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The effect of maximal isometric training on doublet-induced force enhancement and its relationship with changes in voluntary rate of force development

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ABSTRACT

Motor unit double discharges (i.e. doublets), which are excitatory potentials that occur at shorter-than-normal intervals (e.g. 5-10 ms) during normal muscle activation, are known to cause muscle force to exceed that predicted from a standard, linear summation of twitch forces. However, although a marked increase in the occurrence of motor unit doublets at the onset of a contraction has been observed after explosive-type exercise training, and has been correlated with changes in RFD (Van Cutsem et al., 1998), little is known about the influence of strength training on the physiological and biomechanical benefits derived from the phenomenon. The present research examined the effects of 4 weeks of 'explosive' isometric knee extensor strength training on voluntary and electrically-evoked contractile RFD (calculated as the time derivative of the moment-time curve) in 8 untrained male participants. Electrical stimulation (NMES) trains were delivered to the muscle at 20 Hz and 40 Hz and incorporated short (5 and 10 ms) inter-pulse intervals (IPIs) at the onset of stimulation (i.e. variable-frequency trains; VFT). The influence of the short inter-pulse interval was assessed by comparison to a constant frequency train (i.e. the VFT:CFT ratio). Following the training, substantial improvements in maximum isometric knee extensor strength (MVC) (24.3 ± 13.3%, p = 0.002) and RFD measured to time intervals of 50 (55.5 ± 50.3%, p = 0.011), 100 (34.0 ± 47.2%, p = 0.01) and 150 ms (31.9 ± 38.2%, p = 0.02) were observed. RFD normalised to MVC (RFD_{norm}), measured to time intervals of 50 and 100 ms from the onset of contraction, improved by 44.9 ± 38.8% (p = 0.04) and 13.8 ± 12.2% (p = 0.01), respectively. There was a significant reduction in the VFT:CFT ratio after training when a 10-ms IPI preceded a 20-Hz train when measured to 30 (-13.7 ± 11.3%, p = 0.03), 50 (-13.9 ± 8.4%, p = 0.007), 100 (-8.6 ± 10.2%, p = 0.04), and 200 ms (-8.1 ± 5.3%, p = 0.009) as well as in the interval 100-200 ms (-7.4 ± 6.6%, p = 0.02). However, no significant changes were observed for other stimulation frequency-IPI combinations. Moderate-to-very strong positive correlations were observed between changes in RFD_{norm} and changes in VFT:CFT when measured within some time periods, particularly in the early phase of the contraction (r = 0.02 – 0.91). In conclusion, the effect of a high-frequency double discharge at stimulation onset remained unchanged or, under some conditions, was reduced after 4 weeks of explosive-type knee extensor training. Additionally, training-dependent improvements in the ability to rapidly reach a specified torque level relative to peak MVC torque (i.e. RFD_{norm}) were greater for those participants whose VFT:CFT ratio either did not decline or declined the least. These data provide evidence that explosive training may reduce the effect of a high-

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frequency discharge at the onset of a contraction, and that greater increases in RFD may occur in those who most retain this ability.

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CHAPTER 1

Introduction and Aims

The organisation and production of movement is achieved through the cooperation of the nervous and musculoskeletal systems, whereby movement is achieved with the production of muscular force. In human movements, many highly demanding activities are characterised by a limited time to develop force (e.g. less than 200 ms) and are thus too brief for maximum force to be developed (~300 – 600 ms; Suetta et al., 2004; Aagaard, Simonsen, Andersen, Magnusson, & Dyhre-Poulsen, 2002). Therefore, although the maximum voluntary force (MVC) capacity of muscle is an important functional parameter (Buchner & de Lateur, 1993), the capacity to produce force rapidly (i.e. explosive strength) is often considered a more critical factor. Improving the ability to generate explosive force may therefore be considered advantageous for the enhancement of many functional movements.

Explosive strength, typically measured as the rate of force development (RFD), has been shown to increase following certain types of physical training, particularly when contractile force is developed as quickly as possible (see Chapter 1.2). However, whilst numerous physiological and anatomical factors have been shown to influence RFD (see Maffiuletti et al., 2016 for review), there remain a number of gaps in our understanding of the precise physiological determinants of RFD as well as the adaptive mechanisms underpinning training-dependent improvements in RFD. Furthermore, despite the considerable research effort investigating the function and adaptability of the nervous and musculoskeletal systems, there remain critical deficits in our understanding of motor unit physiology, specifically with regards to explaining changes in whole muscle function on the basis of motor unit properties and behaviours (Enoka & Fuglevand, 2000).

One such behaviour, the motor unit double discharge (i.e. a doublet), and the corresponding contractile force enhancement (i.e. doublet-induced force enhancement) has been the topic of numerous studies over the last 75 years. Initially described as a "catchlike effect", the inclusion of two (or more) faster-than-usual pulses at the onset of an otherwise low-frequency stimulation train substantially increases the magnitude and the rate of contraction of the force response (Burke, Rudomin, & Zajac, 1970). Doublet-induced force enhancement was first demonstrated in single motor units (Burke, Rudomin, & Zajac, 1976) and has since been shown in human muscle when contracting both dynamically and isometrically (Binder-Macleod & Kesar, 2005) and the behaviour is now considered to be an implicit property of the motor neurone itself (Garland & Griffin, 1999). Understanding this phenomenon is an attractive goal for many disciplines (including physiotherapy & rehabilitation, and exercise performance), and could potentially provide insight into muscle function in a people with neuromuscular afflictions such as Parkinson's disease, spinal cord injury, Kennedy's disease, and others (Kudina & Andreeva, 2013), which may have functional consequences in human movement performance (Garland & Griffin, 1999).

Much of the existing research investigating the "catchlike effect" in humans has utilised neuromuscular electrical stimulation (NMES), delivered either directly to the nerve or indirectly to the axons overlaying the muscle, to induce muscular contractions. Typically, the response is induced by inserting an additional discharge at the onset of an otherwise constant frequency stimulation train (i.e. two pulses with a brief interpulse interval followed by a normal train of pulses), which produces a dramatic increase in the rate, and sometimes magnitude, of contractile force. However, to date there has been limited investigation of the influence of doublet-induced force enhancement with regards to training-dependent improvements in contractile force production. The seminal paper by Van Cutsem, Duchateau and Hainaut (1998) reported both an increased number of motor units initiating with pairs of

faster-than-usual discharges and a higher occurrence of discharge pairs following 8 weeks of explosive-type training. The authors suggested that the increased occurrence of high-frequency discharges (including doublets) following explosive training could have contributed to the training-dependent improvements in voluntary RFD. While the functional ramifications have not been conclusively elucidated, there is at least some evidence of a relationship between double-discharge occurrences (especially at contraction onset) and RFD in both electrically-induced and voluntary contractions (Van Cutsem & Duchateau, 2005; Van Cutsem et al., 1998). However, it is not yet known whether the influence of a doublet at the onset of a contraction is altered by training, and there is a clear opportunity to determine whether a relationship exists between the magnitude doublet-induced force enhancement and the changes in force-producing capabilities after training. Furthermore, determining the nature of this relationship might help to explain the improvement in force production in response to explosive-type training. In the following review of the literature, the neuromuscular changes associated with improved strength and RFD following explosive strength training, and the mechanisms that are believed to underpin the doublet-induced force enhancement, will be considered with a view to developing testable hypotheses for examining the relationship between training-dependent improvements in voluntary RFD and training-dependent changes (if any) in the response to doublet-initiated electrical stimulation trains.

1.1 Rate of force development

Explosive muscular strength is a critical physiological parameter for successful performance in many sporting disciplines, as well as for the active stabilisation of joints. A standardised method of assessing explosive muscle strength is to measure the contractile rate of force development (RFD), which is typically derived from the force- (or torque-) time curve recorded during explosive isometric and dynamic voluntary contractions (Aagaard et

al., 2002; Kawamori & Newton, 2006; Maffiuletti et al., 2016). As RFD determines the force that can be generated in the early phase of muscle contractions, RFD capabilities have important functional consequences. In the elderly, falls and fall-related injuries can significantly impact life quality, and lower limb muscle strength and RFD are important limiting factors in balance recovery and indicators of injury risk (Pijnappels, Reeves, Maganaris, & Diien, 2008). In sport and other highly demanding physical activities, many movements are characterised by a limited time to develop force (0-200 ms), which is substantially less than the time required to develop maximal force (~300-600 ms; Suetta et al., 2004; Aagaard et al., 2002). Subsequently, the capability to produce contractile RFD is crucial to the success of the movement as higher levels of muscle force can be reached earlier in the contraction. Furthermore, RFD-related variables that describe explosive strength are seemingly more effective at detecting chronic and acute changes in neuromuscular function than maximal strength tests (Penailillo, Blazevich, Numazawa, & Nosaka, 2014) and might therefore be more appropriate in the assessment of sport-specific activities and functional daily tasks, as well as the assessment of the effectiveness of resistance (strength) training.

1.1.1 Determinants of RFD

Although there are a number of neural and muscular variables that determine RFD, rapid muscle activation rates at the onset of contraction seem to be the main factor influencing the ability to attain high levels of RFD (Duchateau & Baudry, 2014; Grimby, Hannerz, & Hedman, 1981; Harridge, 1996), particularly early (e.g. < 75 ms) in the time course of the contraction (Maffiuletti et al., 2016). Rapid contractions are characterised by highly-synchronised bursts of neuronal activity at action onset, with discharge rates being as high as 60-200 Hz (Desmedt & Godaux, 1977; Van Cutsem et al., 1998), compared to more typical discharge rates between 30-60 Hz observed during sustained high-force isometric contractions (Duchateau & Enoka, 2011). Notably, there is some evidence that high

instantaneous discharge rates are associated with transient increases in intracellular Ca^{2+} (Abbate, Bruton, De Haan, & Westerblad, 2002; Bakker, Cully, Wingate, Barclay, & Launikonis, 2017; Cheng, Place, Bruton, Holmberg, & Westerblad, 2013), increasing the rate of occupation of troponin C's second Ca^{2+}-binding site (Bakker et al., 2017) and ultimately increasing the rate of force development (at least in fast-twitch muscle fibres; Bakker et al., 2017). Regardless of the precise mechanism, there appears to be strong intra-individual variability, and there is substantial evidence that muscle activation (often as assessed by electromyography) is a major factor in determining voluntary RFD (Blazevich, Horne, Cannavan, Coleman, & Aagaard, 2008; de Ruiter, Kooistra, Paalman, & de Haan, 2004; Del Balso & Carafelli, 2007).

Muscular determinants also clearly influence the production of rapid force, as inter-individual differences in RFD cannot be completely explained by differences in muscle activation rates (Andersen & Aagaard, 2006). Muscular factors such as MVC force, fibre type composition and musculotendinous stiffness may also influence RFD, particularly later in the contraction (Andersen & Aagaard, 2010; Maffiuletti et al., 2016). In fact, evidence suggests that the main determinants of voluntary RFD may be different during contractions that last longer than 75 ms, (Folland, Buckthorpe, & Hannah, 2014) and that the intrinsic speed-related properties of muscle play an increasingly important role in the production of RFD over the course of the contraction (Andersen & Aagaard, 2010; Folland et al., 2014). There is a significant correlation between RFD and maximal strength (Mirkov, Nedeljkovic, Milanovic, & Jaric, 2004), especially in the later phase of contraction (Andersen & Aagaard, 2006), however, as the main determinants of MVC force are neural drive (i.e. motor unit activation) and the cross-sectional area of the muscle, it is likely that factors impacting MVC force might also impact RFD. Fibre type composition is also impactful, and RFD is faster in type II fires (Harridge, 1996) because of the greater Ca^{2+} release for each action potential as

well as the faster cross-bridge cycling rates compared to slower type I fibres (Baylor & Hollingworth, 1998; Bottinelli, Canepari, Pellegrino, & Reggiani, 1996; Schiaffino & Reggiani, 1996). For example, a moderate, although non-significant, correlation ($r^2 = 0.34$) between vastus lateralis type II percentage and maximal voluntary knee extensor RFD and significant correlation ($r^2 = 0.49$) between vastus lateralis type II fibre area and early phase knee extensor RFD (0-50 ms; Hvid et al., 2010) have been reported (Taylor, Humphries, Smith, & Bronks, 1997). Musculotendinous stiffness is also thought to impact RFD, as the speed of force transmission through a material is dependent upon the material's stiffness. In frog muscle fibres, it was reported that as much as 40% of the variance in early-phase RFD was related to the taking up of slack in the series elastic elements before tension is transmitted (Edman & Josephson, 2007). Tendon stiffness has also been specifically associated with the lag time between muscle activation and force production (i.e., electromechanical delay) (Grosset, Piscione, Lambertz, & Perot, 2009), so changes in tissue stiffness throughout the muscle-tendon unit might hypothetically influence RFD.

1.1.2 Changes in RFD with training

It is well established that strength training elicits marked adaptations both in the muscles and the nervous system (Aagaard et al., 2002; Duchasteau & Enoka, 2002; Enoka, 1996). As little as four weeks of maximal isometric training has been shown to produce substantial improvements in peak MVC force capacity and RFD, and in fact shorter periods of training may elicit the greatest changes in RFD (e.g. see Durbaba, Cassidy, Macaluso, 2013; Ogasawara et al., 2013; Oliveira, Oliveira, Rizatto, & Denadai, 2013; Tillin & Folland, 2014; Tillin, Pain, & Folland, 2011). Notably, improvements in MVC-normalised RFD have been observed after the completion of resistance training programs that involve the rapid production of force (Behm & Sale, 1993). Adaptations in the nervous system appear to be particularly important for the increases in RFD and maximal strength observed with short-

term strength training (Blazevich, Gill, Deans, & Zhou, 2007), largely as a result of the importance of muscle activation (i.e. rate coding) to RFD (Blazevich et al., 2008; de Ruiter et al., 2004). Training-dependent improvements in early-phase RFD are typically explained by changes in discharge rates of the motor neurones (i.e. improved neural drive), consequently increasing the rate of muscle activation. Training-dependent increases in motor neuronal output are likely achieved by increased motor neurone excitability and decreased presynaptic inhibition, down regulation of inhibitory neural pathways, and increased levels of central descending motor drive (Aagaard, 2002), although contributions from other factors such as an increased ability to sustain higher discharge rates during the first several spikes have also been suggested (Van Cutsem et al., 1998).

Although neural adaptations appear to be the primary contributor to training-dependent improvements in RFD, peripheral adaptations have also been shown to influence RFD. Training-dependent increases in electrically-evoked RFD (of 18-31%) following 3 months of explosive-type isometric training (Duchateau & Hainaut, 1984) suggest that changes in RFD capabilities can occur independently of neural adaptations. Changes in muscle size (cross-sectional area and volume) and maximum contractile force production (i.e. MVC) are associated with RFD capabilities (Moss, Refsnes, Abildgaard, Nicolaysen, & Jensen, 1997), although anatomical changes are usually only observed following longer duration heavy-resistance strength training (Aagaard et al., 2001). Selective hypertrophy, specifically in type II fibres, has also been linked to increases in early-phase RFD (Häkkinen, Komi, & Alen, 1985), which is notable given the relationship between the fibre type composition of muscle and Ca^{2+} dynamics (Baylor & Hollingworth, 1998; Close, 1972). Preferential hypertrophy of type II fibres and changes in the fibre type ratio following heavy resistance-training have been positively associated with improvements in RFD independent of changes in MVC (i.e. normalised RFD) (Häkkinen et al., 1985). Lastly, although there is a

lack of evidence investigating the training-induced changes in musculotendinous stiffness, it is reasonable to suggest that adaptations in tendon and/or aponeurosis stiffness might influence RFD capabilities. Several studies have reported substantial increases in tendon stiffness (15-25%) in both patellar and Achilles tendons following prolonged strength-training (Reeves, Maganaris, & Narici, 2003; Waugh, Korff, Fath, & Blazevich, 2014; Seynnes et al., 2009).

In summary, the ability to rapidly produce force is determined by numerous variables, which are dependent on the temporal phase of the contraction and governed by both neural and muscular systems (Aagaard, 2003; Grimby et al., 1981; Harridge, 1996; Maffiuletti et al., 2016). The ability to activate muscle rapidly can be considered the primary determinant of RFD in the early phase of a contraction and, consequently, training induced-changes in neural drive are strongly associated with the improved RFD capability observed after explosive-type resistance training. Muscle-fibre type composition (and the associated Ca^{2+} dynamics) and musculotendinous stiffness may also contribute to changes in rapid force production, and training-induced changes in these factors appear to be associated with improvements in MVC-normalised RFD.

1.2 The effect of doublet discharges on the contractile response

The non-linear force summation that occurs when two (or more) action potentials arrive at the muscle with an unusually brief interpulse interval(s) (IPI), either at the initiation of movement or during a sustained contraction, was first described as a “catchlike property” by Burke, Rudomin, and Zajac (1970), who named it for its similarity to the catch property observed in mollusk muscle fibres (Wilson & Larimer, 1968). Augmented force resulting from unusually high-frequency discharges has been observed in single motor units (Burke, Rudomin, & Zajac, 1976) and in human muscles contracting both dynamically and

isometrically (see Binder-Macleod & Kesar, 2005 for review), and the behaviour is now considered to be an implicit property of the motor neurone itself (Garland & Griffin, 1999). A large and growing body of research has investigated the functional role of doublets using neuromuscular electrical stimulation techniques (NMES; e.g. Binder-Macleod & Barker, 1991; Binder-Macleod & Lee, 1996; Binder-Macleod, Lee, & Baadte, 1997; Karu, Durfee, & Barzilai, 1995; Kesar, Santamore, Perumal, & Binder-Macleod, 2009) by imposing multiple discharge stimuli with brief IPIs (most commonly doublets or triplets) at the onset of an otherwise lower-frequency stimulation train. This methodology has been shown to dramatically influence the rate of force development, and sometimes the sustained peak torque in isometric (Binder-Macleod & Barker, 1991; Burke et al., 1970) and nonisometric (Binder-Macleod & Lee, 1996; Lee, Gerdom, & Binder-Macleod, 1999) muscle contractions.

Although the terms ‘doublet’ and ‘double discharge’ are used inconsistently when describing pairs of motor unit discharges that occur in very short time intervals (e.g. < 10 ms), the term “doublet” was initially used only to describe the motor neurone discharging two pulses with a uniquely short IPI *in vivo* when under conditions of weak synaptic input (Denney-Brown, 1929; Eccles & Hoff, 1932). Doublets arising under these conditions have been referred to as “true” doublets (Bawa & Calancie, 1983) and current evidence suggests that they are a different phenomenon to the double discharges observed at the onset of ballistic contractions (Van Cutsem et al., 1998). True doublets, which were at one time considered statistical anomalies (Bawa & Calancie, 1983), are thought to arise from a delayed depolarisation of the dendrites in the motor neurones (Calvin & Schwindt, 1972; Kudina & Andreeva, 2010). The initial activation of the axon hillock results in subthreshold antidromic signals travelling back towards the dendrites (i.e. delayed depolarisation), which increases the resting membrane potential of the dendrites and makes them more susceptible to synaptic input (i.e. a spike-evoked spike), increasing the likelihood of a second (or even third) action

potential (Calvin & Schwindt 1972; Granit, Kernell, & Shortess, 1963; Kudina & Alexeeva 1992; Kudina & Churikova 1990; Christie & Kamen, 2006). Conversely, the high-frequency double discharges observed during the large excitatory drive achieved during voluntary ballistic contractions could correspond with the motor neurone firing in the secondary range, where the linear curve of firing rate to injected current undergoes a sudden increase in slope (Granit et al., 1963) suggesting they are post-synaptic phenomena. Furthermore, the second discharge is lower in amplitude than the first, indicating that the discharge induces the sarcoplasmic reticulum to release Ca^{2+} while not fully recovered from the first spike (Fatt & Katz, 1951).

Double discharges often occur during initial motor unit recruitment, however if force is increased (via an increased motor unit firing rate) then the motor neurone transitions to a more typical single-discharge firing pattern. For individual motor units that were observed to discharge "true" doublets, the number of doublet discharge events occurring before single discharges are observed decreases as the rate of ramped force increases (Bawa & Calancie, 1983), indicating a fundamentally different behaviour to the double discharges observed during high-intensity ballistic contractions (Desmedt, 1983). It is suggested that this phenomenon of brief IPIs is an example of the regenerative firing mechanisms of the motor neurone as opposed to a large synaptic input in a ballistic movement. Moreover, true doublets have not been found in all motor units, and most doublets are followed by a prolonged interval before another spike (Bawa & Calancie, 1983). Conversely, high-frequency double-discharges at contraction onset have been shown to occur in most motor units studied in rats when subjected to an intracellular injection of rectangular depolarisation current of 1.25 to 4 times the rheobase (Mrówczyński, Krutki, Chakarov, & Celichowski, 2010). Improvement in the ability to recruit motor units with repetitive doublets has been shown, although the IPI durations of true doublets have not been shown to change and the doublet IPI appears to be

completely independent of the voluntary effort and intention of the participant (Bawa & Calancie, 1983; Kudina & Andreeva, 2010). Furthermore, there is evidence that the already brief initial IPI at the onset of ballistic isometric contraction can be further reduced with training using rapid maximal muscular efforts (Van Cutsem, Duchateau, & Hainaut, 1998), which has not been observed for true doublets that are observed during conditions of low excitatory input. Lastly, successive high-frequency discharges arising at the onset of a ballistic contraction have been observed in human muscle without the prolonged post-doublet-associated IPI observed with the "true-doublets" observed under conditions of low excitation (Van Cutsem et al., 1998).

To summarise, instances of discharge groups with unusually brief IPIs observed during conditions of high initial excitatory drive and those during weak synaptic input are unlikely to arise from a common mechanism. However, there is a demonstrably similar contractile consequence, and it is clear that the utilisation of the contractile force enhancement evoked by two closely spaced stimuli (≤ 10 ms) has functionally relevant consequences for the resulting contraction.

1.2.1 Mechanisms influencing doublet-induced force enhancement

The two primary mechanisms that have been proposed to explain doublet-induced enhancement of contractile force are 1) improved Ca^{2+} dynamics (owing to initially increased and sustained levels of myoplasmic Ca^{2+} and enhanced cross-bridge dynamics following the binding of cross bridges) (Bakker et al., 2017; Cheng et al., 2013; Gordon, Homsher, & Regnier, 2000), and 2) a more effective transmission of active tension through the muscle consequent to a more rapid shortening of contractile elements and an increased stiffness (length) in the series elastic elements subsequent to the initial activation (Binder-Macleod & Barrish, 1992; Duchateau & Hainaut, 1986; Mayfield, Cresswell, & Lichtwark, 2016;

Parmiggiani & Stein, 1981). Other factors shown to influence the magnitude of the force enhancement include the parameters describing the stimulation (i.e. the pattern or frequency of the stimulation train and the intensity of the stimulation), muscle fatigue, the degree of potentiation in the muscle, fibre type composition, contraction type, and muscle length (Binder-Macleod & Kesar, 2005). However, the phenomenon cannot be explained by preferential activation of fast muscle, changes at the neuromuscular junction (Slade, Bickel, Warren, & Dudley, 2003), increased myosin light chain phosphorylation (Cheng et al, 2013; Ryder, Lau, Kamm, & Stull, 2007), or an increased axonal recruitment (Karu et al., 1995; Lee et al., 1999). Although there has been substantial exploration of these (and other) mechanisms, it is still not clear what their relative contributions are. Studies attempting to differentiate the relative contributions have reported limited success (Ratkevicius & Quistorff, 2002), although Abbate and colleagues (2002) inferred that only 20% of the force augmentation could be accounted for by passive stiffness mechanisms.

It is well established that Ca^{2+} plays a vital role in the activation of the contractile apparatus, which is achieved through the binding of Ca^{2+} ions to troponin allowing the movement of tropomyosin on the actin filament, strong acto-myosin interaction, and the subsequent development of tension in the muscle. Various studies have provided evidence that a high-frequency burst at the onset of stimulation intensifies the Ca^{2+} release and elevates Ca^{2+} transients, enabling force augmentation (Bakker et al., 2017; Endo, 1977; Duchateau & Hainaut, 1986). This hypothesis is consistent with observations that when the excitation-contraction coupling process is hindered the force enhancement is more pronounced, such as during low-frequency fatigue (Binder-Macleod & Barker, 1991) and when the muscle length is shorter (Abbate, de Ruiter, & de Haan, 2001; Lee et al., 1999). Specifically, the arrival of doublet action potentials at the motor end plate and the subsequent signalling throughout the cell membrane triggers a short-lasting amplification of Ca^{2+} within the myoplasm (Barclay,

2012; Bakker et al., 2017; Cheng et al., 2013; Duchateau & Hainaut, 1986). This subsequently increases myofibrillar sensitivity, probably by increasing the binding rate of Ca^{2+} to the second calcium binding site on troponin C, and thus increases the rate of initiation of cross-bridge binding and force production (Bakker et al., 2017). As a result, the cross-bridge interaction is facilitated, creating an unusually steep rise in contractile force per unit time (Binder-Macleod & Kesar 2005; Cheng et al. 2013) and prolonged force enhancement without sustained elevation of Ca^{2+} concentration (Abbate et al., 2002; Binder-Macleod & Barker, 1991; Bigland-Ritchie, Zijdewind, & Thomas, 2000; Binder-Macleod & Scott, 2001; Ratkevicius & Quistorff, 2002; Bentley & Lehman, 2005).

Series elastic element compliance and the muscle-tendon unit stretch history preceding contractile activity have also been shown to impact the response to doublet stimulations (Lee et al., 1999; Mayfield et al., 2016; Parmiggiani & Stein, 1981). Binder-Macleod and Lee (1996) found stimulation trains with a brief initial IPI (5 ms) were more effective in enhancing force during concentric compared to eccentric contractions and, likewise, isometric contractions were augmented more than concentric. In eccentric contractions, there is little slack in the series elastic elements and the doublet is comparatively less effective (Binder-Macleod & Lee, 1996; Stein & Parmiggiani, 1979). Thus, the rate of tension development by the contractile element stretches the series elastic elements (i.e. removal of slack from within the tendon and aponeurosis) allowing more effective force transmission subsequent to the initial activation (Abbate et al., 2002; Parmiggiani & Stein, 1981).

There has been some suggestion that the primary benefit of the catchlike property is to help to maintain muscular force output under conditions of peripheral fatigue (Bentley & Lehman, 2005; Binder-Macleod & Lee, 1999; Slade et al., 2003). In fatigued muscle, the

augmentation of contractile force caused by a brief initial IPI is particularly exaggerated (Binder-Macleod, Lee, Fritz, & Kucharski, 1998; Van Cutsem et al., 1998; Lee & Binder-Macleod, 2000) and there is evidence that double discharge occurrences are strongly correlated with the severity of fatigue (Griffin, Garland, & Ivanova, 1998). In conditions of low-frequency fatigue, a brief initial interpulse interval largely increases rates of force development but does not completely compensate for the loss in peak force (Bentley & Lehman, 2005). Furthermore, it has been shown that the compliance of the series elastic component of the muscle increases when the muscle is fatigued (Vigreux, Cnockaert, & Pertuzon, 1980), potentially augmenting the doublet-induced force enhancement.

1.3 Summary

The speed with which force can be produced, especially early in the contraction, is often a more important functional parameter than the peak force capacity of the musculoskeletal system. Skeletal muscle force production is controlled primarily through the ordered recruitment of motor units and the modulation of their firing rates, and RFD ultimately depends on the number of motor units that are activated and the rate at which these motor units discharge action potentials (i.e. firing rate). The arrival of two (or more) faster-than-usual action potentials at the motor end plate generates force exceeding the linear summation of the individual twitches, a phenomenon often referred to as the “catchlike property” of muscle. The enhanced force output is likely caused by the short-lived elevation of intracellular calcium (Ca^{2+}) and/or an increased Ca^{2+} sensitivity (Abbate et al., 2002; Barclay, 2012; Nielsen, 2008; Parmiggiani & Stein, 1981) and improved transmission of force by a stiffer muscle (Binder-Macleod & Barrish, 1992; Duchateau & Hainaut, 1986; Mayfield et al., 2016).

A marked increase in the occurrence of high frequency discharges at contraction onset have been observed following training of the tibialis anterior using rapid isometric contractions (Van Cutsem, Duchateau, & Hainaut, 1998), although it is not currently known if other forms of exercise training induce a similar response. However, to our knowledge, there has been no detailed examination of whether the benefit derived from an initial double discharge (i.e. the magnitude of the doublet-induced force enhancement) changes with training, or if a relationship between changes in doublet-induced force enhancement and the improvements in muscular performance observed following periods of physical training (such as strength training) might exist. Given that improvements in RFD observed after short periods (e.g. <5 weeks) of resistance training cannot be entirely accounted for by increases in muscle activation rate, other mechanisms must contribute to the improvements in early RFD (Maffluetti et al., 2016), indicating that physiological factors other than those currently explored (such as muscle activation, rate coding and excitation-contraction coupling) contribute to training-dependent force production improvements. Thus, the questions remain: do adaptive changes in the discharge output of motor neurons take increasing advantage of the intrinsic properties of skeletal muscle that are responsible for the doublet-induced force enhancement? Can we utilise high-frequency double discharges more effectively after a period of specific exercise training? How will training-dependent adaptations in the mechanisms that determine both voluntary force production and doublet-induced force enhancement impact the influence of high frequency doublet discharges at the onset of contraction?

1.4 Aim

The primary aims of this research are to 1) evaluate the torque response to short (5 ms IPI) and long (10 ms IPI) initial double discharges before and after explosive isometric resistance training, 2) evaluate the ability to rapidly produce voluntary force (i.e. RFD) before

and after explosive isometric resistance training, and 3) investigate the relationship between changes in the torque response to short and long double discharges (i.e. the augmentation of RFD) and training-dependent changes in voluntary force and RFD. To this end, changes in the variables describing voluntary RFD were measured and set against the change in the response to a brief initial IPI imposed at the onset of an otherwise constant-frequency electrical stimulation using NMES. This was used to determine if a relationship between doublet-induced force enhancement and voluntary RFD could be observed following training, and to explore how training-induced adaptations in motor neurone function might impact the influence of the doublet. Although intracellular handling of Ca^{2+} and muscle-tendon unit compliance properties are determinants of both voluntary RFD and doublet-induced force enhancement, it is unclear whether training-dependent adaptations in these mechanisms cause a brief initial IPI to be more or less impactful. Nevertheless, it was hypothesised that performing 4 weeks of explosive-type isometric knee extensor training would increase the benefit derived from a brief initial IPI preceding an electrical stimulation, and that it would be positively associated with training-dependent improvements in voluntary rate of force production.

CHAPTER 2

METHODS

2.1 Participants

Eleven healthy male adults who had not engaged in heavy resistance training within 12 months of testing and who had no history of neuromuscular disease began the intervention arm of the study. Eight participants (mean ± SD: age, 20.3 ± 1.6 y; height 1.77 ± 0.08 m; body mass, 75.0 ± 10.8 kg) completed the study as three discontinued prior to completion due to reasons unrelated to the study. Additionally, twelve healthy male adults (age, 20.4 ± 1.6; height, 1.85 ± 0.11 m, body mass 78.2 ± 10.4 kg) completed a non-training control period over a two-week period to assess the between-day reliability of the measurements; control and intervention data were analysed separately. The participants refrained from lower-limb resistance training (other than the training performed during the experiment) but were otherwise allowed to continue their normal daily exercise routines. Sample size estimation, age, and sex were chosen to be similar to previous studies using isometric training to elicit improvements in force production, and muscular electrical stimulation procedures to test the effects of variable frequency trains (Binder-Macleod & Barker, 1991; Binder-Macleod, Lee, & Baadte, 1997; Duchateau, & Hainaut, 1986). Prior to testing, the volunteers were screened for conditions that might prevent safe exercise using the Physical Activity Readiness questionnaire (PAR-Q) (Appendix C), and read and signed an informed consent document (Appendix D). The participants were informed they were free to withdraw from the study at any time. The procedures performed during this research were approved by the Edith Cowan University Human Research Ethics Committee (Appendix E) and were conducted in agreement with the Declaration of Helsinki.

2.2 Experimental design

Participants were required to attend an initial familiarisation session(s) and both pre- and post-training test sessions, with the post-training session being performed 4-5 days after the completion of training and at the same time of day. The familiarisation session allowed the participants to become accustomed to the knee extension tests and electrical stimulation procedures. Participants performed multiple familiarisation sessions until maximum voluntary contractions were reproducible to within ± 5%, and the electrical stimulations could be received without voluntary muscle contraction. For the training, 12 unilateral, isometric knee extensor training sessions were completed across four consecutive weeks (3 sessions per week), with 1-2 non-training days separating each session. The testing and training were completed using the dominant leg, i.e. the leg preferred for kicking a ball (Miller, Downham, & Lexell, 1999). For the reliability (control) testing, the participants were tested on two separate days at the same time of day, with the first session being completed after full familiarisation and no training being imposed between measurement occasions 2 weeks apart. All participants refrained from any stimulant ingestion (including caffeine) in the 24 h prior to each testing session.

2.3 Isometric knee extensor training

Participants trained three times a week with at least 1 rest day between sessions. Each session began with a 5-min warm-up on a cycle ergometer at 60 rpm with 1-kp resistance. The participant was then seated upright on the isokinetic dynamometer with the knee and hip flexed at 70^{o} and 85^{o}, respectively. They thus performed a series of 3-s submaximal isometric contractions at increasingly greater force levels (40, 50... 90% of perceived maximum) with 30 s of between-contraction rest as warm-up. The training consisted of 5 sets of 6 maximal

contractions, held for 5 s with a 5-s rest between contractions and a 1-min rest between sets. Similar programmes have previously been shown to significantly improve maximum isometric strength and RFD (Geertsen, Lundbye-Jensen, & Nielsen, 2008; McDonagh, Hayward, & Davies, 1983; Tillin et al., 2012). The participants were instructed to produce a knee extensor force ‘as fast and then as hard as possible’ in order to generate the highest possible rate of force development (Maffiuletti et al., 2016).

2.4 Testing procedure

Each participant performed a 5-min warm-up on a Monark cycle ergometer at 60 rpm with a 1-kg resistance before being seated on a custom built rigid chair with the hip flexed at 85° (0° = full extension; see Figure 1). The leg was then fixed securely to the lever arm of the custom-built isometric dynamometer (XTran Load Cell S1W, Applied Measurement, Sydney, Australia) above the ankle with a Velcro strap with the knee held at 70° (0° = full knee extension); this angle is ideal for producing peak knee extension torque in most individuals (Aagaard et al., 2002; Bandy & Hanten, 1993; Noorkõiv et al., 2014). In pilot testing it was found that training at this angle minimised patellar tendon soreness and allowed for maximum voluntary exertion during training. Special care was taken to ensure that the lateral femoral condyle was aligned with the axis of rotation of the lever arm. The participant’s positioning was adjusted if there was an observed misalignment between the lateral condyle and the axis of rotation during the most maximal practice trial. The participants were strapped securely across the chest and waist and were able to grip handles situated on the seat to their sides. They then performed single 3-s isometric knee extension contractions at 50%, 70%, and 90% of perceived voluntary effort with 30-s rests to complete the warm-up. Load cell

calibration was performed prior to testing, and based on the amplification range of ± 10 V and a resolution of 313 µV, force increments of 0.01 N could be detected.

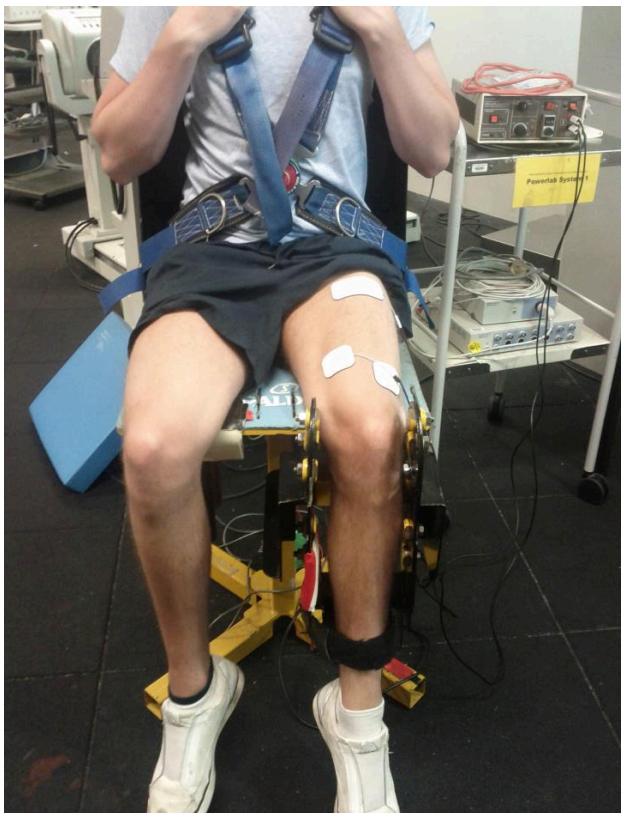

Figure 2. Participants were firmly strapped into the rigid seat across across the hips and chest, with the leg secured above the ankle. Three adhesive neuromuscular electrical stimulation electrodes were placed over the thigh (see text for details).

Following a 2-min passive rest, each participant performed three 3-s maximum voluntary contractions with 3 min of rest between repetitions. Participants were instructed to perform maximal contractions with the greatest possible rate of force development, following instructions to produce force "as fast, and then as hard as possible" (Maffiuletti et al., 2016), to hold for 3 s (indicated by the experimenter) and then to relax as quickly as possible. Participants were provided loud verbal encouragement as well as real-time visual feedback of the knee-extensor joint torque production. Any visible countermovement (negative inflection in joint torque greater than 5 Nm) observed on the joint torque data trace voided the test and the contraction was repeated. Parameters describing maximal voluntary isometric torque production, such as maximum isometric knee extensor torque (MVC), absolute RFD and normalised RFD (RFD_{norm}) (measured from 0-50, 0-100, and 0-150 ms) as well as time to

1/6, 1/2 and 2/3 of MVC (i.e. early, middle and late stage force rise respectively), were calculated from data collected during these MVCs (described below). The repeatability for RFD and RFD_{norm} was investigated on two separate days over a period of 2 weeks in the non-training group.

2.5 Neuromuscular electrical stimulation (NMES) procedure

During testing, three self-adhesive neuromuscular electrical stimulation (NMES) electrodes were used to stimulate the quadriceps muscles (Dura-Stick ® II. Chattanooga Group, Hixon, USA). A large 9×5 cm anode electrode was placed horizontally over the rectus femoris/vastus lateralis muscle area proximally on the thigh while two smaller 5×5 cm cathode electrodes were placed distally on vastus lateralis and vastus medialis approximately at their motor points (Figure 1). Electrode positions were adjusted to find the location that produced the maximum force for a given (low) stimulation intensity, and this procedure was performed in all testing sessions. The distance of the electrodes relative to a straight line measured between the anterior superior iliac spine and the apex of the patella was recorded and used to assist with repeated placement of electrodes for each testing session. Series' of 11 stimuli with pulse width 100 μs at a frequency of 20 Hz were delivered via a constant current electric stimulator (DS7, Digitimer Ltda. Welwyn Garden City, UK). The stimulation intensity (amplitude current) was set to elicit 30% (± 3%) of the maximum voluntary isometric torque (MVIC) when using a constant frequency train of stimuli. After training, the intensity was set to elicit 30% (± 3%) of the new MVIC. This was done to account for any changes in strength following the training protocol.

Two types of electrical stimulation trains were used, a constant frequency train (CFT) of 20 or 40 Hz, and a variable frequency train (VFT) identical in formation to its CFT counterpart except with the first two pulses closely spaced (either 200 Hz [5 ms doublet] or

100 Hz [10 ms doublet] apart). Muscle stimulation trials were subsequently completed with the order of VFTs and CFTs being randomised between participants. Each contraction lasted ~ 500 ms, and both the VFT and CFT contained an identical number of pulses. Four different variable frequency stimulation trains were delivered during the procedure: a 20-Hz train preceded by two pulses with a 5 ms IPI, a 20-Hz train preceded by two pulses with a 10 ms IPI, a 40-Hz train preceded by two pulses with a 5 ms IPI and a 40-Hz train preceded by two pulses with a 10 ms IPI. Three constant frequency trains of 20, 40 and 80-Hz were also delivered. RFD comparisons were made using the impulse (N·s) measured during different phases of the force development (up to 200 ms) to examine the influence of the double discharge (VFT:CFT) (Binder-Macleod & Lee, 1996). Impulse was chosen as it provided a more reliable measure of the contraction history of the sub-tetanic contractions. Repeated stimulations at these frequencies and intensities at 60-s intervals were observed to produce reliable joint torque in pilot testing. The 5- and 10-ms initial IPIs have been shown to augment muscle force output, and are initial IPI durations commonly observed in normal human movement (Binder-Macleod, Kesar, 2005; Griffin, Garland, Ivanova, 1998). 20- and 40-Hz train frequencies were chosen as they are within the observed discharge rates for most human muscles during dynamic contractions (Bellemare et al., 1983; Pucci, Griffin, Cafarelli, 2005). Changes in excitation-contraction coupling (EC) efficiency (influenced potentially by factors such as changes in intracellular Ca^{2+} release and sensitivity) can be quantified using the 20:80 Hz ratio, i.e. the difference in torque in response to 20-Hz and 80-Hz stimulations (Martin, Millet, Martin, Deley & Lattier, 2004; Slade et al., 2003). The 20:80 Hz ratio is considered to provide information regarding Ca^{2+} release from the sarcoplasmic reticulum into the myoplasm as well as sensitivity to Ca^{2+} at the actomyosin complex (Jones, 1996; MacIntosh, 2003; Martin et al., 2004). A change in force response to either a higher or lower frequency stimulation following training can be considered indicative of a change in the ratio

of contributions from central (i.e. pre-motor) or peripheral (i.e. muscular; E-C coupling) factors (Millet & Lepers, 2004). Reliability of these methods has been previously demonstrated across a four-week period (Clark, Cook, & Ploutz-Snyder, 2007), and repeatability was assessed on 2 separate days over a 2-week period in the non-training group (see Table 2).

2.6 Voluntary and evoked peak torque, rate of force development and impulse

Contractile RFD (voluntary contractions) and impulse (electrically evoked) were measured from the contractions with the greatest peak torque and calculated as the average slope of the moment-time curve (Δmoment/Δtime). Contraction onset was defined as an increase of 0.5 N·m from baseline (Soda, Mazzoleni, Cavallo, Guglielmelli, & Iannello, 2010) which did not return to baseline within 100 ms, and onset was checked manually for each trial (Maffiuletti et al., 2016). Contractile impulse (i.e. the normalised torque-time integral) was calculated as the area under the moment-time curve (derivative of moment-time curve) in time intervals of 0-30, 0-50, 0-100, 0-200, and 100-200 ms (0 ms = contraction onset). The area under the moment-time curve can be considered as the kinetic impulse if the limb was allowed to move and reflects the entire contractile history, including various time-related RFD parameters as well as the continued effect of a short initial IPI if present (Aagaard et al., 2002). Voluntary RFD was measured in time intervals of 0-50, 0-100, and 0-150 ms relative to the onset of contraction, and also to one-sixth, one-half, and two-thirds of peak torque allowing evaluation of RFD at an early, middle and late phase (Aagaard et al., 2002; Andersen & Aagaard, 2006; Andersen, Andersen, Zebis & Aagaard, 2010). Analysis beyond time points of 150 ms relative to onset introduced errors due to many subjects exhibiting a non-linear torque decrement near the plateau of contractile torque. Normalised RFD (RFD_{norm}) was calculated as the ratio of the slope of the moment-time curve to the peak isometric joint moment achieved in the same testing session. RFD_{norm} indicates the ability to

rapidly produce force regardless of peak force capacity, i.e. it removes the effects of changes in maximum force that might occur with training and detraining (Blazevich et al., 2008). As there is a high interindividual variability in the magnitude of muscle activation during rapid contractions, especially during the early (0-50 ms) phase (de Ruiter et al., 2004; Folland et al., 2014), it is likely that neural factors contribute substantially to the between-participant variance (Folland et al., 2014). Knee joint force was recorded using LabChart v.6.1.3 Software (PowerLab System, ADInstruments, NSW, Australia) and multiplied by the lever arm length to obtain knee joint torque.

2.7 Statistical analysis

Data are presented as mean ± SD or SE, as appropriate. Separate, one-way, repeated measures ANOVAs were performed to compare RFD responses between VFT and CFT trials to determine whether there was an augmented contractile torque in response to a brief initial IPI both before and then after the training. To evaluate the effect of the duration of the initial interpulse interval (5 ms vs. 10 ms) and the effect of the frequency of the subsequent train (20 or 40 Hz) on RFD at different time intervals from the onset of torque, separate two-way mixed-model ANOVAs (ISI × frequency) were used, both before and also after training. Two-way, repeated measures ANOVA with Bonferroni's post hoc tests were performed to compare the VFT:CFT ratio pre- to post-training. Separate analysis was performed on the non-training (control) group data, including one-way, repeated measures ANOVAs, and Intraclass Correlation Coefficient (ICC) to assess the reliability of the VFT:CFT ratio, and the coefficient of variation (SD/mean) of the change scores was calculated to assess the relative size of variability.

As the voluntary RFD data proved not to be normally distributed, all pre- to post-training changes (measured to the various time intervals) were evaluated with the Wilcoxon

signed-rank test for paired samples (two tailed), and the effect size was calculated if $p \leq 0.1$. Pearson's product-moment correlation coefficients with 90% confidence intervals were then computed to quantify the linear relationships between changes in voluntary torque production (expressed as both absolute and normalised RFD) and changes in response to a variable frequency train (i.e. changes in the VFT:CFT ratio). The following criteria was used to assess the strength of the relationships: trivial ($r < 0.1$), small ($r = 0.1$-0.29), moderate ($r = 0.3$-0.49), large ($r = 0.5$-0.69), very large ($r = 0.7$-0.89) and nearly perfect ($r \geq 0.9$) (Cohen, 1988). Finally, changes in the RFD responses to 20 Hz versus 80 Hz stimulation trains (i.e. 20:80-Hz RFD ratio) were examined using paired t-tests. Statistical significance was set at an α level of 0.05.

CHAPTER 3

RESULTS

3.1 Electrically-evoked torque and RFD

As described in Methods, the reliabilities of the impulses measured to 30, 50, 100, 200 ms as well as in the interval 100-200 ms during electrically-evoked contractions were higher than RFD variables calculated to the same time intervals. Therefore, impulses measured to discrete time intervals were used in subsequent analyses as indicators of RFD during evoked contractions.

In the training group, the imposition of a brief initial interpulse interval (IPI) of either 5 or 10 ms was typically associated with a significantly faster RFD during both 20 Hz and 40 Hz stimulus trains. Before training, mean doublet-induced RFD augmentations ranged from 4 ± 5% to 107 ± 26% depending on the interval duration (see Table 1), although RFD was greater when calculated to shorter (0-30, 0-50, and 0-100 ms) than longer or later (0-200 and 100-200 ms) time intervals. Post-hoc tests revealed a strong statistical effect of NMES train frequency on the VFT:CFT impulse ratio at all measured time intervals, with a larger augmentation of RFD observed during 20-Hz than 40-Hz stimulations ($p < 0.001$ for all comparisons), however there was no effect of initial IPI duration (i.e. no difference between the effect of 5- vs. 10-ms intervals; Table 1).

Following the training intervention, doublet-induced RFD augmentations ranged from -1 ± 5% to 89 ± 39% depending on the interval duration (also see Table 1). The augmentation resulting from a 10-ms IPI preceding a 20-Hz train was significantly reduced compared to pre-training when measured from 0-30 ms (-13.7 ± 11.3%, $p = 0.03$), 0-50 ms (-13.9 ± 8.4%, $p = 0.007$), 0-100 ms (-8.6 ± 10.2%, $p = 0.04$), 0-200 ms (-8.1 ± 5.3%, $p = 0.009$), and 100-200 ms (-7.4 ± 6.6%, $p = 0.02$). However, there were no significant changes in the response

to a 5-ms IPI preceding a 20-Hz train or a 5- or 10-ms IPI preceding a 40-Hz train. Post-hoc tests revealed a strong statistical effect of NMES train frequency on the VFT:CFT impulse ratio at post-training, although statistical significance was only observed at the intermediate duration intervals, i.e. there was no difference in the augmentation provided by either the 5- or 10-ms initial IPI during either a 20- or 40-Hz train when measured from 0-30 ms or from 0-200 ms. No significant changes were observed in the ratio of the peak torque produced during 20- and 80-Hz trains ($p = 0.17$).

Table 1. The influence of a brief initial IPI (5 or 10 ms) imposed at the start of stimulation trains compared to consistent frequency trains (i.e. VFT:CFT) during 20-Hz and 40-Hz trains measured in the intervals 0-30, 0-50, 0-100, 0-200, and 100-200 ms, before and after training. Data are expressed as the percentage difference in contractile impulse. * VFT is significantly different to CFT ($p \leq 0.05$). † significantly different influence of the VFT after training ($p \leq 0.05$).

20 Hz

	5-ms initial IPI			**10-ms initial IPI**		
Time (ms)	Pre (%)	Post (%)	Change (%)	Pre (%)	Post (%)	Change (%)
30	67 ± 29*	64 ± 39*	-2 ± 14	96 ± 40*	66 ± 27*	-15 ± 12†
50	92 ± 31*	89 ± 39*	-1 ± 15	107 ± 26*	78 ± 27*	-14 ± 9†
100	65 ± 19*	66 ± 24*	1 ± 14	74 ± 16*	58 ± 17*	-9 ± 11†
200	36 ± 6*	33 ± 13*	-2 ± 6	41 ± 4*	30 ± 9*	-8 ± 5†
100-200	27 ± 8*	22 ± 9*	-4 ± 6	30 ± 6*	20 ± 7*	-8 ± 7†
40 Hz						
Time (ms)	Pre (%)	Post (%)	Change (%)	Pre (%)	Post (%)	Change (%)
30	41 ± 22*	57 ± 45*	14 ± 39	45 ± 28*	57 ± 39*	12 ± 39
50	42 ± 14*	50 ± 25*	5 ± 27	42 ± 20*	46 ± 25*	3 ± 27
100	20 ± 5*	20 ± 6*	0 ± 7	19 ± 8*	19 ± 9*	0 ± 12
200	9 ± 5*	4 ± 5	-4 ± 7	9 ± 6*	5 ± 5	-3 ± 8
100-200	4 ± 5*	-1 ± 5	-5 ± 7	5 ± 5*	0 ± 4	-4 ± 7

In the non-training control group, no changes were observed in the rate of torque development within any measured time interval ($p = 0.37 – 0.89$). Coefficients of variation calculated on stimulations measured 2 weeks apart ranged from 2.9 – 4.3% and 1.4 – 2.1% for 20 and 40 Hz trains, respectively, when the trains commenced with a 5-ms IPI (see Table 3). Coefficients of variation ranged 5.5 – 7.6% and 2.0 – 6.1% for 20 and 40 Hz trains when

these trains commenced with a 10-ms IPI. For tests completed 2 weeks apart, moderate-to-very high ICCs were observed for torque responses measured in the 20-Hz trains (ICC = 0.87 – 0.97 for 5-ms IPI, and ICC = 0.60 – 0.92 for 10-ms IPI; see Table 3) and in the 40-Hz trains with 5-ms IPI (ICC = 0.66 – 0.99). However, the repeatability of measurements to longer time intervals in particular were lower for 40 Hz trains commencing with a 10-ms interpulse interval (ICC = 0.35, 0.12 and 0.06 for 100, 200, and 100-200 ms, respectively).

Table 2. Coefficients of variation (%) and ICC scores calculated for the control group data measured over the 2-week non-training period.

Coefficients of Variation (%)				
Time (ms)	**20:20-5**	**20:20-10**	**40:40-5**	**40:40-10**
30	4.3	7.6	1.4	2.0
50	3.7	6.5	1.9	2.8
100	4.3	5.2	2.6	4.4
200	2.9	5.5	2.1	4.6
100-200	3.1	6.2	2.1	6.1
ICC				
Time (ms)	**20:20-5**	**20:20-10**	**40:40-5**	**40:40-10**
30	0.97	0.89	0.99	0.98
50	0.97	0.92	0.98	0.94
100	0.94	0.87	0.77	0.35
200	0.90	0.68	0.66	0.12
100-200	0.87	0.60	0.69	0.06

3.2 Effect of training on voluntary torque production and RFD

In the training group, the maximum voluntary isometric knee extensor torque (MVC) increased by 24.3 ± 13.3% ($p = 0.002$) over the training period, from 228.5 ± 39.2 Nm to 282.4 ± 48.4 Nm, with no statistical change being observed in the non-training group (242.5 ± 61.8 Nm to 247.5 ± 62.8 Nm, $p = 0.29$, ICC = 0.99). In addition, changes in RFD (measured as the slope of the moment-time curve) were also observed following training. Expressed in absolute terms (as shown in Figure 2), RFD increased by 55.5 ± 50.3% (from 1082.0 ± 752.5 $Nm \cdot s^{-1}$ to 1451.1 ± 748.0 $Nm \cdot s^{-1}$, $p = 0.011$) when measured from 0-50 ms,

by 34.0 ± 47.2% (from 1375.6 ± 452.7 Nm·s^{-1} to 1694.1 ± 347.9 Nm·s^{-1}, p = 0.01) when measured from 0-100 ms, and by 31.9 ± 38.2% (from 1174.0 ± 399.6 Nm·s^{-1} to 1448.5 ± 274.1 Nm·s^{-1}, p = 0.018) when measured from 0-150 ms (p < 0.01 for all comparisons). No statistical change was detected in peak RFD irrespective of whether it was measured using a 10- or 50-ms window in either the training (1837.4 ± 463.5 Nm·s^{-1} to 2206.5 ± 653.8 Nm·s^{-1}, p = 0.12) or non-training (1825.2 ± 838 Nm·s^{-1} and 1637.6 ± 866 Nm·s^{-1}, p = 0.33) periods.

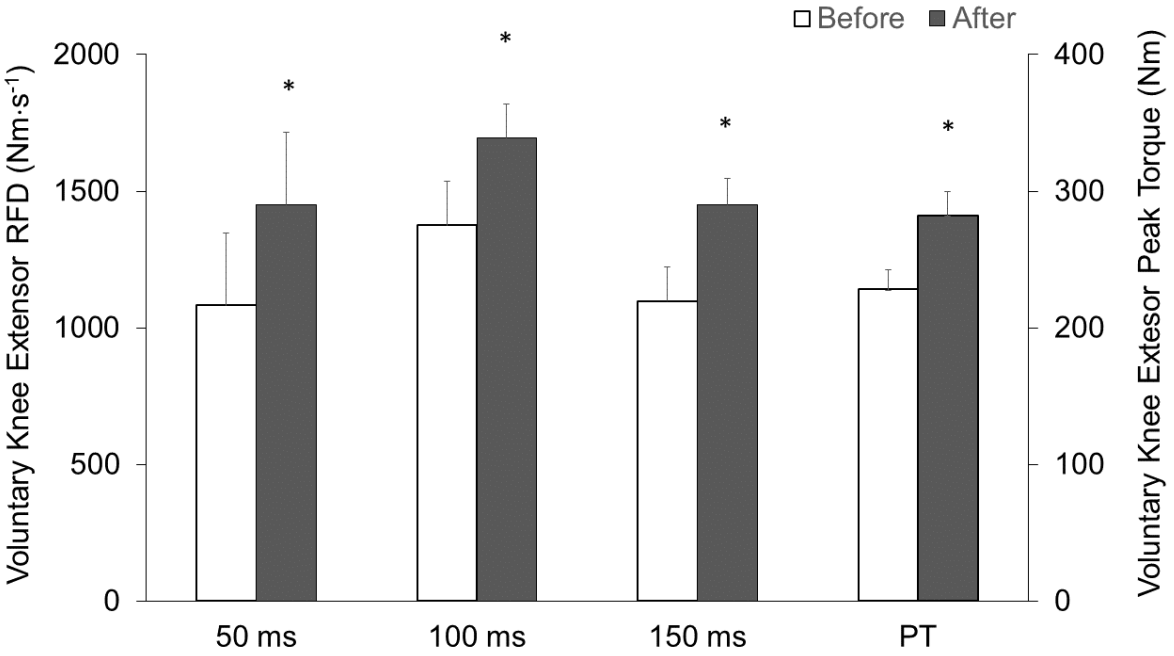

Figure 2. Rates of force development (RFD, mean ± SE) measured to 50, 100, and 150 ms, and maximum voluntary torque achieved (MVC) before and after training. Increases in peak torque, and improvements in absolute RFD were observed in all measured time intervals. * significantly different to pre-training (p ≤ 0.05).

In the training group, normalised RFD (RFD_{norm}), calculated as the ratio of absolute RFD to MVC peak torque, increased by 44.9 ± 38.8% (p = 0.04) when measured in the first 50 ms of contraction (384.1 ± 257.6 %MVC·s^{-1} to 512.7 ± 227.1 %MVC·s^{-1}) and 13.8 ± 12.2% (p = 0.01) in the first 100 ms (538.5 ± 148.3 %MVC·s^{-1} to 623.9 ± 100.8 %MVC·s^{-1}) (see Figure 3). RFD_{norm} was unchanged with training when measured to 150 ms and during the intervals 50-100 ms and 100-150 ms. RFD measured to one-sixth of MVC increased 30 ±

28% (332.8 ± 117.5 %MVC·s^{-1} to 422.2 ± 150.4 %MVC·s^{-1}, p = 0.04) but no statistical changes were observed when measured to one-half or two-thirds of MVC torque (p = 0.08, effect size = 0.12; and p = 0.075, effect size = 0.15, respectively). Similarly, there were no observed changes in time to reach one-half or two-thirds of MVC torque (110 ± 44 ms to 92 ± 23 ms and 177 ± 81 ms to 140 ± 38 ms, respectively). No significant changes were observed in the normalised peak RFD (i.e. peak RFD/MVC) irrespective of whether it was measured using a 10- or 50-ms window. No significant changes were observed in RFD_{norm} in the non-training control group when measured during 50 ms (372.2 ± 132.5 %MVC·s^{-1} to 336.56 ± 118.32 %MVC·s^{-1}, p = 0.18, ICC = 0.93), 100 ms (588.6 ± 148.8 %MVC·s^{-1} to 545.6 ± 143.7 %MVC·s^{-1}, p = 0.17, ICC = 0.93) and 150 ms (496.8 ± 136.0 %MVC·s^{-1} to 503.0 ± 144.8 %MVC·s^{-1}, p = 0.86, ICC = 0.88).

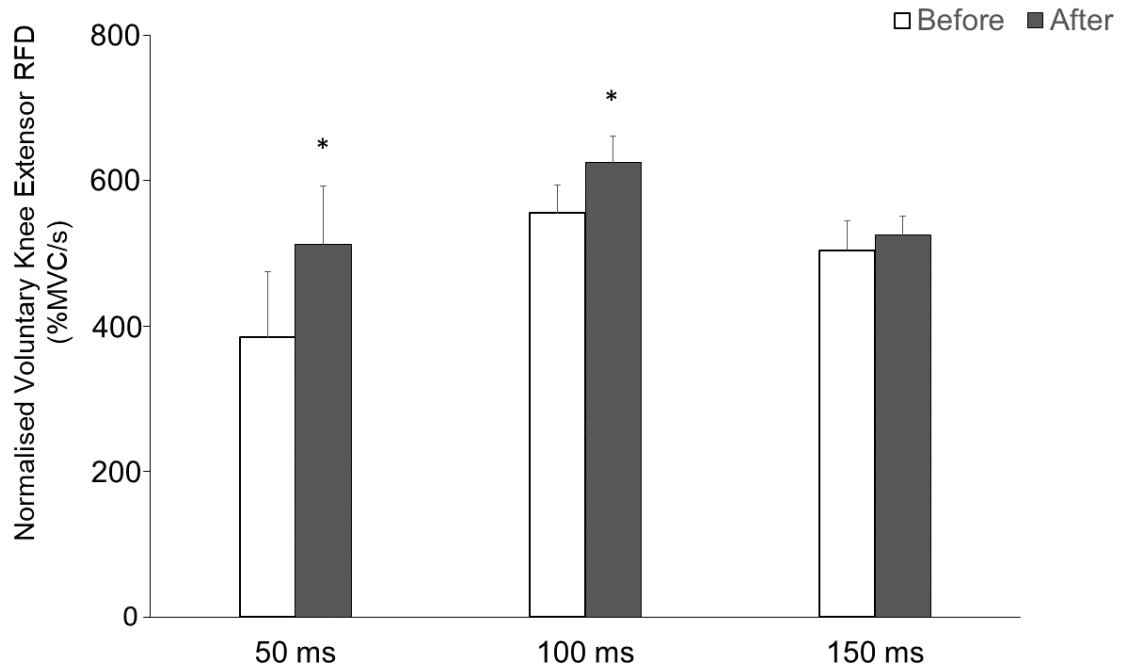

Figure 3. Normalised RFD (mean ± SE) measured from 0-50, 0-100, and 0-150 ms before and after training. Statistically significant improvements were observed in RFD_{norm} to 50 and 100 ms, but not to 150. * significant change from pre- to post-training ($p \leq 0.05$).

3.3 Relationships between changes in VFT:CFT and changes in voluntary RFD

Training-dependent improvements in voluntary RFD and changes in the VFT:CFT ratio were compared using bivariate correlation. Very large correlations were observed between the VFT:CFT impulse ratio measured from 100-200 ms and RFD_{norm} measured in the interval 0-100 ms (r = 0.75, CI = 0.23 -0.94, p = 0.03), one-half MVC (r = 0.80, CI = 0.35-0.95, p = 0.02) and two-thirds MVC (r = 0.77, CI = 0.28 – 0.94 , p = 0.025) (see Table 3 and Figure 4). Furthermore, there was evidence of a relationship between early phase RFD_{norm} and early phase VFT:CFT impulse ratio with a nearly perfect correlation being observed between the improvements in RFD_{norm} measured to one-sixth of MVC and the changes in response to a 10-ms IPI measured from 0-50 ms during a 20-Hz train, despite a statistically significant decrease being observed in the latter variable (r = 0.91, 90% CI = 0.66 – 0.98, p = 0.002) (Figure 5). Furthermore, a very large correlation, but with a wide confidence interval, was observed between changes in RFD_{norm} to one-half of MVC and the change in response to a 5-ms IPI measured during a 20-Hz train (r = 0.76, 90% CI = 0.24-0.94, p = 0.02). In contrast, there was a strong negative relationship between training-dependent changes in the VFT:CFT ratio measured during higher frequency stimulations (40 Hz) and changes in RFD_{norm}. However, closer examination of the data revealed that a single outlier strongly influenced these results (see Figure 6). Reanalysis after the removal of the data point revealed no evidence of a relationship. It is therefore not reasonable to suggest that there is a statistical relationship between changes in voluntary RFD and changes in the response to a 5-ms doublet during a 40-Hz train.

Table 3. Pearson's correlations between training-dependent changes in the 20-Hz VFT:CFT ratio (rows) and RFD_{norm} (columns). * $p \leq 0.05$. † $p \leq 0.10 \geq 0.05$.

	5-ms VFT:CFT					
Interval	50 ms	100 ms	150ms	⅙ MVC	½ MVC	⅔ MVC
30 ms	0.02	0.34	0.72*	0.34	0.62†	0.22
50 ms	0.20	0.24	0.61	0.55	0.59	0.11

100 ms	0.09	-0.04	0.15	0.30	0.22	0.16
200 ms	0.40	0.54	0.66†	0.40	0.75*	0.69†
100 – 200 ms	0.44	0.73*	0.66†	0.27	0.78*	0.78*
10-ms VFT:CFT						
30 ms	0.80*	-0.01	0.03	0.64†	0.33	0.16
50 ms	0.85*	0.20	0.05	0.91*	0.47	0.12
100 ms	0.15	-0.09	0.20	0.22	0.22	0.22
200 ms	0.00	0.36	0.65†	-0.22	0.44	0.64†
100 – 200 ms	-0.11	0.51	0.59	-0.38	0.37	0.60

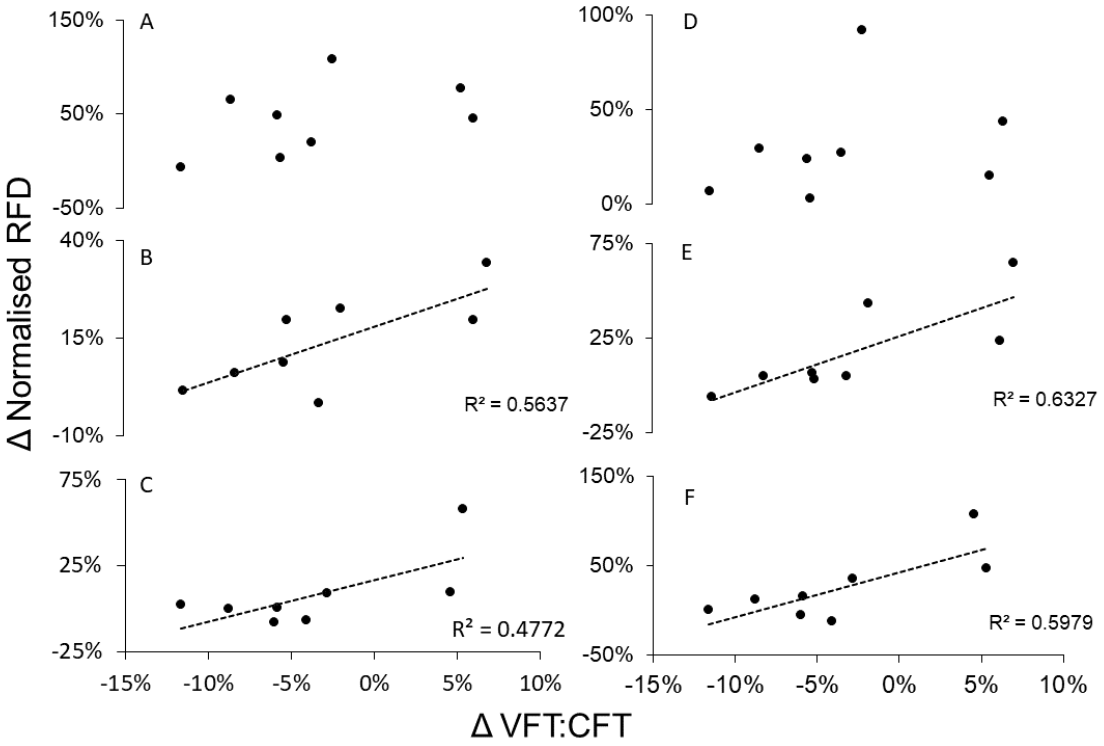

Figure 4. Relationships between training-dependent changes in the VFT:CFT ratio measured from 100-200 ms during a 20 Hz train using a 5-ms IPI and changes in RFD_{norm} measured to A) 50 ms, B) 100 ms, C) 150 ms, D) one-sixth of peak torque, E) one-half of peak torque, and F) two-thirds of peak torque. Statistically significant Pearson's correlation coefficients were found between changes in normalised RFD when measured to later time points in the contraction, and changes in VFT:CFT ratio when measured from 100-200 ms.

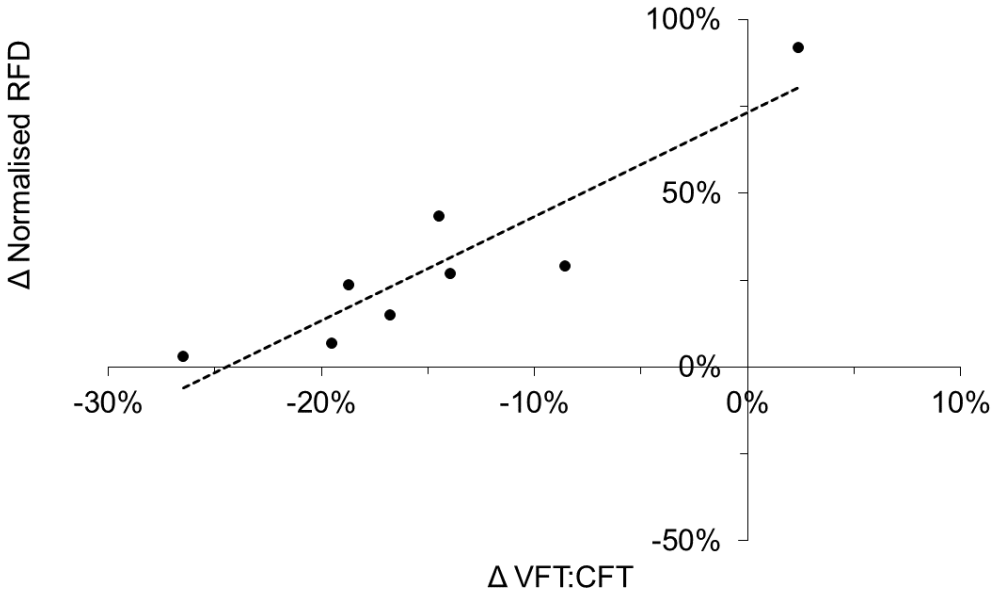

Figure 5. Relationship between the change in the VFT:CFT ratio with a 10-ms brief initial IPI measured during the first 50 ms of a 20-Hz train, and the training-dependent changes in RFD measured to one-sixth of MVC (r = 0.91).

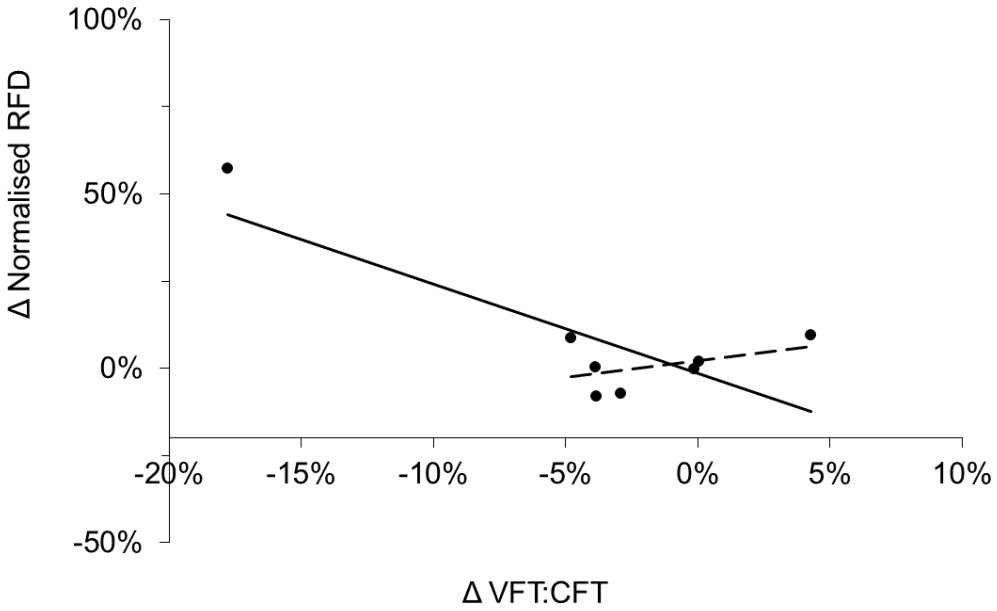

Figure 6. Relationship between the training-dependent changes in the VFT:CFT ratio with a 5-ms initial IPI during the first 200 ms of a 40-Hz train and the changes in RFD during the first 150 ms of MVC. In this example, the negative correlation (r = -0.72; solid line) results from the inclusion of a single outlier and its removal leaves a non-significant and small correlation between the variables (dashed line).

CHAPTER 4

DISCUSSION

4.1 Overview

The main purpose of the present study was to examine the relationship between changes in the force-enhancing effect of a high frequency double discharge (a doublet) at the onset of contraction (assessed using neuromuscular electrical stimulation [NMES]) and changes in voluntary rate of force development in response to a 4-week explosive-type, isometric knee extension training program. This study design was considered to provide *in vivo* information regarding the effect of training on the benefit derived from high-frequency motor unit discharges at the onset of contraction. Testing this relationship required two conditions to be satisfied: (1) that a greater RFD would be evoked by NMES trains commencing with a brief (5 ms or 10 ms) initial interpulse interval (IPI) than trains without, and (2) that the exercise training would prompt significant changes in voluntary RFD. In relation to the first condition, an increased force response was clearly observed when trains of stimuli commenced with two pulses at 5- or 10-ms IPIs (i.e. variable-frequency train, VFT) than when a constant frequency (CFT) was maintained (see Figure 7). Based on existing methodology, a faster evoked RFD response to a short-interval double discharge at the beginning of an electrical stimulation train was taken to demonstrate a benefit of inducing the "catchlike response" of the muscle during voluntary contraction (discussed in detail below). In relation to condition 2, substantial improvements in peak voluntary knee extensor torque (MVC) as well as both absolute and MVC-normalised RFD (RFD_{norm}) were observed following training; there was also a notable inter-individual variability in these responses which allows a more detailed examination of relationships between changes in voluntary RFD and changes in the ability to utilise high-frequency discharges at the onset of contraction.

Based on these results, it was considered appropriate to examine the relationships between the improvements in voluntary RFD and changes in the VFT:CFT ratio (i.e. the augmentation of RFD resulting from either a 5- or 10-ms IPI at contraction onset). The main outcomes of this examination are that the effect of a high-frequency double discharge after explosive-type knee extensor training remained unchanged or, under some conditions (20-Hz train with 10-ms initial IPI), was reduced. Additionally, training-dependent improvements in the ability to rapidly reach a specified torque level relative to peak MVC torque (i.e. RFD_{norm}) were greater for those participants whose VFT:CFT ratio either did not decline or declined the least (see Figure 5). Thus, those who maintained or improved their ability to make use of a doublet at the onset of muscular contractions showed greater improvements in RFD_{norm} after the training, although this was not the case for absolute RFD or voluntary peak MVC torque.

4.2 Effect of an initial doublet on evoked RFD

The present data provide further evidence that shortening of the interval between the first two pulses of an otherwise-constant electrical stimulation train results in a more rapid and forceful muscle contraction (see Table 1) (Binder-Macleod & Barker, 1991; Binder-Macleod & Barrish, 1992; Burke, Rudomin, & Zajac, 1976). Before training, electrical stimulations initiated with a high-frequency double discharge (i.e. a doublet) significantly increased the RFD (measured as impulse; N.s) in all measured time intervals (0-30 ms, 0-50 ms, 0-100 ms, 0-150 ms and 100-200 ms) for each tested combination of interval duration and train frequency in all test subjects. The magnitude of the augmentation was consistent with previous studies evaluating doublet-induced force enhancement (Binder-Macleod & Kesar, 2005; Slade et al., 2003). No statistical difference was observed in the magnitude of the augmentation achieved using 5- or 10-ms initial IPIs, although there was a strong effect of the frequency of the ongoing train, where the doublet had a significantly greater effect when

preceding 20-Hz than 40-Hz stimulation trains. The likely explanation for this is that the lower frequency of muscle activation triggers less Ca^{2+} release than the higher frequency (Szent-Gyorgyi, 1975), resulting in a lower proportion of strongly bound cross bridges and titin activation (Karatzaferi, Chinn, & Cooke, 2004; Metzger & Moss, 1990; Wahr & Metzger, 1999) and thus allowing for a greater effect of the high-frequency double discharge. This is evident when examining the torque records from trains of different frequency, where there are clear torque oscillations at the plateau of the torque-time curve in 20-Hz but not 40-Hz trains (CFT and VFT, e.g. see Figure 7). This suggests that if the intensity of the stimulus causes Ca^{2+} release (and binding) sufficiently to result in a fully-fused tetanus, double discharge-induced improvements in Ca^{2+} sensitivity may be less beneficial to the rate of contractile force development. Furthermore, the increased speed of internal shortening in the contractile elements likely work to inhibit the impact of the double discharge (Mayfield et al., 2016).

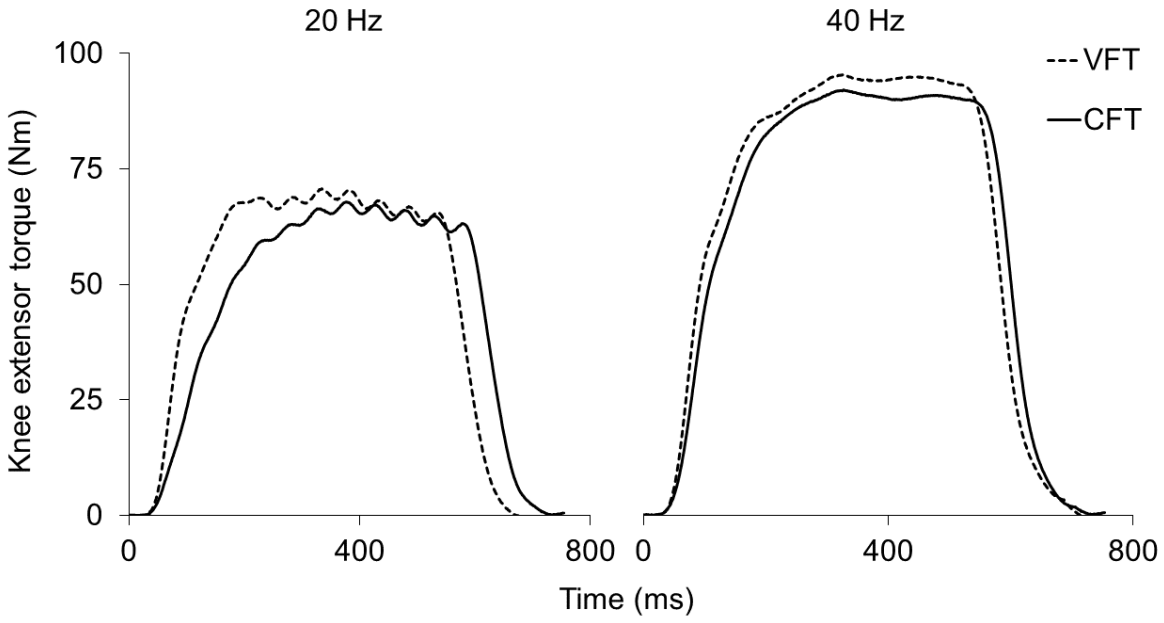

Figure 7. Torque records for 20- and 40-Hz electrically-induced contractions for a single subject. VFT, dashed line; CFT, solid line. A torque oscillation is visible in the 20-Hz records, indicating that the tetanus is not fully fused. Stimulations were delivered with the same amplitude and pulse width, lasting ~500 ms.

4.3 Changes in voluntary RFD and electrically-evoked torque with training

Substantial increases in peak MVC torque and voluntary rate of force development (RFD) were observed following the 4-week period of explosive-type resistance training (24% increase in peak torque, and 30 – 45% increase in RFD). Improvements in knee extensor peak torque were as large, or larger, than those reported in previous knee extensor training studies (18 - 24%; Durbaba et al., 2013; Oliveira et al., 2013; Tillin, 2011; Tillin & Folland, 2014). Commensurate increases in voluntary RFD were also observed (55%, 34%, and 40% increases in the intervals 0-50, 0-100, and 0-150 ms, respectively). Training-dependent improvements in the rates of force development early in a contraction are typically explained by changes in discharge rates of motor neurones especially at the onset of muscle activation. Although muscle activity (EMG) was not recorded in the present study, given the short duration of training it is unlikely that muscle hypertrophy (or significant architectural changes) could be responsible for observed improvements, which are usually observed after longer periods of explosive-type training (Duchateau & Hainaut, 1984). Considering that significant improvements in peak torque and RFD were observed without significant increase in electrically evoked RFD or peak torque, and the evidence demonstrating a causal relationship between training-induced improvements in RFD parameters and increases in muscle activation rates (Blazevich et al., 2008; de Ruiter, Vermeulen, Toussaint, & De Haan, 2007; Duchateau & Baudry, 2014; Maffiuletti et al., 2016), it is likely that neural adaptations largely underpinned the increases in voluntary RFD in the current study.

Significant improvements were also observed when voluntary RFDs were normalised to peak MVC torque (RFD_{norm}), especially during the early phase of the contraction (i.e. 0-50 ms, 0-100 ms and to one-sixth of MVC, see Figure 3). A large interindividual variability was observed in most variables describing the training-dependent changes in voluntary RFD, which has previously been reported in similar investigations (Blazevich et al., 2008;

Mourselas & Granat, 1998). Also, training-dependent increases in RFD_{norm} have been observed in similarly-designed explosive-training experiments, with these increases also typically being detected in the early phases of the contraction (Aagaard et al., 2002; Van Cutsem, Duchateau, & Hainaut, 1998). Improvements in voluntary RFD_{norm} indicate that qualitative adaptations, potentially involving altered double-discharge behaviours, changes in the time-course of the muscle twitch, an improvement of Ca^{2+} handling within the cell, a shift towards greater proportions of type II muscle fibres, or increased passive stiffness in the series elastic element might have occurred (Aagaard et al., 2002; Duchateau & Enoka, 2011; Duchateau & Baudry, 2014; Maffiuletti et al., 2016; Van Cutsem et al., 1998).

The current results did not provide any support for the hypothesis that a training-dependent increase might occur in the contractile augmentation caused by a doublet with a brief initial IPI when preceding an otherwise-constant-frequency electrical stimulation train. In fact, after training, RFD during a variable frequency train (VFT) with a 10-ms initial IPI was significantly reduced when it preceded a 20-Hz stimulation train, indicating that under some stimulus conditions a reduction in the ability to utilise a doublet might occur. To the author's knowledge, this is the first report of a training-induced change in the magnitude of the contractile augmentation resulting from the imposition of a doublet during the onset of electrical stimulation, and suggests that the ability to benefit from a high frequency burst at the onset of contraction may, under some conditions, be reduced after training using explosive contractions with a fast rate of force development. It is important to note, however, that although the effect was diminished, VFTs with a 10-ms initial IPI still augmented RFD by 20 – 78% (depending on the measured interval) during a 20-Hz stimulation train. Furthermore, VFTs continued to significantly augment muscular rate of force development under all conditions except in the 0-200 ms interval when a doublet with a 10-ms IPI preceded a 40-Hz train (see Table 1). It must also be noted that the influence of training-

related changes in the discharge behaviour of the motor unit at the onset of voluntary contraction was not taken into account. In summary, no evidence was found in the present study that the benefit derived from the imposition of an initial brief IPI during electrical stimulation is improved after 4 weeks of explosive isometric knee-extensor training, and indeed there is some evidence that the effect may be attenuated.

Although no significant improvements were observed in the benefit derived from the doublet after training, this does not preclude further examination of the relationship between changes in the VFT:CFT ratio and changes in RFD, as it could provide insight into the adaptive mechanisms that give rise to increased strength and RFD after training. Indeed, the present results provide evidence of a positive linear relationship between training-dependent changes in RFD_{norm} and training-dependent changes in the VFT:CFT ratio measured during 20-Hz stimulation trains; therefore, under some conditions at least, those participants whose VFT:CFT ratio declined the least (or even improved) following training tended to show greater improvements in RFD_{norm} (see Figures 3 and 4). As the electrically induced double discharge was imposed on all recruited fibres and was identical in both test sessions, any training-related changes in the force response are indicative of changes in the intrinsic properties of the muscle (i.e. the ability to utilise the double discharge rather than the number that reach the muscle). However, because of the high inter-individual variability and subsequently wide confidence intervals of the correlation change scores, these data should be verified in future studies.

4.4 Adaptive mechanisms in response to explosive-type strength training

One reasonable hypothesis is that training-induced adaptations in the mechanisms underpinning explosive force production might be responsible for the increases in early-phase RFD_{norm} while simultaneously attenuating the force enhancement derived from the doublet, in

particular the 10-ms IPI when preceding the slower (20 Hz) trains. If true, this would suggest a negative relationship between RFD_{norm} and the VFT:CFT ratio might exist, however the data presented here contradicts this. Therefore, an additional explanation is that large inter-individual variations in training-dependent changes in RFD_{norm} are indicative of inter-individual differences in the training-induced adaptations of mechanisms underpinning rapid force production, which is reflected in the magnitude of the force enhancement induced by a doublet. Although the current data only allows speculation, there are several factors that are most likely responsible.

Given that Ca^{2+} dynamics strongly mediate the activation of the contractile apparatus, and that the primary mechanism of doublet-induced force enhancement appears to be an amplified Ca^{2+} concentration in the myoplasm, it is likely that adaptive changes in the mechanisms of Ca^{2+} handling would substantially influence the double discharge. Adaptations in Ca^{2+} dynamics (i.e. more Ca^{2+} released from SR, lower thresholds for cross-bridge interaction, faster cross-bridge cycling, etc.) clearly have the potential to impact RFD, and the rate of release of Ca^{2+} from the sarcoplasmic reticulum has previously been reported to increase following high-intensity exercise training in cyclists (Ørtenblad, Lunde, Levin, Andersen, & Pedersen, 2000), resistance-training in elderly women (Hunter at al., 1999) and aerobic training in mouse muscle (Ferreira et al., 2010), although it is currently not clear if this meaningfully impacts RFD *in vivo*. Theoretically, adaptations in the magnitude of Ca^{2+} efflux, and/or a shift towards a more Ca^{2+} sensitive actin-myosin complex after training, might render doublet-induced, transient improvements in Ca^{2+} release and binding (i.e. sensitivity) less influential in a more Ca^{2+}-sensitive system. Indeed, partial inhibition of the sarcoplasmic reticulum Ca^{2+} release has been shown to cause a more pronounced doublet-induced force enhancement (Stein & Parmiggiani, 1981), so it may be the case that training-dependent enhancement of Ca^{2+} dynamics might have attenuated the doublet-induced force

enhancement in the present study. However, although Ca^{2+} sensitivity has been previously reported to improve following strength (Godard, Gallagher, Raue, & Trappe, 2002) and high-intensity plyometric training (Malisoux, et al., 2006), it appears limited to type I fibres, and may not strongly influence RFD capabilities (Hvid et al., 2011; Malisoux et al., 2006).

Muscle fibre type composition influences both RFD capabilities and doublet-induced force enhancement. Although type I fibres exhibit a more pronounced response to initial double discharges (Burke, Rudomin, & Zajac, 1970; George, Binder-Macleod, Delosso, & Santamore, 1997; Thomas, Johansson, & Bigland-Ritchie, 1999), they are expected to contribute less strongly to RFD than type II fibres (see Maffiuletti et al., 2016 for review), and greater RFD is observed in muscles (or individuals) with a higher type II fibre content. Preferential hypertrophy of type II fibres is associated with improvements in MVC-normalised RFD and has been reported in response to heavy resistance training (Häkkinen et al., 1985), although with a substantially longer training period than the present study (14 weeks). It could therefore be possible that if the training induced a greater contribution of type II fibres and/or the type I fibres are more sensitive to Ca^{2+}, the effect of a double discharge might be relatively less pronounced. Additionally, it has also been shown that muscles with a higher portion of fast-twitch fibres are more likely to exhibit firing patterns initiated with brief IPIs and exhibit more doublet occurrences (Van Cutsem et al., 1998).

The compliance of the contractile apparatus as well as potential training-induced changes in tendon stiffness might offer a possible mechanical explanation for the relationship between changes in the VFT:CFT ratio and the changes in RFD_{norm}. Muscle-tendon unit stiffness has previously been shown to increase after resistance training in young adults (e.g. 50 – 84%; Kubo, Kanehisa, & Fukunaga, 2001; Malliaras et al., 2013) and an association between normalised RFD and tendon stiffness has been observed in both patellar and

Achilles tendons (Bojsen-Moller, Magnusson, Rasmussen, Kjaer, & Aagaard, 2005; Kubo et al., 2001; Waugh et al., 2014). Doublet-induced force enhancement is influenced by the compliance of the series elastic components, and although it appears that the interaction between the in-series components influences doublet-induced force enhancement, it is not yet fully understood. Abbate and colleagues (2002) estimated that series elastic element compliance accounted for ~20% of the VFT-induced force enhancement (using a triplet applied to intact fibres of mouse flexor digitorum brevis), however increased stiffness was achieved by stretching the muscle fibre. Given that Ca^{2+} sensitivity is influenced by the length of the muscle (Edman, 1975; Endo, 1972; Herzog & Leonard, 1997; Maréchal & Plaghki, 1979; Stephenson & Williams, 1982) it is possible that stretching the muscle to change compliance may not be an appropriate method when evaluating the doublet-induced force enhancement, which is influenced by both Ca^{2+} dynamics and muscle-tendon unit stiffness. Indeed, when stiffness in the muscle-tendon unit is reduced by adding artificial in-series compliance, the magnitude of the doublet-induced force enhancement has been shown to be reduced (Mayfield, Launikonis, Cresswell, & Lichtwark, 2016). The authors posit that a more compliant tendon permits internal shortening of the contractile element, interfering with the activation and cross-bridge processes governing doublet-induced force enhancement. Considering the present data, it is possible that training-dependent increases in tendon stiffness in some subjects might explain the linear relationship between RFD_{norm} and the VFT:CFT ratio and a more critical evaluation is warranted.

Significant work remains to fully elucidate the impact that varying training-related adaptations have on the magnitude of doublet-induced force enhancement, however the present data provide no evidence that improvements in the utilisation of a single doublet contribute to improved RFD capabilities following training. In fact, the observed positive linear relationship between changes in RFD_{norm} and the changes in the VFT:CFT ratio are

likely explained by an inter-subject variability in training-dependent adaptations whereby those individuals whose RFD_{norm} improved the most may have been less susceptible to changes in mechanisms that influence Ca^{2+} sensitivity or series stiffness, and thus retained their ability to make best use of high-frequency motor unit firing at contraction onset. However, this hypothesis remains to be explicitly tested.

CHAPTER 5

Conclusion

5.1 Restatement of aims

The main aim of the present research was to investigate the potential relationship between the magnitude of doublet-induced force enhancement, and training-dependent adaptations in voluntary rates of force development. This required two conditions be satisfied: (1) that a greater RFD would be evoked by NMES trains commencing with a brief (5 ms or 10 ms) initial interpulse interval than trains without, and (2) that the exercise training would prompt significant changes in voluntary RFD. Upon satisfying these conditions it was considered appropriate to examine the relationship between the training-dependent improvements in voluntary RFD and training-dependent changes in the VFT:CFT ratio (i.e. relative effect of a variable frequency train of stimulation), and to explore how adaptations in motor unit function might impact this response.

5.2 Findings

The main findings were that, even alongside training-dependent improvements in voluntary torque production including peak torque, RFD, and RFD_{norm}, no significant improvement in the VFT:CFT ratio was observed, and in fact a statistically significant decrease was observed in the response to a 10-ms initial IPI during lower-frequency (20 Hz) evoked contractions. It is therefore likely that a training-dependent improvement in the voluntary activation of muscle (e.g. earlier onset of muscle activity, increased firing frequency, increased initial discharge frequency (Van Cutsem et al., 1998)) is largely responsible for the improved capacity to quickly develop force rather than changes at the muscular level. Additional evidence was provided that shortening of the interval between the first two spikes of an otherwise-constant frequency electrical stimulation train results in a

more rapid and forceful muscle contraction in the human quadriceps muscle. Furthermore, evidence was found for a positive-linear relationship between improvements in RFD_{norm} and changes in the VFT:CFT ratio, such that those who retained more of the benefit of the variable frequency stimulation showed the greatest improvements in RFD_{norm}. These results did not support the hypothesis that utilisation of a single doublet discharge imposed at the onset of an electrically-induced contraction would improve after training, but in fact indicate that the opposite is true. However, evidence of a relationship between changes in the magnitude of the doublet-induced force enhancement and improvements in MVC-normalised RFD was observed following training, suggesting that retaining the ability to utilise double discharges is beneficial for rapidly producing force. As far as the author is aware, this is the first examination of the magnitude of the doublet-induced force enhancement and changes in the utilisation of double discharges after exercise training. These findings add to our understanding of effect of high-frequency discharge firing at the onset of contractions, and it is believed that further investigation into the mechanisms underpinning the changes in response to variable frequency stimulation trains (i.e. with high initial stimulation rates) may provide information relating to the adaptive response to resistance training.

With respect to the present thesis some limitations should be highlighted. Electrically-evoked contractions were set to elicit as close to 30% of the MVC peak torque during the 20-Hz CFT, however, force output is not linearly related to the amplitude of the stimulation intensity, thus preventing the inducement of precise force values. Although this was inherently controlled by the study design (values were normalised) and the differences were relatively small, it is unknown if the intensity difference meaningfully impacted the magnitude of the doublet force enhancement. A second limitation is that a moderate sample size was used, which necessitates cautious interpretation; subjects were selected based on

training history and familiarised extensively to improve reliability, however the project suffered a high attrition rate (3 of 11 subjects) due to circumstances unrelated to the study.

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APPENDIX A - ADVERTISEMENT

School of Exercise Biomedical and Health Sciences

AUSTRALIA ECU EDITH COWAN UNIVERSITY

Exercise and Sport Science Research Group

Is seeking

Males aged 18-35 years

Who have not participated in any formal training (strength or otherwise) in the last 12 months.

And would like to

Participate in an isometric (stationary leg) single-leg training study, and 4 testing sessions assessing maximum thigh strength, neural activation and how our muscles respond to different patterns of activation.

To enquire about participating please contact

David Murray: (08) 6304 5819 or d.murray@ecu.edu.au

Contact: David Murray
Phone: 08 6304 5819
Email: d.murray@ecu.edu.au

APPENDIX B – INFORMATION LETTER

Information Letter for Participant

Project title

The effect of maximal isometric training on rate of force development during voluntary and 'catch-inducing' muscle stimulations tests

Researchers

This research project is being performed as part of a Masters candidature (Exercise and Sports Science) at Edith Cowan University (ECU).

MSc candidate David Murray (d.murray@ecu.edu.au) 6304 5819

Supervisor A/Prof. Anthony Blazevich (a.blazevich@ecu.edu.au) 6304 5472

Further details of supervisors and the School of Exercise and Health Sciences are available at: http://www.sebhs.ecu.edu.au

Purpose

The purpose of this study is to investigate the role that a special type of nervous system activation of muscles (called motor unit double discharges, or doublets) plays in allowing fast rates of force development in the quadriceps (thigh) muscles in fatigued and non-fatigued states, and specifically whether the mechanism they exploit is trainable. The testing will therefore consist of a series of electrical impulses being delivered directly to the muscle both with (doublet stimulation) and without (non-doublet stimulation) the doublets, in non-fatigued and fatigued muscles, before and after a 4-week isometric strength training intervention. The study will demonstrate whether the training allows our nervous system to make better use of this naturally occurring muscle activation strategy.

Research

To participate in the study you (the volunteer) will be required to fill out a medical questionnaire and refrain from any intensive exercise the day prior to any of the testing days. You will be required to refrain from taking any stimulants, or depressants (or other state altering drugs) for at least 6 hours,

and alcohol at least 24 hours, prior to testing.

As a participant you will be required to attend an initial familiarisation session, 2 testing sessions performed two weeks apart on your weakest leg, a testing session performed with your strongest leg, 12 single-leg (strongest leg) isometric (i.e. non-moving) knee extensor training sessions, and finally 2 more testing sessions using the strongest leg 2 weeks apart. Thus there will be a total of 5 testing and 12 training sessions. Each testing session will be performed at the same time of day, and will be immediately preceded by a 5-minute bicycle warm-up and a series of submaximal isometric knee extension contractions.

The first session will be used to familiarise you (the volunteer) with the testing procedures including: maximal voluntary isometric knee extensions, muscle stimulations, and electromyography (recording of the small electrical signals from your muscles) preparation. Electrical stimulation will require a small electrical current to be applied to the quadriceps muscles through three electrodes placed passively and painlessly on the skin. All stimulations will begin at very low intensities, and increase progressively until the desired intensity is achieved (producing 30% of MVC). The intensity required is generally considered mildly uncomfortable, but SHOULD NOT BE PAINFUL.

The following three sessions comprise the full testing sessions, with the first two performed with the weaker leg, and the data being used to establish a control for the training (i.e. show that there are no changes in testing results when there is no training intervention).

<u>Testing</u>

The testing will be done both with (non-fatigued) and without (fatigued) the performance of a prolonged fatiguing muscle contraction. After a 5-minute warm-up on a bicycle, you will be seated in a rigid force chair to perform a series of submaximal isometric (i.e. non-moving) knee extension contractions, followed by 2 isometric maximal contractions.

The testing will then begin, and will consist of nine different electrical stimulation series' being delivered to the muscle, with and without doublets, delivered twice (18 total) in a randomised order, with 60 seconds rest between each stimulation series.

Following the non-fatigued testing there will be a 5 minute rest (where you will be free to walk around) and the 'fatigued state' testing will begin. This testing will consist of a fatiguing isometric contraction (held at 80% of maximum, lasting 30s), a 10-s rest, followed by one of the same nine stimulation series' (delivered only once each).

A typical testing session should last between 55 and 65 minutes.

Training

The training will be done over 12 sessions and performed across 4 weeks (3 sessions per week with at least 1 day between sessions) using your strongest leg. Each session will begin with a 5-minute warm-up on a bicycle, following which you will be seated upright in an isokinetic dynamometer, and complete the warm-up by performing a series of submaximal contractions. The training itself consists of 5 sets of 6 maximal isometric knee extensions held for 5 s, with a 5-s rest between reps and a 1-min rest between sets. You will be asked to produce knee extensor force 'as fast and as hard as possible' in order to generate the highest possible rate of force development.

A typical training session will last 15-20 minutes.

Table 1: Example timetable for a single participant.

	Monday	**Tuesday**	**Wednesday**	**Thursday**	**Friday**
Week 1	Familiarisation		Control		
Week 2					
Week 3			Control		Pre-Testing
Week 4	Training		Training		Training
Week 5	Training		Training		Training
Week 6	Training		Training		Training
Week 7	Training		Training		Training
Week 8		Post-Testing			
Week 9					
Week 10		Post-Testing			

Eligibility

To volunteer for this study you must:

Be a male aged between 18 and 35 years.
Have engaged in no heavy resistance training the last 12 months.
Have no history of neuromuscular diseases, or any other issue preventing maximal exercise.

Risks

As the training will consist of maximal efforts, there is a small chance of muscular strain, however given that the contractions will be performed isometrically it is extremely unlikely that any strain will exceed minor.

Though electrical stimulations may cause an uncomfortable sensation, the intensities are much too low to be painful, and the researcher will ask continuously for feedback.

The skin abrasion performed prior to the placement of the skin adhesive electromyogram electrodes (to record small electrical signals for your muscles) will be wiped with alcohol wipes in order to further minimise the already small risk of infection.

Benefits

Participation in the project will provide a unique opportunity to learn about the function of the neuromuscular system, and to see high-level data collection techniques. It will also provide the opportunity to ask questions about how humans adapt to strength training.
You will also receive free isometric knee extension strength and force production assessment.

Confidentiality of Information

Anonymity will be protected as much as possible during the investigation. Codes will be assigned to replace names and all information provided will be treated with full confidentiality by the investigator. Contact information will only be accessible during the period of the study and only by the chief researcher, and all raw data collected will only be accessible by the chief researcher and supervisor. Data will be stored in a password-protected computer only accessible by the chief researcher, and hard copy data will be kept in a locked filing cabinet in the researcher's office. The information and data that is collected during the study will be used to answer the research questions, and all data will be stored according to ECU policy and regulations following the completion of the study.

Results of Study

The results of the study will be used for completion of a Masters by Research thesis and may be presented at conferences and published in peer-reviewed journals, as magazine articles, as an online article or part of a book or report. Published results will not contain information that can be used to identify participants unless specific consent has been obtained from the participant. A copy of published results is obtainable by request from the investigator.

Voluntary Participation

Your participation in this study is entirely voluntary. No monetary reward will be provided, however $50 remuneration will be provided if you complete the training to help pay for parking and fuel costs. No explanation or justification is required if you decide not to continue, and it will not disadvantage you in any way or offer any penalty.

Withdrawing Consent

You are free to withdraw from the project at any time. You also have the right to withdraw any personal information that has been collected for the purposes of the study.

Questions/Further Information

If you have any questions or require any further information about the research project, please do not hesitate to contact:

David Murray (MSc Student – Researcher)
School of Exercise and Health Sciences, Edith Cowan University
270 Joondalup Drive, Joondalup, WA 6027, Australia - Office 19.384
Ph: (+61 8) 6304 5819
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Or

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Ph: (+61 8) 6304 5472
E-mail: a.bleazevich@ecu.edu.au

If you have any concerns or complaints about this research project you may contact an independent research ethics officer:

Research Ethics Officer
Edith Cowan University
270 Joondalup Drive
JOONDALUP WA 6027
Phone: (08) 6304 2170
Email: research.ethics@ecu.edu.au

This project has been approved by the ECU Human Research Ethics Committee.

_____ _____

Signature Date

APPENDIX C – MEDICAL QUESTIONNAIRE

Pre-exercise Medical Questionnaire

This questionnaire is designed to establish your medical history background, and identify any injury or illness that may influence the testing and your physical performance. If you are under 18 years of age, then a parent or guardian should complete the questionnaire on your behalf, or check your answers and then sign in the appropriate section to verify that they are satisfied the questions are correctly answered, to the best of their knowledge.

Please answer all questions as accurately as possible. If you are unsure about any of the questions, please ask for clarification. All information provided is strictly confidential.

Personal Details

Name:_____

Date of birth (DD/MM/YYYY):_____

Part A		If YES, please provide details
1. Are you a male over the age of 45, or female over the age of 55 who has had a hysterectomy or is postmenopausal?	Y N	_____
2. Are you a regular smoker or have you quit in the last 6 months?	Y N Unsure	_____
3. Has a close family member had heart disease or surgery, or a stroke before the age of 60 years?	Y N Unsure	_____
4. Do you have, or have you ever been told you have, blood pressure exceeding 140/90 mmHg, or do you currently take blood pressure medication?	Y N Unsure	_____
5. Do you have, or have you ever been told you have, a total cholesterol level exceeding 5.2 mmol/L (200 mg/dL)?	Y N Unsure	_____

6. Is your BMI (weight/height^2) greater than 30 kg/m^2? Y N Unsure _____

Part B

1. Have you ever had a serious asthma attack during exercise? Y N _____

2. Do you have asthma that requires medication, preventative or otherwise? Y N _____

3. Have you had an epileptic seizure in the last 5 years? Y N _____

4. Do you have any moderate or severe allergies? Y N _____

5. Do you, or could you reasonably, have an infectious disease? Y N _____

6. Do you, or could you reasonably, have a disease or infection that could be aggravated by exercise? Y N _____

7. Are you, or could you reasonably be, pregnant? Y N _____

Part C

1. Are you currently taking any prescribed or non prescribed medications? Y N _____

2. Have you had, or do you currently have any of the following? Y N _____

Rheumatic fever Y N _____

Heart abnormalities Y N _____

Diabetes Y N _____

Y N _____

Epilepsy	Y N	_____
Recurring back pain that could make exercise problematic, or that could be aggravated by exercise		
Recurring neck pain that could make exercise problematic, or that could be aggravated by exercise	Y N	_____
Any neurological disorders that would make exercise problematic, or that could be aggravated by exercise	Y N	_____
Recurring muscle or joint injuries that could make exercise problematic, or that may be aggravated by exercise	Y N	_____
A burning or cramping sensation in your legs when walking short distances	Y N	_____
Chest discomfort, unreasonable breathlessness, dizziness of fainting, or blackouts during exercise	Y N	_____

Part D

Have you had flu in the last week?	Y N	_____
Do you currently have an injury that might affect, or be affected by exercise?	Y N	_____

*Is there any other condition not previously mentioned that may affect your ability to participate in the study?

Y N _____

Have you ever been told by a medical practitioner or health care professional that you have a nerve or muscle disorder?

Y N

Do you have a heart pacemaker?

Y N

Do you have any metallic implants (e.g. bone pins)? Y N

Declaration (to be signed in the presence of the researcher)

I acknowledge that the information provided on this form, is to the best of my knowledge, a true and accurate indication of my current state of health.

Participant

Name:_____ Date (DD/MM/YYYY):_____

Signature:_____

Researcher

Signature:_____

Date (DD/MM/YYYY):_____

Parent/Guardian (only if applicable)

I, _____, as parent/guardian of Mr/Miss _____, acknowledge that I have checked the answers provided to all the questions in the medical questionnaire and verify that they are correct, to the best of my knowledge.

Signature:_____

Date (DD/MM/YYYY):_____

Practitioner (only if applicable)

I, Dr._____ Have read the medical questionnaire and information/consent form provided to my patient Mr/Miss _____, and clear him/her medically for involvement in exercise testing.

Signature:_____

Date (DD/MM/YYYY):_____

APPENDIX D – INFORMED CONSENT

DECLARATION

I [PRINT NAME] _____ have read the information provided and any questions I have asked have been answered to my satisfaction. I agree to participate in this activity, realising that I may withdraw at any time without reason or prejudice.

I understand that all information provided is treated as strictly confidential and will not be released by the investigator unless required to by law. I have been advised as to what data is being collected, what the purpose is, and what will be done with the data upon completion of the research. I agree that research data gathered for the study may be published provided my name or other identifying information is not used.

I fully understand that this research will involve my participation in:

1- Maximal isometric knee extensor training.

2- Delivery of electrical stimulation to the quadriceps muscles.

_____ _____

Signature Date

APPENDIX E – ETHICS APPROVAL

Dear David

Project Number: 8111 MURRAY
Project Name: The effect of maximal isometric training on rate of force development during voluntary and 'catch-inducing' muscle stimulation tests

Supervisors: - Anthony Blazevich

Ethics approval for your research project was granted from 23 May 2012 to 01 December 2013.

The *National Statement on Ethical Conduct in Human Research* requires that all approved projects are subject to monitoring conditions. This includes completion of an annual report (for projects longer than one year) and completion of a final report at the completion of the project.

A **FINAL REPORT** is due on **01 December 2013**.

A copy of the ethics report form can be found on the Ethics Website

Please complete the ethics report form and return the signed form to the Research Ethics Office.

Regards
Kim

Kim Gifkins
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