7.4 ± 1.8	9.8 ± 2.6$^{\#}$
Global fatigue		
Pre-exercise MVC, N·m	202.5 ± 16.2	209.3 ± 14.9
Peak MVC at task failure, N·m	105.8 ± 6.7*	110.8 ± 7.1*
Mean MVC at task failure, N·m	85.2 ± 4.6*	91.8 ± 5.6*
ΔMVC/Δt, N·m·min^{-1}	–22.1 ± 5.7	–17.8 ± 4.7$^{\#}$
Peripheral fatigue		
Pre-exercise doublet, N·m	94.4 ± 8.8	91.6 ± 7.7
Doublet at task failure, N·m	61.3 ± 5.0*	57.9 ± 5.7*
% Change at task failure	33.1 ± 2.3	35.5 ± 3.4
Δdoublet/Δt, N·m·min^{-1}	–8.2 ± 2.1	–6.5 ± 1.6$^{\#}$
Central fatigue		
Pre-exercise VA, %	91.6 ± 0.4	92.4 ± 1.0
VA at task failure, %	64.0 ± 3.3*	72.0 ± 4.2*
% Change at task failure	30.0 ± 3.6	22.2 ± 4.7
ΔVA/Δt, %/min	–5.7 ± 1.3	–3.7 ± 1.1$^{\#}$
Surface EMG		
arEMG at task beginning, % MVC	55.0 ± 3.1	56.9 ± 3.9
arEMG at task failure, % MVC	73.1 ± 5.4*	78.8 ± 7.5*
ΔarEMG/Δt, % MVC/min	3.7 ± 1.2	3.4 ± 1.0
Heart rate		
Pre-exercise heart rate, bpm	72 ± 3	71 ± 4
Heart rate at task failure, bpm	133 ± 5*	136 ± 5*
ΔHeart rate/Δt, bpm·min^{-1}	12.0 ± 2	10.0 ± 2$^{\#}$
RPE		
RPE at task beginning	12.9 ± 0.4	11.9 ± 0.4$^{\#}$
RPE at task failure	19.7 ± 0.2*	19.5 ± 0.2*
ΔRPE/Δt	1.3 ± 0.2	1.2 ± 0.2$^{\#}$

Values are means ± SEM. MVC, maximal voluntary contraction; VA, voluntary activation; EMG, electromyogram; arEMG, average rectified EMG of the vastus lateralis; Δ, change; t, time. Symbols indicate a statistically significant difference compared to the following: *pre-exercise value/value at task beginning, $^{\#}$placebo.

Table 7.3. Changes in variability, complexity and fractal scaling responses during contractions in the placebo and caffeine trials.

Parameter	Placebo	Caffeine
SD		
SD at task beginning, N·m	2.8 ± 0.3	2.7 ± 0.2
SD at task failure, N·m	7.2 ± 0.8*	6.8 ± 1.0*
ΔSD/Δt, N·m·min^{-1}	1.0 ± 0.3	0.8 ± 0.2#
CV		
CV at task beginning, %	2.8 ± 0.2	2.7 ± 0.2
CV at task failure, %	7.7 ± 0.4*	7.1 ± 0.7*
ΔCV/Δt, %/min	1.1 ± 0.2	0.8 ± 0.2#
ApEn		
ApEn at task beginning	0.40 ± 0.03	0.42 ± 0.03
ApEn at task failure	0.13 ± 0.02*	0.15 ± 0.02*
ΔApEn/Δt	–0.06 ± 0.01	–0.04 ± 0.01#
SampEn		
SampEn at task beginning	0.37 ± 0.03	0.38 ± 0.03
SampEn at task failure	0.12 ± 0.02*	0.14 ± 0.02*
ΔSampEn/Δt	–0.05 ± 0.01	–0.03 ± 0.02#
DFA α		
DFA α at task beginning	1.42 ± 0.02	1.43 ± 0.02
DFA α at task failure	1.60 ± 0.01*	1.60 ± 0.03*
ΔDFA α /Δt	–0.04 ± 0.01	–0.03 ± 0.01

Values are means ± SEM. SD, standard deviation; CV, coefficient of variation; ApEn, approximate entropy; SampEn, sample entropy; DFA α, detrended fluctuation analysis; Δ, change; t, time. Symbols indicate a statistically significant different compared to the following: *pre-exercise value/value at task beginning, #placebo.

Discussion

The present study sought to investigate the effects of caffeine on knee extensor torque complexity during neuromuscular fatigue. The major novel finding of the present study was that caffeine ingestion slowed the loss of torque complexity seen with neuromuscular fatigue. This response was accompanied by increased time to task failure and a slowed rate of fatigue development. Both the placebo and caffeine conditions were associated with a loss of MVC torque and the development of significant central and peripheral fatigue, which were accompanied by increasingly Brownian (DFA α = 1.50) fluctuations in torque output. At task failure in each condition, common values were observed for torque complexity, MVC torque, and central and peripheral fatigue. Caffeine ingestion, though, significantly attenuated the rate at which all of these fatigue parameters decreased. These results provide further support for the contention that changes in torque

complexity with neuromuscular fatigue are linked to task demands, torque generating capacity and the fatigue process; provide the first evidence that torque complexity is sensitive to pharmacological intervention; and provide further insight into the mechanisms of caffeine's ergogenic effect.

Effect of caffeine on fresh knee extensor contractile properties

Caffeine ingestion has previously been demonstrated to potentiate contractile properties in fresh muscle (Plaskett and Cafarelli, 2001; Meyers and Cafarelli, 2005; Park et al., 2008). A recent meta-analysis concluded that caffeine has a significant effect on MVC torque, increasing it by ~4%, with this figure raised to ~7% when the knee extensors alone were considered (Warren et al., 2010). The present study demonstrated a small, but significant, increase in knee extensor MVC torque of ~3% following caffeine ingestion (Table 7.1). The increase in MVC torque following caffeine ingestion has largely been attributed to improved voluntary activation, due to a release of inhibition in the motor cortex brought about by caffeine's antagonistic effect on adenosine (Kalmar and Cafarelli, 1999; Kalmar et al., 2006). The notion that increased MVC torque following caffeine ingestion is mediated by voluntary activation is supported by the meta-analysis of Warren et al. (2010), which found that caffeine had a significant effect on voluntary activation (effect size = 0.670, $P < 0.001$), but not on electrically evoked strength (effect size 0.115, $P = 0.24$). Though the present study observed no effect of caffeine on voluntary activation, a significant increase in vastus lateralis EMG_{max} was observed (Table 7.1), which could be indicative of increased muscle activity. However, it must be noted that the amplitude of the EMG signal does not provide a direct index of neural drive to the muscle (Farina et al., 2010) and such an increase in EMG_{max} could be due to factors such as crosstalk, increased electrode conductance or increased blood flow (Farina et al., 2004).

Effect of caffeine on fatigue and torque complexity

In line with its hypotheses, the present study demonstrated that the loss of torque complexity previously observed with neuromuscular fatigue (Chapters 4, 5 and 6) can be significantly slowed by the ingestion of caffeine (Figure 7.5; Table 7.3). There were no differences in complexity between the conditions at the start of the tests, and as fatigue developed ApEn and SampEn significantly decreased (indicating increased signal regularity) and DFA α significantly increased (indicating increasingly Brownian

fluctuations), reaching common values at task failure in each condition (Figure 7.5; Table 7.3). However, the rates at which ApEn and SampEn decreased were significantly attenuated following caffeine ingestion (Table 7.3). Moreover, at isotime in the caffeine condition ApEn was significantly higher, SampEn had a tendency to be higher and DFA α had a tendency to be lower than at task failure in the placebo condition (Figure 7.5). Post-hoc power calculations revealed the power of the test for SampEn was 0.71 and for DFA α was 0.63. Low complexity values are associated with compromised motor control, impaired performance and eventually task failure, with the fatigue-induced loss of complexity appearing to reflect increased relative task demands and to serve as a sensitive surrogate index of fatigue (Chapter 5). Thus, the higher complexity at the time equivalent to task failure is suggestive of a significant reserve in exercise capacity. Such a conclusion is supported by the observation that MVC torque also remained significantly higher at isotime in the caffeine condition compared to task failure in the placebo condition (Figure 7.1; Table 7.2). These findings indicate that pre-exercise caffeine ingestion slows the loss of adaptability of motor control and narrowing of system responsiveness, which decreases in complexity measures are thought to reflect (Lipsitz and Goldberger, 1992; Goldberger et al., 2002).

The ergogenic ability of caffeine has long been established, with studies dating back over 100 years demonstrating its performance enhancing capabilities across a variety of exercise modalities and tasks (Rivers and Webb, 1907; Costill et al., 1977; Graham et al., 1998; Graham, 2001). The results of the present study provide further support for this ergogenic effect, demonstrating that caffeine ingestion increased the time to task failure of the knee extensors during single leg intermittent isometric exercise by ~28% (Table 7.2). This performance enhancement is of greater magnitude than the ~18% improvement documented for open ended neuromuscular tasks by Warren et al. (2010) in their meta-analysis, but is of similar magnitude to that previously observed in studies of the knee extensors (Kalmar and Cafarelli, 1999; Meyers and Cafarelli, 2005). Though the ability of caffeine to improve performance has long been recognised, the exact mechanism(s) behind this ergogenesis has remained obscure. Though there is considerable evidence to suggest caffeine's action is on the central nervous system (Fredholm et al., 1999; Kalmar and Cafarelli, 2004a), it has been suggested that caffeine is likely to have more than one mechanism of action (Graham, 2001; Doherty and Smith, 2005).

The increase in time to task failure following caffeine ingestion was accompanied by a significant slowing in the rates of global, peripheral and central fatigue development (Figures 7.1, 7.3 and 7.4; Table 7.2). The values for MVC torque and potentiated doublet torque at task failure, though, were not significantly different between the two conditions (Table 7.2), indicating that participants continued exercising to the same limiting values in each condition and that it simply took longer to reach these values following caffeine ingestion. Furthermore, at isotime in the caffeine condition MVC torque remained significantly higher than at task failure in the placebo condition (Figure 7.1; Table 7.2), indicating that participants still had a significant reserve of maximal torque, and thus of exercise capacity. These findings suggest that task failure in both conditions was caused by the inability of the neuromuscular system to deliver the required torque, rather than by task disengagement (Marcora and Staiano, 2010), as participants were able to exercise to the same limiting values (that is, the point at which task demands equalled their maximal output) regardless of whether they ingested a placebo or caffeine. These results indicate that task failure in each condition was caused by central and peripheral perturbations, which led to an inability to meet task demands. That global, central and peripheral fatigue were all slowed suggests that caffeine may act not only through traditionally accepted central mechanisms (Fredholm et al., 1999; Kalmar and Cafarelli, 2004a) but also through peripheral mechanisms (Tarnopolsky and Cupido, 2000; Meyers and Cafarelli, 2005).

Physiological bases for changes in neuromuscular system behaviour

Knee extensor torque complexity has been repeatedly demonstrated to decrease with the development of neuromuscular fatigue and the reduction of torque generating capacity (Chapters 4, 5 and 6). That the rates of decrease of both MVC torque (Table 7.2) and torque complexity (Table 7.3) with fatigue were slowed following the ingestion of caffeine indicates a slowing of the increase in relative task demand, and therefore further links torque complexity to changes in task demands, be they absolute (i.e. increased torque requirement; Chapter 5) or relative (i.e. increased fatigue). Such a finding also further supports the notion that complexity measures can act as a sensitive surrogate index of fatigue (or force generating capacity), which could be used when maximal contractions and/or electrical stimulation are not possible or are undesirable. These findings also suggest that the mechanisms responsible for the slowed loss of torque complexity and the ergogenic effect of caffeine could be intrinsically linked.

It has long been thought that caffeine's ergogenic action is on the central nervous system, where it acts as an adenosine antagonist (Biaggioni et al., 1991) preventing adenosine from exerting its inhibitory effects on the α-motoneuron pool (Fredholm, 1995; Fredholm et al., 1999). Indeed, caffeine has been demonstrated to increase spinal excitability; that is, the net effect of membrane properties and the summation of all synaptic input to α-motoneurons (Walton et al., 2003; Kalmar et al., 2006). The results of the present study indicate that the rate of central fatigue development, as measured by a decrease in voluntary activation, is slowed following caffeine ingestion (Table 7.2). This could be indicative of a greater ability to excite the motor unit pool in the face of fatigue, be it through an increase in spinal excitability (Walton et al., 2003) or maintenance of optimal descending drive (Taylor et al., 2000). It should be noted, however, that while caffeine ingestion has been demonstrated to offset a decline in spinal excitability, increased spinal excitability did not offset the central activation failure seen with fatigue (Kalmar et al., 2006). Though voluntary activation was not significantly different at isotime in the caffeine condition compared to task failure in the placebo condition, it was, nevertheless, ~12% higher (Figure 7.4). Post-hoc power analysis revealed the power of this test to be 0.52. As MVC torque (Figure 7.1) and ApEn (Figure 7.5A) were significantly higher at isotime, in the absence of a difference in the level of peripheral fatigue, this suggests a centrally-mediated link between the maintenance of voluntary torque output and complexity. Better maintenance of voluntary activation, through an effect on spinal excitability or descending drive, could be responsible for the slowed rate of fatigue development, which in turn is associated with a slowed loss of complexity.

As caffeine has been demonstrated to effect synaptic input to motoneurons (Walton et al., 2003; Kalmar et al., 2006), another central factor that may relate to caffeine's ergogenesis, and that has been speculated to influence torque complexity (Chapter 5), is the common synaptic input to motoneurons, known as common drive (De Luca and Erim, 1994; Farina and Negro, 2015). As previously mentioned (Chapter 5), motor unit synchronisation is a necessary consequence of common drive (Farina and Negro, 2015). Common drive, and therefore motor unit synchronisation, have been demonstrated to increase as a function of both increased absolute task demands (i.e. increased contraction intensity) and increased relative task demands (i.e. fatigue; Castronovo et al., 2015); and common drive has been proposed to be the biggest determinant of force fluctuations (Dideriksen et al., 2012; Farina et al., 2014). As the loss of MVC torque was slower with

caffeine ingestion, and MVC torque remained higher at isotime compared with task failure in the placebo condition, this is indicative of a slower increase in relative task demands and potentially a lower level of common drive. If the increase in common drive with fatigue was attenuated by caffeine ingestion, this could explain the slower rates of loss of complexity (Table 7.3), as well as the significantly higher complexity (Figure 7.5) and lower variability seen at isotime. Though the functional role of motor unit synchronisation in force production is unclear (Farina and Negro, 2015), increased levels of motor unit synchronisation are thought to disrupt motor performance (Yao et al., 2000; Holtermann et al., 2009), which could be reflected in the form of reduced complexity. Motor unit synchronisation has previously been speculated to be the cause of the increased regularity of tremor complexity seen with ageing (Sturman et al., 2005) and has been associated with a decrease in the complexity of sEMG output during fatiguing contractions (Mesin et al., 2009; Beretta-Piccoli et al., 2015). Recordings of motor unit action potentials would be necessary to confirm whether common drive influences torque complexity, and also whether caffeine influences common drive.

It was previously asserted (Chapter 5) that only metabolically-mediated peripheral fatigue is capable of inducing the chain of events leading to a loss of torque complexity. It therefore seems reasonable to suggest that the slower rate of peripheral fatigue development observed following caffeine ingestion in the present study (Table 7.2) could account for the slower loss of complexity (Table 7.3). Such a slowing of peripheral fatigue development with caffeine ingestion has previously been observed by Kalmar and Cafarelli (1999), and could also account for caffeine's ergogenic effect. It has been speculated, based on the observations of a slowing of peripheral fatigue (Kalmar and Cafarelli, 1999) and the potentiation of contraction force during low, but not high, frequency electrical stimulation (Lopes et al., 1983; Tarnopolsky and Cupido, 2000), that caffeine acts to decrease sarcoplasmic reticulum Ca^{2+} uptake, which would increase Ca^{2+} availability and serve to maintain submaximal force production (Kalmar and Cafarelli, 1999; Tarnopolsky and Cupido, 2000). Measurable effects on Ca^{2+} release or reuptake have, however, generally only been observed during caffeine administration in the millimolar range in animal models (e.g., Howlett et al.,2005), which would be toxic to humans (Fredholm et al., 1999). If caffeine does positively influence Ca^{2+} handling, this would suggest that it could modulate the ATP cost of force production and the metabolic cost of constant load exercise (Doherty and Smith, 2005). If this were the case, it provides

a further potential link with complexity, and support for Seely and Macklem's (2012) hypothesis of an inverse relationship between a system's prevailing metabolic rate and the complexity of its outputs. However, if caffeine does influence complexity through peripheral mechanisms, it likely only does so late in the task, since the potentiated doublet torque did not differ between the caffeine and placebo conditions at isotime (Figure 7.3). Thus, the reduction in the rate of torque complexity loss and the higher torque complexity at isotime in the caffeine condition are more likely caused by the effects of caffeine on processes upstream in the central nervous system described above.

Conclusion

In summary, this study has demonstrated that the increased endurance time brought about by caffeine ingestion is accompanied by a slowing of the rate of loss of torque complexity, measured using ApEn, SampEn and DFA α. Caffeine ingestion also slowed the rates of global, central and peripheral fatigue development, with participants reaching the same limiting values in each condition. The slowing of both central and peripheral fatigue development suggests that caffeine's ergogenic action is not limited to the central nervous system, but also extends to the periphery, potentially influencing Ca^{2+} handling. The slowed rate of loss of complexity provides further indication that complexity can be used as a surrogate index of fatigue. Given the previous association of metabolically-mediated peripheral fatigue with the loss of complexity, it seems possible that the slowed rate of peripheral fatigue development with caffeine ingestion is, at least partly, responsible for the slowed loss of complexity. However, given the slowed rates of change of compensatory central adjustments and that both voluntary activation and complexity were higher at isotime, it is likely that central mechanisms predominate in the slowed loss of torque complexity seen with caffeine ingestion.

Chapter 8 – Study 5

Prolonged depression of knee extensor torque complexity following eccentric contractions of the knee extensors

Introduction

The findings presented within the first four studies of this thesis (Chapters 4, 5, 6 and 7) have demonstrated that fatiguing isometric contractions of the knee extensors result in a progressive reduction in the complexity of torque output, with values becoming increasingly Brownian (DFA α = 1.50) as task failure approaches. The previous study (Chapter 7) sought to investigate the influence of caffeine, an ergogenic aid thought to exert a beneficial effect largely through central mechanisms, on torque complexity. The purpose of the present study was to investigate the effect of eccentric exercise-induced muscle damage, which results in prolonged impairment of force production largely through peripheral mechanisms, on torque complexity and recovery kinetics.

Eccentric contractions are a common part of many everyday activities (e.g. descending stairs) and sporting events (e.g. those involving stretch-shortening cycles), and occur when muscles are forcibly lengthened (Enoka, 1996). Though eccentric contractions are capable of producing greater force than either isometric or concentric contractions (Katz, 1939; Doss and Karpovich, 1965), the lengthening the muscle fibres must undergo places high stress on the structures involved in force production, leading to prolonged muscle damage, delayed onset muscle soreness and functional impairment (Asmussen, 1956; Fridén et al., 1981; Clarkson et al., 1992). The exact mechanisms responsible for this muscle damage are not entirely clear. It is believed that the damage is initially caused by mechanical disruption of the sarcomeres (Proske and Morgan, 2001), due to the weakest sarcomeres in a myofibril being overstretched (Morgan, 1990; Talbot and Morgan, 1996). As contractions progress, this process is repeated and the disruption spreads across the myofibril and to other myofibrils. When this region of disruption is large enough, membrane damage occurs, beginning with the tearing of the t-tubules (Takekura et al., 2001). This is followed by damage to the sarcoplasmic reticulum and the uncontrolled release of calcium (Ca^{2+}) into the sarcoplasm, causing impairment of the excitation-

contraction coupling process by decreasing the amount of Ca^{2+} available for release (Proske and Morgan, 2001; Warren et al., 2001).

Such muscle damage is persistent, with mechanical disruption and increased creatine kinase still observed several days after the initial event (Fridén et al., 1983); considerably longer following either isometric or concentric exercise (Newham et al., 1983; Lavender and Nosaka, 2006). A consequence of this muscle damage is a decrease in maximal force generating capacity, which is of greater extent and duration than after the performance of either isometric or concentric contractions (Jones et al., 1989; Smith and Newham, 2007). While maximal force following fatiguing isometric contractions recovers to >90% of its fresh value after approximately 60 minutes (Sahlin and Ren, 1989; Allman and Rice, 2001), significant decrements in maximal force following eccentric exercise persist for several days, and in some cases have still been evident for up to two weeks (Cleak and Eston, 1992; Sayers and Clarkson, 2001). Interestingly, it has also been observed that voluntary activation can be decreased for up to four days following eccentric exercise (Endoh et al., 2005), indicating a central effect of muscle damage.

An additional, and often overlooked, effect of eccentric exercise-induced muscle damage is on the ability to control force. Several studies have demonstrated that eccentric contractions result in an increase in force fluctuations, as measured by the coefficient of variation (CV), during subsequent low, moderate and high force isometric contractions (Weerakkody et al., 2003; Lavender and Nosaka, 2006; Semmler et al., 2007; Skurvydas et al., 2010). This effect has typically been observed an hour after the cessation of exercise, though in some cases has persisted for 24 hours (Leger and Milner, 2001; Dartnall et al., 2008), and has not been observed following isometric or concentric contractions (Lavender and Nosaka, 2006; Semmler et al., 2007). The cause of this increased variability is currently unknown. However, enhanced motor unit synchronisation, a phenomenon linked with common synaptic input to muscles (Farina and Negro, 2015) and associated with increased force variability in fatigued muscle (Yao et al., 2000; Holtermann et al., 2009), has been demonstrated to occur during isometric contractions performed following eccentric exercise (Dartnall et al., 2008). Increased motor unit synchronisation has also been speculated to be a potential cause of the reduced complexity of torque output seen with fatigue (Chapters 5, 6 and 7).

The aims of the present study were, therefore, to determine whether eccentric contractions, a form of exercise that impairs muscle function through peripheral mechanisms, influences torque complexity, and to track and compare recovery kinetics from muscle damaging exercise with those following fatiguing isometric contractions. The specific experimental hypotheses tested were: 1) that eccentric exercise will reduce the complexity of isometric knee extension torque (as measured by decreased approximate entropy [ApEn] and sample entropy [SampEn], and increased detrended fluctuation analysis [DFA] α); 2) that isometric knee extension torque complexity will still be depressed 60 minutes after the cessation of eccentric exercise; and 3) that the reduction in knee extension torque complexity seen with isometric exercise will have recovered 60 minutes after the cessation of fatiguing isometric exercise.

Methods

Participants

10 healthy participants (8 male, 2 female; mean ± SD: age 24.8 ± 6.2 years; height 1.75 ± 0.08 m; body mass 69.5 ± 10.6 kg) provided written informed consent to participate in the study, which was approved by the ethics committee of the University of Kent, and which adhered to the Declaration of Helsinki. None of the participants had been involved in any lower limb strength training for ≥ 3 months. Participants were instructed to arrive at the laboratory in a rested state (having performed no strenuous exercise in the preceding 24 hours) and not to have consumed any food or caffeinated beverages in the three hours before arrival. Participants attended the laboratory at the same time of day (± 2 hours) during each visit.

Experimental design

Participants were required to visit the laboratory on seven occasions over a four to six-week period. During their first visit, participants were familiarised with all testing equipment and procedures, and the settings for the dynamometer and stimulator were recorded. The second visit involved performance of fatiguing intermittent isometric knee extension contractions (Isometric exercise; see below); with the third visit, 24 hours later, assessing recovery. The contractions in these visits were performed with the dominant leg (the leg participants would instinctively use to kick a football). At least one week after the third visit, the fourth visit involved performance of intermittent eccentric knee

extension contractions (Eccentric exercise; see below); with the fifth, sixth and seventh visits, 24 hours, 48 hours and one week later, assessing recovery. The contractions in these visits were performed with the non-dominant leg. The methods used for the setup of the dynamometer, femoral nerve stimulation and surface EMG are described in the General Methods (Chapter 3).

Protocol

All visits began with the instrumentation of the participants, the (re-)establishment of the correct dynamometer seating position and supramaximal stimulation response. Participants then performed a series of brief (3 second) MVCs to establish the maximum torque of the leg to be used in that visit. The MVCs were separated by 60 seconds rest, and continued until three consecutive peak torques were within 5% of each other. Participants were given a countdown, followed by very strong verbal encouragement to maximise torque. The first MVC was used to establish the fresh maximal EMG signal, against which the subsequent EMG signals were normalised (Data analysis; see below). The second and third MVCs were performed with peripheral nerve stimulation. In all instances where MVCs were performed with stimuli, the stimuli were delivered 1.5 seconds into the contraction to coincide with maximal torque, and 2 seconds after the contraction to provide a resting potentiated doublet (see General Methods; Chapter 3). Following this, participants performed a series of five isometric contractions at a target torque of 50% MVC. These contractions were 6 seconds long and separated by 4 seconds rest. The target torque was based on the highest instantaneous MVC torque recorded during the pre-test MVCs.

Participants were additionally asked to rate their muscle soreness and capillary whole-blood was sampled from a finger tip. Muscle soreness was measured using a visual analog scale consisting of a horizontal line 10 cm long, with 0 and 10 marked at each end (see Appendix). On this scale, zero corresponded to no muscle soreness and 10 corresponded to the most intense soreness imaginable. Participants performed a squat down to ~ 90° of knee flexion and were asked to draw a line marking their subjective soreness, with the distance to the mark (in centimetres) being used to quantify soreness. A finger tip blood sample was then taken, and centrifuged for 10 minutes to obtain plasma. Plasma samples were then stored at –80°C for later analysis of creatine kinase (CK). Plasma CK was determined using a commercially available kit (CKNAC, Randox Laboratories Ltd.,

Crumlin, County Antrim, UK) and standard spectrophotometric-colorimetric procedures with a Randox Monza (Randox Laboratories Ltd., Crumlin, County Antrim, UK).

Following this, participants rested for 10 minutes. In the second and fourth visits, participants then performed the isometric exercise and eccentric exercise, respectively (see below). Immediately at task end/failure and then after 10, 30 and 60 minutes recovery, a measure of MVC torque, accompanied by peripheral nerve stimulation, was taken and the 50% MVC task was performed. At each of these time points, the 50% MVC task was performed twice, with 5 minutes rest separating each set of contractions. The first set of contractions was normalised to 50% of the MVC obtained at that time point (50% relative), while the second set was performed at 50% of the pre-exercise MVC (50% absolute). Measures of muscle soreness and finger tip blood samples were obtained immediately at task end/failure and after 60 minutes recovery. All of these measures (MVC torque, 50% relative, 50% absolute, muscle soreness and blood sample) were also recorded 24 hours after isometric exercise, and 24 hours, 48 hours and one week after eccentric exercise.

Isometric exercise

In their second visit, participants performed intermittent isometric knee extension contractions at a target torque of 50% MVC until task failure. The target torque of 50% MVC was based on the highest instantaneous torque recorded during the pre-test MVCs. The duty cycle for the contractions was 0.6; with contractions lasting 6 seconds and being followed by 4 seconds rest. The contractions were performed until task failure, the point at which the participant failed to reach the target torque on three consecutive occasions, despite strong verbal encouragement. Participants were not informed of the elapsed time during the trials, but were informed of each “missed” contraction. After the third missed contraction, participants were instructed to immediately produce an MVC, which was accompanied by peripheral nerve stimulation. Further details of the contraction protocol can be found in the General Methods (Chapter 3) and a graphical representation can found in Chapter 5 (Figure 5.2).

Eccentric exercise

Eccentric exercise with the non-dominant leg was used to induce a minimum 40% reduction of isometric MVC torque (Prasartwuth et al., 2006; Dartnall et al., 2008). This

protocol was used to induce a similar amount of muscle damage in all participants, compared to the large variation in strength loss that can be seen following a fixed number of eccentric contractions (Hubal et al., 2007). Participants were seated with their non-dominant leg strapped to the dynamometer and raised their leg to an angle of 20° extension (with full extension being 0°). The dynamometer then flexed the participant's knee to an angle of 90° extension, at a constant angular velocity of $60°\cdot s^{-1}$, whilst the participant resisted this motion by attempting to maximally extend their knee. Each eccentric contraction was separated by a minimum of 3 seconds rest. Contractions were performed in sets of 10, followed by a 60 second rest period. At the start of each 60 second rest period, participants performed an isometric MVC. The eccentric contractions continued until there was a reduction in isometric MVC torque exceeding 40%. At this point, participants performed another isometric MVC, this time accompanied by peripheral nerve stimulation.

Data analysis

The data analysis focused on three specific areas: 1) basic measures of torque and EMG; 2) measures of central and peripheral fatigue; and 3) the variability and complexity of the torque output of the isometric contractions. All data were analysed using code written in MATLAB R2013a (The MathWorks, Massachusetts, USA).

Torque and EMG: The mean and peak torques, and vastus lateralis EMG were calculated for each isometric contraction at 50% MVC as described in the General Methods (Chapter 3). Details of how task failure was calculated in the isometric test are given in the General Methods (Chapter 3).

Central and peripheral fatigue: Measures of central and peripheral fatigue were calculated based on the stimuli delivered during and after the MVCs performed pre-test, at task end/failure and throughout the recovery periods. Further details are given in the General Methods (Chapter 3). Muscle contractility was assessed for each peripherally derived resting twitch as contraction time to peak torque (TPT) and one-half relaxation time ($RT_{0.5}$). Further details are given in the Methods section of Chapter 5.

Variability and complexity: All measures of variability and complexity were calculated using the steadiest five seconds of each isometric contraction at 50% MVC. The amount

of variability in the torque output of each isometric contraction was measured using the standard deviation (SD) and CV. The temporal structure of variability was examined using multiple time domain analyses. ApEn and SampEn were used to determine the complexity of the torque output; while DFA was used to estimate the temporal fractal scaling. Further details are given in the General Methods (Chapter 3).

Statistics

All data are presented as means ± SEM unless otherwise stated, and results were deemed statistically significant when $P < 0.05$. One-way ANOVAs with repeated measures were used to test for differences between time points for MVC torque, arEMG, potentiated doublet torque, voluntary activation, measures of variability, measures of complexity, muscle soreness and plasma CK in the isometric and eccentric conditions. When main effects were observed, Student's paired samples t-tests and Bonferroni-adjusted 95% confidence intervals were then used to determine specific differences.

Results

Preliminary measures

The contractile properties of the knee extensors, along with muscle soreness and plasma CK, measured prior to the isometric and eccentric conditions are shown in Table 8.1. The variability and complexity of torque output prior to the isometric and eccentric conditions are shown in Table 8.2. There were no significant differences between the conditions prior to exercise for any of the variables.

Muscle soreness and plasma creatine kinase

Muscle soreness increased over time in both the eccentric, muscle damage ($F_{5,45} = 27.48$, $P < 0.001$) and isometric, fatigue conditions ($F_{3,27} = 13.42$, $P < 0.001$; Table 8.1). By the end of the eccentric test, soreness had increased from 0.37 ± 0.12 to 6.92 ± 0.96 cm (95% paired samples confidence intervals (CIs): 3.4, 9.7 cm) and remained significantly elevated over the next 48 hours (5.25 ± 0.51 cm; CIs 3.3, 6.5 cm). It had recovered and was not significantly different to its pre-test value one week after exercise (CIs –0.7, 0.2 cm). At the end of the isometric test, soreness had increased from 0.47 ± 0.13 to 4.96 ± 0.72 cm (CIs 2.2, 6.8 cm). Soreness decreased over the next 24 hours, but still remained significantly elevated at this time point (1.72 ± 0.41 cm; CIs 0.3, 2.2 cm).

Plasma CK increased over time in the eccentric condition ($F_{5,45}$ = 19.68, P < 0.001; Table 8.1). 60 minutes after exercise, plasma CK had increased from 172.1 ± 51.8 U/L to 378.0 ± 63.8 (CIs 47.8, 364.1 U/L). It peaked 24 hours after exercise (893.4 ± 122.8 U/L; CIs 299.1, 1143.5 U/L) and remained significantly elevated 48 hours after exercise (CIs 202.7, 997.8 U/L). It had recovered and was not significantly different from its pre-test value one week after exercise (CIs –160.8, 71.0 U/L). There was also a significant effect of time on plasma CK in the isometric condition ($F_{2,37}$ = 7.42, P = 0.023), though subsequent t-tests revealed no significant differences between time points.

Torque and EMG

There was a significant effect of time on MVC torque in the both the eccentric ($F_{7,63}$ = 64.37, P < 0.001) and isometric conditions ($F_{5,45}$ = 93.21, P < 0.001; Figure 8.1; Table 8.1). Task end in the eccentric condition occurred when the isometric MVC performed at the end of a set had decreased by 40%. This occurred after 182 ± 24 contractions and resulted in a change in MVC torque from 233.6 ± 23.5 to 137.7 ± 14.4 N·m; a significant decrease of 41.0 ± 1.7% (CIs –46.7, –35.2%). MVC torque exhibited limited recovery (to 166.6 ± 16.2 N·m) throughout the subsequent 60 minutes, and remained significantly depressed, by 28.1 ± 2.3%, at the end of this period (CIs –36.2, –20.0%). There was further partial recovery after 24 hours and 48 hours, though MVC torque still remained significantly depressed at these time points, by 23.9 ± 3.1% (CIs –34.6, –13.1%) and 19.7 ± 3.0% (CIs –30.1, –9.3%), respectively. MVC torque had recovered and was not significantly different from its pre-test value one week after exercise (CIs –6.1, 10.2%).

Task failure in the isometric condition occurred when participants were no longer able to achieve the target torque (123.0 ± 12.2 N·m), despite a maximal effort. This occurred after 4.3 ± 0.5 minutes and resulted in a change in MVC torque from 246.1 ± 24.4 to 130.6 ± 11.5 N·m; a significant decrease of 46.2 ± 1.4% (CIs –50.9, –41.5%). MVC torque exhibited partial recovery throughout the subsequent 60 minutes, though still remained significantly depressed, by 11.9 ± 2.1%, at the end of this period (CIs –18.8, –5.0%). MVC torque had recovered and was not significantly different from its pre-test value 24 hours after exercise (CIs –0.3, 4.3%).

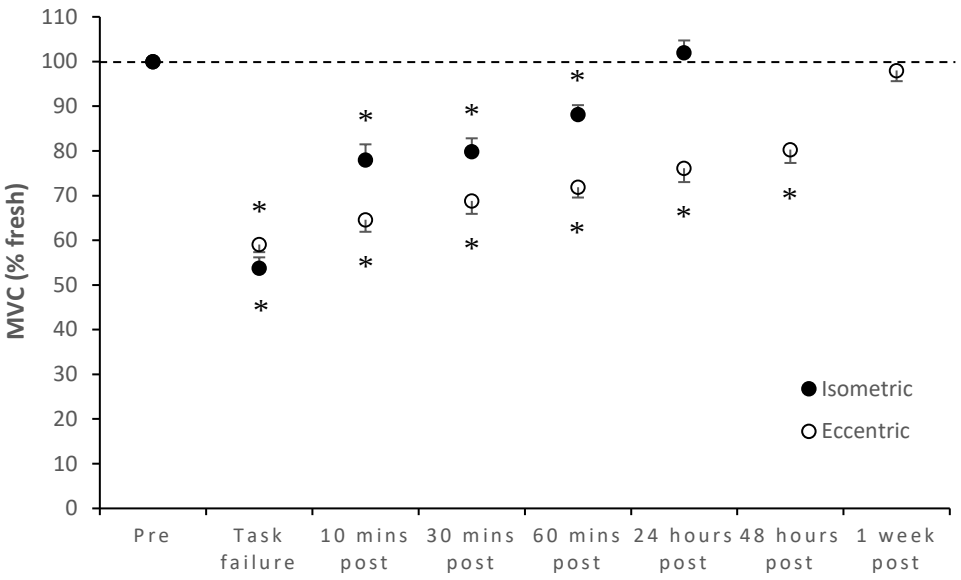

Figure 8.1. Group peak MVC torque (± SEM) at each time point of the isometric and eccentric conditions. * = Significantly different from the pre-test value.

The mean arEMG, normalised to a fresh pre-test MVC, during the contractions at 50% absolute ($F_{7,63}$ = 24.59, P < 0.001) and 50% relative ($F_{7,63}$ = 11.03, P < 0.001) changed over time in the eccentric condition (Figure 8.2; Table 8.1). By the end of the eccentric test, the arEMG for 50% absolute had increased from 51.2 ± 2.3 to 66.5 ± 4.9% (CIs 1.1, 29.2%). Throughout the subsequent 60 minutes, this increased further, reaching 89.7 ± 3.6% at the end of this period. arEMG started to recover thereafter, but still remained significantly elevated after 48 hours (68.4 ± 1.5%; CIs 4.5, 30.0%). It had recovered and was not significantly different from its pre-test value one week after exercise (CIs –13.2, 4.9%). The arEMG for 50% relative increased during the 60 minutes after exercise, and was significantly greater at the end of this period (67.1 ± 2.9%; CIs 7.0, 24.9%). It had recovered and was not significantly different from its pre-test value 24 hours after exercise (CIs –11.9, 7.4%).

The mean arEMG for 50% absolute ($F_{5,45}$ = 18.33, P < 0.001) and 50% relative ($F_{5,45}$ = 5.87, P < 0.001) also changed over time in the isometric condition (Figure 8.2; Table 8.1). Over the course of the isometric test, the arEMG for 50% absolute increased from 52.9 ± 2.0 to 88.3 ± 5.8% (CIs 18.5, 52.2%). arEMG decreased over the subsequent 60 minutes, but still remained significantly elevated at the end of this period (66.2 ± 1.9%; CIs 7.3, 19.2%). It had recovered and was not significantly different from its pre-test value 24 hours after exercise (CIs –10.9, 7.1%). The arEMG for 50% relative was significantly

lower at task failure than pre-test (CIs –24.5, –3.4%), but had recovered and was not significantly different from its pre-test value 10 minutes after exercise (CIs –17.9, 15.8%).

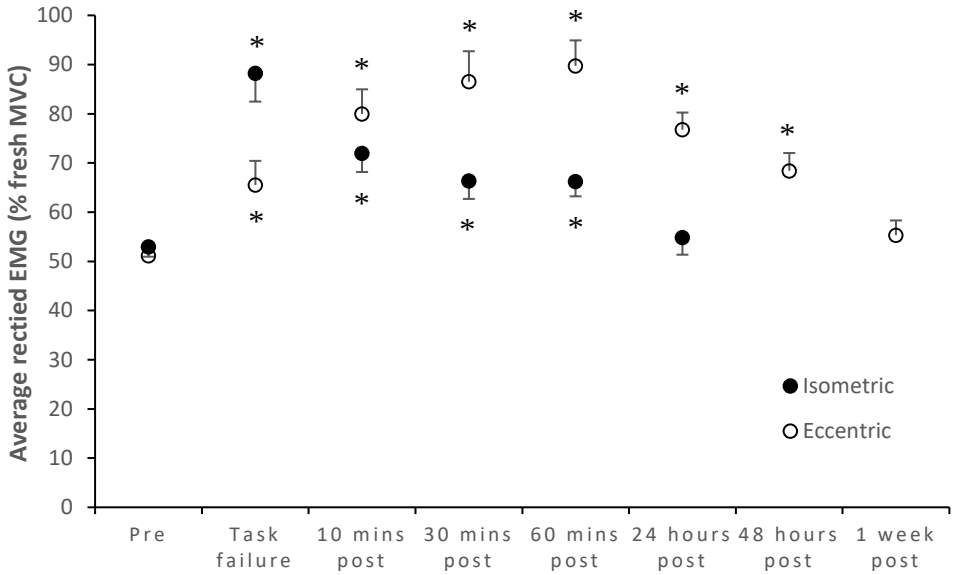

Figure 8.2. Group mean arEMG for the contractions at 50% absolute (± SEM) at each time point of the isometric and eccentric tests. * = Significantly different from the pre-test value.

Peripheral and central fatigue

Both the eccentric ($F_{7,56}$ = 33.22, P < 0.001) and isometric conditions ($F_{5,40}$ = 26.52, P < 0.001) resulted in significant reductions in potentiated doublet torque (Figure 8.3; Table 8.1), indicating the presence of peripheral perturbations. By the end of the eccentric test, potentiated doublet torque had decreased from 109.2 ± 9.1 to 84.8 ± 7.6 N·m (CIs –34.0, –14.9 N·m). Throughout the subsequent 60 minutes, it continued to decrease, reaching 70.8 ± 5.7 N·m at the end of this period. Potentiated doublet torque started to recover thereafter, but still remained significantly decreased after 24 hours (91.6 ± 7.8 N·m; CIs –34.3, –1.0 N·m). It had recovered and was not significantly different from its pre-test value 48 hours after exercise (CIs –2.6, 29.1 N·m). Over the course of the isometric test, potentiated doublet torque decreased from 107.9 ± 8.3 to 63.3 ± 5.3 N·m (CIs –69.0, –20.2 N·m). Throughout the subsequent 60 minutes it exhibited partial recovery, but still remained significantly decreased at the end of this period (87.6 ± 6.9 N·m; CIs –31.7, –9.0 N·m). It had recovered and was not significantly different from its pre-test value 24 hours after exercise (CIs –5.8, 13.9 N·m).

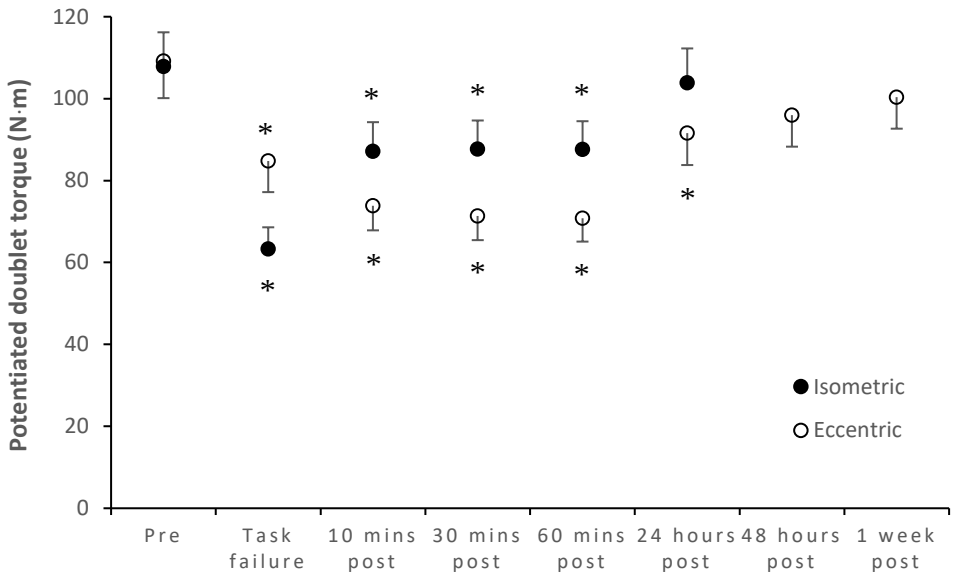

Figure 8.3. Group mean potentiated doublet torque (± SEM) at each time point of the isometric and eccentric tests. * = Significantly different from the pre-test value.

Both the eccentric ($F_{7,56}$ = 16.05, P < 0.001) and isometric conditions ($F_{5,40}$ = 12.70, P < 0.001) also resulted in significant reductions in voluntary activation (Figure 8.4; Table 8.1), indicating the presence of central perturbations. By the end of the eccentric test, voluntary activation had decreased from 92.0 ± 0.8 to 68.3 ± 3.2% (CIs –37.3, –10.0%). It remained significantly decreased after 30 minutes of recovery (82.0 ± 2.6%; CIs –18.8, –1.2%), but had recovered and was not significantly different from its pre-test value 60 minutes after exercise (CIs –2.4, 8.6%). Over the course of the isometric test, voluntary activation decreased from 91.7 ± 0.6 to 77.3 ± 3.2% (CIs –25.5, –3.3%). It remained significantly decreased after 30 minutes of recovery (83.9 ± 2.2%; CIs –15.4, –0.3%), but had recovered and was not significantly different from its pre-test value 60 minutes after exercise (CIs –1.3, 9.7%).

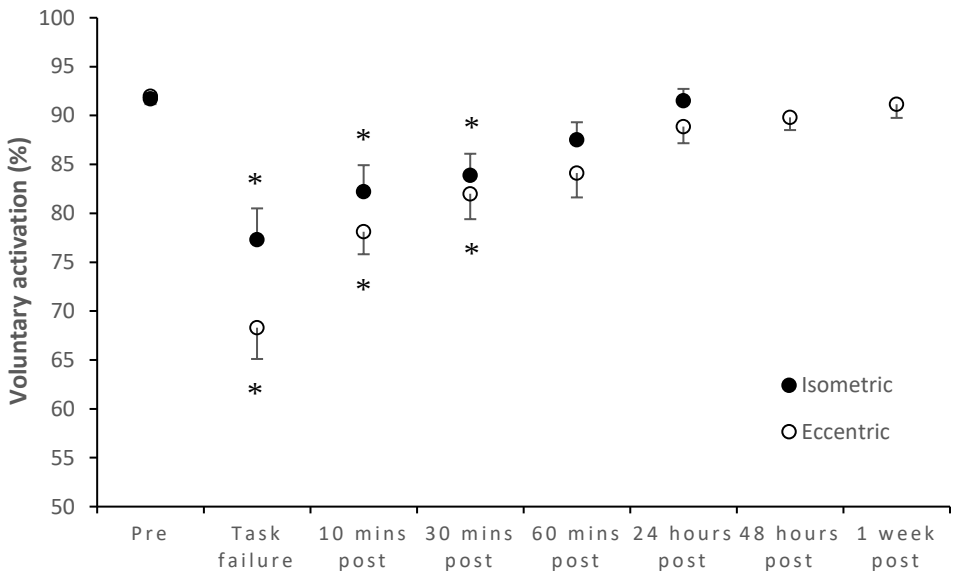

Figure 8.4. Group mean voluntary activation (± SEM) at each time point of the isometric and eccentric tests. * = Significantly different from the pre-test value.

Variability and complexity

There was a significant effect of time on the amount of variability in the eccentric condition, as measured by the SD and CV at 50% absolute (SD, $F_{7,63} = 8.39$, $P < 0.001$; CV, $F_{7,63} = 7.88$, $P < 0.001$) and 50% relative (CV, $F_{7,63} = 14.26$, $P < 0.001$; Table 8.2). By the end of the eccentric test, the SD for 50% absolute had increased from 3.5 ± 0.3 to 8.0 ± 1.4 N·m (CIs 0.09, 9.3 N·m), while the CV had increased from 2.8 ± 0.3 to 7.4 ± 1.3% (CIs 0.05, 9.0%). The CV remained significantly higher 10 minutes after exercise (CIs 0.06, 6.0%), and had a tendency to remain higher 60 minutes after exercise (4.4 ± 0.8; CIs –3.6, 0.5%). The CV of the 50% relative contractions was increased at the end of exercise (4.6 ± 0.2%; CIs 0.5, 3.0%), and remained significantly higher after 60 minutes (3.9 ± 0.4%; CIs 0.06, 2.1%). It had recovered and was not significantly different from its pre-test value after 24 hours (CIs –0.9, 0.8%). There was also a significant effect of time on the SD and CV in the isometric condition at 50% absolute (SD, $F_{5,45} = 19.39$, $P < 0.001$; CV, $F_{5,45} = 24.70$, $P < 0.001$; Table 8.2). Over the course of the isometric test, the SD for 50% absolute increased from 3.3 ± 0.5 to 10.4 ± 1.7 N·m (CIs 1.9, 12.4 N·m), while the CV increased from 2.6 ± 0.1 to 9.6 ± 1.3% (CIs 2.5, 11.4%). Both had recovered and were not significantly different from the pre-test values after 10 minutes of recovery (SD, CIs –0.7, 0.7 N·m; CV CIs –0.5, 0.4%).

Complexity, as measured by ApEn and SampEn, changed over time in both the eccentric (ApEn, $F_{7,63}$ = 17.16, P < 0.001; SampEn, $F_{7,63}$ = 16.78, P < 0.001) and isometric conditions (ApEn, $F_{5,45}$ = 28.27, P < 0.001; SampEn, $F_{5,45}$ = 26.21, P < 0.001) for the contractions at 50% absolute (Figure 8.5; Table 8.2). By the end of the eccentric test, ApEn had decreased from 0.39 ± 0.04 to 0.20 ± 0.03 (CIs –0.3, –0.08), while SampEn had decreased from 0.36 ± 0.04 to 0.18 ± 0.03 (CIs –0.3, –0.07). ApEn (0.25 ± 0.04; CIs –0.2, –0.07) and SampEn (0.22 ± 0.03; CIs –0.2, –0.06) remained significantly depressed 60 minutes after exercise. Both had recovered and were not significant different from their pre-test values 24 hours after exercise (ApEn, CIs –0.06, 0.2; SampEn, CIs –0.05, 0.2). Over the course of the isometric test, ApEn decreased from 0.41 ± 0.04 to 0.09 ± 0.01 (CIs –0.4, –0.2), while SampEn decreased from 0.37 ± 0.04 to 0.08 ± 0.01 (CIs –0.4, –0.2). Both had recovered and were not significantly different from their pre-test values 10 minutes after exercise (ApEn, CIs –0.006, 0.1; SampEn, CIs –0.00004, 0.1). There were also significant effects of time on ApEn and SampEn for 50% relative in the eccentric (ApEn, $F_{7,63}$ = 3.41, P = 0.004; SampEn, $F_{7,63}$ = 3.22, P = 0.006) and isometric conditions (ApEn, $F_{5,45}$ = 4.12, P = 0.003; SampEn, $F_{5,45}$ = 4.53, P = 0.002), though subsequent t-tests revealed no significant differences between time points.

DFA α changed over time in both the eccentric ($F_{7,63}$ = 16.21, P < 0.001) and isometric conditions ($F_{5,45}$ = 32.45, P < 0.001) for the contractions at 50% absolute (Figure 8.5; Table 8.2). By the end of the eccentric test, DFA α had increased from 1.43 ± 0.03 to 1.54 ± 0.03 (CIs 0.04, 0.2). DFA α remained significantly elevated 60 minutes after exercise (1.55 ± 0.02; CIs 0.04, 0.2). It had recovered and was not significantly different from its pre-test value 24 hours after exercise (CIs –0.1, 0.03). Over the course of the isometric test, DFA α increased from 1.39 ± 0.03 to 1.64 ± 0.02 (CIs 0.2, 0.3). It was still significantly elevated 10 minutes after exercise (1.46 ± 0.02; CIs 0.02, 0.1), but had recovered and was not significantly different from its pre-test value 30 minutes after exercise (CIs –0.1, 0.02). There was also a significant effect of time on DFA α for 50% relative in the eccentric condition ($F_{7,63}$ = 4.51, P < 0.001), though subsequent t-tests revealed no significant differences between time points.

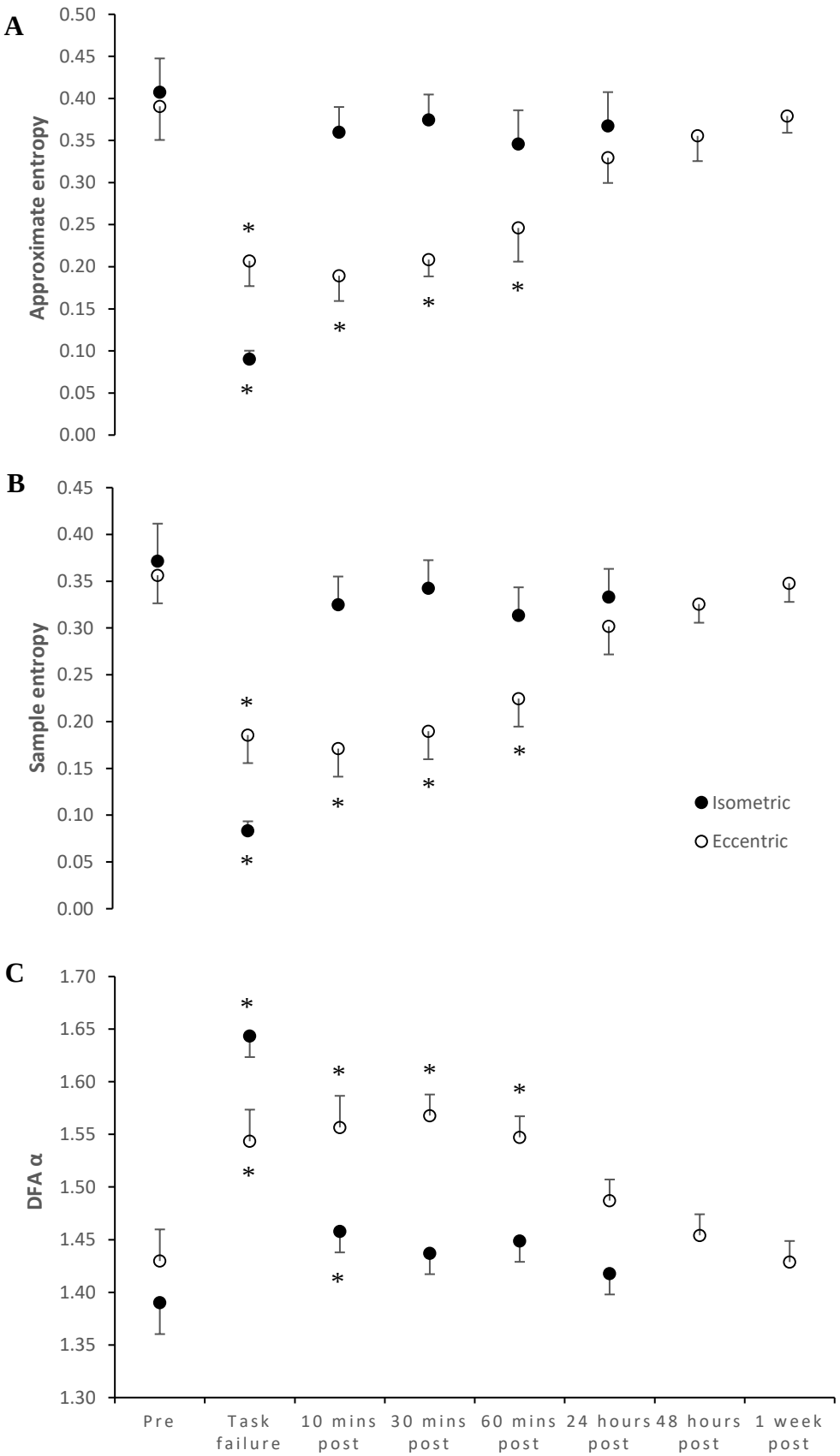

Figure 8.5. Group mean (± SEM) changes in torque ApEn (A), SampEn (B) and DFA α (C) at each time point of the isometric and eccentric tests. * = Significantly different from the pre-test value.

Table 8.1. Voluntary torque, potentiated doublet torque, voluntary activation, EMG, muscle soreness and plasma creatine kinase responses over the course of the isometric and eccentric tests.

Parameter		Pre	Task end/failure	10 mins post	30 mins post	60 mins post	24 hours post	48 hours post	1 week post
MVC torque,	Iso	100	53.8 ± 1.4*	78.0 ± 3.5*	79.8 ± 3.0*	88.1 ± 2.1*	102.0 ± 0.7	–	–
% pre	Ecc	100	59.0 ± 1.7*	64.6 ± 2.7*	68.8 ± 2.9*	71.9 ± 2.3*	76.1 ± 3.1*	80.3 ± 3.0*	98.0 ± 2.4
Doublet,	Iso	107.9 ± 8.3	63.3 ± 5.3*	87.2 ± 7.1*	87.7 ± 7.0*	87.6 ± 6.9*	103.9 ± 8.4	–	–
N·m	Ecc	109.2 ± 9.1	84.8 ± 7.6*	73.8 ± 6.0*	71.4 ± 5.9*	70.8 ± 5.7*	91.6 ± 7.8*	96.0 ± 7.7	100.4 ± 7.7
VA,	Iso	91.7 ± 0.6	77.3 ± 3.2*	82.2 ± 2.7*	83.9 ± 2.2*	87.5 ± 1.8	91.5 ± 1.2	–	–
%	Ecc	92.0 ± 0.8	68.3 ± 3.2*	78.1 ± 2.3*	82.0 ± 2.6*	84.1 ± 2.5	88.9 ± 1.7	89.8 ± 1.3	91.2 ± 1.4
arEMG Abs,	Iso	52.9 ± 2.0	88.3 ± 5.8*	72.0 ± 2.1*	66.4 ± 1.7*	66.2 ± 1.9*	54.9 ± 1.4	–	–
% MVC	Ecc	51.2 ± 1.4	65.6 ± 4.9*	80.0 ± 4.1*	86.5 ± 3.8*	89.7 ± 3.6*	76.8 ± 2.5*	68.4 ± 1.5*	55.3 ± 1.5
arEMG Rel,	Iso	52.9 ± 2.0	39.0 ± 1.2*	53.6 ± 1.5	50.2 ± 1.6	55.7 ± 2.2	52.4 ± 1.8	–	–
% MVC	Ecc	51.2 ± 1.4	48.3 ± 2.7	58.0 ± 2.8	62.5 ± 2.4*	67.1 ± 2.9*	53.4 ± 1.7	49.0 ± 2.0	51.90 ± 2.4
Soreness,	Iso	0.47 ± 0.13	4.96 ± 0.72*	–	–	3.50 ± 0.82*	1.72 ± 0.41*	–	–
cm	Ecc	0.37 ± 0.12	6.92 ± 0.96*	–	–	6.00 ± 5.28*	5.28 ± 0.45*	5.25 ± 0.51*	0.62 ± 0.18
CK,	Iso	165.8 ± 34.1	168.1 ± 34.9	–	–	196.4 ± 40.4	200.1 ± 41.1	–	–
U/L	Ecc	172.1 ± 51.8	316.6 ± 80.7	–	–	378.0 ± 62.7*	893.4 ± 122.8*	722.3 ± 92.6*	217.0 ± 31.8

Values are means ± SEM. MVC, maximal voluntary contraction; doublet, potentiated doublet torque; VA, voluntary activation; arEMG, average rectified EMG of the vastus lateralis; CK, plasma creatine kinase; Iso, isometric condition; Ecc, eccentric condition; Abs, 50% absolute; Rel, 50% relative. * indicates a statistically significant difference from the pre-test value.

Table 8.2. Variability, complexity and fractal scaling responses over the course of the isometric and eccentric tests.

Parameter		Pre	Task end/failure	10 mins post	30 mins post	60 mins post	24 hours post	48 hours post	1 week post
SD Abs, N·m	Iso	3.3 ± 0.5	10.4 ± 1.7*	3.3 ± 0.2	3.2 ± 0.3	3.5 ± 0.4	3.0 ± 0.3	–	–
	Ecc	3.5 ± 0.3	8.0 ± 1.4*	6.2 ± 0.8	5.5 ± 0.7	5.0 ± 0.8	3.6 ± 0.4	3.3 ± 0.3	2.9 ± 0.2
SD Rel, N·m	Iso	3.3 ± 0.5	1.8 ± 0.2*	2.6 ± 0.2	2.7 ± 0.2	2.9 ± 0.2	2.9 ± 0.2	–	–
	Ecc	3.5 ± 0.3	3.5 ± 0.4	3.4 ± 0.3	3.7 ± 0.5	3.5 ± 0.4	2.7 ± 0.2	2.7 ± 0.3	3.2 ± 0.3
CV Abs, %	Iso	2.6 ± 0.1	9.6 ± 1.3*	2.6 ± 0.2	2.6 ± 0.2	2.9 ± 0.3	2.4 ± 0.2	–	–
	Ecc	2.8 ± 0.3	7.4 ± 1.3*	5.9 ± 0.9*	5.1 ± 0.9	4.4 ± 0.8	3.0 ± 0.5	2.7 ± 0.4	2.4 ± 0.1
CV Rel, %	Iso	2.6 ± 0.1	2.5 ± 0.3	2.6 ± 0.2	2.7 ± 0.2	2.6 ± 0.2	2.3 ± 0.2	–	–
	Ecc	2.8 ± 0.3	4.6 ± 0.2*	4.2 ± 0.4*	4.5 ± 0.5	3.9 ± 0.4*	2.9 ± 0.2	2.8 ± 0.2	2.7 ± 0.2
ApEn Abs	Iso	0.41 ± 0.04	0.09 ± 0.01*	0.36 ± 0.03	0.37 ± 0.03	0.35 ± 0.04	0.37 ± 0.04	–	–
	Ecc	0.39 ± 0.04	0.20 ± 0.03*	0.19 ± 0.03*	0.21 ± 0.02*	0.25 ± 0.04*	0.33 ± 0.03	0.36 ± 0.03	0.38 ± 0.02
ApEn Rel	Iso	0.41 ± 0.04	0.53 ± 0.06	0.44 ± 0.04	0.45 ± 0.03	0.40 ± 0.03	0.39 ± 0.03	–	–
	Ecc	0.39 ± 0.04	0.36 ± 0.05	0.33 ± 0.03	0.30 ± 0.03	0.34 ± 0.03	0.40 ± 0.03	0.38 ± 0.04	0.35 ± 0.03
SampEn Abs	Iso	0.37 ± 0.04	0.08 ± 0.01*	0.33 ± 0.03	0.34 ± 0.03	0.31 ± 0.03	0.33 ± 0.03	–	–
	Ecc	0.36 ± 0.04	0.18 ± 0.03*	0.17 ± 0.03*	0.19 ± 0.02*	0.22 ± 0.03*	0.30 ± 0.03	0.33 ± 0.02	0.35 ± 0.02
SampEn Rel	Iso	0.37 ± 0.04	0.49 ± 0.05	0.40 ± 0.04	0.41 ± 0.03	0.37 ± 0.03	0.35 ± 0.03	–	–
	Ecc	0.36 ± 0.04	0.33 ± 0.04	0.30 ± 0.03	0.27 ± 0.03	0.31 ± 0.03	0.37 ± 0.03	0.36 ± 0.03	0.32 ± 0.02
DFA α Abs	Iso	1.39 ± 0.03	1.64 ± 0.02*	1.46 ± 0.02*	1.44 ± 0.02	1.45 ± 0.02	1.42 ± 0.02	–	–
	Ecc	1.43 ± 0.03	1.54 ± 0.03*	1.56 ± 0.03*	1.57 ± 0.02*	1.55 ± 0.02*	1.49 ± 0.02	1.45 ± 0.02	1.43 ± 0.02
DFA α Rel	Iso	1.39 ± 0.03	1.44 ± 0.02	1.42 ± 0.03	1.41 ± 0.02	1.42 ± 0.02	1.41 ± 0.02	–	–
	Ecc	1.43 ± 0.03	1.47 ± 0.03	1.49 ± 0.02	1.50 ± 0.03	1.48 ± 0.02	1.43 ± 0.02	1.43 ± 0.02	1.44 ± 0.02

Values are means ± SEM. SD, standard deviation; CV, coefficient of variation; ApEn, approximate entropy; SampEn, sample entropy; DFA α, detrended fluctuation analysis; Iso, isometric condition; Ecc, eccentric condition; Abs, 50% absolute; Rel, 50% relative. * indicates a statistically significant difference from the pre-test value.

Discussion

The present study aimed to investigate changes in knee extensor torque complexity following muscle-damaging eccentric contractions. The major novel finding of the present study was that eccentric exercise resulted in a prolonged loss of torque complexity, which was of greater duration than that induced by fatiguing isometric exercise. Both the eccentric and isometric conditions were associated with a loss of MVC torque and the development of significant central and peripheral perturbations, which were accompanied by increasingly Brownian (DFA α = 1.50) fluctuations in torque output. Recovery from these perturbations and of complexity variables was, however, significantly delayed following eccentric exercise. Torque complexity recovered back to baseline levels after 10 minutes of recovery in the isometric condition, but required 24 hours recovery in the eccentric condition. These results indicate that eccentric exercise negatively influences torque complexity; provide the first insight into the recovery kinetics of torque complexity following muscle-damaging and fatiguing exercise; and provide further evidence that the effects of eccentric exercise not only lead to physiological and mechanical alterations, but also neural impairment.

Effect of eccentric exercise on fatigue and torque complexity

It has long been established that eccentric exercise results in a prolonged decrement in torque generating capacity (Davies and White, 1981; Newham et al., 1987; Jones et al., 1989). More recently, it has been shown that eccentric exercise also results in a prolonged increase in torque variability (Semmler et al., 2007; Dartnall et al., 2008). The present study, in line with its hypotheses, is the first study to demonstrate that such responses also apply to torque complexity (Figure 8.5; Table 8.2). Eccentric exercise resulted in a reduction in isometric knee extension torque complexity, as measured by significantly decreased ApEn and SampEn (indicating increased signal regularity) and significantly increased DFA α (indicating increasingly Brownian fluctuations). Over the next 60 minutes, complexity exhibited no recovery and remained at the same level as at the cessation of exercise. It was only after 24 hours that complexity had recovered and was not significantly different from its pre-test value. Such findings are similar to those investigating the magnitude of variability, which have shown increased CV during the 60 minutes following eccentric exercise (Lavender and Nosaka, 2006; Semmler et al., 2007; Skurvydas et al., 2010). Given the purported significance of complexity (Lipsitz and

Goldberger, 1992; Goldberger et al., 2002), these results suggest that eccentric exercise results in a prolonged narrowing of system responsiveness and loss of adaptability in motor control.

As previously observed (Chapters 4, 5, 6 and 7), torque complexity significantly decreased over the course of isometric exercise performed to task failure. In contrast to the eccentric exercise, though, recovery of torque complexity following isometric exercise began immediately and was complete 10 minutes after the cessation of exercise (Figure 8.5; Table 8.2). Given that both the eccentric and isometric conditions resulted in significant global, central and peripheral perturbations, it is likely that the losses in complexity in each condition have, to some extent, similar causes. For example, it has previously speculated that increased motor unit synchronisation may be responsible for reduced torque complexity (Chapters 5, 6 and 7), and increases in motor unit synchronisation have been observed during both fatiguing isometric contractions (Castronovo et al., 2015) and eccentric contractions (Semmler et al., 2002). However, that complexity recovers almost immediately upon the cessation of isometric exercise, but takes 24 hours following eccentric exercise suggests a specific effect of eccentric exercise is responsible for this delayed recovery. It has been speculated that the delayed recovery of the magnitude of variability following eccentric contractions is of central origin, and could be due to increased motor unit recruitment and rate coding to compensate for losses from damaged motor units (Semmler et al., 2007) or due to enhanced motor unit synchronisation (Dartnall et al., 2008); both of which have been associated with the fatigue-induced loss of complexity observed in the previous chapters of this thesis.

Recovery of MVC torque has been shown to be ~90% complete 60 minutes after fatiguing isometric exercise (Sahlin and Ren, 1989; Allman and Rice, 2001), but takes several days, if not longer, to recover following muscle damaging eccentric exercise (Jones et al., 1989; Sayers and Clarkson, 2001). The present study provides further support for such recovery kinetics; with MVC torque reaching ~88% of its fresh value after 60 minutes recovery from fatiguing isometric exercise, but still being significantly decreased after 48 hours recovery from muscle damaging eccentric exercise (Figure 8.1; Table 8.1). Interestingly, the recovery kinetics of MVC torque, in both conditions, differed from those of complexity; with torque generating capacity recovering significantly more slowly (Table

8.1; Table 8.2). Such a delayed recovery of torque generating capacity in comparison to complexity suggests that there is a dissociation between global fatigue and complexity during recovery from exercise, and that changes in variability and complexity during recovery are not related to MVC torque (Lavender and Nosaka, 2006). Thus, while the loss of complexity during exercise mirrors the loss of torque generating capacity and provides a useful surrogate index of fatigue (Chapter 5), it appears the same is not true during recovery from exercise. Whilst the dissociation between complexity and global fatigue during recovery from eccentric exercise is likely due to an effect, be it mechanical or neural, of muscle damage, the mechanism behind the rapid recovery of complexity in comparison to global fatigue following isometric exercise is not presently clear.

Previous research has indicated that eccentric exercise results in an increase in the amplitude of submaximal EMG during recovery (Semmler et al., 2007; Dartnall et al., 2008). In the present study, EMG amplitude following isometric exercise was significantly increased at task failure, but decreased throughout the subsequent 60 minutes of recovery. However, following eccentric exercise the EMG amplitude continued to increase throughout that 60 minutes for both the contractions at 50% of the absolute and relative MVCs (Figure 8.2; Table 8.1). Indeed, it was not until 60 minutes after eccentric exercise that EMG amplitude reached its peak; and it remained significantly increased 48 hours after exercise. That EMG starts to recover immediately upon cessation of isometric exercise, but continues to increase during the 60 minutes following eccentric exercise may be of importance to the recovery of complexity. It has been suggested that adjustments in motor unit activation play a role in the increased EMG amplitude following eccentric exercise (Semmler et al., 2007; Dartnall et al., 2008). Specifically, increased motor unit synchronisation has been observed immediately following eccentric exercise (Dartnall et al., 2008), with this increase lasting as long as one week (Dartnall et al., 2011). Several computer simulation studies have suggested that increased motor unit synchronisation substantially increases EMG amplitude (Yao et al., 2000; Zhou and Rymer, 2004). Moreover, motor unit synchronisation has previously been speculated to be a potential cause of the fatigue-induced loss of torque complexity (Chapters 5, 6 and 7).

Physiological bases for changes in neuromuscular system behaviour

Eccentric exercise is well known for impairing neuromuscular function through peripheral mechanisms, i.e. the muscle damage it induces (Allen, 2001). It is, therefore, possible that the prolonged reduction in complexity following eccentric exercise could also have a peripheral mechanism. Eccentric exercise results in low-frequency fatigue (Balnave and Allen, 1995; Dundon et al., 2008), caused by an impairment of excitation-contraction coupling, most likely due to a decrease in the amount of Ca^{2+} available for release (Balnave and Allen, 1995; Allen, 2001). The previous study of this thesis (Chapter 7) speculated on a link between Ca^{2+} handling, metabolic rate and complexity. It was hypothesised that caffeine potentially acts to decrease sarcoplasmic reticulum Ca^{2+} uptake, leading to increased Ca^{2+} availability, which would serve to maintain submaximal force production (Kalmar and Cafarelli, 19990. Such a potential positive effect on Ca^{2+} handling suggested that caffeine may be able to decrease the ATP cost of force production and the metabolic cost of constant load exercise. As the loss of complexity was also slowed by caffeine ingestion, this provided a link to Seely and Macklem's (2012) hypothesis of an inverse relationship between a system's prevailing metabolic rate and the complexity of its output. In contrast, eccentric exercise has been shown to result in decreased Ca^{2+} availability (Balnave and Allen, 1995; Ingalls et al., 1998), increased energetic cost of force production (Warren et al., 1996) and now a prolonged reduction in torque complexity. It must be noted, though, that the studies indicating decreased Ca^{2+} availability after eccentric exercise were performed on isolated single or whole mouse muscles (Balnave and Allen, 1995; Warren et al., 1996 Ingalls et al., 1998), and although Ca^{2+} handling may be impaired, cytoplasmic Ca^{2+} may actually be elevated in the early stages of damage due to sarcoplasmic reticulum disruption (Proske and Morgan, 2001). The present results further suggest a possible link between Ca^{2+} handling, metabolic rate and complexity, though it must be noted that no direct evidence linking Ca^{2+} handling with complexity has yet been found.

Based on the observations of decreased complexity as a result of both increased contraction intensity and increased fatigue, it was previously speculated that decreased torque complexity is simply a function of the engagement of a greater proportion of the motor unit pool (Chapter 4 and 5). The present findings provide some support for such a contention. The muscle damage brought about by eccentric exercise results in some muscle fibres contributing little to force production (Proske and Morgan, 2001). Thus, in

order to compensate for losses from damaged motor units, increased recruitment and rate coding would be necessary to achieve the target torque (Semmler et al., 2002), as indicated by the increasing EMG during the first 60 minutes of recovery (Figure 8.2; Table 8.1). Such an increased activation of the motor unit pool may potentially contribute to the observed prolonged reduction in complexity. However, the muscle damage experienced and decreased force generating capability persist for longer than the decreased complexity, suggesting that the damage-induced activation of a greater proportion of the motor unit pool may play only a minor role in the prolonged reduction in torque complexity. Furthermore, during recovery from isometric exercise the continued presence of peripheral fatigue would likely indicate fibres contributing less to force production, necessitating greater activation of the motor unit pool, yet complexity recovers within 10 minutes of the cessation of exercise.

Increased motor unit synchronisation has previously been observed following eccentric exercise and has been speculated to be a cause of the increased EMG amplitude and torque variability seen after such exercise (Saxton et al., 1995; Semmler et al., 2007; Dartnall et al., 2008). Motor unit synchronisation is a consequence of a common synaptic input to motoneurons (common drive; De Luca and Erim 1994; Farina and Negro, 2015), and has been linked, in the previous chapters of this thesis, with the fatigue-induced loss of torque complexity. Common drive, and therefore motor unit synchronisation, have been proposed to be the main determinant of force variability (Dideriksen et al., 2012; Farina et al., 2014), and have been demonstrated to increase as a function of fatigue (Castronovo et al., 2015), just as complexity has been demonstrated to decrease as a function of fatigue. Though the EMG setup used in the present study (a single set of bipolar surface electrodes) precludes the measurement of motor unit synchronisation, previous studies have observed an increase immediately after and 24 hours after (Dartnall et al., 2008) eccentric exercise. Furthermore, the increasing EMG amplitude (Figure 8.2; Table 8.1) and increased amount of variability (Table 8.2) in the 60 minutes following eccentric exercise suggest a role for adjustments in motor unit activation (Dartnall et al., 2008), and are both typical of increased motor unit synchronisation (Yao et al., 2000; Zhou and Rymer et al., 2004). Given the previous association between increased motor unit synchronisation and decreased complexity during fatiguing contractions, it would seem reasonable to suggest that the prolonged loss of complexity in the present study is related, at least partly, to an eccentric exercise-induced increase in motor unit synchronisation.

However, the previously observed increases in motor unit synchronisation have been observed 24 hours (Dartnall et al., 2008) and even up to a week after (Dartnall et al., 2011) eccentric exercise, longer than the presently observed decrement in complexity. Further research, utilising high density sEMG to investigate motor unit synchronisation in the knee extensors following eccentric exercise is required to firmly establish any relationship with complexity.

Conclusion

In summary, this study has demonstrated that muscle-damaging eccentric exercise results in a decrease in isometric knee extensor torque complexity, as measured using ApEn, SampEn and DFA α; with this decrement being considerably more prolonged than that resulting from fatiguing isometric exercise. This provides the first evidence that eccentric exercise affects the adaptability of motor control. Eccentric exercise was also associated with more prolonged decreases in global and peripheral perturbations than isometric exercise, which are attributed to the effects of muscle damage. As torque complexity recovered immediately following isometric exercise, the more prolonged reduction following eccentric exercise was also likely due to an effect of this muscle damage. However, whether this was due to the mechanical disruption itself or due to the mechanical disruption impairing and/or influencing neural drive is yet to be fully elucidated. Whatever the cause of the prolonged reduction in complexity, it appears that the effects of eccentric exercise are not limited to the periphery, but also extend to the central nervous system and the ability to control torque output.

Chapter 9 – General Discussion

Healthy physiological outputs, such as heart rate, respiration, gait and muscle torque output, are characterised by non-stationarity and non-linearity; that is, they are not stable, but instead exhibit constant complex fluctuations (Goldberger, 1996; Goldberger et al., 2002; Peng et al., 2002; Vaillancourt and Newell., 2003). Traditionally, such fluctuations have been regarded as unwanted noise from both a physiological perspective (i.e. the classic concept of homeostasis; Cannon, 1929) and a sporting perspective (i.e. variability leads to poor performance; Slifkin and Newell, 1998). However, over the last 30 years, the use of measures derived from the field of non-linear dynamics has made it increasingly apparent that these complex fluctuations are not unwanted noise; rather, they are fundamental to healthy function and reflect the adaptability and functionality of the system of origin (Goldberger, 1991; Goldberger et al., 2002; Lipsitz, 2002).

Reductions in complexity and changes in fractal scaling are a common occurrence in the outputs of many physiological systems in healthy ageing (Lipsitz and Goldberger, 1992; Goldberger et al., 2002; Manor and Lipsitz, 2013). Of importance to the present thesis are the previously observed decreases in the complexity of torque output with ageing (Vaillancourt et al., 2003; Challis, 2006; Sosnoff and Newell, 2008). Such reductions in torque complexity, especially when combined with an increase in the amplitude of force fluctuations (Hunter et al., 2005), imply system dysfunction and a loss of motor control (Lipsitz and Goldberger, 1992; Vaillancourt et al., 2003; Peng et al., 2009). An increase in the amplitude of force fluctuations is also a frequently observed phenomenon in neuromuscular fatigue (Furness et al., 1977; Galganski et al., 1993; Contessa et al., 2009). However, changes in the complexity of fluctuations represent changes in temporal structure and provide greater insight into underlying system organisation (Goldberger and West, 1987a), but have received little attention.

Despite considerable evidence of the potential importance of physiological complexity for system functionality (Lipsitz and Goldberger, 1992; Lipsitz, 2004; Peng et al., 2009), only a handful of studies have investigated changes in the complexity of various outputs in response to neuromuscular fatigue (Cignetti et al., 2009; Cashaback et al., 2013; Cortes et al., 2014). Indeed, prior to the commencement of work on the present thesis, there had been no reports in the literature describing changes in the complexity and fractal scaling

of isometric muscle torque output in response to neuromuscular fatigue. Any change towards a less complex torque output with neuromuscular fatigue could have many have important functional implications. It could, for example, indicate that the neuromuscular system has become less adaptable and more unstable (Lipsitz and Goldberger, 1992; Cignetti et al., 2009) and potentially more energetically expensive (Glenny, 2011; Seely and Macklem, 2012); both of which may significantly influence exercise tolerance. Research into such potential changes in complexity with neuromuscular fatigue is, therefore, of fundamental importance.

9.1 – Summary of main findings

The principal aim of the present thesis was to investigate changes in the complexity and fractal scaling responses of isometric knee extension torque output during neuromuscular fatigue. The first study of this thesis (Chapter 4) provided novel evidence that neuromuscular fatigue perturbs the complexity of torque output. The subsequent studies then sought to expand on this relationship between neuromuscular fatigue and complexity, and to elucidate the mechanism(s) for the observed fatigue-induced loss of complexity.

Neuromuscular fatigue reduces the complexity of isometric torque output

The first experimental study of this thesis (Chapter 4) sought to determine the effect of neuromuscular fatigue on the complexity of joint torque during isometric contractions. The results obtained were the first to indicate that the complexity of isometric knee extension torque is perturbed by neuromuscular fatigue. Specifically, it was demonstrated that the complexity of torque output progressively decreased as fatigue developed, regardless of whether the muscle was driven maximally or submaximally. Torque complexity, therefore, systematically decreased as torque generating capacity decreased and the relative demands of the task increased. This loss of torque complexity was quantified by significant decreases in approximate entropy (ApEn) and sample entropy (SampEn), both of which indicate increased signal regularity (Pincus, 1991; Richman and Moorman, 2000); and by a significant increase in detrended fluctuation analysis (DFA) α, indicating increasingly Brownian (DFA $\alpha = 1.50$) noise (Seely and Macklem, 2004).

These findings extended the “loss of complexity” hypothesis in ageing (Lipsitz and Goldberger, 1992) to the neuromuscular fatigue process in otherwise healthy humans. Such a loss of torque complexity is likely to be the result of mechanisms directly affecting the output of the motor unit pool. However, the observed reductions in torque complexity in both the maximal and submaximal conditions were accompanied by the development of significant central and peripheral fatigue, precluding the isolation of a mechanism responsible for the loss of complexity. Nevertheless, based on the purported significance of physiological complexity (Lipsitz and Goldberger, 1992; Goldberger et al., 2002), the results suggested that the impact of neuromuscular fatigue is not limited to torque-generating capacity but extends to the adaptability of the neuromuscular system to external perturbations.

Neuromuscular fatigue reduces torque complexity only above the critical torque

The second experimental study (Chapter 5) sought to expand upon the findings of the first study by investigating fatigue-induced changes in complexity in relation to the critical torque (CT). It was demonstrated that significant decreases in ApEn and SampEn, and a significant increase in DFA α, were observed when the CT was exceeded, but that no changes were evident during 30 minutes of contractions below the CT. These changes indicated that it is only above the CT that a progressive reduction in the complexity of torque output occurs, implying that it is fatigue mechanisms particular to contractions above the CT that are responsible for the loss of complexity. The dominant fatigue mechanism above the CT is believed to be metabolite-mediated peripheral fatigue, as progressive phosphocreatine (PCr) depletion, and inorganic phosphate (P_i) and proton (H^+) accumulation occur only above the CT (Jones et al., 2008; Burnley et al., 2010). Though the fatigue-induced loss of complexity occurred exclusively above the CT, suggesting that metabolite-mediated peripheral fatigue plays an essential role, significant central fatigue was also observed for contractions above the CT. It therefore remained unclear whether the loss of complexity was due to a direct effect of peripheral fatigue or to the compensatory central adjustments that are required to continue exercise in the face of developing peripheral fatigue.

The observation of a decrease in complexity above, but not below the CT demonstrated that metrics derived from non-linear dynamics are able to identify changes in

neuromuscular system behaviour coincident with the CT. This provides a parallel between torque complexity and measures such as $\dot{V}O_2$, blood [lactate], [P_i] and pH, which are all able to attain steady states below critical power/CT, but rise inexorably until task failure above it (Poole et al., 1988; Jones et al., 2008). It is unclear why complexity and the CT are linked, but one possible explanation relates to changes in metabolic rate. Muscle $\dot{V}O_2$ rises as a function of time only above critical power, just as complexity was demonstrated to fall as a function of time only above the CT; observations in line with the inverse relationship between the metabolic rate of a system and the complexity of its output hypothesised by Seely and Macklem (2012).

Another key finding of the second experimental study was that task failure in the trials above the CT was characterised by common values of complexity, regardless of the intensity of the contractions or the rate of change. Such an observation could have important implications for exercise tolerance. As task failure was also characterised by similarly low and common values of complexity in the various fatigue conditions in the third and fourth studies of this thesis (Chapters 6 and 7), it would appear that low complexity may be a prerequisite for task failure. The fact that complexity progressively decreases as fatigue develops and relative task demands increase, reaching common values at task failure regardless of exercise intensity or rate of change also suggested that torque complexity can provide a sensitive surrogate index of fatigue. This could be of particular importance in situations when the use of traditional fatigue measures, such as maximal contractions or electrical stimulation, are not possible.

Torque complexity is intensity dependent

Based on a previously observed inverted-U shaped relationship between contraction intensity and complexity (Slifkin and Newell, 1999; Slifkin and Newell, 2000), the first study of this thesis (Chapter 4) hypothesised that submaximal contractions would exhibit greater complexity than maximal contractions. This proved to be the case, with the initial contractions of the submaximal test (at 40% MVC) being significantly more complex than the initial contractions of the maximal test. The greater complexity during the submaximal contractions was thought to be reflective of a more adaptable torque output, which could be easily modulated up or down in order to meet changing task demands.

The relationship between contraction intensity and complexity was extended in the second study (Chapter 5), with contractions across the whole working range of the knee extensors being investigated. It was demonstrated that complexity was highest at 10% MVC and decreased with increasing contraction intensity, reaching its lowest value during maximal contractions. Though discrepant with the inverted-U shaped relationship (Slifkin and Newell, 1999; Svendsen and Madeleine, 2010), these results were similar to those of Forrest et al. (2014), who criticised previous studies for their use of inappropriate signal acquisition and ApEn processing parameters. The observed relationship makes some intuitive sense, given the purported role of complexity as an index of a system's adaptability (Lipsitz and Goldberger, 1992; Goldberger et al., 2002), and is in line with Seely and Macklem's (2012) hypothesis of an inverse relationship between work rate and complexity.

The observed decrease in complexity with increasing contraction intensity must be due to an effect on the motor unit pool. Submaximal contractions rarely achieve tetanic rates of discharge (Enoka and Fuglevand, 2001), meaning the force exerted by a muscle is characterised by complex fluctuations, due to submaximal activation of motor units (Taylor et al., 2003). Increasing contraction intensities require higher discharge rates (Bigland and Lippold, 1954; Moritz et al., 2005). Such higher discharge rates are more likely to result in an output approximating a fused tetanus (Buller and Lewis, 1965), which could account for the smoother torque output with fewer fluctuations observed during high intensity and maximal contractions. In this case, complexity systematically decreases as torque requirement increases, reaching its lowest point when recruitment and rate coding reach their peaks. Alternatively, the decreased complexity could be related to common drive; that is, the common synaptic input to motoneurons (De Luca and Erim, 1994; Farina and Negro, 2015). Common drive has been proposed to be the main determinant of force variability (Negro et al., 2009; Dideriksen et al., 2012; Farina et al., 2014) and has been demonstrated to increase with increasing contraction intensity (Castronovo et al., 2015). Furthermore, motor unit synchronisation, a necessary consequence of a shared synaptic input, has been proposed to be a likely contributor to the time and frequency domains of torque output (Taylor et al., 2003) and has been speculated to influence the complexity of loaded postural tremor with ageing (Sturman et al., 2005).

Pre-existing neuromuscular fatigue influences torque complexity

After having established the intensity dependence of changes in torque complexity with neuromuscular fatigue (Chapters 4 and 5), the next step was to attempt to determine the mechanism(s) responsible for the fatigue-induced reduction in complexity. The third experimental study of this thesis (Chapter 6) hypothesised that increasing central and peripheral fatigue, in isolation, at the start of an exercise bout would influence complexity, therefore providing an indication as to whether any change was central or peripheral in origin. Separating central and peripheral fatigue, though, proved impossible; there was no significant "cross-over" of central fatigue from the exercised limb to the unexercised contralateral limb during the central fatigue condition, and sustained peripheral fatigue likely mediated a sustained decrease in voluntary activation during the peripheral fatigue condition. This meant that isolating whether peripheral alterations or the resulting central adjustments were the cause of the fatigue-induced loss of complexity was not possible. Such findings did, however, suggest that central and peripheral fatigue are inextricably linked and that both contribute to the fatigue-induced loss of complexity.

Whilst isolating central and peripheral fatigue at the start of an exercise bout proved impossible, pre-existing neuromuscular fatigue did, nonetheless, have an effect on complexity. Although the complexity values at the start of the exercise bouts with various forms of pre-existing fatigue were not always significantly different from that of fresh muscle, they were, in every case, lower, as measured by decreased ApEn and SampEn, and increased DFA α. This reduced complexity was accompanied by both decreased MVC torque (indicating increased relative task demands) and decreased time to task failure (indicating decreased exercise tolerance). These findings, combined with the inability to separate central and peripheral fatigue, led to the suggestion that changes in complexity may be brought about by both central and peripheral mechanisms, and act as indicators of overall system function in the presence of fatigue.

Caffeine slows the loss of torque complexity with neuromuscular fatigue

The fourth experimental study (Chapter 7) sought to investigate whether caffeine, an ergogenic aid thought to act primarily through central mechanisms (Fredholm et al., 1999; Kalmar and Cafarelli, 2004a), influenced torque complexity during neuromuscular

fatigue. It was demonstrated that the ingestion of 6 $mg \cdot kg^{-1}$ caffeine significantly increased time to task failure and significantly slowed the previously observed fatigue-induced reduction in torque complexity, as measured by attenuated rates of decrease in ApEn and SampEn, and an attenuated rate of increase in DFA α. The slowing of the reduction in torque complexity mirrored that of the loss of MVC torque, providing further evidence that changes in complexity reflect relative task demands and can be used as a surrogate index of fatigue, system functionality and exercise capacity. These findings indicated that pre-exercise caffeine ingestion slows the narrowing of system responsiveness decreases in complexity measures are thought to reflect (Lipsitz and Goldberger, 1992; Goldberger et al., 2002), thus serving to preserve the adaptability of motor control for longer.

Not only did caffeine ingestion attenuate the rate of loss of complexity, but it also significantly attenuated the rates of development of global, central and peripheral fatigue. Caffeine's ergogenic effect has largely been attributed to central mechanisms, and its inhibitory effect on adenosine, which preferentially inhibits the release of excitatory neurotransmitters (Fredholm et al., 1999). The slowing of the development of both central and peripheral fatigue, though, suggests that caffeine's effect on fatigue, and indeed complexity, may not be limited to traditionally accepted central mechanisms, but may extend to peripheral mechanisms. The slowing of the development of central fatigue could be indicative of caffeine increasing spinal excitability (Walton et al., 2003), maintaining optimal descending drive (Taylor et al., 2000) or influencing common drive (Farina and Negro, 2015), any of which could exert an effect on the motor unit pool and complexity. The slowing of the development of peripheral fatigue could be reflective of caffeine positively influencing calcium (Ca^{2+}) handling and reducing the ATP cost of force production (Kalmar and Cafarelli, 1999; Tarnopolsky and Cupido, 2000), which could be of importance to fatigue-induced changes in complexity given the previous association between metabolic rate and complexity (Seely and Macklem, 2012). However, that both central and peripheral fatigue were slowed by caffeine ingestion, again, precluded the isolation of a specific mechanism responsible for the reduced complexity. The evidence does suggest, though, that any influence of caffeine through peripheral mechanisms occurs only late in exercise and that a central effect is responsible for most of the attenuation of the loss of complexity.

Eccentric exercise causes a prolonged reduction in torque complexity

The final experimental study of this thesis (Chapter 8) sought to investigate the effect of eccentric exercise, which results in prolonged impairment of force production through peripheral mechanisms (Proske and Morgan, 2001), on complexity and subsequent recovery. It was demonstrated that eccentric exercise resulted in a reduction in torque complexity, as measured by significantly decreased ApEn and SampEn, and significantly increased DFA α. More importantly, though, over the next 60 minutes, complexity exhibited no recovery, with ApEn, SampEn and DFA α all remaining at the same level as at the end of exercise. It was only after 24 hours recovery that complexity had returned back to its baseline level; a finding similar to that previously obtained when examining the magnitude of variability, as measured by the coefficient of variation, following eccentric exercise (Lavender and Nosaka, 2006; Semmler et al., 2007). These findings suggest that eccentric exercise not only delays the recovery of torque generating capacity, but also results in a prolonged loss of adaptability and narrowing of system responsiveness in motor control.

Just as was observed in the preceding studies (Chapters 4, 5, 6 and 7), fatiguing isometric exercise also resulted in a decrease in torque complexity. However, in contrast to the eccentric exercise, the recovery of torque complexity was complete after only 10 minutes. Both the isometric and eccentric exercise resulted in significant global, central and peripheral perturbations suggesting that the loss of complexity in each condition had, to some extent, similar causes. However, the prolonged decrement in complexity following eccentric exercise compared to the near instantaneous recovery following isometric exercise suggests that some specific effect of eccentric exercise has a significant impact on complexity. Though eccentric exercise is typically associated with muscle damage and the inhibition of neuromuscular performance through peripheral mechanisms (Allen, 2001), recent evidence has suggested that its impact may extend to the central nervous system, where it negatively effects neural drive and motor unit activation (Semmler et al., 2007; Dartnall et al., 2008). However, it was not possible to identify whether the prolonged loss of torque complexity was due to mechanical disruption at the periphery, or this mechanical disruption influencing neural drive.

9.2 – Potential mechanisms of the fatigue-induced loss of torque complexity

The fatigue-induced reduction of torque complexity and shift to Brownian noise observed in the five experimental studies of this thesis was, in every case, accompanied by the development of both significant central and peripheral fatigue. The observed loss of torque complexity must, by definition, be caused by mechanisms directly affecting the motor unit pool. However, the development of both central and peripheral fatigue in tandem prevented the isolation of the mechanism(s) responsible. Alternatively, it may be that central and peripheral fatigue are inextricably linked, and it is a combination of the peripheral alterations and the central adjustments aimed at combating such alterations that cause complexity to be reduced.

Peripheral mechanisms

The fatigue-induced reduction in torque complexity is closely associated with an increase in relative task demands, as measured by decreasing MVC torque and increasing EMG amplitude. As fatigue develops, there is a fall in the force output of fatiguing motor units, consequent to metabolite-mediated peripheral fatigue (Allen et al., 2008), along with a slowing of twitch time to peak tension and half-relaxation time (Cady et al., 1989b; Jones et al., 2009). Though the latter was not observed in the present thesis, the net effect of these responses could be a smoothing of the torque time series, and a reduction in complexity. However, the fall in the force output of fatiguing motor units necessitates increased central drive, and thus motor unit recruitment and rate coding, in order to maintain torque output. These responses are reflected in the increased EMG amplitude, and their net effect could also be a smoothing of the torque time series. This presence of both increased peripheral fatigue and increased central drive makes the identification of a mechanism responsible for the loss of complexity very difficult using contractions in vivo alone.

The observation, in the second experimental study (Chapter 5), that complexity only decreases as a function of time above the CT provided the first real mechanistic evidence, suggesting that fatigue mechanisms specific to contractions above the CT are responsible for the reduction in torque complexity. It is thought that metabolite-mediated peripheral fatigue predominates above the CT, on the basis that progressive PCr depletion, and P_i

and H^+ accumulation only occur above the CT (Jones et al., 2008; Vanhatalo et al., 2010). P_i accumulation, in particular, has been associated with fatigue, both through a direct effect on cross-bridge force (Debold et al., 2006) and, perhaps more importantly, through a depressive effect on Ca^{2+} kinetics (Westerblad et al., 2000; Dutka et al., 2005). It was, therefore, speculated that metabolite-mediated peripheral fatigue plays an integral role in reducing torque complexity. However, as significant central fatigue also developed above the CT, it remained unclear whether metabolite-mediated peripheral fatigue, perhaps through an effect of increased $[P_i]$ on Ca^{2+}, directly reduces torque complexity, or whether the central adjustments such peripheral fatigue necessitates, are responsible. Given the difficulty of separating central and peripheral fatigue, studies of muscle in vitro may be necessary in order to confirm a direct effect of metabolite-mediated peripheral fatigue on complexity. At the very least, though, these findings suggest that metabolite-mediated peripheral fatigue initiates the chain of events that leads to a loss of torque complexity.

An interesting observation of the second, third and fourth experimental studies (Chapters 5, 6 and 7) was that task failure was accompanied by common values for both peripheral fatigue, as measured by potentiated doublet torque, and complexity. This was despite varying task demands, experimental manipulation, rates of change and times to task failure. That potentiated doublet torque had declined to similar levels is in line with previous data indicating a consistent level of metabolic disturbance and/or peripheral fatigue at task failure (Amann et al., 2007; Burnley, 2009; Burnley et al., 2010). That complexity also declined to similarly low levels at task failure is a major novel finding, and indicates that task failure is characterised not only by consistent levels of metabolic disturbance and peripheral fatigue, but also by consistently low levels of torque complexity. This bolsters the link between peripheral fatigue and complexity, as it seems that the loss of complexity is coupled to the magnitude of peripheral fatigue, rather than simply task duration. Further to this, circulatory occlusion at task failure in the third experimental study (Chapter 6) resulted in maintained peripheral fatigue and no recovery of complexity. This lack of recovery was likely mediated by a lack of perfusion, resulting in no recovery of the metabolic milieu.

Seely and Macklem (2012) have hypothesised that there is an inverse relationship between a system's prevailing metabolic rate and its output complexity. The results of the second experimental study provide some support for this hypothesis, since it is only above

the critical power/CT that muscle $\dot{V}O_2$ rises inexorably as a function of time (Poole et al., 1988), just as it is only above the CT that torque complexity decreases as a function of time. Moreover, the accumulation of P_i, which is likely to occur only above the CT (Jones et al., 2008; Vanhatalo et al., 2010), has a negative effect on muscular efficiency (Sahlin et al., 1998; Grassi et al., 2015). Further potential links between complexity and metabolic rate were highlighted in the fourth and fifth experimental studies (Chapters 7 and 8). In Chapter 7 it was demonstrated that caffeine ingestion slowed the fatigue-induced loss of torque complexity, as well as the rate of development of peripheral fatigue. It has previously been speculated that caffeine acts peripherally to decrease sarcoplasmic reticulum Ca^{2+} uptake, which would increase Ca^{2+} availability and serve to maintain submaximal force production (Kalmar and Cafarelli, 1999; Tarnopolsky and Cupido, 2000). This suggested that caffeine may be able to modulate the ATP cost of force production and the metabolic cost of constant load exercise (Doherty and Smith, 2005). Thus, the slowing of the loss of complexity may have been due to a slowing in the rise in metabolic rate. However, measurable effects on Ca^{2+} release or reuptake have only been observed with caffeine administration in the millimolar range in animals (Howlett et al., 2005), with this level of caffeine being toxic to humans. Conversely, the eccentric exercise-induced muscle damage in Chapter 8 likely acted to decrease the amount of Ca^{2+} available for release (Balnave and Allen, 1995; Ingalls et al., 1998) and increased the energetic cost of force production (Warren et al., 1996). Thus, the prolonged reduction in complexity may have been due to a prolonged increase in metabolic rate. However, Ca^{2+} may actually increase in the initial stages of muscle damage, due to disruption of the sarcoplasmic reticulum (Proske and Morgan, 2001). Further research into the metabolic basis of torque complexity is required to firmly establish a relationship between changes in metabolic rate and complexity.

The available evidence suggests that peripheral fatigue plays an important role in initiating the loss of torque complexity. Whether this is due to a direct metabolic effect, or due to the central adjustments necessary to continue exercise in the face of developing peripheral fatigue, though, remains unclear. If peripheral fatigue is directly responsible, it seems reasonable to suggest it is via an effect of metabolites on Ca^{2+} handling. Ca^{2+} plays a crucial role in skeletal muscle activation, with an increase in free myoplasmic $[Ca^{2+}]$ being the trigger for contraction (Westerblad et al., 2000; Allen et al., 2008b). The metabolite-mediated peripheral fatigue seen above the CT in the second experimental

study would have involved increased [P_i] (Jones et al., 2008), which is likely to have influenced Ca^{2+} kinetics, decreasing the amount of Ca^{2+} available for release (Westerblad et al., 2000; Allen and Westerblad, 2001), potentially increasing metabolic rate and decreasing efficiency. The postulated effects of caffeine (Kalmar and Cafarelli, 1999; Chapter 7) and eccentric exercise (Warren et al., 1996; Chapter 8) on metabolic rate, and changes in complexity, were also likely mediated by an effect on Ca^{2+} kinetics.

Central mechanisms

As previously mentioned, the fatigue-induced reduction in torque complexity is closely associated with increased relative task demands. During fatiguing submaximal contractions, the neuromuscular system maintains torque output in the face of reduced muscle fibre twitch force (Allen et al., 2008a) by increasing central drive, thus increasing motor unit recruitment and rate coding (Bigland-Ritchie et al., 1986a; Adam and De Luca, 2003). As fatiguing contractions progress, a greater proportion of the motor unit pool is therefore necessarily engaged in the task. The observed reduction in complexity could simply relate to such an increase in the overall excitatory drive to the muscle; with complexity progressively decreasing as recruitment and rate coding increase, and reaching its lowest value when recruitment and rate coding presumably reach their peaks. Further evidence for this supposition comes from the results of the second and fifth experimental studies (Chapters 5 and 8). In Chapter 5 it was observed that complexity also decreased as a function of absolute task demands (i.e. increasing contraction intensity). As absolute demands increase, greater recruitment and rate coding are necessary to achieve the increasingly higher target torques. In Chapter 8 it was speculated that the muscle damage incurred would have led to some muscle fibres contributing little to force production (Proske and Morgan, 2001), necessitating increased recruitment and rate coding to compensate and achieve the target torque (Semmler et al., 2002). In line with the present hypothesis, such greater activation of the motor unit pool could have been responsible for the prolonged loss of complexity following eccentric exercise.

Another central adjustment potentially related to the fatigue-induced loss of complexity is the common synaptic input to motoneurons and modulation of motor unit discharge rates, known as common drive (De Luca and Erim, 1994; Farina and Negro, 2015). It has been postulated that common drive represents the effective neural drive delivered to

muscles and is the main determinant of force variability in a contraction (Dideriksen et al., 2012; Farina et al., 2014). More recently it has been demonstrated that the relative strength of common synaptic input to motoneurons increases when the net excitatory input to motoneurons increases, whether this is due to an increase in contractile intensity or to the progression of fatigue (Castronovo et al., 2015). The results of the present thesis have demonstrated that both increasing contraction intensity and neuromuscular fatigue are also associated with decreased torque complexity. Thus, if common synaptic input explains the magnitude of force fluctuations (Farina and Negro, 2015), this suggests that it may also impact the structure of fluctuations.

Common drive may influence the complexity of torque output through the modulation of motor unit discharge rates. A necessary consequence of a common synaptic input to all motoneurons is the correlated discharge of action potentials, known as motor unit synchronisation (Semmler, 2002; Farina and Negro, 2015). As common synaptic input increases as neuromuscular fatigue develops, so must motor unit synchronisation increase. Motor unit synchronisation has previously been suggested to be a likely contributor to the time and frequency domains of torque output (Taylor et al., 2003). Indeed, increased motor unit synchronisation has been postulated to be the cause of decreased complexity of postural tremor (as measured using ApEn) with ageing (Sturman et al., 2005), has been linked to decreases in the fractal dimension (indicating greater regularity) of surface EMG with fatigue (Mesin et al., 2009; Beretta-Piccoli et al., 2015) and has been speculated to be the cause of the increased magnitude of variability seen following eccentric exercise (Saxton et al., 1995; Semmler et al., 2007; Dartnall et al., 2008). Furthermore, motor unit synchronisation has been described as “phase-locking” (De Luca et al., 1982b), which may be analogous to “mode-locking,” a phenomenon in which outputs oscillate at a single dominant frequency and is associated with a pathological loss of complexity and functional responsiveness in ageing and disease (Peng et al., 1998; Peng et al., 2009). Though the functional role of motor unit synchronisation in force production is unclear (Farina and Negro, 2015), excessive levels are thought to disrupt motor performance (Yao et al., 2000; Holtermann et al., 2009), which could be reflected in the form of reduced complexity.

9.3 – Implications

Complexity is thought to be a hallmark of a healthy system; reflecting the ability to adapt to internal and external perturbations (Pincus and Goldberger, 1994; Lipsitz, 2004; Peng et al., 2009). In the case of the neuromuscular system, the complexity of torque output is thought to reflect the ability to adapt motor output rapidly and accurately in response to alterations in demand (Vaillancourt and Newell, 2003). Thus, the fatigue-induced reduction in complexity observed in the five experimental studies of this thesis has important implications for motor control.

It was observed in the second, third and fourth experimental studies (Chapters 5, 6 and 7) that torque complexity declined to similar values at task failure, despite different task demands, experimental manipulation and rates of change. That task failure was characterised by similarly low values of complexity suggested that complexity may have an influence on exercise tolerance, with low values being a prerequisite for task failure. High levels of complexity reflect greater adaptability and functionality in a system (Lipsitz and Goldberger, 1992; Lipsitz, 2004), allowing it to modulate the excitatory and inhibitory properties of the motor unit pool to match task demands (Kilner et al., 1999). Low levels of complexity are reflective of increasing system dysfunction (Goldberger et al., 2002; Peng et al., 2009), and an increasing inability to match task demands (Sosnoff et al., 2004). The low values of complexity attained at task failure, therefore, may have been so low as to compromise motor control and limit task performance, in accord with the proposed functional significance of physiological complexity (Vaillancourt et al., 2003; Peng et al., 2009). Whether low torque complexity plays a direct role in precipitating task failure in unclear. Nevertheless, low complexity is clearly associated with reduced motor control and neuromuscular performance, and a role in the mechanisms of task failure is plausible. Further research is required to directly test this hypothesis.

Expanding on the link between torque complexity and exercise tolerance, the progressive decline of complexity as fatigue develops, combined with the common values attained at task failure, suggests that complexity can act as a surrogate index of fatigue. This supposition is further bolstered by the observation that both complexity (Chapter 5) and the tolerable duration of exercise (Hill and Lupton, 1923) decrease as contraction intensity

increases. Such a relationship is not wholly surprising, given that complexity is purported to reflect the adaptability and functionality of the system of origin (Lipsitz, 2002; Peng et al., 2009); both of which necessarily decrease as fatigue develops. If, indeed, complexity does act as a surrogate index of fatigue, or an index of system functionality in the face of fatigue, this raises the possibility that it could be used to provide a real-time assessment of the fatigue process, instead of simply being used as a post-hoc analysis measure. This could be particularly advantageous in situations where the use of traditional fatigue measures, such as maximal contractions and electrical stimulation, are not possible or are undesirable, as may be the case when assessing elderly subjects or working with athletes in the field. The present thesis has only examined isometric contractions of the knee extensors, and further research is required to establish the relationship between fatigue and neuromuscular output complexity in a range of tasks and muscle groups. Such research, though, could have important implications for the assessment of fatigue in laboratory settings, and given the rise of wearable and equipment mounted devices to measure neuromuscular output during exercise, such work could pave the way to real-time assessment of the fatigue process in free running conditions.

Low values of torque complexity may not only affect exercise tolerance, but also the quality and co-ordination of skilled movements (Singh et al., 2010; Cortes et al., 2014). The reduced complexity observed with neuromuscular fatigue indicates an output that became more periodic and rigid (Forestier and Nougier, 1998; Goldberger, 2006), which would signify a decreased ability to adapt to any further stressor (Peng et al., 2009). Elite athletes do not exhibit motor invariance, even after years of practice (Bauer and Schöllhorn, 1997; Davids et al., 2004), and healthy movements exhibit an optimal state of variability (Stergiou and Decker, 2011). A reduction in complexity and a more periodic output have, therefore, been postulated to result in decreased co-ordination (Cortes et al., 2014). Such a loss of co-ordination would have a detrimental effect on the performance of skilled movements in athletic and sporting events (Forestier and Nougier, 1998), and could have a detrimental effect on functional movements, which would be of particular concern for older adults.

Fatigue-induced changes in variability and complexity have also been speculated to relate to injury (Cortes et al., 2014). Neuromuscular fatigue is associated with increased risk of certain types of injury (Kernozek et al., 2008; McLean and Samorezov, 2009), and several

studies have reported that individuals exhibiting lower complexity and variability in lower limb mechanics during dynamic exercise are at increased risk of anterior cruciate ligament injury (Hamill et al., 1999; Hamill et al., 2005). Variability and complexity provide an adaptive mechanism to external perturbations, and their presence may be an important factor in reducing injury risk (Bartlett et al., 2007; Stergiou and Decker, 2011). Thus, the presently observed fatigue-induced reduction in torque complexity, and potential loss of co-ordination, could be a precursor for increased likelihood of injury or damage (Cortes et al., 2014).

9.4 – Directions for future research

The results of the present thesis have demonstrated that neuromuscular fatigue decreases the complexity of isometric knee extension torque, shifting it to an output that is increasingly Brownian in nature. Research into complexity and neuromuscular fatigue is very much in its infancy, and further research is undoubtedly warranted to expand the present findings. It is clear that the principal aim of future research investigating complexity and fractal scaling responses to neuromuscular fatigue should be to identify the mechanism(s) responsible for the presently observed changes.

Establishing the mechanism for the fatigue-induced loss of complexity

The evidence obtained in the present thesis suggests several peripheral and central mechanisms which could be responsible for the fatigue-induced loss of torque complexity. The challenge of future studies will be demonstrating a direct causal link between one of these mechanisms and changes in complexity. However, given the difficulty in separating peripheral and central fatigue in vivo, the isolation of a mechanism may be best determined through the use of mathematical modelling and simulation.

Peripheral mechanisms

The metabolic basis for changes in torque complexity could be examined through the use of ergogenic aids and nutritional interventions that act through peripheral mechanisms. Supplements such as sodium bicarbonate, ß-alanine and ammonium chloride all have the potential to alter pH and muscle buffering capacity; exercise-induced glycogen depletion

reduces our energy stores and potentially effects Ca^{2+} handling; while chronic supplementation of creatine increases PCr resynthesis, boosting short-term energy stores. Any one of these has the potential to alter torque complexity; either through an effect on baseline values or by altering the rate of change during exercise.

Sodium bicarbonate has long been demonstrated to enhance exercise performance (Dennig et al., 1931); slowing the rate of force decline (Hunter et al., 2009a) and increasing time to task failure in a variety of exercise modalities (Raymer et al., 2004; Sostaric et al., 2006; McNaughton et al., 2008). Acute ingestion of sodium bicarbonate induces metabolic alkalosis, which may improve performance through a number of proposed mechanisms, including preserving membrane excitability (Sostaric et al., 2006), accelerating glycogenolysis (Hollidge-Horvat et al., 2000) and increasing extracellular proton buffer capacity (Street et al., 2005). Similarly, chronic supplementation of ß-alanine is also thought to enhance performance through inducing alkalosis (Sale et al., 2010). Chronic supplementation of ß-alanine improves exercise capacity and performance (Stout et al., 2007), with this increase hypothesised to be due to increased muscle carnosine concentrations improving intracellular buffering capacity (Hill et al., 2007). Conversely, metabolic acidosis, brought about by acute ammonium chloride ingestion (Dennig et al., 1931; George and MacLaren, 1988; Carr et al., 2011) or chronic dietary intervention (Greenhaff et al., 1988), has been repeatedly demonstrated to decrease exercise performance.

Metabolic alkalosis and acidosis could thus be used to manipulate exercise capacity and investigate any subsequent change in complexity, be it an increased/decreased baseline value or an increased/decreased rate of change. The effects of metabolic alkalosis and acidosis on changes in torque complexity with fatigue could be easily examined with a similar methodology to that used in the present thesis to investigate the effects of caffeine (Chapter 7). Such studies could be improved by also making make use of ^{31}phosphorus magnetic resonance spectroscopy to examine real-time changes in metabolites and correlate these with changes in complexity; an approach which would be able to rule in or out different metabolic changes as potential causes of the fatigue-induced loss of complexity.

It is well-established that our ability to exercise is seriously compromised when our glycogen stores are reduced (Bergström et al., 1967; Hermansen et al., 1967), and it has been speculated that muscle glycogen content may affect excitation-contraction coupling and Ca^{2+} handling (Stephenson et al., 1999; Ørtenblad et al., 2011). Studies on both single muscle fibres and exercising humans have demonstrated that glycogen depletion and an absence of carbohydrate intake during recovery results in lower sarcoplasmic reticulum Ca^{2+} release (Chin and Allen, 1997; Ørtenblad et al., 2011). Given this effect of glycogen depletion on Ca^{2+} and the speculation (above) that Ca^{2+} handling could play a role in changes in torque complexity, it would be of interest to investigate whether glycogen depletion effects torque complexity during a subsequent bout of exercise. Given that the experimental protocols used in the present thesis were not sufficient to deplete glycogen stores, this would likely need to be accomplished through the use of intense cycle ergometry exercise, such as that used by Duhamel et al. (2006). Following this, the complexity of isometric knee extension torque could be assessed during recovery in the presence and absence of carbohydrate feeding.

If torque complexity is, as speculated above, related to metabolic rate and the energetic cost of force production, another ergogenic aid that could be used to potentially manipulate complexity is creatine. PCr aids in the rapid phosphorylation of adenosine diphosphate back to adenosine triphosphate (ATP), by the creatine kinase CK reaction (Bemben and Lamont, 2005). It has been demonstrated that chronic supplementation of creatine can substantially increase muscle creatine concentration and PCr resynthesis (Greenhaff et al., 1994), and that this can lead to enhanced fatigue resistance in short duration, high-intensity exercise (Birch et al., 1994; Balsom et al., 1995). It has also been shown that creatine supplementation increases W′, the finite amount of work that can be performed above the CT (Miura et al., 1999). Given the potential for creatine to influence ATP and to increase the amount of work done above the critical torque, it may be that supplementation increases baseline complexity values, or allows a greater amount of work to be done before complexity starts to decline. The investigation of an effect of increased muscle creatine and PCr on complexity could be enhanced by the use of ^{31}phosphorus magnetic resonance spectroscopy, to correlate changes in PCr with those in complexity.

Central mechanisms

One of the central adjustments proposed to be a likely contributor to the fatigue-induced loss of torque complexity is an increase in the common synaptic input to motoneurons (i.e. common drive), and the increased motor unit synchronisation that is a necessary consequence of this. The surface EMG (sEMG) setup used in the experimental studies of the present thesis (a single set of bipolar electrodes) did not allow for the measurement of motor unit action potentials, from which measures of common drive and motor unit synchronisation are derived. In order to quantify these, future studies would need to use a high-density sEMG array, comprised of many closely spaced surface electrodes on several locations across the muscle (Merletti et al., 2008; Negro et al., 2009; Castronovo et al., 2015). From this it is possible to decompose the sEMG signal, in order to measure motor unit discharge timings, from which information can then be extracted regarding the synaptic input received by corresponding motoneurons (Farina et al., 2010; Farina and Holobar, 2016).

It may be useful to replicate some of the studies of the present thesis with the addition of a high-density sEMG array, in order to obtain measures of common drive and motor unit synchronisation. Firstly, it would be of interest to determine whether the fatigue-induced increases in common drive and motor unit synchronisation previously observed (Castronovo et al., 2015) exhibit the same intensity specific behaviour as complexity measures with regard to the CT. The sustained fatiguing contractions performed by Castronovo et al. (2015) ranged from 20-75%, and were likely to have been above the CT, since the CT typically occurs at ~15% MVC for sustained contractions (Monod and Scherrer, 1965; Hendrix et al., 2009). Secondly, it would be of interest to determine whether the ingestion of caffeine has any effect on common drive and motor unit synchronisation. Caffeine has previously been demonstrated to increase spinal excitability, the net effect of membrane properties and the summation of synaptic input to α-motoneurons (Walton et al., 2003; Kalmar et al., 2006), so an effect on common drive and motor unit synchronisation is plausible. And finally, it would be beneficial to examine changes in the complexity of motor unit discharge rates with fatigue. Previously, the ApEn of the discharge rate of single motor units has been shown to linearly increase with an increase in motor unit discharge rate (Vaillancourt et al., 2002; Vaillancourt et al., 2003); though changes with fatigue have yet to be examined.

Modelling and simulation

Given the difficulty in determining whether fatigue-induced changes in complexity are a function of peripheral alterations or the subsequent central adjustments required to continue exercise, it may be that computer modelling and simulation studies, rather than in vivo studies, provide the best hope of discerning a mechanism. Fuglevand et al. (1993) developed a widely-used model of motor unit recruitment and rate coding, which simulates the chain of events from the excitation of the motoneurons to the forces exerted at the tendon; and which has proved useful in the examination of force variability (Moritz et al., 2005; Barry et al., 2007). Yao et al. (2000) extended this model to address the influence of motor unit synchronisation, finding that increased synchronisation increased EMG amplitude and the magnitude of force variability. Dideriksen et al. (2010) also extended the model, this time to include adjustments that occur during fatiguing contractions, such as varying motor unit twitch force, recruitment and discharge rate and variability. Now it is known that fatigue results in an increase in common drive and motor unit synchronisation (Castronovo et al., 2015) and a decrease in torque complexity (Chapters 4, 5, 6, 7 and 8), it would be of great benefit to extend the Dideriksen et al. (2010) model to investigate these changes.

Generalisation

The experimental studies presented in this thesis investigated only the complexity of isometric knee extension torque. Future studies on changes in complexity with neuromuscular fatigue in other muscle groups and as a result of dynamic exercise would extend and enhance the present findings, and could lead to complexity statistics becoming a staple laboratory measure of fatigue.

Other muscle groups

Although the knee extensors represent an easy and functionally important muscle group to test, fatigue-induced changes in torque complexity need to be examined in other muscle groups, in order to extend the present findings and confirm torque complexity as a useful surrogate measure of fatigue. Given the different fibre type compositions and fatigue properties of other muscle groups (Bigland-Ritchie et al., 1986a; Harridge et al., 1996), it may be that they exhibit subtly different complexity responses, which would provide further information regarding the underlying mechanisms of fatigue, and the dynamics

and adaptability of different muscle groups. Furthermore, fatigue-induced changes in complexity may have different functional implications, depending on the muscle group tested. For example, low complexity is associated with task failure in exhaustive exercise, but may be associated with a loss of manual dexterity in exercise involving skilled performance.

Other muscle groups could be easily tested using a similar protocol to that used in the studies of the present thesis (i.e. 6s contractions, 4s rest), thus enabling direct comparison with the present results. Muscle groups that may be of interest to test include the triceps surae, elbow flexors and first dorsal interosseous. The triceps surae is a more fatigue resistant muscle group, with a larger proportion of type I muscle fibres than the knee extensors (Bigland-Ritchie et al., 1986a; Harridge et al., 1996). This enhanced fatigue resistance could be reflected by a slower rate of change in complexity or by higher values of complexity when fresh. The elbow flexors have frequently been studied in terms of changes in the magnitude of variability with fatigue (Hunter and Enoka, 2001; Hunter et al., 2004) and a recent study has examined the complexity of fresh contractions (Svendsen and Madeleine, 2010). Any change in the complexity of torque in the elbow flexors could have important implications for skilled performance and co-ordination in, for example, racket sports. The first dorsal interosseous has long been studied in terms of complexity, with Slifkin and Newell's initial studies on complexity and force production having been conducted on it (Slifkin and Newell, 1999; Slifkin and Newell, 2000), along with several studies on the age-induced loss of complexity (Vaillancourt and Newell, 2003; Vaillancourt et al., 2003). Research on fatiguing contractions of the first dorsal interosseous would provide excellent insight into the effects of fatigue on complexity in fine motor tasks.

Whole body dynamic exercise

If complexity measures are to be used for the real-time assessment of fatigue in free running conditions, the results of the present thesis need to be extended beyond isometric exercise to a range of dynamic, whole body tasks. Several studies have already investigated changes in complexity arising from dynamic tasks, though have focused on the complexity of whole body motion rather than muscular output. In contrast to the present results, these studies have observed an increase in complexity with neuromuscular fatigue (Cignetti et al., 2009; McGregor et al., 2011; Cortes et al., 2014). To date, no

study has investigated the effect of dynamic exercise on the complexity of muscle torque output. This could be easily accomplished through an exhaustive running or cycling task, and taking pre-and post-test measures of isometric torque complexity. Alternatively, and perhaps more importantly for the potential application to real time assessment of fatigue, the complexity of the force applied to the pedals during cycling could be assessed. A recent study by Coelho et al. (2015), though providing no direct measure of the magnitude of variability, clearly indicated that there was an increase in the variability of the power applied to the pedals during an exhaustive ramp cycle ergometry test, which could be indicative of a change in complexity.

9.5 – Conclusions

Physiological systems, even under resting conditions, exhibit constant complex fluctuations in their outputs (Goldberger et al., 1990; Lipsitz, 2002). This complexity has come to be regarded as a hallmark of healthy systems, endowing them with the ability to adapt to internal and external perturbations (Pincus and Goldberger, 1994; Peng et al., 2009). A loss of this complexity, frequently evident in ageing, is associated with a loss of system control and increased dysfunction (Lipsitz, 2004; Peng et al., 2009). The present thesis aimed to investigate changes in the complexity of isometric muscle torque output with neuromuscular fatigue, and has provided substantial evidence that the complexity of torque output is significantly perturbed by fatigue.

The experimentation within this thesis has demonstrated that the complexity of muscle torque output is progressively reduced, and that torque fluctuations become increasingly Brownian (DFA $\alpha = 1.50$), as neuromuscular fatigue develops. This reduction in complexity was found to be intensity dependent, only occurring during contractions performed above the CT. It was also found to be consistent, with values at task failure being common despite different task demands. Muscle torque complexity was also demonstrated to be sensitive to pre-existing fatigue, pharmacological intervention (i.e. caffeine ingestion) and muscle damage (i.e. eccentric exercise). These findings suggest that muscle torque complexity is significantly affected by the central and peripheral perturbations contributing to neuromuscular fatigue and provides a sensitive index of muscle functionality in the face of such perturbations.

In summary, this thesis has provided the first experimental evidence that the complexity of isometric muscle torque output decreases with neuromuscular fatigue. This extends the "loss of complexity" hypothesis in ageing (Lipsitz and Goldberger, 1992) to the neuromuscular fatigue process in otherwise healthy adults. Such a loss of complexity is indicative of a loss of system adaptability and functionality, and could have important implications for exercise tolerance, the mechanisms of task failure and future real time assessment of the fatigue process.

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Appendix

Ethical approval codes

Study 1 (Chapter 4): Prop_52_2013_2014

Study 2 (Chapter 5): Prop_152_2013_2014

Study 3 (Chapter 6): Prop_54_2014_2015

Study 4 (Chapter 7): Prop_117_2014_2015

Study 5 (Chapter 8): Prop_02_2015_2016

HEALTH QUESTIONNAIRE

Name……

Date of Birth…….. Age……

Please answer these questions truthfully and completely. The sole purpose of this questionnaire is to ensure that you are in a fit and healthy state to complete the exercise test.

ANY INFORMATION CONTAINED HEREIN WILL BE TREATED AS CONFIDENTIAL.

Section 1 – General Health

Please read the questions below and answer each one honestly with YES or NO.

		YES	NO
1.	Has your doctor ever said that you have a heart condition OR high blood pressure?		
2.	Do you feel pain in your chest at rest, during your daily activities of living, OR when you do physical activity?		
3.	Do you lose balance because of dizziness OR have you lost consciousness in the last 12 months?		
4.	Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)?		
5.	Are you currently taking prescribed medications for a chronic medical condition?		
6.	Do you have a bone or joint problem that could be made worse by becoming more physically active?		
7.	Has your doctor ever said that you should only do medically supervised physical activity?		
8.	Have you suffered from any lower limb injury in the past 3 months?		
9.	Do you smoke?		
10.	Are you currently pregnant?		

If you answered NO to all of the questions above, you are cleared to take part in the exercise test and can go to SECTION 3 to sign the form.

If you answered YES to one or more of the questions in Section 1 – PLEASE GO TO SECTION 2.

Section 2 – Chronic Medical Conditions

SECTION 2 - CHRONIC MEDICAL CONDITIONS

		Please read the questions below carefully and answer each one honestly: check YES or NO.	YES	NO
1.		Do you have Arthritis, Osteoporosis, or Back Problems?	☐ If yes, answer questions 1a-1c	☐ If no, go to question 2
	1a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	1b.	Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebra (e.g., spondylolisthesis), and/ or spondylolysis/pars defect (a crack in the bony ring on the back of the spinal column)?	☐	☐
	1c.	Have you had steroid injections or taken steroid tablets regularly for more than 3 months?	☐	☐
2.		Do you have Cancer of any kind?	☐ If yes, answer questions 2a-2b	☐ If no, go to question 3
	2a.	Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head, and neck?	☐	☐
	2b.	Are you currently receiving cancer therapy (such as chemotherapy or radiotherapy)?	☐	☐
3.		Do you have Heart Disease or Cardiovascular Disease? This includes Coronary Artery Disease, High Blood Pressure, Heart Failure, Diagnosed Abnormality of Heart Rhythm	☐ If yes, answer questions 3a-3e	☐ If no, go to question 4
	3a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	3b.	Do you have an irregular heart beat that requires medical management? (e.g. atrial fibrillation, premature ventricular contraction)	☐	☐
	3c.	Do you have chronic heart failure?	☐	☐
	3d.	Do you have a resting blood pressure equal to or greater than 160/90 mmHg with or without medication? (Answer YES if you do not know your resting blood pressure)	☐	☐
	3e.	Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?	☐	☐
4.		Do you have any Metabolic Conditions? This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes	☐ If yes, answer questions 4a-4c	☐ If no, go to question 5
	4a.	Is your blood sugar often above 13.0 mmol/L? (Answer YES if you are not sure)	☐	☐
	4b.	Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, and the sensation in your toes and feet?	☐	☐
	4c.	Do you have other metabolic conditions (such as thyroid disorders, pregnancy-related diabetes, chronic kidney disease, liver problems)?	☐	☐
5.		Do you have any Mental Health Problems or Learning Difficulties? This includes Alzheimer's, Dementia, Depression, Anxiety Disorder, Eating Disorder, Psychotic Disorder, Intellectual Disability, Down Syndrome)	☐ If yes, answer questions 5a-5b	☐ If no, go to question 6
	5a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	5b.	Do you also have back problems affecting nerves or muscles?	☐	☐

Please read the questions below carefully and answer each one honestly: check YES or NO.			YES	NO
6.	Do you have a Respiratory Disease? This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary High Blood Pressure		☐ If yes, answer questions 6a-6d	☐ If no, go to question 7
	6a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	6b.	Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy?	☐	☐
	6c.	If asthmatic, do you currently have symptoms of chest tightness, wheezing, laboured breathing, consistent cough (more than 2 days/week), or have you used your rescue medication more than twice in the last week?	☐	☐
	6d.	Has your doctor ever said you have high blood pressure in the blood vessels of your lungs?	☐	☐
7.	Do you have a Spinal Cord Injury? This includes Tetraplegia and Paraplegia		☐ If yes, answer questions 7a-7c	☐ If no, go to question 8
	7a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	7b.	Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting?	☐	☐
	7c.	Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as Autonomic Dysreflexia)?	☐	☐
8.	Have you had a Stroke? This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event		☐ If yes, answer questions 8a-c	☐ If no, go to question 9
	8a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	8b.	Do you have any impairment in walking or mobility?	☐	☐
	8c.	Have you experienced a stroke or impairment in nerves or muscles in the past 6 months?	☐	☐
9.	Do you have any other medical condition not listed above or do you live with two chronic conditions?		☐ If yes, answer questions 9a-c	☐ If no, read the advice on page 4
	9a.	Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months OR have you had a diagnosed concussion within the last 12 months?	☐	☐
	9b.	Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, kidney problems)?	☐	☐
	9c.	Do you currently live with two chronic conditions?	☐	☐

Is there any other reason why you cannot take part in this exercise test?

Yes ☐ **No ☐**

If yes please provide details below:

…….

If you answered NO to all of the follow-up questions about your medical condition, you are cleared to take part in the exercise test.

If you answered YES to one or more of the follow-up questions about your medical condition it is strongly advised that you should seek further advice from a medical professional before taking part in the exercise test.

Section 3 - Declaration

Please read and sign the declaration below:

I, the undersigned, have read, understood and completed this questionnaire to the best of my knowledge.

NAME: ……

SIGNATURE: ……DATE: …….....

SIGNATURE OF PARENT/GUARDIAN: ……

This health questionnaire is based around the PAR-Q+, which was developed by the Canadian Society for Exercise Physiology www.csep.ca

Muscle soreness

Please draw a line through the scale to indicate how sore your quadriceps are. 0 represents not sore at all and 10 represents the most intense soreness you can imagine.