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## Mechanisms affecting post-activation potentiation following voluntary isokinetic knee extension

Laurent B. Seitz  
*Edith Cowan University*

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Mechanisms affecting post-activation  
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**Mechanisms Affecting Post-activation Potentiation  
Following Voluntary Isokinetic Knee Extensions**

**By**

**Laurent B. Seitz, M.Sc.**

**A thesis submitted in partial fulfilment of the requirements for the degree of  
Doctor of Philosophy**

**Principal Supervisor: Associate Professor Anthony J. Blazeovich**

**Co-supervisor: Doctor G. Gregory Haff**

**School of Exercise and Health Sciences**

**Edith Cowan University, Australia**

**August 31, 2014**

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*Laurent B. Seitz*

*August 31, 2014*

## **ABSTRACT**

The present research was designed to: 1) determine whether the voluntary PAP effects commonly observed after conditioning activity (CA; i.e. muscular contraction prior to a ‘test’ contraction) are a consequence of acute neuromuscular alterations relating to the CA itself, or whether they simply reflect warm-up and/or familiarisation effects; 2) clarify the influence of the contraction velocity, duration and total work characteristics of the CA on voluntary PAP; 3) determine the factors allowing stronger individuals to express higher level of voluntary PAP; and 4) determine the peripheral and central mechanisms of voluntary PAP in human skeletal muscle. In Study 1, the effects of different contraction velocity, duration and total work characteristics on PAP were examined after a complete warm-up. The contributions of peripheral and central mechanisms to PAP were also examined. Voluntary and electrically-evoked torques and electromyogram (EMG) data were captured before and after five different dynamic (isokinetic) CAs, after the participants had completed an extensive warm-up including extensive task-specific practice to the point where maximal voluntary contractile capacity was achieved. Vastus lateralis (VL) EMG amplitude normalised to the muscle compound action potentiation (M-wave) amplitude (EMG:M), was taken as a measure of central drive whereas twitch peak torque and M-wave amplitude were recorded to assess peripheral function. Even after a plateau in voluntary contractile capacity was achieved after the complete warm-up, the imposition of CAs elicited significant increases in both voluntary and twitch torques (i.e., PAP). CAs with longer total contraction duration (6 s) and a minimum total work of ~750-900 J produced PAP, regardless of the velocity of the CA. No changes in EMG:M were detected after any CA suggesting that central drive was not a major factor influencing PAP under the present experimental

conditions. However, the increases in twitch peak torques with lack of change in M-wave amplitude suggest that peripheral function, possibly including changes in myosin regulatory light chain (RLC) phosphorylation and increased intracellular  $\text{Ca}^{2+}$  release and sensitivity may have contributed to the observed PAP.

It is clear from the literature and the results of Study 1 that there is a significant inter-individual variability in the PAP phenomenon. Typically, stronger individuals are able to express higher levels of PAP but it is unclear why this occurs. Therefore, in Study 2 peak knee extensor torque at  $60^{\circ}\cdot\text{s}^{-1}$ , quadriceps and VL cross-sectional area (CSA) and volume, and the type II myosin heavy chain (MHC) isoform percentage (VL) were measured to determine their relative contribution to PAP elicited under voluntary conditions. There were large to very large correlations between PAP magnitude and peak knee extensor torque at  $60^{\circ}\cdot\text{s}^{-1}$  ( $r=0.62$ ), quadriceps ( $r=0.68$ ) and VL ( $r=0.62$ ) CSA, and quadriceps ( $r=0.63$ ) and VL ( $r=0.65$ ) volume. Nonetheless, these correlations were not statistically significant after adjusting for the influence of type II MHC percentage (using partial correlation analysis). By contrast, the strongest correlation was observed for type II MHC percentage ( $r=0.77$ ), and this correlation remained significant ( $r=0.56-0.66$ ) after adjusting for other variables. This finding suggests that PAP magnitude is most clearly associated with the type II MHC isoform percentage in the human quadriceps femoris. This might be explained by the fact that myosin RLC phosphorylation, one proposed mechanism responsible for PAP, has been shown to be greater in type II MHC isoforms. The results of Study 1 and Study 2 suggest that changes at the peripheral level, possibly including changes in myosin RLC phosphorylation (and increased intracellular  $\text{Ca}^{2+}$  release and sensitivity) may be a primary candidate mechanism of PAP induced by a voluntary CA, although more direct measurements are required to test this assumption.



Therefore, tetanic stimulations and maximal isokinetic knee extensions at  $180^{\circ}\cdot\text{s}^{-1}$  were used in Study 3 to provide a more detailed investigation of the role of changes in the excitation-contraction (E-C) coupling process (i.e. changes in myosin RLC phosphorylation or increased intracellular  $\text{Ca}^{2+}$  release and sensitivity) to the PAP response induced by a voluntary CA. Torques produced during voluntary knee extensions, 20 Hz and catch-inducing (20-Hz train preceded by a double pulse with 5-ms interval) stimulation trains, the 20- vs. 80-Hz torque ratio (20:80) as well as the force-augmenting effect of the catch-inducing train were recorded before and after a voluntary CA or a control condition (no CA, rest). Statistically significant increases in voluntary torque, torques elicited by 20-Hz and catch-inducing trains, and 20:80 were observed 1, 4 and 7 min after the CA. Moreover, the force-augmenting effects of the catch-inducing train diminished as the magnitude of PAP increased and then increased as the magnitude of PAP diminished. Statistically significant correlations ( $r=0.50-0.81$ ) were also found between the changes in voluntary torque production (i.e. PAP) and the changes in these variables. These results suggest that increases in PAP following a voluntary CA are strongly associated with changes in peripheral function, most probably changes in the E-C coupling efficiency.

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*Allez plus que 2 mois et demi et on s'y met un cassoulet dans le gossier !!!!!*

# **LIST OF PUBLICATIONS INCLUDED AS PART OF THE THESIS**

## **Original papers**

Seitz LB, Trajano GS, Dal Maso F, Haff GG, and Blazevich AJ. Post-activation potentiation during voluntary contractions after continued knee extensor task-specific practice. *Applied Physiology Nutrition and Metabolism*. In press (Appendix 8).

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# CHAPTER 1

## Introduction and Overview

There is significant practical interest in the idea that the performance of a maximal, or near maximal, muscle contraction (i.e. a conditioning activity; CA) might improve muscular performance in a subsequent contraction, a phenomenon termed post-activation potentiation (PAP) (Sale, 2002). In the present thesis, voluntary PAP refers to the improvement in muscular performance during a voluntary contraction in response to a CA whereas twitch PAP is the increase in the twitch response following a CA. Despite considerable literature examining the effects of CAs on subsequent muscular performance including explosive sporting activities such as jumping, throwing and sprinting, conflicting evidence has been reported with respect to the extent of improvement and the mechanisms that underpin the effect, particularly when voluntary contractions are performed during both the CA and subsequent contraction (i.e. as opposed to electrical nerve or muscle stimulation). For example, increases in voluntary plantar flexor peak torque at  $180^{\circ}\cdot s^{-1}$  (Miyamoto, Kanehisa, Fukunaga, & Kawakami, 2011a) and countermovement jump height (Young, Jenner, & Griffiths, 1998) were observed 1 to 5 minutes following a 6-s isometric maximal voluntary contraction (MVC) of the plantar flexors and 5 repetitions maximum (RM) in the back squat exercise, respectively. Nonetheless, other studies have reported significant decreases in muscular performance following the execution of a voluntary CA (Chiu et al., 2003; Duthie et al., 2002). Also, significant decreases in 10, 30 and 40 m sprint times were observed 3 to 5 minutes following the performance of 10 sets of 1 repetition back squat performed at 90% 1-RM (Chatzopoulos et al., 2007) or 2 sets of 4 back squats at 80% 1-RM (Rahimi, 2007). Additionally, studies have also reported a lack of significant effect of a voluntary CA on

vertical jump height (Mangus et al., 2006; Scott & Docherty, 2004) and dynamic knee extension torque (Gossen & Sale, 2000). Collectively, these findings indicate a lack of conclusive evidence as to whether voluntary PAP can be reliably elicited. Thus, more research is required to determine the factors that influence PAP, when elicited under voluntary conditions, so that clear protocols for its development might be implemented in clinical, athletic and other populations.

It has been suggested that the characteristics of both the CA and the individual as well as the type of subsequent activity influence the magnitude of voluntary PAP (Tillin & Bishop, 2009). For instance, increases in muscular performance following fast- but not slow speed (isokinetic) CAs have been previously reported (Chaouachi et al., 2011). However, the two CAs provided different contraction durations (3 and 15 s, respectively), so the relative influence of isokinetic CA velocity on the development of voluntary PAP could not be specifically determined. There is also evidence that the contraction duration of an isometric CA influences twitch PAP (Vandervoort, Quinlan, & McComas, 1983), but it has yet to be determined whether this occurs under dynamic contractions. Moreover, the total work performed during the CA might also influence the voluntary PAP response because too little work may not trigger the mechanism(s) responsible for PAP whereas too much work may induce high levels of fatigue, thus masking the potentiation effects. Nonetheless, the influence of the total work of the CA on voluntary PAP has not yet been examined. Although there is also considerable evidence suggesting that an individual's strength level influences the voluntary PAP response (Chiu et al., 2003; Jo et al., 2009; Ruben et al., 2010; Seitz et al., 2014a; Seitz, Trajano, & Haff, 2014b), it is not clear why this occurs. One possibility is that stronger individuals tend to have a greater percentage of type II muscle fibres (Aagaard & Andersen, 1998; Maughan, Watson, & Weir, 1983b; Thorstensson, Grimby, & Karlsson, 1976), which has been associated with greater twitch PAP responses (Hamada, Sale, MacDougall, & Tarnopolsky, 2000; Hamada, Sale, MacDougall, & Tarnopolsky, 2003),



although this has not always been observed in human skeletal muscle (Stuart, Lingley, Grange, & Houston, 1988). A final important consideration is that PAP studies have used different research designs, and in particular different warm-up procedures, which might have affected voluntary PAP responses. With respect to the influence of the type of subsequent activity on PAP, it has been hypothesised that whilst a specific CA might enhance a particular subsequent activity, it might decrease or have no effect on the performance of a different activity (Tillin & Bishop, 2009). For instance French et al. 2003 reported increases in drop jump and knee extension performance following a 3-s isometric knee extension but no changes in 5-s cycle sprint performance. These findings suggest therefore that the kinematics of the CA should closely match those of the subsequent activity.

Overall, the mechanisms of the voluntary PAP phenomenon's and the factors influencing them are not completely defined. This has ensured that strategies to elicit PAP cannot be specifically developed. With regards to mechanisms, increases in EMG and H- (Hofmann) reflex amplitudes have been traditionally taken as evidence of an influence of central (neural) mechanisms on voluntary PAP (Folland, Wakamatsu, & Fimland, 2008; Gullich & Schmidtbleicher, 1996; Hough, Ross, & Howatson, 2009; Trimble & Harp, 1998). However, several methodological limitations exist that have prevented clear conclusions being drawn. For example, Hough et al. (2009) associated the potentiation effects during a vertical jump task with increases in EMG activity, thus providing reasonable evidence that increased motor unit recruitment contributed to the observed potentiation. However, changes in EMG activity during potentiation might result from both central and/or peripheral mechanisms (Arabadzhev, Dimitrov, Dimitrova, & Dimitrov, 2010; Thomas, Johansson, & Bigland-Ritchie, 2006), so it cannot specifically be determined whether an increase in central drive occurred. Furthermore, the increases in H-reflex amplitude reported in several studies can be taken as good evidence of an increased excitability of the motor neurone pool, which could improve motor unit recruitment and thus induce a PAP effect (Folland et al., 2008;

Gullich & Schmidtbleicher, 1996; Trimble & Harp, 1998). However, in these studies the H-reflex amplitude was assessed in non-contracting muscle, which may not adequately reflect changes occurring at the spinal level during contraction (Aagaard, Simonsen, Andersen, Magnusson, & Dyhre-Poulsen, 2002b; Voigt, Chelli, & Frigo, 1998). Thus, there is still some question as to the magnitude of increase in the H-reflex during contraction after a voluntary CA. As yet no studies have examined changes in motor neurone excitability during muscle contraction and other more direct measures of cortico-spinal drive (e.g. cortico-electrical/magnetic stimulation or V- and F-wave measurement) have not been taken. Therefore, the relative contribution of central mechanisms to the voluntary PAP effect remains unclear.

PAP is also thought to occur through alterations at the peripheral (muscular) level, as suggested by increased myosin regulatory light chain (RLC) phosphorylation in response to a PAP-inducing CA (Grange, Vandenboom, & Houston, 1993; Moore & Stull, 1984; Palmer & Moore, 1989; Sweeney, Bowman, & Stull, 1993). However, despite such a mechanism being relatively well defined in animal models (Grange et al., 1993; Moore & Stull, 1984; Palmer & Moore, 1989; Sweeney et al., 1993) there is a lack of evidence for humans (Stuart et al., 1988). For example, Stuart et al. (1988) reported no association between myosin RLC phosphorylation and twitch potentiation after a 10-s isometric MVC. Therefore, more detailed investigation is required to more clearly determine the influence of changes in peripheral function on voluntary PAP. In the following literature review, the influence of central and peripheral mechanisms on PAP will be considered with a view to developing specific testable hypotheses for future research.

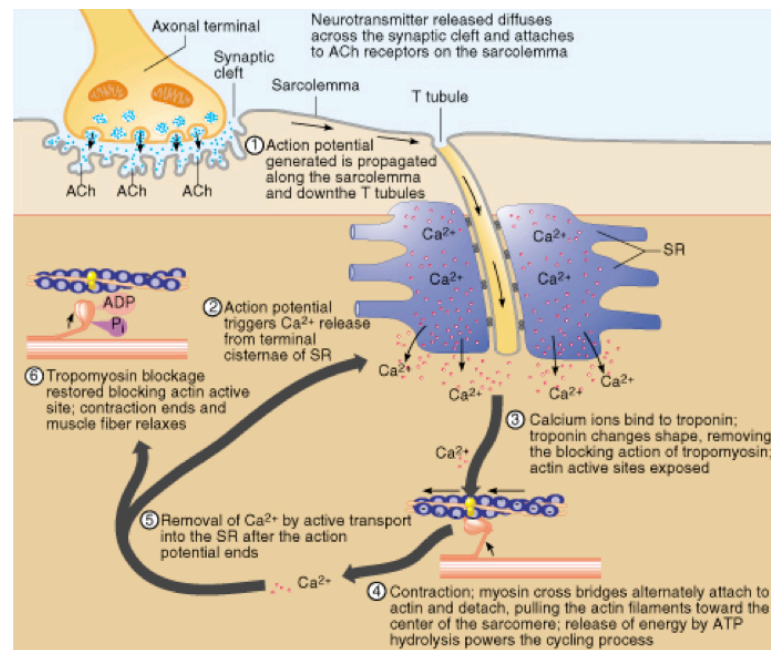
## **1.1 Process of voluntary muscle force production**

The development of skeletal muscle force is a complex process involving a series of events occurring at both the central (neural) and peripheral (muscular) levels. When a nerve

impulse emanating from the motor cortex and triggering motor neurone activity reaches the motor end plate, voltage-dependent  $\text{Ca}^{2+}$  channels located in the axonal membrane open, allowing  $\text{Ca}^{2+}$  to flow from the extracellular fluid into the presynaptic neuronal terminal. The entry of  $\text{Ca}^{2+}$  into this terminal allows the release of the neurotransmitter acetylcholine into the synaptic cleft that binds to acetylcholine receptors on the sarcolemma. This binding causes the ion channels located in the acetylcholine receptors to open and allow the passage of sodium (inward) and potassium (outward) causing the depolarisation of the membrane. Once the membrane is depolarised, a membrane potential is generated and then propagates along the sarcolemma and down the transverse (t)-tubules, initiating the excitation-contraction (E-C) coupling process in the muscle cell (Figure 1.1). Depolarisation of the T-tubules triggers the opening of voltage-dependent  $\text{Ca}^{2+}$  channels (i.e. dihydropyridine receptors) located on the sarcolemma and the paired L-type voltage-dependent calcium channels (i.e. ryanodine receptors), allowing sarcoplasmic release of  $\text{Ca}^{2+}$  into the myoplasm which is then available to bind to troponin C (Balog, 2010). This binding ultimately modulates tropomyosin activity and shifts the actin filament from a blocked to a closed state allowing weak interaction between myosin heads and actin in a process known as crossbridge cycling. This process improves actin affinity for  $\text{Ca}^{2+}$  thus allowing additional crossbridges to be formed. As a result, tropomyosin is relocated from actin binding sites thus uncovering binding sites for the myosin head on the actin filament (Vandenboom, 2004). As myosin heads bind to actin, ATP is hydrolysed releasing inorganic phosphate that initiates a crossbridge power stroke (Vandenboom, 2004), during which the myosin heads rotate towards actin and pull Z-lines toward the centre of the sarcomere to produce force (Huxley & Hanson, 1954). It is commonly considered that the phosphorylation of the myosin RLC, especially in type II muscle fibres, is an important step influencing  $\text{Ca}^{2+}$  sensitivity of the myofibres, and thus force development (Grange, Cory, Vandenboom, & Houston, 1995; Grange, Vandenboom,

Xeni, & Houston, 1998). Therefore, interventions aimed at increasing phosphorylation might be expected to also influence muscular force.

The ability to generate force during a contraction is also governed by the ability of the central system to appropriately activate the muscles involved (Marieb & Hoehn, 2007). The activation of muscles is primarily controlled through changes in motor unit recruitment, rate coding, motor unit synchronisation and neural inhibition (Marieb & Hoehn, 2007). The number and type of motor units recruited influence the force produced by a muscle. According to the Henneman's size principle motor units are recruited in a systematic order during voluntary contractions: small  $\alpha$ -motoneurons innervating type I fibres are initially activated at low force levels while large  $\alpha$ -motoneurons activating type IIa and IIx are typically activated later at high force levels (Henneman & Olson, 1965). Another important determinant for force production is related to the rate coding of motor units. Rate coding can influence the muscle force generation capacity by enhancing the magnitude of force generated during contraction and by impacting the rate of force development (Marieb & Hoehn, 2007). The synchronisation of motor units involves the simultaneous activation of numerous motor units and has hypothetically been associated with increased force production and to positively influence the rate of force development during a contraction (Marieb & Hoehn, 2007). The last component influencing force production is the inhibition of neuromuscular activation that could be caused by inhibitory feedback via sensory group I and II afferents (Aagaard et al. 2002b).



**Figure 1.1.** Excitation-contraction coupling process (from Marieb & Hoehn, 2007).

## 1.2 Effect of warm-up activities on performance

Researches have assumed that warm-up activities, prior to exercise or sport participation, have the potential to improve subsequent performance via several physiological and psychological changes (Bishop, 2003; Fradkin, Zazryn, & Smoliga, 2010). For example, increases in peak power output (Dolan, Greig, & Sargeant, 1985; Stewart, Macaluso, & De Vito, 2003) and MVC (Stewart et al., 2003) have been observed following the completion of an active warm-up. From a physiological perspective, the proposed benefits of warm-up appear to be largely attributable to an increase in muscle temperature, as indicated by increases in maximal isokinetic torque, vertical jump height and sprint performance subsequent to an increase in muscle temperature (Bergh & Ekblom, 1979). Increases in muscle temperature may improve subsequent performance by decreasing muscle stiffness (Buchthal, Kaiser, & Knappeis, 1944), increasing nerve conduction velocity (Karvonen, 1992) and rate of force development (Young & Behm, 2003), and reducing the time to peak force (Davies & Young, 1983). In addition, increasing muscle temperature may also improve

the rate of myosin ATPase activity (Binkhorst, Hoofd, & Vissers, 1977), ultimately increasing both cross-bridge cycling rate and maximum fibre/muscle shortening velocity (Bárány, 1967). Warm-up may also have some psychological effects that contribute to improved performance, since it may increase motivation or provide time to concentrate on or plan for a task (Bishop, 2003). Additionally, learning effect can also influence subsequent performance if an individual has not previously been given the opportunity to practice the test (i.e. familiarisation) (MacIntosh, Robillard, & Tomaras, 2012).

The voluntary PAP phenomenon has been explored in studies that have used different warm-up activity strategies. Some researchers have imposed standardised warm-up activities including cycling or running, light stretching and sub-maximal repetitions of the performance test (Duthie, Young, & Aitken, 2002; French, Kraemer, & Cooke, 2003; Jo et al., 2009). Others studies did not appear to use a warm-up before the baseline testing (Gossen & Sale, 2000; Hamada et al., 2000; Jubeau, Gondin, Martin, Van Hoecke, & Maffiuletti, 2010; Miyamoto et al., 2011a; Miyamoto, Yanai, & Kawakami, 2011b). While the findings of the aforementioned studies are important from a practical standpoint, since they show that muscular performance can be improved after a CA, it is not known whether the increase in muscular performance commonly observed after a PAP-inducing CA is a consequence of acute neuromuscular alterations relating to the CA itself or whether it simply reflects warm-up and/or familiarisation effects (MacIntosh et al., 2012). Therefore, it has yet to be determined whether performing a voluntary CA contributes to improved voluntary muscular functional performance after maximal muscle contractile capacity has been attained through a complete warm-up and task practice, (MacIntosh et al., 2012). Such a finding would be of significant practical interest to those who commonly complete a full warm-up routine prior to sport or exercise participation, and thus seek an additional strategy to enhance muscular performance. Repeating previous studies after a complete warm-up period would allow relevant conclusions to be drawn.

### **1.3 Neuromuscular mechanisms of the PAP effect**

Although PAP may occur through an increased recruitment of higher-order (Type II) motor units and myosin RLC phosphorylation (Tillin & Bishop, 2009), the relative contribution of these mechanisms to the voluntary PAP effect remain unclear. Determining the impact of these mechanisms will help indicate the appropriate site(s) for targeted interventions to enhance the voluntary PAP response, and therefore how to elicit PAP in individuals who do not seem to respond to a CA.

#### **1.3.1 Central factors influencing PAP**

An increased recruitment of higher-order (Type II) motor units has traditionally been accepted as one mechanism responsible for PAP under voluntary contraction conditions (Hodgson, Docherty, & Robbins, 2005; Tillin & Bishop, 2009). Specifically, increases in H-reflex amplitude, which is a function of the size and number of motor units recruited during an electrically-elicited afferent volley, have been reported after completing a voluntary CA (Folland et al., 2008; Gullich & Schmidtbleicher, 1996; Trimble & Harp, 1998). It is assumed that these increases reflect reflex transmission between Ia afferent terminals and the post-synaptic membranes of the  $\alpha$  motor neurons of homologous muscle. This would occur through an increase in motor neurone excitability or decreased pre-synaptic inhibition and thus increased action potential propagation from Ia terminals across synaptic junctions at the spinal cord (Luscher, Ruenzel, & Henneman, 1983). Because the recruitment of motor units by Ia afferents follows an orderly pattern from smallest to largest diameter (Henneman's size principle; Henneman & Olson, 1965), the increases in H-reflex amplitude after a voluntary CA might reflect the increased activation of subsequently larger, higher threshold motor units. Given that higher-order motor units, including large type II, are more difficult to recruit, it is assumed that a voluntary CA may increase the contribution of higher-order motor units to muscular contraction and thus increase muscular performance during a subsequent contraction

(Gullich & Schmidtbleicher, 1996; Hodgson et al., 2005). The belief that increased recruitment of higher-order (Type II) motor units contributes to voluntary PAP is based on the initial work of Gullich and Schmidtbleicher (1996), who reported significant correlations ( $r=0.90$ ) between the time course of changes in H-reflex amplitude and voluntary torque production (i.e. voluntary PAP after a 5-s isometric leg press CA). However, this result may be partly influenced by inherent methodological constraints associated with H-reflex measurements. First, the H-wave amplitude was not normalised to the compound muscle action potential (M-wave) amplitude and therefore increases in peripheral conductance, including increased  $\text{Na}^+\text{-K}^+$  pump activity (Thomas et al., 2006), may have influenced the H-reflex response. Second, the H-reflex response appears to have been elicited in resting muscle, which may not adequately reflect changes occurring at the spinal level during muscular contraction (Aagaard et al., 2002b; Voigt et al., 1998). Other studies that have assessed changes in H-reflex amplitudes after a voluntary CA (Folland et al., 2008; Trimble & Harp, 1998) also provide good evidence for an increased recruitment of higher-order (Type II) motor units given that the researchers normalised the H-wave amplitudes to M-wave amplitudes. However, H-reflex appears to have been elicited at rest (Folland et al., 2008), although this is not made explicitly clear in the study by Trimble and Harp (1998). Furthermore, the relative contribution of increased H-reflex amplitude to voluntary PAP remains undetermined because no attempt has been made to examine the relationship between changes in the normalised H-reflex amplitude measured during muscle contraction, and changes in voluntary PAP. It is likely that increases in both spinal excitability and, ultimately, recruitment of higher-order motor units, may have a finite influence on voluntary PAP because the upper limit of motor unit recruitment is approximately 85% of the maximal force in most muscles (Duchateau, Semmler, & Enoka, 2006). Therefore, there may be minimal scope for an increased recruitment of higher-order (Type II) motor units contributing to voluntary PAP after a maximal voluntary CA. Taken collectively, these findings suggest that



the increases in H-reflex observed in these studies cannot be taken as evidence of increased motor unit recruitment contributing to voluntary PAP.

Voluntary PAP was also associated with an increase in EMG amplitude in human skeletal muscle following a voluntary CA (Hough et al., 2009). The authors concluded that increased motor unit recruitment contributed to the observed potentiation given the commonly observed increase in surface EMG amplitude with increases in motor unit recruitment (Farina, Fosci, & Merletti, 2002). However, such findings should be interpreted with caution, since changes in EMG activity might result from both central and/or peripheral mechanisms, such as lengthening of the intracellular action potential profile (Arabadzhev et al., 2010) or increases in  $\text{Na}^+$ - $\text{K}^+$  pump activity (Thomas et al., 2006). Normalisation of the EMG amplitude to the M-wave amplitude is considered to largely eliminate the effect of peripheral changes in membrane excitability and thus provide a reasonable indicator of central drive (Arabadzhev et al., 2010; Lepers, Maffiuletti, Rochette, Brugniaux, & Millet, 2002; Millet & Lepers, 2004). However, no changes in EMG:M following the performance of a voluntary CA inducing voluntary PAP were observed in the plantar flexors (Fukutani, Miyamoto, Kanehisa, Yanai, & Kawakami, 2013). This finding suggests that changes in central drive may not be a major factor influencing voluntary PAP. However, additional experiments are required to confirm these findings under various experimental conditions. Ultimately, experiments using more detailed methodologies, such as cortical or cortico-medullary stimulation, will probably be required to determine more clearly the contribution of central mechanisms to voluntary PAP.

### **1.3.2 Peripheral factors influencing PAP**

#### ***1.3.2.1 Neuromuscular transmission and excitability of the muscle fibre's sarcolemma***

The analysis of the M-wave amplitude would provide information regarding changes in neuromuscular transmission and excitability of the muscle fibre's sarcolemma (Lepers et al., 2001). While increases in M-wave amplitude have been reported following a voluntary CA that induced twitch PAP (Hamada et al., 2000; Jubeau et al., 2010), several studies also report no change in the M-wave amplitude in the presence of either twitch (Miyamoto et al., 2011a; Miyamoto et al., 2011b) or voluntary (Miyamoto et al., 2011a) PAP after a voluntary CA. One explanation for these contrasting findings is that the participants in these studies may or may not have completed a warm-up to the point where no further performance enhancement could be possible and thus differences in muscle temperature etc. may be present. Therefore, at this time there is no clear evidence as to whether M-wave amplitude can be taken as a reliable indicator of changes in peripheral function after a PAP-inducing voluntary CA. Future research should assess changes in M-wave amplitude after a complete task-specific warm-up is performed.

#### ***1.3.2.2 Excitation-contraction (E-C) coupling process***

Changes at the muscular level, and specifically in the E-C coupling process may be important to the observed PAP process because of the strong correlation observed between the magnitude of phosphorylation of the myosin RLC and increases in twitch force in response to a PAP-inducing CA in animals (Grange et al., 1993; Moore & Stull, 1984; Palmer & Moore, 1989; Sweeney et al., 1993). Increased myosin RLC phosphorylation, through activation of  $\text{Ca}^{2+}$ -calmodulin-dependent myosin kinase (Stull, Nunnally, Moore, & Blumenthal, 1985), is thought to potentiate subsequent muscular contraction by increasing the sensitivity of actin-myosin to  $\text{Ca}^{2+}$  released from the sarcoplasmic reticulum by the ryanodine receptors (Grange et al., 1993; Palmer & Moore, 1989; Vandenboom, Grange, & Houston,

1995). This subsequently increases the likelihood of myosin interaction with actin and thus the number of strongly bound cross-bridges (Levine, Kensler, Yang, Stull, & Sweeney, 1996; Sweeney, Yang, Zhi, Stull, & Trybus, 1994), resulting in an increase in muscle tension (Barany, Barany, Gillis, & Kushmerick, 1980; Manning & Stull, 1982; Metzger, Greaser, & Moss, 1989). As a consequence, myosin RLC phosphorylation has its greatest effect in circumstances in which  $\text{Ca}^{2+}$  concentrations are relatively low, such as during twitches, sub-tetanic frequencies of stimulation (Baudry et al. 2008). Although these studies have been essential in defining the influence of myosin RLC phosphorylation on twitch PAP in rodent muscle, there is a lack of evidence for human skeletal muscle as indicated by the lack of association between the phosphorylation of the myosin RLC and twitch potentiation after a 10-s isometric MVC (Stuart et al., 1988). Furthermore, Smith & Fry (2007) reported that individuals who responded positively to a voluntary CA exhibited an increase in myosin RLC phosphorylation whereas those who had a significant decrease in myosin RLC phosphorylation did not improve their voluntary muscular performance post-CA. Evidence for changes in the E-C coupling process in animal models is also supported by increases in the ratio of the forces produced during 60- vs. 200-Hz frequency trains during constant frequency nerve stimulation in the type II twitch muscles of rodents after a PAP-inducing CA (Rijkelijkhuizen, De Ruiter, Huijing, & De Haan, 2005). Such findings suggest that a similar technique might be used in humans, where direct measurement of the phosphorylation status of the myosin RLC has proved problematic. Collectively, these findings indicate that changes in the E-C coupling process may underpin PAP in skeletal muscle, although more detailed investigation is required to confirm this assumption in humans. Changes in the E-C coupling process may be detectable through the analysis of (i) the characteristics of twitch in response to muscle or nerve electrical stimulation and (ii) the ratio of the forces (or joint torques) produced at 20- vs. 80-Hz frequency trains (20:80) during constant frequency stimulation. The 20:80 ratio provides information about the release of  $\text{Ca}^{2+}$  from the sarcoplasmic

reticulum into the myoplasm and about  $\text{Ca}^{2+}$  sensitivity (Jones, 1996; MacIntosh & Rassier, 2002; Martin, Millet, Martin, Deley, & Lattier, 2004). Although fatiguing exercise in humans has been reported to cause a decrease in this ratio (Jones, 1996; Martin et al., 2004), it has not been determined whether the ratio is altered after a PAP-inducing voluntary CA in humans. Collectively, changes in the characteristics of the twitch and 20:80 measured after a complete task-specific warm-up might be taken considered important evidence for an influence of the E-C coupling process on the voluntary PAP phenomenon.

### ***1.3.2.3 Muscle “catchlike” properties***

Theoretically, changes in the muscle’s catchlike properties may also influence muscular force production after a PAP-inducing CA, since this phenomenon is thought to be underpinned by increased sarcoplasmic  $\text{Ca}^{2+}$  release (Cheng, Place, Bruton, Holmberg, & Westerblad, 2013) and/or increased  $\text{Ca}^{2+}$  sensitivity (Abbate, Bruton, De Haan, & Westerblad, 2002; Nielsen, 2009). The catchlike property of skeletal muscle refers to the tension enhancement occurring when an initial, short-interval (e.g. 5 ms) double-spike stimulus precedes a train of constant-frequency stimuli; trains of stimuli that take advantage of the catchlike property are called catch-inducing trains (Burke, Rudomin, & Zajac, 1970). The muscle’s catchlike property can be assessed through the analysis of catch-inducing trains and of the force-augmenting effects of the catch-inducing train (Ding, Storaska, & Binder-Macleod, 2003). The latter variable represents the extra force produced during a catch-inducing train (e.g. 20-Hz train preceded by a doublet) in comparison to an identical constant frequency train without a doublet. Increases in force produced during a catch-inducing train have been reported in human skeletal muscle following a PAP-inducing electrically-evoked CA (Stuart, Binder-Macleod & Lee, 1996; Ding et al., 2003), providing evidence that such changes may also influence voluntary PAP after voluntary CAs. However, no attempt has been made to determine whether such changes occur after voluntary PAP-inducing CAs.

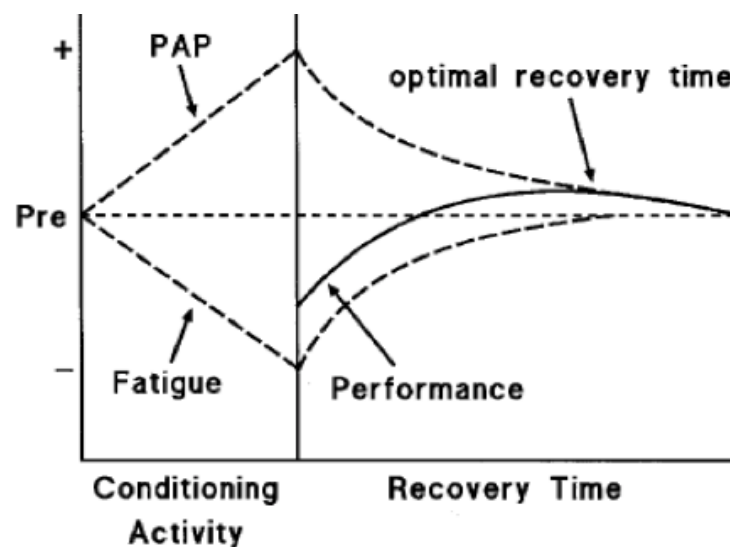
Moreover, it has also been shown that the extent of force produced during a catch-inducing train, in comparison to a constant low frequency train, is reduced in the presence of potentiation but increased in the presence of fatigue in both animals (Bevan, Laouris, Reinking, & Stuart, 1992; Burke, Rudomin, & Zajac, 1976) and humans (Ding et al., 2003). This finding suggests that the mechanisms of PAP (induced by electrically-evoked CAs) and the catchlike property are not mutually exclusive. Nevertheless, the effect of a voluntary PAP-inducing CA on the catchlike property of skeletal muscle has yet to be determined. Thus, in addition to analyses of the M-wave amplitude and 20:80 ratio, quantification of the changes in force production during catch-inducing trains and the force-augmenting effects of catch-inducing trains before and after a voluntary PAP-inducing CA could help explain the contribution of peripheral mechanisms to PAP elicited under voluntary conditions.

## **1.4 Factors influencing PAP**

### **1.4.1 Balance between force depression and potentiation**

Increases in muscular performance after a CA appear to depend on the net balance between fatigue (i.e. force depression) and potentiation co-existing (Rassier & Macintosh, 2000). Therefore, muscular performance may decrease if the effects of fatigue dominate the effects of potentiation, be enhanced if potentiation dominates, or remain unchanged if fatigue and potentiation are at similar levels. This relationship between fatigue and potentiation is illustrated in Figure 1.2. It is generally accepted that fatigue tends to dominate potentiation in the early stages of the recovery after a CA, therefore masking the PAP effect and ultimately decreasing muscular performance. However, because the effects of fatigue tend to dissipate at a faster rate than changes causing potentiation, muscular performance can be improved at some point during the recovery period. However, if the time between the CA and the subsequent performance is too long, the ability to express PAP will decrease and no

potentiation effect will be observed. Therefore, there is probably an optimal recovery time during which muscular performance is maximised (Sale, 2002). Although this temporal profile of fatigue and potentiation is largely theoretical, it has been highlighted by several studies reporting a decrease in vertical jump performance 15 s after a CA but increases 3-16 minutes into recovery (Crewther et al., 2011; Kilduff et al., 2007; Seitz et al., 2014a; Seitz et al., 2014b). However, it is worth noting that it has not always been observed (French et al., 2003; Gourgoulis, Aggeloussis, Kasimatis, Mavromatis, & Garas, 2003). One explanation for this finding is that the balance between fatigue and potentiation is likely to be influenced by the characteristics of both the CA and the individual.

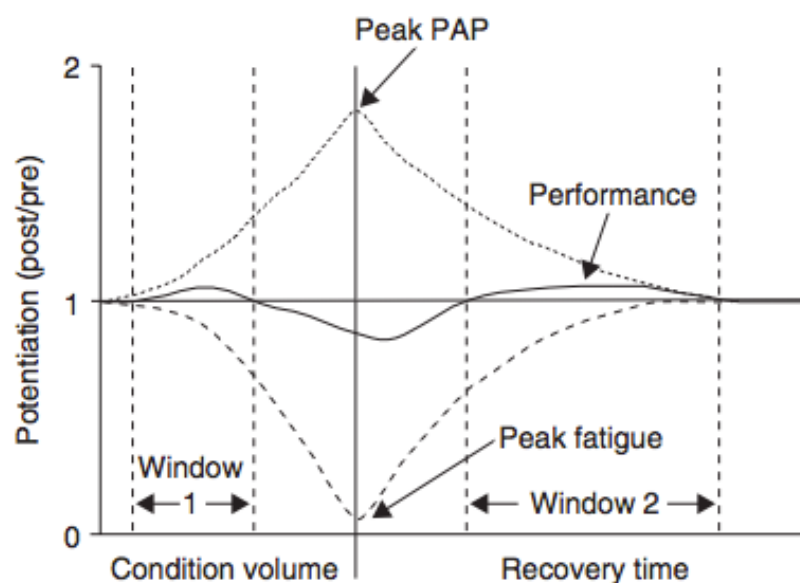


**Figure 1.2.** A theoretical strategy for explaining the relationship between fatigue, PAP and performance. The conditioning activity induces both fatigue and potentiation and subsequent muscular performance will depend on the balance between these two parameters. Generally, fatigue dominates potentiation during the early stages of recovery after the condition activity. However, the effects of fatigue may dissipate more rapidly than the effects of potentiation, and muscular performance may thus increase (from Sale, 2002).

#### 1.4.2 Influence of the loading volume and intensity of the conditioning activity on PAP

Researchers have assumed that the magnitude and time course of voluntary PAP can be influenced by the loading volume and intensity of the CA (Wilson et al., 2013). Generally, the lower the loading volume of the CA the less fatigue will be induced and the sooner a

potentiation of muscular force might be observed (i.e. Window 1 in Figure 1.3). However, if the loading volume is too low, the mechanism(s) responsible for voluntary PAP may not be triggered. Conversely, greater loading volume should induce greater fatigue and thus increase the time before a performance gain might be observed (Window 2 in Figure 1.3), although too much fatigue may offset potentiation. A recent meta-analysis by these authors determined that CAs comprising multiple sets may produce greater voluntary PAP effects than single sets in trained individuals whereas single sets may be more beneficial in less experienced individuals. Research also indicates that CAs of moderate intensities (60-85% 1-RM) may be more beneficial than those of higher intensities (>85% 1-RM) (Wilson et al., 2013).



**Figure 1.3.** A model of the hypothetical influence of the loading volume of the conditioning activity on PAP (from Tillin & Bishop, 2009). PAP can be realised sooner after a low-volume conditioning activity (Window 1) or later after a high-volume conditioning activity (Window 2).

### 1.4.3 Influence of contraction velocity, duration and total work of the conditioning activity on PAP

Little is known about the influence of the contraction velocity, duration and total work of a voluntary CA on voluntary PAP. One study assessed voluntary PAP following isokinetic

CAs of different velocities, which allows accurate CA velocity to be achieved and therefore for an accurate assessment of the effects of CA contraction velocity to be studied (Chaouachi et al., 2011). The results show that a fast-speed ( $300^{\circ}\cdot\text{s}^{-1}$  knee extension) CA increased subsequent voluntary knee extensor torque production in fast- ( $300^{\circ}\cdot\text{s}^{-1}$ ) but not slow-speed ( $60^{\circ}\cdot\text{s}^{-1}$ ) tests. Surprisingly, however, a slow-speed CA ( $60^{\circ}\cdot\text{s}^{-1}$ ) had no effect on either fast- or slow-speed voluntary peak torque production. These results are indicative of a velocity-specific effect of the CA on subsequent voluntary muscular performance. However, a warm-up to the point where voluntary torque production was stable (i.e. complete warm-up) was not performed, so the possibility exists that this result reflects a learning effect from the performance of velocity-specific practice. It would be appropriate to replicate this study using a complete task-specific warm-up to ensure that baseline voluntary muscle performance is stable prior to the performance of the CA. Moreover, the slow- and fast- velocity CAs provided different contraction durations (15 vs. 3 s, respectively). Therefore, the precise influence of the velocity of dynamic CAs on voluntary PAP remains unclear. It seems likely that altering the velocity of a CA will also alter other parameters such as the contraction duration and total work performed during the CA. The contraction duration of an isometric CA appears to influence twitch PAP responses (Vandervoort et al., 1983). For example, Vandervoort et al. (1983) compared the influence of the duration of isometric MVCs of the dorsiflexors on twitch PAP. The results show that a 10-s CA induced greater twitch PAP responses than 1-, 3- and 30-s CAs, whilst a 60-s CA resulted in force depression, probably because it induced greater levels of fatigue (Vandervoort et al., 1983). Regardless, determining whether the contraction duration of dynamic (isokinetic) CAs influences the voluntary PAP response may be worthwhile.

As an additional factor, the total work performed during a CA may also influence voluntary PAP but this hypothesis has yet to be explicitly tested. It is likely that the contraction duration and total work of CAs may influence voluntary PAP because shorter



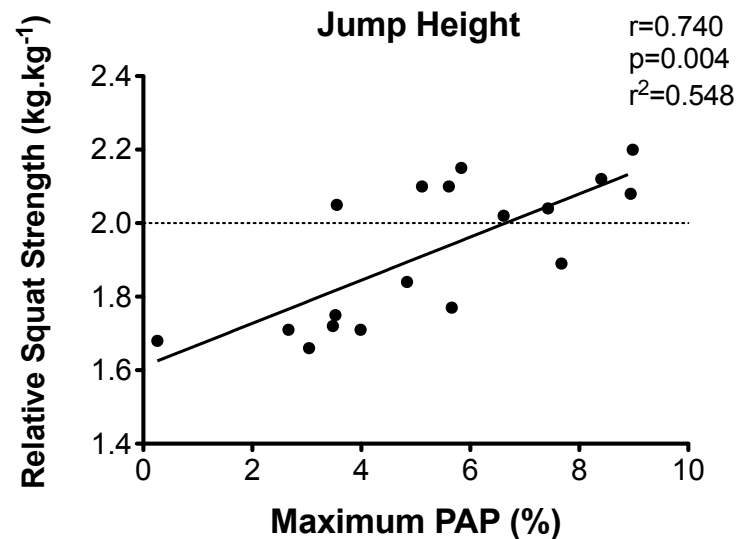
contraction durations (or inadequate volume of work) may prevent the triggering of the mechanism(s) responsible for potentiation, whilst longer contraction durations or (too much work) may induce excessive fatigue and therefore offset potentiation effects (Rassier & Macintosh, 2000). The testing of CAs with both different contraction velocities and durations would allow for their effects to be examined simultaneously with the effect of total work.

#### **1.4.4. Influence of strength level on voluntary PAP**

Numerous studies indicate that an individual's strength level may partly dictate the voluntary PAP response (Chiu et al., 2003; Gourgoulis et al., 2003; Jo et al., 2009; Kilduff et al., 2007; Ruben et al., 2010; Seitz et al., 2014a; Seitz et al., 2014b). For example, a significant correlation was observed between relative back squat strength and the magnitude of potentiation in vertical (Seitz et al., 2014a) and horizontal (Ruben et al., 2010) jump tests, suggesting that stronger individuals (i.e. those who can squat  $>2\times$  body mass) might express greater voluntary PAP responses when compared to their weaker counterparts (Figure 1.4). A correlation between strength level and voluntary PAP was revealed in a study examining the relationship between voluntary PAP magnitude in 20-m sprint test and both back squat and power clean strength (Seitz et al., 2014b). Collectively, these studies indicate that the magnitude of the voluntary PAP response achieved after the performance of a voluntary CA is related to an individual's strength level, however it is not clear why this occurs.

One hypothesis is that stronger individuals may have a greater percentage of type II muscle fibres (Aagaard & Andersen, 1998; Maughan et al., 1983b; Thorstensson et al., 1976) and therefore are more likely to exhibit PAP effects (Hamada et al., 2000) since phosphorylation of the myosin light chain is greater in type II muscle fibres (Moore & Stull, 1984). A greater muscle cross-sectional area (Ikai & Fukunaga, 1968; Maughan, Watson, & Weir, 1983a) or volume (Fukunaga et al., 2001) may characterise stronger individuals, and therefore any increase in tissue-specific force elicited by a CA will be amplified in them.

Nevertheless, whether a relationship exists between voluntary PAP and muscle size has yet to be tested. However, it is not yet clear whether the level of muscular strength of an individual is causative, or only correlative, of the magnitude of voluntary PAP exhibited. To determine why stronger individuals benefit from the performance of a voluntary CA, each of these factors should be studied in detail.



**Figure 1.4.** The correlation between relative back squat strength and maximum voluntary PAP response (from Seitz et al., 2014a). A greater PAP effect was observed in stronger individuals (e.g. who could squat  $>2\times$  body mass) than weaker individuals.

## 1.5 Conclusions

While this review provides evidence that increases in muscular performance are possible after the performance of CAs, the response is not consistent between studies or between individuals within studies. One explanation for this is that factors such as muscle temperature and task familiarisation (in the period immediately before a test) may vary. These factors are influenced by the warm-up procedures, which appear to vary significantly between studies (or are not reported). Thus, there is a need to complete studies where a full warm-up, including extensive test practice, is performed. Such studies would also be of significant practical interest to individuals who complete warm-ups prior to sports or exercise.

Importantly, the relative importance of central and peripheral factors to the PAP effect is still debated especially when elicited under voluntary conditions. Changes in peripheral function (such as increased intracellular  $\text{Ca}^{2+}$  release and sensitivity) may be a major factor of voluntary PAP whereas the contribution of central mechanisms (i.e. increased central drive) may be limited. However, to date no research has examined in detail the relative influence of peripheral vs. central mechanisms to voluntary PAP. Determining the impact of these mechanisms will help indicate the appropriate site(s) for targeted interventions to enhance the voluntary PAP response, and therefore how to elicit PAP in individuals who do not seem to respond to a CA.

It is also likely that the contraction velocity, duration and total work characteristics of dynamic CAs would influence the voluntary PAP response but this requires further investigation. Finally, it remains unclear why stronger individuals are able to express higher level of potentiation than their weaker counterparts and further research is again required to determine why this is the case.

## **1.6 Aim of the thesis**

The major aims of the present thesis are to: 1) determine whether the voluntary PAP effects commonly observed after CA are a consequence of acute neuromuscular alterations relating to the CA itself, or whether they simply reflect warm-up and/or familiarisation effects; 2) clarify the influence of the contraction velocity, duration and total work characteristics of the CA on voluntary PAP; 3) determine the factors (i.e. muscle fibre type composition and muscle size) allowing stronger individuals to express higher level of voluntary PAP; and 4) determine the influence of peripheral and central mechanisms on voluntary PAP in human skeletal muscle.

## **CHAPTER 2**

### **Study 1: Post-activation potentiation during voluntary contractions after continued knee extensor task-specific practice**

## **2.1 Introduction**

There is significant practical interest in the idea that the performance of maximal, or near maximal, voluntary muscle contractions (i.e. a voluntary CA) might evoke an increase in muscular performance in a subsequent contraction and this phenomenon is commonly termed post-activation potentiation (PAP); voluntary PAP can be defined as the improvement in muscular performance during a voluntary contraction in response to a CA whereas twitch PAP refers to the increase in twitch force production.

There is a significant body of evidence demonstrating an increase in voluntary muscle performance after a CA (the reader is directed to the reviews by Hodgson et al., 2005; Tillin & Bishop, 2009). However, voluntary PAP responses elicited by voluntary CAs have often been explored in subjects who have performed only standardised warm-up activities consisting of cycling or running, light stretching and sub-maximal repetitions of the performance test (Duthie et al., 2002; French et al., 2003; Jo et al., 2009), and other studies did not appear to use a warm-up before the baseline measurements (Gossen & Sale, 2000; Hamada et al., 2000; Jubeau et al., 2010; Miyamoto et al., 2011a; Miyamoto et al., 2011b). Therefore, it is not known whether the PAP effect observed in the aforementioned studies is a consequence of acute neuromuscular alterations relating to the CA itself, or whether it simply reflects warm-up and/or familiarisation effects (MacIntosh et al., 2012). Such a finding would be important to those who commonly complete a full warm-up routine prior to sport or exercise participation, and seek an additional strategy to enhance muscular performance. To address this question a complete warm-up during which continued task-specific practice results in no further torque enhancement can be provided, and a voluntary CA with different force-time or movement pattern characteristics can be imposed to determine whether it enhances subsequent torque production.

A considerable body of evidence supports the possibility of extra muscle force or joint torque development even after maximal muscle contractile capacity (i.e. maximal torque production) has been attained (Collins, Burke, & Gandevia, 2001; Frigon et al., 2011). For example, variable-frequency muscle or nerve stimulation techniques, such as triangular-frequency stimulation ramps (greater force is produced at the same stimulation frequency on the descending slope of a triangular frequency ramped contraction) (Stuart, Binder-Macleod & Clamann, 1989), top hat stimulations (e.g., 20-80-20 Hz frequency trains, where greater force is produced at the same stimulation frequency in the second 20-Hz train; Frigon et al., 2011) and staircase stimulations (constant low-frequency stimulation progressively increases force production; Rack & Westbury, 1969) have been shown to increase force generation above that elicited using standard stimulation parameters. However, it remains to be determined whether incidences of extra muscle force or joint torque can occur under voluntary conditions (i.e., PAP) by use of voluntary contractions (i.e., a voluntary CA) with altered force-time characteristics.

Studies previously examining the effects of voluntary CAs on voluntary force/torque production have revealed significant increases (French et al., 2003; Miyamoto et al., 2011a; Seitz et al., 2014a; Seitz et al. 2014b), no changes (Gossen & Sale, 2000; Gourgoulis et al., 2003) or decreases (Chiu et al., 2003; Duthie et al., 2002) in muscular function (i.e. jump and sprint performances, isokinetic peak velocity and isokinetic torque production). Therefore, there is some contention as to whether PAP can be reliably elicited in voluntary contractions, even when extensive task practice is not provided. Based on previous evidence it is certain that a voluntary CA that elicits PAP after a complete warm-up would have particular contraction velocity, duration (i.e. time under tension) and/or total work characteristics (Chaouachi et al., 2011; Vandervoort et al., 1983). It has been previously demonstrated that a fast-speed ( $300^{\circ}\cdot\text{s}^{-1}$  knee extension) CA benefited subsequent performance in fast- ( $300^{\circ}\cdot\text{s}^{-1}$ ) but not slow-speed ( $60^{\circ}\cdot\text{s}^{-1}$ ) tests, whilst a slow-speed CA had no effect on either fast- or

slow-speed performance (Chaouachi et al., 2011). These findings are indicative of a velocity-specific effect, however some interpretive caution is required since baseline muscle performance may not have been stable prior to the CA being undertaken as a complete warm-up was not provided. Also, the fast- and slow-velocity CAs provided different contraction durations (3 vs. 15 s, respectively), and probably a different total work was performed. Therefore, the relative influences of voluntary CA velocity on the development of voluntary PAP could not be clearly determined.

Altering parameters such as the velocity of a voluntary CA clearly alters the contraction duration in addition to the total work performed, and these effects have not been separated in previous studies. Although, it has been previously demonstrated that the contraction duration of an isometric CA influences twitch PAP (Vandervoort et al., 1983), the influence of the contraction duration of dynamic CAs on voluntary PAP has yet to be determined. The contraction duration and total work of dynamic CAs may influence voluntary PAP because the performance of CAs of shorter contraction duration or of too little work might prevent the triggering of the mechanism(s) responsible for voluntary PAP whilst longer contraction durations or too much work might induce excessive fatigue and therefore reduce the ability to express potentiation (Rassier & Macintosh, 2000). The testing of dynamic CAs with both different contraction duration and velocity characteristics would allow for their effects to be examined simultaneously with the effect of total work. Nonetheless, given the above, it could be hypothesised that dynamic CAs that elicit voluntary PAP after a complete task-specific warm-up, and thus replicating the phenomena observed during some electrical stimulation protocols, might have specific contraction velocity, duration and total work characteristics.

One difficulty with determining the optimum conditions under which PAP is evoked is that the mechanisms contributing to the effect are still not well understood. For example, increases (Hamada et al., 2000; Jubeau et al., 2010) or no changes (Miyamoto et al., 2011a;

Miyamoto et al., 2011b) in M-wave amplitude, which can be taken as a reasonable indicator of changes in neuromuscular transmission and the excitability of the muscle fibre's sarcolemma (Lepers et al., 2001), have been reported following voluntary CAs inducing twitch PAP. A lack of change in M-wave amplitude in the presence of twitch PAP may be suggestive of an influence of changes in the E-C coupling process on PAP. An increased phosphorylation of the myosin RLC has also been demonstrated to be an important mechanism of PAP in animal studies (Grange et al., 1993; Moore & Stull, 1984; Palmer & Moore, 1989; Sweeney et al., 1993). These findings are consistent with the observation of twitch PAP in humans (Hamada et al., 2000; Vandervoort et al., 1983) and suggest that an improved E-C coupling may be an important mechanism in humans. However, the time course of twitch PAP, which can last up to 5-6 min and with a twitch PAP peak occurring immediately after completing a CA, appears to be different to the time course of voluntary PAP, which has been shown to persist for up to 18.5 min and with a peak occurring at 7-10 min (Wilson et al., 2013). These results suggest that the mechanisms of twitch and voluntary PAP may be at least partly divergent. Also, it is not known whether changes in the E-C coupling efficiency can be elicited when a complete task-specific warm-up is imposed before the CA. At the central level, increases in the recruitment of higher-order (Type II) motor units are thought to contribute to voluntary PAP (Folland et al., 2008; Gullich & Schmidtbleicher, 1996; Trimble & Harp, 1998). However, this result may be at least partly influenced by inherent methodological constraints associated with H-reflex measurements. For example, in the studies of Gullich and Schmidtbleicher (1996) and Folland et al. (2008), an enhancement of H-wave amplitude was observed indicating an increase in Ia synaptic efficiency, motor neurone excitability, or a reduction in inhibitory interneurone activity (Aagaard, Simonsen, Andersen, Magnusson, & Dyhre-Poulsen, 2002a; Pierrot Deseilligny, 1997; Pierrot-Deseilligny & Burke, 2005). However, the H-reflex response appears to have been elicited in resting muscle, which may not adequately reflect changes occurring at the spinal level during



muscular contraction (Aagaard et al., 2002b; Voigt et al., 1998). Furthermore, the relative contribution of an increased H-reflex amplitude to voluntary PAP remains undetermined because no attempt was made to examine the relationship between changes in the normalised H-reflex amplitude and voluntary PAP. Alternatively, Hough et al. (2009) reported an increase in EMG activity with voluntary PAP following a voluntary CA and concluded that an increased motor unit recruitment contributed to the observed potentiation. However, changes in EMG activity might result from both central and/or peripheral mechanisms, such as lengthening of the intracellular action potential profile (Arabadzhev et al., 2010) or increases in  $\text{Na}^+$ - $\text{K}^+$  pump activity (Thomas et al., 2006). Therefore such findings must be interpreted with caution. Normalisation of the EMG amplitude to the M-wave amplitude is considered to largely eliminate the effect of peripheral changes in membrane excitability and thus provide a reasonable indicator of central drive (Arabadzhev et al., 2010; Lepers et al., 2002; Millet & Lepers, 2004). Using this method Fukutani et al. (2013) reported no changes in EMG:M following the performance of a voluntary CA inducing voluntary PAP, although a complete warm-up was not provided in the study. Nevertheless, this result suggests that changes in central drive may not be a major candidate influencing voluntary PAP. Assessments of EMG:M after a complete task-specific warm-up may provide important information regarding the potential influences of central drive on voluntary PAP under these specific conditions.

Therefore, the purposes of the present study were: 1) to determine whether performing dynamic CAs could contribute to induce both voluntary and twitch PAP in individuals who have performed a complete warm-up during which extensive task-specific practice were performed to the point where no further improvement in voluntary torque production could be achieved by continued task practice; 2) to examine the influence of dynamic CA velocity, total contraction duration and total work characteristics on the changes in torque production; and 3) determine the peripheral and central mechanisms of PAP. It was hypothesised that performing a knee extension CA would increase both voluntary and twitch PAP, that the

velocity of the CA would have little impact on changes in torque production as long as a minimum of total contraction duration and total work is provided, and that PAP would be associated with changes at both the peripheral (twitch torque and M-wave) and central (EMG:M) levels.

## **2.2 Methods**

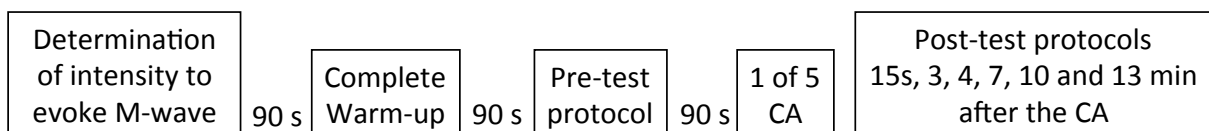
### **2.2.1 Participants**

Seventeen resistance-trained men (mean  $\pm$  SD: age,  $25.4 \pm 3.9$  yr; height,  $1.82 \pm 0.4$  m; body mass,  $84.3 \pm 10.5$  kg) volunteered for the study. They volunteered on the basis that they were involved in a lower body resistance-training program for power and/or muscular strength for at least 6 months. They were required to abstain from taking any stimulants or depressants, including caffeine for at least 6 hours and alcohol for at least 24 hours, prior testing and to refrain from performing sports or hard exercise training for one day prior to the experimental day. The procedures of the investigation were approved by the Edith Cowan University Human Research Ethics Committee and were in agreement with the Declaration of Helsinki. An informed consent was obtained from each participant.

### **2.2.2 Study design and overview**

The participants visited the laboratory on six separate occasions at the same time of the day separated by 3-4 days. The purpose of the first visit was to familiarise them with the isokinetic knee extension and the electrical stimulation procedures. During the subsequent visits, the participants completed an experimental procedure consisting of: (1) the determination of the current intensity required to evoke the maximum M-wave amplitude, (2) a complete warm-up including extensive task-specific practice, and (3) a test protocol that was performed before and 1, 4, 7, 10 and 13 min after completion of one of five knee

extension CAs. The participants were seated on a dynamometer (Biodex System 3 Pro, Biodex Medical System, Shirley, New York) during the electrical twitch stimulations and voluntary contractions, with their dominant (strongest) thigh strapped to the dynamometer's chair and the ankle fixed to the dynamometer lever arm. The lateral femoral epicondyle was aligned to the axis of rotation of the dynamometer, and the knee and hip joints were flexed at 90° and 85°, respectively. Gravity correction and calibration of the dynamometer were made at the beginning of each testing session. The participants were unaware of the study hypotheses as well as task-specific warm-up and test protocol scores.



**Figure 2.1.** The experimental design of the study and time course of measurements. CA: conditioning activity.

### 2.2.3 Nerve stimulation procedure (twitch torque)

On arrival at the laboratory the participants cycled on a Monark cycle ergometer for five minutes at 60 rpm with a 1-kg resistance (300 W in total). They were then seated on the dynamometer chair and the stimulation intensity required to evoke the maximal M-wave amplitude was determined by delivering single 0.2-ms square-wave pulses to the femoral nerve using a constant current stimulator (DS7H, Digitimer Ltd, Welwyn Garden City, UK). The cathode electrode (Kendall Medi-Trace 200 series electrode; 10 mm diameter, USA) was positioned immediately beside the femoral artery in the inguinal region and the anode electrode was positioned over the greater trochanter. The cathode was moved above the inguinal ligament to find the location that produced the greatest M-wave amplitude at a low stimulation intensity. The maximal intensity was then determined by increasing the current until a further increase in stimulus intensity failed to elicit a greater M-wave amplitude. To ensure that a supramaximal current was used during the twitch stimulation, an intensity of 120% of maximal M-wave amplitude was used. The peak-to-peak amplitude of the M-wave

was calculated from the VL EMG data recorded during the evoked twitches. Single twitch measurements proved to be reliable during pilot testing (coefficient of variance and intra-class correlation coefficients were  $1.7 \pm 1.1\%$  and 0.89, respectively).

#### **2.2.4 Measurement of muscle activity (EMG)**

After careful preparation of the skin by shaving, lightly abrading and cleaning with alcohol to reduce the impedance below  $5\text{ k}\Omega$ , the surface EMG signals from VL, vastus medialis and rectus femoris were recorded at a 4 kHz analogue-digital conversion rate using a Bagnoli-8 Main Unit EMG system (DelSys, Inc., MA, USA) and band-pass filtered (10–500 Hz) using LabChart Software (PowerLab System, ADInstruments, v.6.1.3, NSW, Australia). The interelectrode distance was 1 cm and the reference electrode was positioned on the patella. Surface EMG was also recorded from VL using a pseudo-monopolar configuration (sample rate 4 kHz) using the BioAmp EMG system (PowerLab System, ADInstruments, NSW, Australia) in order to obtain M-wave data. The active electrode was positioned over the VL, the dispersive electrode positioned over the quadriceps tendon proximal to the superior border of the patella and the reference electrode placed on the tibia. This configuration, which is similar to that used previously for the triceps surae (Pinniger, Nordlund, Steele, & Cresswell, 2001; Trajano, Seitz, Nosaka, & Blazevich, 2013), gave larger and more reliable responses in pilot testing (coefficient of variance and intra-class correlation coefficient were  $1.4 \pm 1.0\%$  and 0.92, respectively). Muscle activity was expressed as root mean square EMG amplitude (100-ms averaging window) and normalised to the M-wave amplitude to obtain EMG:M.

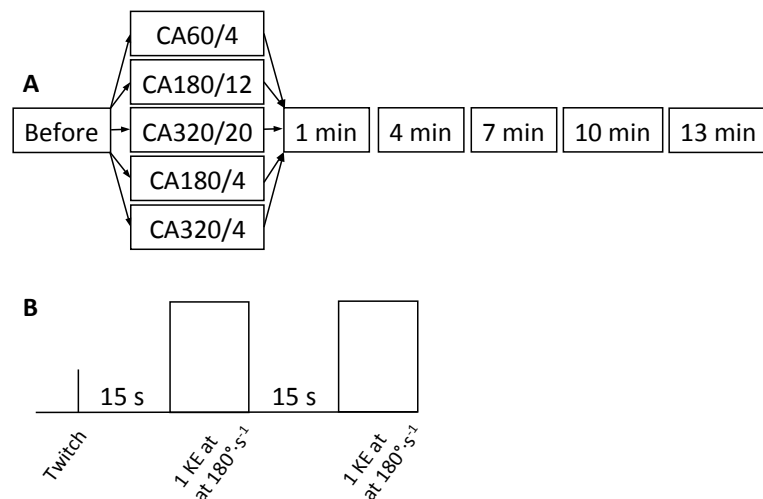
#### **2.2.5 Complete task-specific warm-up procedure**

Following the determination of the stimulation parameters, the participants performed a complete task-specific warm-up procedure to warm the muscles, provide extensive task-

specific practice and to achieve maximal muscle contractile capacity. It comprised two isokinetic knee extensions at  $180^{\circ}\cdot\text{s}^{-1}$  at 20, 40, 60 and 80% of their perceived maximal force at 45-s intervals (8 knee extensions in total). Isokinetic knee extensions (at  $180^{\circ}\cdot\text{s}^{-1}$ ) at 100% of maximum were then performed ‘as fast and hard as possible’ every minute until peak torque production in three consecutive contractions differed by less than 2%. This was used to ensure that the warm-up was complete and that further contractions had no additional effect on voluntary torque production. A 90-s rest period, chosen based on pilot data showing that this rest interval did not affect torque production, was imposed prior to start the test protocol. Isokinetic knee extensions measurements proved to be reliable during testing (coefficient of variance and intra-class correlation coefficients were  $1.1 \pm 0.8\%$  and 0.93, respectively).

## 2.2.6 Test protocol

A test protocol was completed 90 s before (pre-test) and 1, 4, 7, 10 and 13 minutes after (post-test) each CA. For each test, one supramaximal stimulus was delivered at rest (isometric) to the femoral nerve with the knee angle set at  $60^{\circ}$  ( $0^{\circ}$  = full knee extension) 15 s before two maximal dynamic knee extensions at  $180^{\circ}\cdot\text{s}^{-1}$ , each separated by a 15 s-rest period. A  $180^{\circ}\cdot\text{s}^{-1}$  angular velocity was chosen based upon research demonstrating that the magnitude of PAP is greater during tests at fast (i.e.,  $180^{\circ}\cdot\text{s}^{-1}$ ) vs. slow ( $30^{\circ}\cdot\text{s}^{-1}$ ) joint angular velocities (Fukutani et al., 2013).



**Figure 2.2.** A) Experimental design and time course of measurements. The test protocol was completed 90 s before and 1, 4, 7, 10 and 13 min after one of the five conditioning activities (CA). B) Order of measurements. CA60/4 = 4 knee extensions at  $60^{\circ}\cdot s^{-1}$ , CA180/12 = 12 knee extensions at  $180^{\circ}\cdot s^{-1}$ , CA300/20 = 20 knee extensions at  $300^{\circ}\cdot s^{-1}$ , CA180/4 = 4 knee extensions at  $180^{\circ}\cdot s^{-1}$ , CA300/4 = 4 knee extensions at  $300^{\circ}\cdot s^{-1}$ , KE = voluntary knee extension.

For the dynamic knee extensions, the participants received verbal encouragement to extend their knee ‘as fast and as hard as possible’ throughout the whole range of motion. The range of motion was set to  $90^{\circ}$  to  $0^{\circ}$  ( $0^{\circ}$  = full knee extension) and participants were asked to move through the entire range of motion with a repetition completed when the lever arm was stopped at the mechanical stop position of the Biodex. The participants were asked to relax their leg before extending their knee so the knee angle was at  $90^{\circ}$  before initiating the knee extension. The leg was passively returned to the starting position after the shortening contraction. The knee extension resulting in the highest voluntary peak torque at  $180^{\circ}\cdot s^{-1}$  at each time point (i.e. pre-test and 1, 4, 7, 10 and 13 minutes after each CA) was selected for further analysis. Voluntary PAP was calculated as:

$$\% \text{ Voluntary PAP} = [(\tau_{\text{vol,post-CA}} - \tau_{\text{vol,pre-CA}}) / \tau_{\text{vol,pre-CA}}] \times 100$$

where  $\tau_{\text{vol,post-CA}}$  is the voluntary peak torque at  $180^{\circ}\cdot s^{-1}$  measured during the test protocol after the CA (i.e. post-CA) and  $\tau_{\text{vol,pre-CA}}$  is the voluntary peak torque at  $180^{\circ}\cdot s^{-1}$  measured before the CA. Twitch PAP was calculated as:

$$\% \text{ Twitch PAP} = [(\tau_{\text{tw,post-CA}} - \tau_{\text{tw,pre-CA}}) / \tau_{\text{tw,pre-CA}}] \times 100$$

where  $\tau_{\text{tw,post-CA}}$  is the elicited peak twitch torque measured at rest during the test protocol after the CA and  $\tau_{\text{tw,pre-CA}}$  is the elicited peak twitch torque measured before the CA. The torque signal was recorded at a 4 kHz analogue-digital conversion rate and filtered at 14 Hz

with a low pass Butterworth filter using LabChart Software (PowerLab System, ADInstruments, v.6.1.3, NSW, Australia). The reliability of the isokinetic knee extensions (coefficient of variance and intra-class correlation coefficients were  $1.2 \pm 1.0\%$  and 0.93, respectively) and twitch measurements (coefficient of variance and intra-class correlation coefficients were  $1.4 \pm 0.9\%$  and 0.92, respectively) during test protocol proved to be high.

### 2.2.7. Conditioning activity protocols

CAs involved the performance of a series of maximal dynamic contractions as outlined in Table 2.1. A 10-s interval was afforded between each knee extension effort. The effect of the CA at one knee extension velocity was examined in each of the five randomised sessions. Two CAs (i.e., CA180/4: 4 knee extensions at  $180^\circ \cdot s^{-1}$ ; and CA300/4: 4 knee extensions at  $300^\circ \cdot s^{-1}$ ) of shorter contraction duration were performed to test the hypothesis that the contraction duration of a CA is an important factor influencing PAP; CA180/4 also served as the control condition since it included only a small number of extra repetitions of the test contraction.

**Table 2.1:** Conditioning activities used in the experimental protocol.

Conditioning activity	Repetitions/angular velocity	Total contraction time (s)
CA60/4	4 repetitions at $60^\circ \cdot s^{-1}$	6
CA180/12	12 repetitions at $180^\circ \cdot s^{-1}$	6
CA300/20	20 repetitions at $300^\circ \cdot s^{-1}$	6
CA180/4	4 repetitions at $180^\circ \cdot s^{-1}$	2
CA300/4	4 repetitions at $300^\circ \cdot s^{-1}$	1.2

CA: conditioning activity; CA60/4, CA180/12, CA300/20, CA180/4, CA300/4: angular velocity [ $(^\circ \cdot s^{-1})$ /repetitions].

### 2.2.8. Calculation of total contraction time and total work

Total contraction time was calculated as:  $\left(\frac{ROM}{v_{CA}}\right) \times n$ , where  $ROM$  is the range of motion of the knee extensions performed during the CA (i.e.,  $90^\circ$ ),  $v_{CA}$  is the velocity ( $^\circ.s^{-1}$ ) of the CA,  $n$  is the number of knee extensions performed during the CA.

Total work was calculated as:  $[\sum(\tau_{mean,KE})/n] \times \theta$ , where  $\tau_{mean,KE}$  is the average torque of each knee extension repetition performed during the CA,  $n$  is the number of knee extensions performed during the CA and  $\theta$  is the total angular displacement (in radians) of the knee extension repetitions performed during the CA. This calculation includes the acceleration and deceleration phases, and is thus a measure of all work completed across all repetitions.

### 2.2.9 Statistical analyses

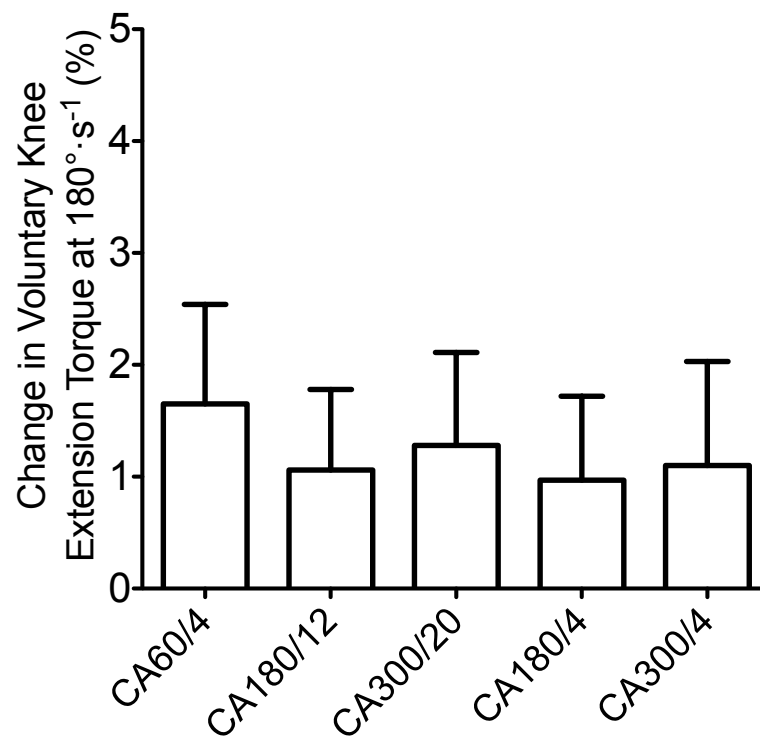
One-way repeated measures ANOVAs were performed to compare: 1) the difference between the greatest and least voluntary torques produced during the last three knee extensions during warm-up and the knee extension before each conditioning activity (i.e. pre-CA) to determine whether the task-specific warm-up was complete; and 2) the work done during the different CAs. Separate two-way (time  $\times$  condition) repeated measures ANOVAs were performed to compare changes in all variables before and 1, 4, 7, 10 and 13 minutes after each CA. Pairwise comparisons with Bonferroni corrections were performed when significant interaction effects were detected. Pearson's correlation analyses were used to assess the relationship between total work and the maximal voluntary PAP response. For all statistical analyses, the level of significance was set at an  $\alpha$  level of 0.05.



## 2.3 Results

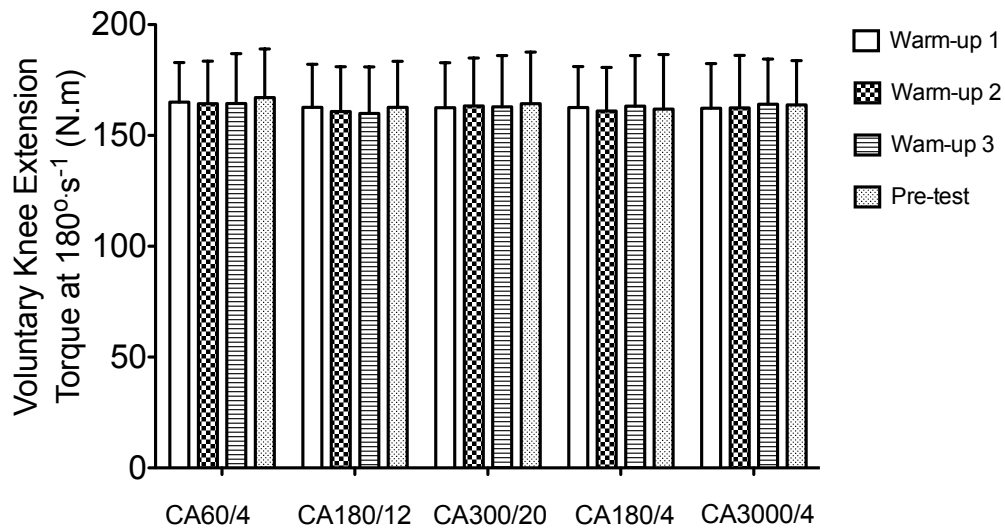
### 2.3.1 Complete task-specific warm-up

On average, the participants required  $4.6 \pm 1.1$  knee extensions at their maximal capacity to produce less than 2% difference in voluntary torque in three consecutive knee extensions. There were no differences for any condition between the greatest and least torques produced during the last three knee extensions at  $180^\circ \cdot s^{-1}$  during warm-up and the knee extension before each CA (Figure 2.3).



**Figure 2.3.** Percent difference between the greatest and least voluntary torques produced during the last three knee extensions at  $180^\circ \cdot s^{-1}$  during warm-up and the knee extension before each conditioning activity. There were no differences in any condition, indicating that the warm-up was complete.

Similarly, no differences were observed between the peak voluntary torques produced during the last three knee extensions at  $180^\circ \cdot s^{-1}$  in warm-up and the knee extension during pre-test in any condition (Figure 2.4).



**Figure 2.4.** Voluntary torques produced during the last three knee extensions at  $180^{\circ}\cdot s^{-1}$  in warm-up (i.e. warm-up 1, warm-up 2, warm-up 3) and the knee extension during pre-test.

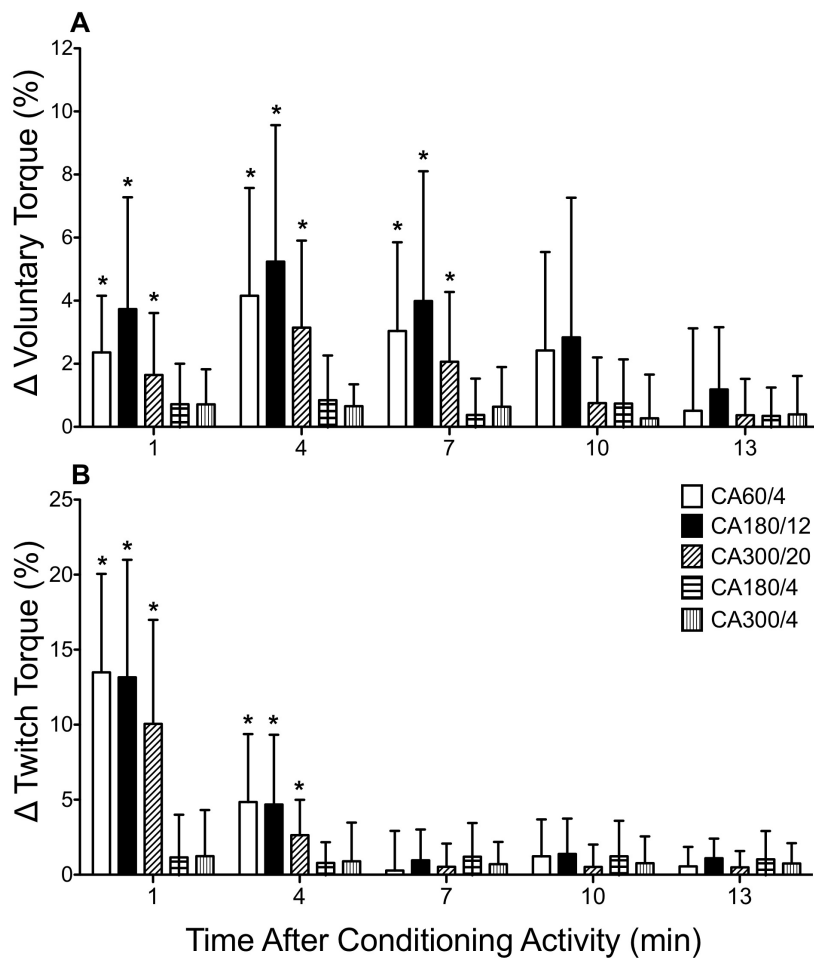
The lack of statistical difference in voluntary torque production at  $180^{\circ}\cdot s^{-1}$  among these contractions indicates that maximal voluntary contractile capacity was achieved before undertaking the CA and the task-specific warm-up was complete since no further improvement in voluntary torque production could be elicited by further practice using a 45-s rest interval.

### 2.3.2 Voluntary PAP

Figure 2.5A shows the significant interaction (time  $\times$  condition) effect for voluntary PAP ( $p < 0.04$ ). Post-hoc analyses revealed a significant increase in voluntary torque from 1 to 7 min in CA60/4 (e.g., 4 knee extensions at  $60^{\circ}\cdot s^{-1}$ ), CA180/12 (e.g., 12 knee extensions at  $180^{\circ}\cdot s^{-1}$ ) and CA300/20 (e.g., 20 knee extensions at  $300^{\circ}\cdot s^{-1}$ ) with no significant difference from the pre-test value being found at 10 and 13 min. There was no change in voluntary torque after CA180/4 (control) and CA300/4 ( $p > 0.1$ ). No differences in voluntary PAP were observed between CA60/40, CA180/12 and CA300/20 (Figure 6A).

### 2.3.3. Twitch PAP

Similarly, Figure 2.5B shows the significant interaction (time  $\times$  condition) effect for twitch PAP ( $p < 0.05$ ). Post-hoc analyses revealed a significant increase in twitch torque from 1 to 4 min in CA60/4, CA180/12 and CA300/20 with no significant difference from the pre-test value being found at 7, 10 and 13 min. There was no change in twitch torque following CA180/4 and CA300/4.



**Figure 2.5.** Time course of (A) voluntary and (B) twitch torques after the five conditioning activities. \*significantly different from baseline ( $p < 0.05$ ).

### 2.3.4 Contraction velocity

As shown in Figures 2.5A and 2.6A, CA60/4 ( $+4.8 \pm 3.0\%$ ), CA180/12 ( $+5.9 \pm 4.1\%$ ) and CA300/20 ( $+3.6 \pm 2.4\%$ ) induced similar ( $p > 0.05$ ) changes in peak voluntary torque post-

CA. Likewise, changes in post-CA peak twitch torque were similar ( $p>0.05$ ) after CA60/4 ( $+13.5 \pm 6.6\%$ ), CA180/12 ( $+13.4 \pm 7.4\%$ ) and CA300/20 ( $+10.6 \pm 6.2\%$ ). These results suggest that the CA contraction velocity was not an important factor for both voluntary and electrically evoked PAP.

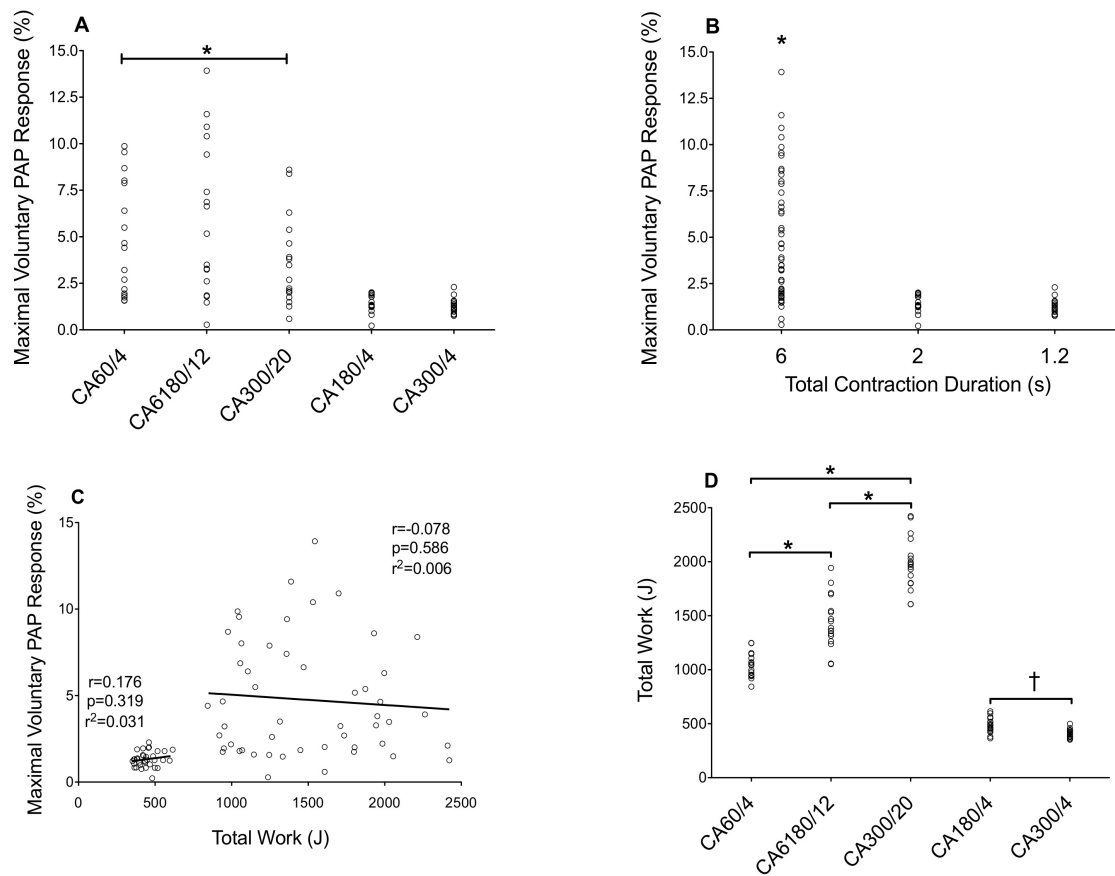
### **2.3.5 Contraction duration**

62.5B shows that CA60/4, CA180/12 and CA300/20, i.e. the CAs with the longest contraction duration (i.e. 6 s), elicited significant maximal voluntary and twitch PAP responses ( $p<0.05$ ). The results also indicate that there was no statistical difference in either maximal voluntary or twitch PAP responses between these three CAs. On the contrary, CA180/4 and CA300/4, the two CAs with shortest contraction durations (2 and 1.2 s, respectively), did not elicit voluntary or twitch PAP ( $p>0.05$ ).

### **2.3.6. Total work**

As shown in Figure 2.6C, no correlation ( $r=0.18$ ;  $p=0.32$ ;  $r^2=0.03$ ) was found between the total work done during the CAs in which PAP was not induced (CA180/4 and CA300/4; left side) and the PAP response elicited by these activities. Similarly, no correlation ( $r=-0.08$ ;  $p=0.59$ ;  $r^2=0.01$ ) was found between the total work done during the CAs inducing PAP (CA60/4, CA180/12 and CA300/20; right side) and the PAP response elicited by these activities. Note, however, that a correlation existed for all data ( $r=0.42$ ;  $p=0.0001$ ;  $r^2=0.18$ ), indicating at least some importance of total work, however the heteroscedastic nature of the data invalidates the use of standard correlation procedures. At least, a minimum amount of work appeared to be required for PAP to be induced (i.e.  $\sim 750$ -900 J in the present experiments; Figure 2.6C). As shown in Figure 2.6D, significant differences ( $p=0.0001$ ) were observed in the total work produced during CA60/4, CA180/12 and CA300/20, although these CAs induced similar PAP responses. In addition, the total work produced during

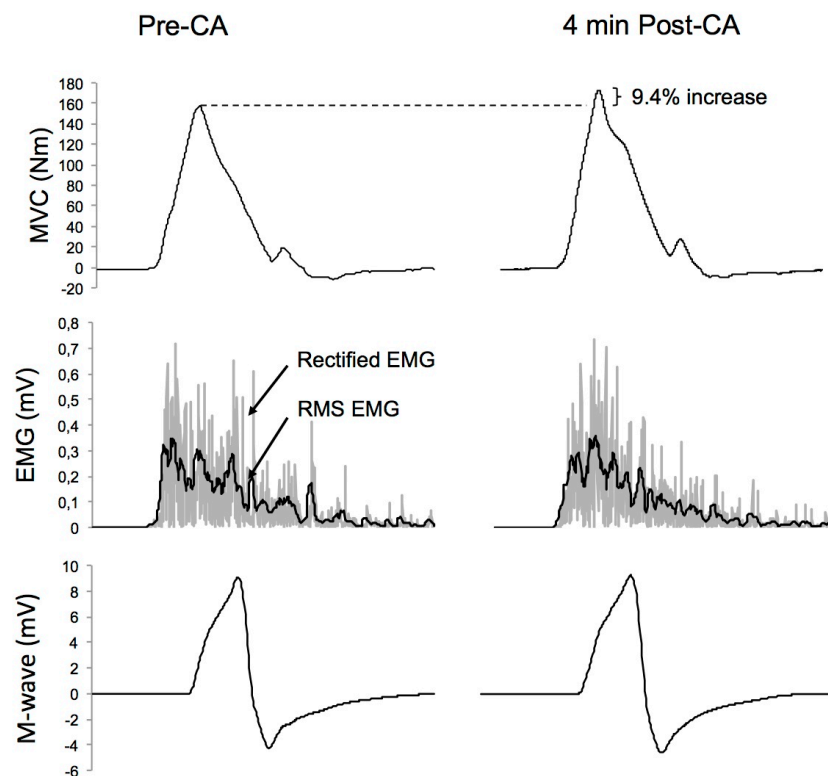
CA180/4 and CA300/4 was significantly ( $p=0.001$ ) smaller than during CA60/4, CA180/12 and CA20/300.



**Figure 2.6.** Maximum voluntary PAP response (%): A) for all participants after each conditioning activity ( $n=85$ ; \*significantly different from CA180/4 and CA300/4) and B) for all participants after conditioning activities of different contraction durations (\*significantly different from 2 and 1.2 s). C) Correlations between maximum voluntary PAP response (%) and total work done during the conditioning activities. D) Total work done after each conditioning activity (\*significantly different, † significantly different from CA60/4, CA180/12, and CA300/20).

### 2.3.7. Electromyogram and M wave

When compared to the pre-test values, no significant interaction effect was found for EMG:M or M-wave amplitude at any time points for any CA, indicating a lack of change in central drive and muscle excitability following the CAs ( $p>0.05$ ) (raw data for one participant are shown in Figure 2.7).



**Figure 2.7.** Example of data obtained from one participant before (left) and 4 min after a conditioning activity (right). An increase in voluntary torque (first row) but no changes in EMG amplitude (second row) or M-wave amplitude (last row) are visible 4 min after the conditioning activity. CA = conditioning activity.

## 2.4 Discussion

The acute effects of dynamic (isokinetic) CAs of different contraction velocity, duration and total work characteristics on subsequent torque production were examined in the knee extensors after individuals performed a complete warm-up including extensive task-specific practice to the point where no further improvement in voluntary torque production could be achieved with repeated test practice (i.e., maximal voluntary contractile capacity was achieved). The main findings were that: 1) performance of dynamic CAs could contribute to improve subsequent voluntary and electrically-evoked torques, even when maximal voluntary contractile capacity was achieved after completing a full warm-up; 2) dynamic CAs of longer contraction duration (i.e. time under tension) elicited significant increases in torque production regardless of the movement velocity and total work done during a CA, although a

minimum of work appears to have been required for the PAP effect to be elicited; and 3) a lack of change in both M-wave and EMG:M was observed after the CAs.

Consistent with previous published research (Babault, Maffiuletti, & Pousson, 2008; Chaouachi et al., 2011; Fukutani et al., 2013) the present investigation demonstrates that the performance of isokinetic CAs can contribute to improve both voluntary and electrically-evoked torques. Because a complete task-specific warm-up was completed in which additional practice resulted in no further effect on voluntary torque production (i.e. maximal voluntary contractile capacity was achieved under those conditions), the present data demonstrate that increases in voluntary and electrically-evoked torque production following a CA with force-time characteristics different to the performance test may result specifically from acute physiological changes in response to the CA rather than being either a warm-up or familiarisation effect. Our findings indicate that the participants' maximal voluntary muscle contractile capacities were achieved before performing the CA since the final 3 contractions in the warm-up differed by <2% in peak torque and no statistical difference was found between the voluntary torque produced during the last three knee extensions in warm-up and that produced during the knee extension at pre-test (baseline) (Figures 2.3 and 2.4). The increases in torque production (i.e., PAP) after the performance of voluntary CAs displaying different force-time characteristics than the performance test (i.e. different number of contractions and/or velocities) is in line with previous studies reporting the development of extra muscles forces or joint torques (e.g., 'extra torque') after the imposition of varying stimulation frequencies during (involuntary) muscle or nerve stimulation (Stuart et al., 1989; Frigon et al., 2011; Rack & Westbury, 1969). From a practical standpoint, the present findings have important implications because they demonstrate the ability to elicit supra-maximal voluntary torques through the performance of voluntary muscular contractions even when maximal voluntary contractile capacity (i.e. peak torque) was previously achieved under specific practice conditions.

Our hypothesis that movement velocity would not be a decisive factor for PAP was supported by the present data as three CAs performed at different velocities (i.e., 60, 180 and  $300^{\circ}\cdot\text{s}^{-1}$ ) but displaying similar contraction duration characteristics induced similar PAP effects (see Figure 2.5A). Our findings contrast those of a previous study that reported increases in voluntary knee extensor peak torque following the performance of fast- (i.e.  $300^{\circ}\cdot\text{s}^{-1}$ ) but not slow-velocity ( $60^{\circ}\cdot\text{s}^{-1}$ ) CAs (Chaouachi et al., 2011). However, the results should be interpreted differently to the present study since a complete task-specific warm-up was not provided and the fast- and slow-velocity CAs provided different contraction durations (3 vs. 15 s, respectively). Therefore, the relative influences of dynamic CA velocity and contraction duration on the development of voluntary PAP could not be clearly determined. However, it is important to note that the dynamic knee extensions during testing in the present study were performed at only one velocity (i.e.  $180^{\circ}\cdot\text{s}^{-1}$ ) and the degree of potentiation seems to be dependent on the velocity of the test protocol (Babault et al., 2008; Chaouachi et al., 2011; Fukutani et al., 2013). Therefore, further testing is required to estimate potential velocity-specific test effects. We chose a  $180^{\circ}\cdot\text{s}^{-1}$  angular velocity based upon research demonstrating greater PAP responses during fast- (i.e. 150, 180,  $300^{\circ}\cdot\text{s}^{-1}$ ) vs. slow (30 or  $60^{\circ}\cdot\text{s}^{-1}$ ) performance tests (Babault et al., 2008; Chaouachi et al., 2011; Fukutani et al., 2013).

Although it has been previously shown that the magnitude of voluntary PAP is influenced by the duration of an isometric CA (Vandervoort et al., 1983), the present investigation is the first to demonstrate that the contraction duration of a dynamic (isokinetic) CA may have been a factor influencing the PAP response. Indeed, CAs of longer duration (i.e. CA 60/4, CA180/12 and CA300/20; contraction duration = 6 s) induced both voluntary and twitch PAP, whereas no PAP was observed following CAs of shorter duration (i.e. 180/4 and 300/4; contraction duration  $\leq 2$  s). These findings suggest that a minimum contraction duration threshold must be reached during a dynamic CA to trigger the mechanisms underpinning PAP. Our findings contrast those of a previous study reporting increases in



voluntary knee extensor peak torque following the performance of short (i.e. 3 s) but not long contraction duration (i.e. 15 s) isokinetic CAs (Chaouachi et al., 2011). This discrepancy might be explained by the fact that the long contraction duration CA may have produced greater level of fatigue, resulting in the masking of the PAP effects (Chaouachi et al., 2011). Interestingly, the short contraction duration imposed by Chaouachi et al. (2011) (i.e. 3 s) induced PAP whereas no PAP effect was observed in the present study after a 2-s CA (i.e. CA180/4). The possibility exists that the results of Chaouachi et al. (2011) may have been influenced by the absence of complete task-specific warm-up and thus maximal voluntary contractile capacity may not have been achieved before baseline testing. Because a complete warm-up including extensive task-specific practice was provided in the present study, it is more likely that the two CAs with the shortest times under tension (i.e. 1.2 and 2 s) did not produce a sufficient additional stimulus to trigger the mechanism(s) responsible for PAP.

The total work done does not appear to be an important determinant of PAP as (1) no correlation ( $r=-0.08$ ;  $p=0.59$ ) was found between the total work done during CA60/4, CA180/12 and CA300/20 and the PAP response elicited by these activities (Figure 2.5C, right side), and (2) significant ( $p=0.0001$ ) differences were observed in the total work produced during CA60/4, CA180/12 and CA300/20 although these CAs induced similar PAP responses (Figure 2.5D). Nonetheless, as shown in Figure 2.5C, there appears to be a minimum amount of work required to elicit PAP under the present conditions ( $\sim 750$ - $900$  J in the present experiments). This again aligns with the hypothesis that a minimum contraction duration (or work) must be reached during an dynamic CA to trigger the mechanisms responsible for PAP.

Consistent with a recent study (Fukutani et al., 2013), we were unable to detect a change in EMG:M under the present experimental conditions, suggesting that changes in central drive were not a major factor influencing the PAP response. Previous research associated PAP with the increases in H-reflex (Folland et al., 2008; Gullich & Schmidtbleicher, 1996; Trimble & Harp, 1998) and EMG amplitudes (Hough et al., 2009).

Nevertheless, measurements of H-reflex amplitude, and indeed the EMG:M response may be influenced by inherent methodological constraints. For example, the H-wave (Gullich & Schmidtbleicher, 1996) and EMG amplitudes (Hough et al. (2009) in some studies were not normalised to the compound muscle action potential (M-wave) amplitude, and therefore increases in peripheral conductance, including increased  $\text{Na}^+\text{-K}^+$  pump activity (Thomas et al., 2006) might have influence the H-wave and EMG amplitudes. Furthermore, the H-reflex elicited in resting muscle (Folland et al., 2008; Gullich & Schmidtbleicher, 1996) may not always adequately reflect changes occurring during muscular contraction (Aagaard et al., 2002b; Voigt et al., 1998). Taken collectively, the present data and those of Fukutani et al. (2013) suggest that central mechanisms, at least when measured with EMG:M, may not influence PAP. However, direct measures of cortico-spinal drive (e.g. cortico-electrical/magnetic stimulation or V- and F-wave measurement) may be required to detect small changes at the central level.

Consistent with previous research (Miyamoto et al., 2011b), the ability to drive action potentials to the muscle and along the t-tubules was unaltered after the CAs, as no change in M-wave amplitude was observed. This lack of change in M-wave amplitude and the significant increases in twitch torque at 1 and 4 min following three of the PAP-inducing CAs are in line with previous research (Miyamoto et al., 2011b). These results are suggestive that changes in peripheral muscle function influencing twitch PAP, possibly including alterations in force transmission efficiency (Mahlfeld, Franke, & Awiszus, 2004) and myosin RLC phosphorylation (Grange et al., 1993; Sweeney et al., 1993) may occur. As in other studies (Baudry et al. 2007; Hamada, Sale 2000) our data show a rapid and short-lived change in twitch torque but a prolonged voluntary torque change. Also, the time of peak increase in voluntary torque (at 4 min) did not coincide with the peak twitch torque increase (at 1 min). This difference in temporal responses suggests that the mechanisms of twitch and voluntary PAP may be at least partly divergent. Changes in peripheral function such as

increased intracellular  $\text{Ca}^{2+}$  release and sensitivity may have also contributed to the observed PAP (Hamada et al., 2000) but these changes should be confirmed using trains of stimuli to allow accumulation or increased sensitivity of  $\text{Ca}^{2+}$  to be detected. For instance, changes in the ratio of the forces produced at high- (e.g. 80 Hz) vs. low- (e.g. 20 Hz) frequency trains of stimuli (MacIntosh & Rassier, 2002) may provide information regarding  $\text{Ca}^{2+}$  homeostasis (i.e. changes in steady-state  $\text{Ca}^{2+}$ ) (Jones, 1996; MacIntosh & Rassier, 2002; Martin et al., 2004).

In conclusion, because the participants in the current study performed a complete task-specific warm-up where further contractions had no additional effect on voluntary torque production, the present data provide the clearest evidence of the presence of voluntary and twitch PAP following dynamic CAs of different force-time characteristics. Therefore, CAs may be used even in conditions where a complete warm-up is performed, such as in exercise and sporting environments. In addition, the results show that a minimum contraction time (time under tension) and total work may be required in order to elicit PAP under these conditions whereas the velocity of the CA may not be an important factor influencing PAP. Changes in central drive did not appear to be a significant factor influencing the PAP response as the EMG:M amplitude remained unaltered following the CAs, although more detailed examinations may be required to identify small changes within the neuromuscular pathway. At the peripheral level, increases in twitch torque were evident and no changes in M-wave amplitude were observed, suggesting that changes in peripheral function may have contributed to the observed PAP.

## **CHAPTER 3**

**Study 2: Relationships between muscular strength level, muscle cross-sectional area and volume, myosin heavy chain isoform composition and voluntary post-activation potentiation.**

### **3.1. Introduction**

Post-activation potentiation, or PAP, is the phenomenon in which a preceding contraction (i.e., a CA) elicits an acute improvement in muscular performance during a subsequent test contraction (Tillin & Bishop, 2009). Voluntary PAP can be defined as the improvement in muscular performance during a voluntary contraction in response to a CA whereas twitch PAP is defined by the increase in electrically-elicited (twitch) muscular force production. PAP has been ubiquitously reported in studies using electrical or voluntary contractions as the CA and/or the test contraction (the reader is directed to the reviews by Hodgson et al., 2005 and Tillin & Bishop, 2009). However, an important and consistent finding in these studies is a high inter-individual variability in the voluntary PAP response. Overall, increases (Wilson et al., 2013), no change (Gossen & Sale, 2000; Gourgoulis et al., 2003) or decreases (Chiu et al., 2003; Duthie et al., 2002) in muscular performance after the CA have been reported, which is indicative of a responder versus non-responder phenomenon. This responder versus non-responder phenomenon is also highlighted in Chapter 2 in Figures 2.6.A, 2.6.B, 2.6.C. Thus, despite the practical benefits of improving muscular performance with the use of CAs, some individuals do not seem able to utilise the strategy effectively. This might be explained by the fact that the PAP response is influenced by the characteristics of both the CA and individual.

Studies have shown that the ability to exhibit voluntary PAP is influenced by the volume and intensity of the CA and by the rest period between the CA and the subsequent muscular performance (Wilson et al., 2013 and Study 1 of Chapter 2). Several individual's characteristics that potentially differ between responders and non-responders might also explain the difference in voluntary PAP susceptibility. First, twitch PAP may be more clearly elicited in individuals with a high proportion of type II muscle fibres, as indicated by the strong relationship between type II muscle fibres proportion and twitch PAP magnitude

(Hamada et al., 2000; Hamada et al., 2003). This makes sense from the perspective that twitch PAP has been shown to occur through an increase in myosin RLC phosphorylation in response to a CA, and that this process is most notable in fibres with a greater proportion of the type II-myosin heavy chain (MHC) isoform in animals (Klug, Botterman, & Stull, 1982; Manning & Stull, 1982; Moore & Stull, 1984). However, this has not always been observed in human skeletal muscle (Stuart et al., 1988). The relationship between type II fibres proportion and PAP magnitude also makes sense from the perspective that voluntary PAP may be accompanied by increases in H-reflex (Folland et al., 2008; Gullich & Schmidtbleicher, 1996; Trimble & Harp, 1998) and EMG (Hough et al., 2009) amplitudes. Given that higher-order motor units, including the large type II motor units, are more difficult to recruit, it is assumed that a voluntary CA may increase the contribution of higher-order motor units to muscular contraction and increase muscular performance during a subsequent voluntary contraction (Gullich & Schmidtbleicher, 1996; Hodgson et al., 2005). Nonetheless, methodological constraints are associated with H-reflex measurements and recent studies (Fukutani et al., 2013 and Study 1) did not find changes in EMG:M despite a notable PAP response after voluntary CAs so it is unclear whether changes in fibre recruitment underpin the PAP phenomenon.

There is also a large body of literature indicating that stronger individuals may be able to express higher level of voluntary potentiation (Chiu et al., 2003; Jo et al., 2009; Ruben et al., 2010; Seitz et al., 2014a; Seitz et al., 2014b). For example, Seitz et al. (2014b) found a significant correlation ( $r=0.67$ ) between back squat strength and the magnitude of potentiation during a sprint task. Similarly, Ruben et al. (2010) reported a greater PAP effect on average peak power produced during a horizontal jump task in stronger individuals in comparison to their weaker counterparts. One explanation for this is that stronger individuals are often shown to have a higher percentage of type II muscle fibres (Aagaard & Andersen, 1998; Maughan et al., 1983b; Thorstensson et al., 1976) and therefore would be likely to exhibit

greater increases in myosin RLC phosphorylation in response to the CA or respond more to increases in the ability to recruit type II muscle fibres, resulting in a greater voluntary PAP response. It could also be argued that a greater muscle cross-sectional area (CSA) (Ikai & Fukunaga, 1968; Maughan et al., 1983a) or volume (Fukunaga et al., 2001) would be a characteristic of stronger individuals, whereby any increase in tissue-specific force elicited by voluntary PAP will be amplified in these individuals. However, although muscle size might in some way be a causative factor influencing voluntary PAP, the possibility exists that its relationship with voluntary PAP is also explicable by its relationship with fibre (motor unit) type. Nonetheless, to our knowledge no attempt has been made to quantify the relationships between voluntary PAP and muscle strength, muscle size, and fibre type (i.e. percentage of type II MHC isoform).

Given the above, the purpose of the present study was to examine the relationship between knee extensor voluntary PAP magnitude and maximal voluntary knee extensor torque, quadriceps and vastus lateralis (VL) CSA, quadriceps and VL volume and type II MHC isoform percentage in VL. It was hypothesised that participants displaying a greater voluntary PAP magnitude would also display a greater maximal voluntary knee extensor torque, quadriceps and VL CSA, quadriceps and VL volume and type II MHC isoform percentage, but that type II MHC isoform would be the strongest correlate. The CA used in the present study was chosen based upon the results of Study 1.

## **3.2. Methods**

### **3.2.1. Participants**

Thirteen resistance-trained men (mean  $\pm$  SD: age,  $24.1 \pm 3.0$  yr; height,  $1.85 \pm 0.11$  m; body mass,  $86.1 \pm 10.1$  kg) volunteered for the study. They were recruited on the basis that they had been involved in a lower body resistance-training program for muscular strength

and/or power for at least 6 months. Each participant signed an informed consent form, and the procedures of the investigation were approved by the Edith Cowan University Human Research Ethics Committee and were in agreement with the Declaration of Helsinki.

### **3.2.2. Study design and overview**

The participants visited the laboratory on four separate occasions (familiarisation, experimental, magnetic resonance imaging (MRI) and muscle microbiopsy sessions), each separated by 5 to 7 days. The participants were familiarised with an isokinetic knee extension task during the first visit. During the experimental session, the participants completed a procedure consisting of: (1) a task-specific warm-up procedure (described below), and (2) a test protocol that was performed before and 1, 4, 7 and 10 minutes after completing a knee extension CA. During the task-specific warm-up procedure, test protocol and CA, the participants were seated on an isokinetic dynamometer (Biodex System 3 Pro, Biodex Medical System, Shirley, New York) with their dominant (strongest) thigh strapped to the chair and the ankle fixed to the dynamometer's lever arm. The lateral femoral epicondyle was aligned to the axis of rotation of the dynamometer, and the knee and hip joints were flexed at 90° and 85°, respectively. The participants were unaware of the study hypotheses as well as task-specific warm-up and test protocol scores.

### **3.2.3. Task-specific warm-up procedure**

Based on the results of Study 1 a task-specific warm-up procedure was used to ensure that any increases in voluntary knee extensor torque after completing a CA could be specifically attributed to acute physiological changes in response to the CA rather than warm-up or familiarisation effects. The participants performed two isokinetic knee extensions at  $180^{\circ}\cdot\text{s}^{-1}$  at 20, 40, 60 and 80% of their perceived maximal force through a 90° range of motion at 45 s intervals. Isokinetic knee extensions at 100% of maximum were then



performed ‘as fast and hard as possible’ every minute until peak torque production in three consecutive contractions differed by less than 2%. A 90-s rest period, chosen based on the results of Study 1, was then imposed prior starting the test protocol.

### **3.2.4. Test protocol and conditioning activity**

A test protocol requiring the performance of 2 isokinetic knee extensions at  $180^{\circ}\cdot\text{s}^{-1}$  (separated by 15 s) was completed 90 s before (pre-CA) and 1, 4, 7 and 10 minutes after (post-CA) a CA involving 4 isokinetic knee extensions at  $60^{\circ}\cdot\text{s}^{-1}$ . The knee extensions at  $60^{\circ}\cdot\text{s}^{-1}$  were separated by a 10-s rest period. This CA was chosen based on the results of Study 1 showing a significant PAP magnitude following the performance of 4 isokinetic knee extensions at  $60^{\circ}\cdot\text{s}^{-1}$ . The participants received verbal encouragement to extend their knee ‘as fast and as hard as possible’ throughout the range of motion ( $90^{\circ}$ – $0^{\circ}$ ,  $0^{\circ}$ =full knee extension) during the knee extensions. The knee extension resulting in the highest voluntary torque during pre-CA and post-CA testing (irrespective of the time) was selected for further analysis. Voluntary PAP was calculated as:

$$\% \text{ voluntary PAP} = [(\tau_{\text{post-CA}} - \tau_{\text{pre-CA}}) / \tau_{\text{pre-CA}}] \times 100$$

where  $\tau_{\text{post-CA}}$  is the highest voluntary torque measured during the test protocols after the CA and  $\tau_{\text{pre-CA}}$  is the highest voluntary torque measured before the CA.

### **3.2.5. Magnetic resonance imaging**

A 1.5 Tesla MRI scanner (Magnetom Essenza, Siemens Medical Solutions, Erlangen, Germany) was used to measure the cross-sectional area and volume of the quadriceps muscle group of the dominant leg whilst participants lay supine. A proton density turbo spin-echo axial (cross-sectional) slice sequence (field of view =  $256 \times 256 \text{ mm}^2$ , TR = 3720 ms, TE = 25

ms, base resolution = 384 x 384 pixels, voxel size 0.7×0.5×5.0 mm) was utilised to acquire multiple 5-mm thick serial sections contiguously from the anterior superior iliac spine to the tibial tuberosity. VL, vastus medialis, rectus femoris and vastus intermedius total CSA and volume were determined by manually tracing MRI slices from the proximal point of the greater trochanter to the distal region of the femoral lateral condyle using open-source DICOM imaging software (OsiriX MD v.1.4, OsiriX foundation, Geneva). Care was taken to exclude adipose incursions from each slice. When clear delineation of the vastii muscles was not possible because of a lack of observable intermuscular septum, a line was traced from the end of the observable septum to a landmark on the muscle's perimeter where the septum had intersected in distal slices (Blazevich, Cannavan, Coleman, & Horne, 2007). In order to calculate quadriceps CSA (cm<sup>2</sup>), the area of each muscle in each slice was computed automatically by summing the given tissues' pixels and multiplying by the pixel surface area. Muscle volume (cm<sup>3</sup>) was obtained by multiplying muscle tissue area by slice thickness (Lee et al., 2000). The participants were required to abstain from any exercise (including experimental sessions) for at least 72 h prior the MRI procedure.

### **3.2.6. Muscle microbiopsy procedure**

Muscle biopsy samples were taken from VL of the dominant leg by percutaneous needle microbiopsy. After careful preparation of the skin by shaving, lightly abrading and cleaning with alcohol in order to minimise the risk of infection, a eutectic mixture of local anaesthetics (EMLA, Astra Pharmaceuticals, Sydney, Australia) cream was applied to the biopsy area and subsequently covered with a plastic wrap for 30 min. The plastic wrap and cream were then removed, the skin was sterilised with povidine iodine (Betadine solution, Faulding Consume, Virginia, Qld, Australia), and then punctured (2 cm depth) with the 13 gauge insertion cannula at 50% of the distance from the greater trochanter to the lateral epicondyle of the femur in the middle of the muscle belly. A 14 gauge triggered microbiopsy

needle (Bard Biopsy Systems, Covington, GA, USA) was then inserted in the cannula and a muscle sample was taken. The triggered micro biopsy needle was then removed while the 13 gauge insertion cannula remained in place, the tissue was extracted and immediately frozen in liquid nitrogen, and a further two samples were obtained from the same site. A total of ~30 mg of tissue was obtained and stored at -80°C for further analyses. This micro biopsy procedure has been validated by Hayot et al. 2005 who reported a similar MHC isoform distribution in VL using the micro biopsy technique and the traditional, more invasive, Bergström technique. The participants were required to abstain from any exercises (including experimental sessions) for at least 72 h prior the micro biopsy procedure.

### **3.2.7. Determination of myosin heavy chain isoform distribution**

A 4-gel vertical electrophoresis system (Mini-PROTEAN, Tetra Cell for mini precast gel, 165-8004, Bio-Rad Laboratories, Hercules, CA) was used to separate myosin isoform.

*Samples.* Frozen samples were cut into slices using a scalpel and placed in 40 µl of refrigerated homogenisation buffer (250 mM sucrose, 100 mM KCl, 5 mM EDTA and 20 mM Tris, pH 6.8). A micropestle was used to homogenise samples. The protein content of each sample was determined using a spectrophotometer with a wavelength of 595 nm using Bradford reagent (Bio-Rad Laboratories; Hercules, CA) in order to standardise the amount of protein loaded per well (14.5 cm width, 8.3 cm height and 1 mm thickness; Bio-Rad Laboratories, Hercules, CA).

*Stacking and separating gel.* The stacking gel contained 33.6% dH<sub>2</sub>O, 14% Tris-HCl 0.5 (pH 6.7), 13.3% acrylamide:Bis (50:1), 30% glycerol, 4% 100mM EDTA (pH 8.0) and 4% SDS. The separating gel was produced by mixing 15% dH<sub>2</sub>O, 13.3% Tris-HCl 1.5 (pH 8.8), 26.7% acrylamide:Bis (50:1), 30% glycerol, 10% glycine and 4% SDS. In order to initiate

polymerisation, TEMED and ammonium persulfate were added to both the separating and stacking gels to a final concentration of 0.1 and 1%, respectively.

*Running buffers.* The lower running buffer consisted of 100 mM Tris, 150 mM glycine and 0.1% SDS. The upper running buffer contained five times the concentration of the lower running buffer. Lower and upper running buffers were cooled to between 4 and 5°C in a refrigerator before use.

*Electrophoretic runs.* The gel unit was cooled to between 4 and 5°C in a refrigerator for the duration of the electrophoretic runs (14 h at 140 V, constant voltage).

*Staining and densitometry.* All gels were stained with Coomassie Blue stain solution for 30 min and destained three times (30 min each) with 70% dH<sub>2</sub>O, 20% methanol and 10% acetic acid. Gels were then scanned with a computer scanner and the densitometric profile was calculated using ImageJ analysis software for Macintosh (v.1.48, National Institutes of Health, Bethesda, Maryland). The determination of densitometric profile proved to be reliable with coefficient of variance and intra-class correlation coefficient being  $1.9 \pm 1.2\%$  and 0.89, respectively.

### **3.2.8. Statistical analyses**

Pearson's correlation coefficients were computed to determine the relationships between voluntary PAP magnitude and maximal voluntary knee extensor torque (measured at  $60^\circ \cdot s^{-1}$ ), quadriceps and VL CSA, quadriceps and VL volume and type II MHC isoform percentage. Initially, the strength of relationships was quantified by calculating coefficients of determination ( $R^2$ ) of linear regressions. The strength of relationships was then quantified by means of polynomial fits and by calculating  $R^2$  using the method of least squares. The order

of the polynomial was determined in a stepwise fashion. Starting with an order of one,  $R^2$  was ascertained. The order of the polynomial was then increased until the  $R^2$  value did not increase by more than 2% if another order was added (Waugh, Blazeovich, Fath, & Korff, 2012).  $R^2$  values of linear and non-linear regressions were then compared. Because the difference in  $R^2$  values between the linear and non-linear regressions was negligible for all the relationships, only linear models were plotted. Where significant correlations were observed between independent variables, partial correlation analyses were used to determine the relationship between voluntary PAP and maximal voluntary knee extensor torque at  $60^\circ \cdot s^{-1}$ , quadriceps and VL CSA, quadriceps and VL volume and/or type II MHC isoform percentage while controlling for a third, independent variable.

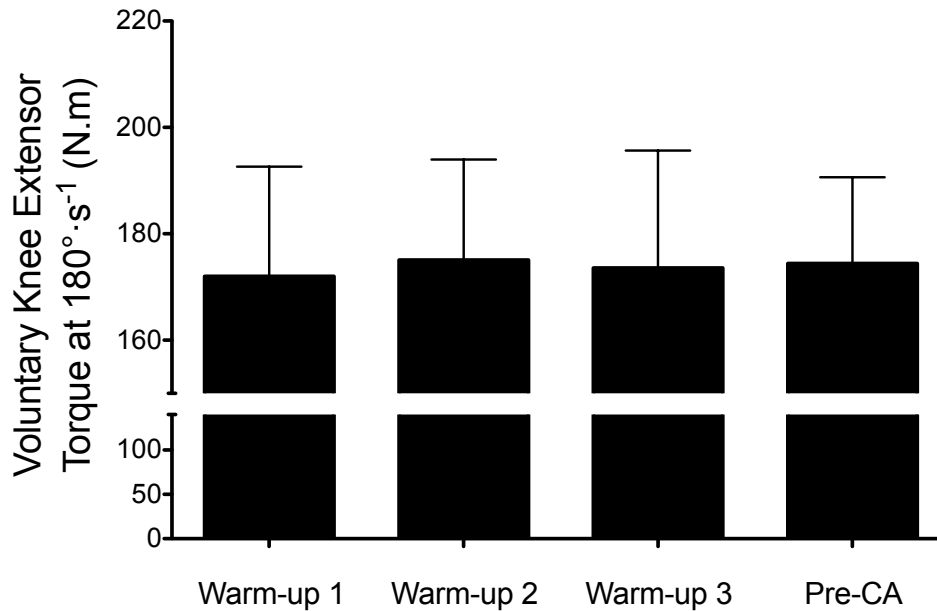
The strength of relationships was assessed using the following criteria (trivial ( $r < 0.1$ ), small ( $r = 0.1-0.29$ ), moderate ( $r = 0.3-0.49$ ), large ( $r = 0.5-0.69$ ), very large ( $r = 0.7-0.89$ ) and nearly perfect ( $r \geq 0.9$ ). The magnitude of the effect size (ES) was considered trivial ( $< 0.20$ ), small ( $0.20-0.50$ ), medium ( $0.50-0.80$ ), large ( $0.80-1.30$ ) or very large ( $> 1.30$ ) {Cohen, 1988 #680}. For all statistical analyses, the level of significance was set at  $p \leq 0.05$ . One-way repeated measures ANOVAs were used to compare the voluntary torque produced during the last three knee extensions of the task-specific warm-up and that produced during the pre-CA knee extensions to determine whether the task-specific warm-up was complete.

### **3.3. Results**

#### **3.3.1 Task-specific warm-up procedure**

No difference was observed between the highest knee extension torques produced during the last three knee extensions of the warm-up and the knee extension during pre-CA testing ( $p = 0.87$ ; Figure 3.1). The lack of statistical difference in torque production between these contractions indicates that maximal muscle contractile capacity was achieved before

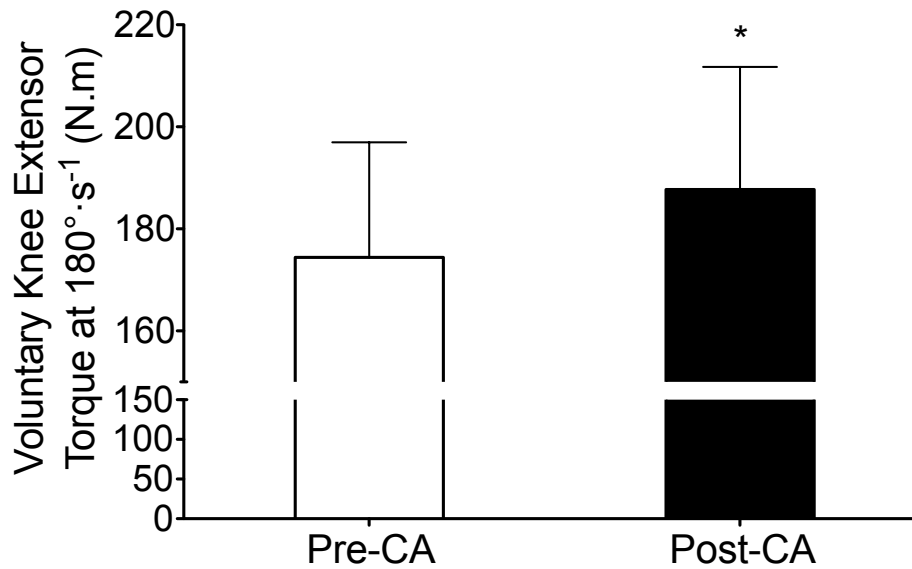
undertaking the CA and the task-specific warm-up was complete since no further improvement in torque production could be elicited by further practice using a 45-s rest interval.



**Figure 3.1.** Voluntary knee extensor torque produced during the last three knee extensions of the warm-up (i.e. warm-up 1, warm-up 2 and warm-up 3) and the pre-CA knee extension. The lack of statistical difference in knee extensor torque production among these contractions indicated that maximal voluntary contractile capacity was achieved before undertaking the conditioning activity (i.e. the task-specific warm-up was complete). CA = conditioning activity.

### 3.3.2. Voluntary PAP magnitude

The voluntary knee extensor torque captured during post-CA testing (i.e. maximum voluntary PAP magnitude irrespective of the time point) was significantly higher ( $+7.2 \pm 4.6\%$ ;  $p < 0.001$ ) than the voluntary knee extensor torque during pre-CA (baseline) testing (Figure 3.2). The magnitude of change ( $ES=0.57$ ) was considered medium.



**Figure 3.2:** Voluntary knee extensor torque before (pre-CA) and after (post-CA) the knee extension conditioning activity. CA = conditioning activity. \* significantly different from Pre-CA ( $p \leq 0.05$ ).

### 3.3.3. Relationships between voluntary PAP magnitude and muscular variables

The muscular variables of the participants are depicted in Table 3.1. There were large to very large correlations between voluntary PAP magnitude and muscular variables including maximal voluntary knee extensor torque at  $60^\circ \cdot s^{-1}$ , quadriceps and VL CSA, quadriceps and VL volume and type II MHC isoform percentage (Column A; Table 3.2). Therefore, a greater voluntary PAP magnitude was observed in participants who could produce higher knee extensor torque, had larger quadriceps and VL CSA and volumes, and had a greater percentage of the fast-MHC isoform.

The relationships between voluntary PAP and maximal voluntary knee extensor strength, quadriceps and VL CSA, and quadriceps and VL volume were not statistically significant after adjusting for the influence of type II MHC isoform percentage using partial correlation analysis (Column B; Table 3.2). By contrast, the correlation between voluntary PAP and type II MHC percentage remained significant after adjusting for the other variables (Column C, Table 3.2).

**Table 3.1.** Voluntary PAP, torque, muscle size and fibre type characteristics of the participants.

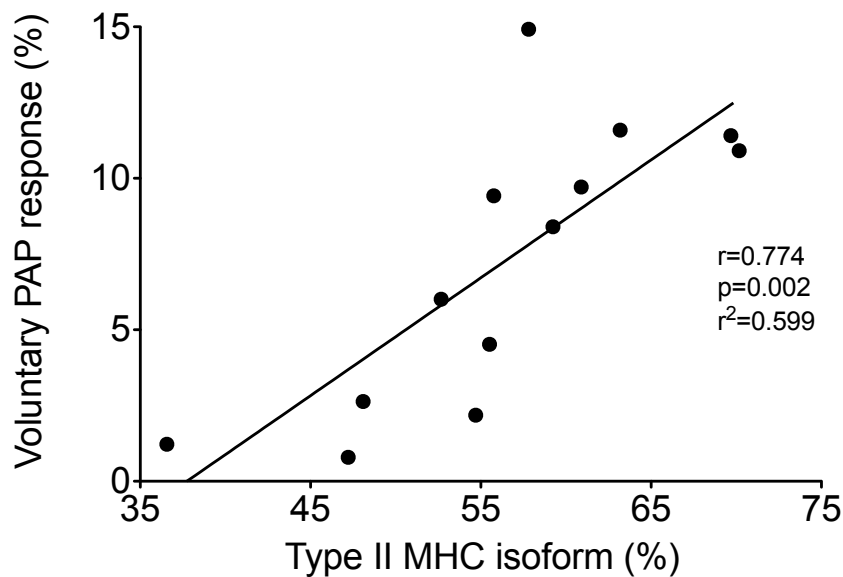
Variables	Mean	±	SD
Voluntary PAP (%)	7.2	±	4.6
Maximal KE Torque at 60°·s <sup>-1</sup> (Nm)	275.4	±	21.4
Quadriceps CSA (cm <sup>2</sup> )	83.3	±	9.36
Quadriceps volume (cm <sup>3</sup> )	2653.9	±	171.5
VL CSA (cm <sup>2</sup> )	24.3	±	4.6
VL volume (cm <sup>3</sup> )	773.7	±	188.3
Type II MHC isoform (%)	56.3	±	9.2

SD = standard deviation; PAP= post-activation potentiation; KE = knee extensor; VL = vastus lateralis; CSA = cross-sectional area; MHC = myosin heavy chain.

**Table 3.2.** Pearson's and partial correlations between muscle torque and size, type II MHC and voluntary PAP.

	(A)		(B)		(C)	
	r	p	r	p	r	p
Maximal Voluntary KE Torque (Nm) at 60°·s <sup>-1</sup>	0.62	0.037	0.38	0.231	0.66	0.019
Quadriceps CSA (cm <sup>2</sup> )	0.68	0.010	0.17	0.605	0.52	0.082
Quadriceps volume (cm <sup>3</sup> )	0.63	0.020	0.23	0.477	0.61	0.037
VL CSA (cm <sup>2</sup> )	0.62	0.024	0.37	0.236	0.66	0.019
VL volume (cm <sup>3</sup> )	0.65	0.016	0.43	0.160	0.66	0.020
Type II MHC isoform (%)	0.77	0.002	-	-	-	-

PAP = post-activation potentiation; KE = knee extensor; CSA = cross-sectional area; VL = vastus lateralis; MHC = myosin heavy chain. (Column A = Correlations between voluntary PAP and various muscular variables. Column B = Partial correlations between voluntary PAP and various muscular variables, adjusted for type II MHC isoform %. Column C = Partial correlations between voluntary PAP and type II MHC isoform (%), adjusted for the various muscular variables).





**Figure 3.3.** Relationship between voluntary PAP response and type II myosin heavy chain isoform percentage. PAP= postactivation potentiation; MHC= myosin heavy chain.

### 3.4. Discussion

The relationships between voluntary PAP magnitude and muscular variables including maximal voluntary extensor strength, quadriceps and VL CSA, quadriceps and VL volume and type II MHC isoform percentage were examined. The main finding was voluntary PAP magnitude was strongly correlated with maximal voluntary knee extensor torque, quadriceps and VL CSA as well as quadriceps and VL volume, however voluntary PAP magnitude was most strongly associated with the type II MHC isoform percentage in the human quadriceps femoris and this correlation remained significant ( $r=0.52-0.66$ ) even after accounting for the influence of muscle strength and size.

The results of the present study are in line with previously published research demonstrating that the performance of an isokinetic CA can contribute to improved voluntary torque production (i.e. voluntary PAP) (Babault et al., 2008; Chaouachi et al., 2011; Fukutani et al., 2013). Because the participants in the present study completed a task-specific warm-up in which maximal contractile capacity was achieved before performing the pre-CA (baseline) testing (Figure 3.1), our data demonstrate that increases in voluntary knee extensor torque production following a CA most likely result from acute physiological changes in response to the CA rather than being either a warm-up or familiarisation effect. This finding is in agreement with the results of Study 1 (Chapter 2) in which the presence of voluntary PAP was observed after maximal voluntary contractile capacity was achieved following the performance of a complete task-specific warm-up.

The large correlation ( $r=0.62$ ;  $p=0.027$ ) between maximal voluntary muscle torque production and voluntary PAP magnitude is also in accordance with previous studies reporting a statistically significant correlation between muscle strength and voluntary PAP (Chiu et al., 2003; Jo et al., 2009; Ruben et al., 2010; Seitz et al., 2014a; Seitz et al., 2014b).

This might be explained by the fact that maximal voluntary knee extensor torque production was significantly, although only moderately, correlated ( $r=0.55$ ;  $p=0.05$ ) with type II MHC isoform percentage, which indicates a link between muscular strength and fibre type (Aagaard & Andersen, 1998; Maughan et al., 1983b; Thorstensson et al., 1976). Therefore, stronger individuals, who tend to have a greater percentage of the type II MHC isoform will also exhibit a greater voluntary PAP response since PAP is most notable in fibres with a greater proportion of the type II MHC isoform (Klug, Botterman, & Stull, 1982; Manning & Stull, 1982; Moore & Stull, 1984). Additionally, the correlation between maximal voluntary knee extensor torque production and voluntary PAP magnitude might also be explained by the fact that individuals producing higher torque levels exhibited a greater quadriceps and VL size (volume and CSA) than their weaker counterparts. Indeed, voluntary peak torque was significantly correlated with voluntary PAP magnitude, although again this correlation was no longer significant when type II MHC was added to a partial correlation analysis. Therefore, it could be argued that any increase in tissue-specific force elicited by voluntary PAP might have been amplified in the strongest individuals although muscle size does not appear to be the most significant factor influencing the voluntary PAP response, at least under the current testing conditions. Future research should attempt to specifically determine whether an increase in tissue-specific force elicited by voluntary PAP is amplified in individuals having larger muscle size.

An important finding of the present study was that the relationships between voluntary PAP and maximal voluntary knee extensor strength, quadriceps and VL CSA, and quadriceps and VL volume were not statistically significant after adjusting for the influence of type II MHC isoform percentage. By contrast, the correlation between voluntary PAP and type II MHC percentage remained significant after adjusting for the other variables (column C, Table 3.2). This finding suggests that voluntary PAP magnitude is most clearly associated with the type II myosin isoform percentage in the human quadriceps femoris. This finding is in

agreement with previous studies showing that individuals with a greater percentage of type II twitch fibers express higher levels of PAP (Hamada et al., 2000; Hamada et al., 2003). These previous studies utilised relatively small sample sizes (i.e. 8 participants) and thus may have been prone to type I error, however our results using a larger (n=13) sample are consistent with their findings. This strong correlation may be explained by the fact that myosin RLC phosphorylation, one proposed mechanism responsible for PAP, has been shown to be greater in type II twitch fibres (Klug et al., 1982; Manning & Stull, 1982; Moore & Stull, 1984). Phosphorylation of myosin RLCs through the activation of MLC kinase is thought to potentiate subsequent contraction by increasing the sensitivity of actin-myosin to  $\text{Ca}^{2+}$  released by the sarcoplasmic reticulum (Grange et al., 1993; Palmer & Moore, 1989; Vandenboom et al., 1995) and thus increasing the likelihood of myosin cross-bridge interaction with actin (Levine et al., 1996; Sweeney et al., 1994). The result is an increase in the number and rate of myosin cross-bridges binding to the actin filament, resulting in an increase in muscle tension (Barany et al., 1980; Manning & Stull, 1982; Metzger et al., 1989). Individuals with a higher percentage of type II twitch fibers may be most likely to exhibit greater myosin RLC phosphorylation because of the higher content of MLC kinase in these fibres (Moore & Stull, 1984). It is worth noting that 13 participants is a small sample size for examining relationships and future research using larger participant cohorts may be required to confirm the present findings. An alternative explanation is that voluntary PAP may be accompanied by increases central neural drive, in line with evidence of increases in H-reflex (Folland et al., 2008; Gullich & Schmidtbleicher, 1996; Trimble & Harp, 1998) and EMG (Hough et al., 2009) amplitudes, which may theoretically increase the contribution of higher-order, type II motor units to muscular contraction (Hodgson et al., 2005). Therefore, individuals with a higher percentage of type II muscle fibres might benefit more from an improved ability to recruit the higher-order motor units, resulting in a greater voluntary PAP response.

In conclusion, the present results show that voluntary PAP magnitude is strongly correlated with maximal voluntary knee extensor torque production (i.e. muscular strength), quadriceps and VL CSA, quadriceps and VL volume (i.e. muscle size) and the percentage of type II MHC isoform (i.e. fibre type). However, the findings that the strongest correlations with voluntary PAP magnitude was observed with the Type II MHC percentage and that this correlation remained significant after accounting for other variables using partial correlations analysis suggest that voluntary PAP magnitude is most clearly associated with the type II myosin isoform percentage. These results are of interest from a practical standpoint as they suggest that interventions leading to an increase in type II MHC isoform content may allow for greater voluntary PAP magnitudes to be achieved. Moreover, the association between type II MHC isoform and voluntary PAP also provides insights about the potential influence of peripheral mechanisms on voluntary PAP. Future research should use more direct techniques to assess potential changes in peripheral function, particularly in the E-C coupling process, following a PAP-inducing CA. A secondary finding was that the voluntary PAP response was also correlated with quadriceps (and VL) muscle size and maximal strength. Nonetheless, these associations were no longer significant after accounting for the possible influence of type II MHC percentage. Therefore, these factors likely play a lesser, or associative, role in the voluntary PAP response.

## **CHAPTER 4**

### **Study 3: Associations between changes in the excitation-contraction coupling process and voluntary post-activation potentiation assessed in the human quadriceps**

#### 4.1. Introduction

The performance of a maximal, or near maximal, voluntary muscle contraction (i.e. a voluntary CA) seems to increase muscular performance in a subsequent task and this phenomenon is termed post-activation potentiation (PAP). One potential explanation for this phenomenon is that peripheral factors, such as changes in the muscle's contractile capacity, may underpin PAP because of the increases in myosin RLC phosphorylation observed in response to a PAP-inducing CA in animal models (Grange et al., 1993; Houston, Green, & Stull, 1985; Klug et al., 1982; Moore & Stull, 1984; Palmer & Moore, 1989; Sweeney et al., 1993). Moreover, in rat muscle increases in the ratio of peak forces produced during 60- vs. 200-Hz frequency trains during constant frequency stimulation have been observed following a PAP-inducing CA (Rijkkelikhuisen et al., 2005), which is indicative of changes in  $\text{Ca}^{2+}$  homeostasis. Nonetheless, these results have yet to be confirmed in humans. It is likely that changes in peripheral function, particularly in the E-C coupling process (i.e. increased intracellular  $\text{Ca}^{2+}$  release and/or  $\text{Ca}^{2+}$  sensitivity) in muscles such as the human quadriceps, may play a role in PAP since no alterations in M-wave amplitude in the presence of twitch PAP (Chapter 2; Miyamoto et al., 2011b) have been observed in this muscle group. This result is suggestive that changes in neuromuscular transmission and excitability of the muscle fibre's sarcolemma (Lepers et al., 2001) are not involved in the PAP process. Additionally, a strong correlation was observed between the magnitude of PAP and the percentage of type II MHC isoform in the knee extensors (Study 2; Chapter 3), which makes sense from the perspective that fibres with a greater proportion of type II MHC isoforms have a greater myosin RLC phosphorylation capacity (Houston et al., 1985) and thus a greater potential for PAP. Collectively, these findings suggest that changes in the E-C coupling process may be influential in the PAP process in human skeletal muscle.

Alterations in the E-C coupling process may be detectable through the analysis of the

ratio of the forces (or joint torques) produced during 20- vs. 80-Hz trains of constant frequency electrical stimulation (20:80). While decreases in 20:80 have been reported in humans after fatiguing exercise (Jones, 1996; Martin et al., 2004), no attempt has been made to quantify changes in this ratio after a PAP-inducing CA in humans. In addition, the muscle's catchlike property refers to the increase in contractile force observed when an initial, short-interval (e.g. 5 ms) double-spike stimulus precedes a train of constant-frequency stimuli; trains of stimuli that take advantage of the catchlike property are referred to as catch-inducing trains (Burke et al., 1970). Changes in the influence of catch-inducing trains after a PAP-inducing CA may also provide information about calcium homeostasis since this phenomenon is thought to be underpinned by increased sarcoplasmic  $\text{Ca}^{2+}$  release (Cheng et al., 2013) and/or increased  $\text{Ca}^{2+}$  sensitivity (Abbate et al., 2002; Nielsen, 2009). Increases in the force produced during a catch-inducing train have been reported in human skeletal muscle following a PAP-inducing, electrically-evoked CA (Ding et al., 2003), however it has yet to be determined whether this occurs after a voluntary PAP-inducing CA. Interestingly, it has also been shown in both animals (Burke et al., 1976) and humans (Stuart, Binder-Macleod, Lee, Fritz, & Kucharski, 1998; Ding et al., 2003) that the force produced during a catch-inducing train, when compared to a constant low-frequency train, is reduced in the presence of potentiation whereas it is increased in the presence of fatigue. This finding suggests that the mechanisms of PAP and the catchlike property of skeletal muscle may be the same. However, these studies have used electrically-evoked CAs to induce PAP, and the effect of voluntary PAP-inducing CAs on the extent of force increase produced during a catch-inducing train has yet to be determined. Together, such analyses would provide new information regarding changes in the E-C coupling process influencing voluntary PAP in human skeletal muscle.

Given the above, the purpose of the present study was to complete a more detailed investigation of the potential role of changes in the E-C coupling process to the voluntary PAP response induced by a voluntary CA in human skeletal muscle. The peak voluntary joint

torques produced during dynamic (isokinetic) knee extensions, isometric torques produced during 20-Hz and catch-inducing trains (using transcutaneous electrical stimulation) as well as during 20- and 80-Hz trains (20:80) were compared before and after a voluntary CA. The force-augmenting effects of the catch-inducing train, defined as the proportional difference between torques produced during a catch-inducing train (i.e. 20-Hz train preceded by a doublet) and a constant frequency train, were also examined.

## **4.2. Methods**

### **4.2.1. Participants**

Fourteen resistance-trained men (mean  $\pm$  SD: age,  $26.4 \pm 4.9$  yr; height,  $1.80 \pm 0.8$  m; body mass,  $81.3 \pm 8.5$  kg) volunteered for the study on the basis that they were involved in a lower body resistance-training program for muscular strength and/or power for at least 6 months. They were required to abstain from taking any stimulants or depressants, including caffeine for at least 6 hours and alcohol for at least 24 hours, prior testing. The procedures of the investigation were approved by the Edith Cowan University Human Research Ethics Committee and were in agreement with the Declaration of Helsinki. An informed consent was obtained from each participant.

### **4.2.2. Study design and overview**

The participants visited the laboratory on three separate occasions (one familiarisation and two experimental sessions) at the same time of the day separated by 5-7 days. The purpose of the first visit was to familiarise them with the isokinetic knee extensions and test procedures. During the subsequent two experimental sessions the participants completed a test protocol incorporating tetanic transcutaneous stimulations and maximal voluntary dynamic knee extensions, which were performed before and 1, 4, 7 and 10 min after one CA or a



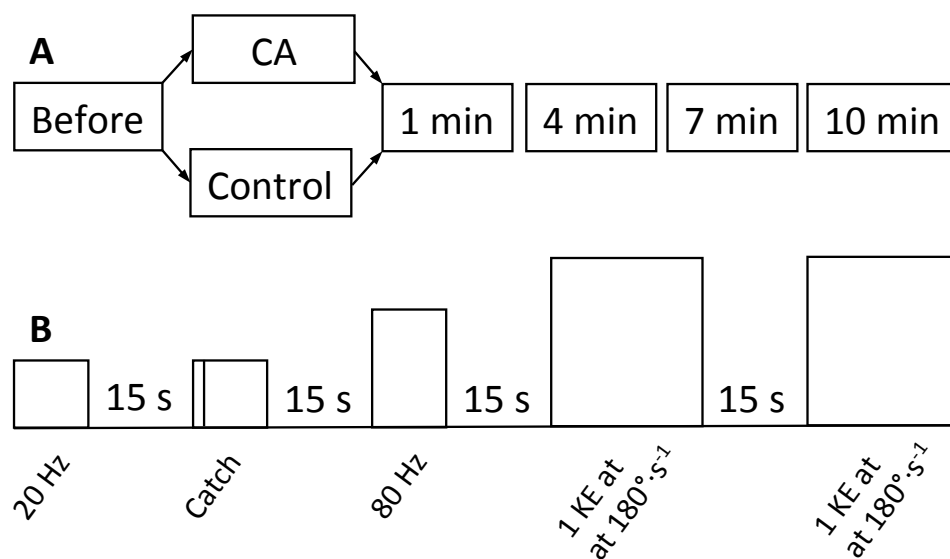
control condition (no CA, rest). The two experimental testing sessions were completed in a randomised order. For all procedures the participants were seated on a dynamometer (Biodex System 3 Pro, Biodex Medical System, Shirley, New York) with their dominant (strongest) thigh strapped to the dynamometer's chair and fixed just above the ankle to the dynamometer lever arm. The lateral femoral epicondyle was aligned with the axis of rotation of the dynamometer, and the knee and hip joints were flexed at 90° and 85°, respectively. The participants were unaware of the study hypotheses as well as test protocol scores. The participants were unaware of the study hypotheses as well as test protocol scores.

#### **4.2.3 Tetanic stimulation procedure**

Electrical square-wave stimuli (0.2-ms pulse width) were delivered to the knee extensor muscle belly through two self-adhesive electrodes (9 × 5 cm, DuraStick II, Chattanooga Group, Hixon, TN) using a constant current stimulator (DS7AH, Digitimer Ltd, Welwyn Garden City, UK). The cathode and anode electrodes were positioned over the motor points of the vastus medialis and rectus femoris/VL, respectively (Ding et al., 2003; Vincent Martin et al., 2004). The electrodes were moved around these areas to find the location that produced the highest torque at a low stimulation intensity (Stuart et al., 1998). For all tetanic stimulations, the stimulation intensity necessary to reach 50% of MVC with a 0.5-s 80-Hz tetanic stimulation was used (Martin et al., 2004). Three evoked contractions of same duration were delivered to test the E-C coupling efficiency: 1) 20-Hz train of 11 pulses (0.05-s interpulse interval); 2) catch-inducing train (i.e., 2 pulses at 0.01-s plus, 10 pulses at 0.05-s interpulse interval); and 3) 80-Hz train of 36 pulses (0.0125-s interpulse interval) (Trajano et al., 2013).

#### 4.2.4. Test protocol

Following the determination of the tetanic stimulation parameters, the participants performed a standardised warm-up consisting of two isokinetic knee extensions at  $180^{\circ}\cdot\text{s}^{-1}$  at 20, 40, 60 and 80% of their perceived maximal force at 45-s intervals. Four contractions at 100% were then performed at 45-s intervals. This procedure was based upon the methods used in Studies 1 and 2 and ensured that the warm-up was complete. A test protocol was then completed 90 s before (pre-CA) and 1, 4, 7 and 10 min after (post-CA) the CA or the control (rest, no CA) interventions. The CA consisted of one set of 4 knee extensions at  $60^{\circ}\cdot\text{s}^{-1}$  with 10 s of rest between each knee extension. The test protocol consisted of the three tetanic stimulations each separated by 15 s (i.e., 20-Hz train, catch-inducing train and 80-Hz train) delivered at a  $60^{\circ}$  knee angle ( $0^{\circ}$ = full knee extension) and two maximal isokinetic knee extensions at  $180^{\circ}\cdot\text{s}^{-1}$ , each separated by a 15 s-rest period (Figure 4.1). For the dynamic knee extensions, the participants received verbal encouragement to extend their knee ‘as fast and as hard as possible’ throughout the whole range of motion ( $90\text{--}0^{\circ}$ ;  $0^{\circ}$ = full knee extension).



**Figure 4.1.** A) Experimental design and time course of measurements. The participants were assessed before and 1, 4, 7 and 10 min after the conditioning activity (CA) protocol or control

(rest) period. B) Order of measurements. 20 Hz = 20 Hz tetanic stimulation, catch = catch-inducing tetanic stimulation, 80 Hz = 80 Hz tetanic stimulation, KE = voluntary knee extension.

#### 4.2.5. Evoked and voluntary torque measurements

The knee extensor torques elicited by 20 Hz and catch-inducing trains, as well as the ratios of 20 Hz vs. 80 Hz (20:80) and the force-augmenting effect of the catch-inducing train were used as measures of the E-C coupling process. The knee extensions resulting in the highest voluntary peak torque at each time point (i.e. pre- and post-CA) were selected to calculate voluntary PAP using the equation:

$$\% \text{ Voluntary PAP} = [(\tau_{\text{vol,post-CA}} - \tau_{\text{vol,pre-CA}}) / \tau_{\text{vol,pre-CA}}] \times 100$$

where  $\tau_{\text{vol,post-CA}}$  is the voluntary peak torque measured during the test protocol after the CA (i.e. post-CA) and  $\tau_{\text{vol,pre-CA}}$  is the voluntary peak torque measured before the CA (i.e. pre-CA).

The force-augmenting effect of the catch-inducing train was calculated at each time point (pre and 1, 4, 7 and 10 min post-CA) using the following equation:

$$y = [(\tau_{\text{CIT}} - \tau_{\text{20Hz}}) / \tau_{\text{20Hz}}] \times 100$$

where  $y$  is the force-augmenting effect of the catch-inducing train,  $\tau_{\text{CIT}}$  is the torque produced during the catch-inducing train and  $\tau_{\text{20Hz}}$  is the torque produced during the 20-Hz constant frequency train (Ding et al., 2003).

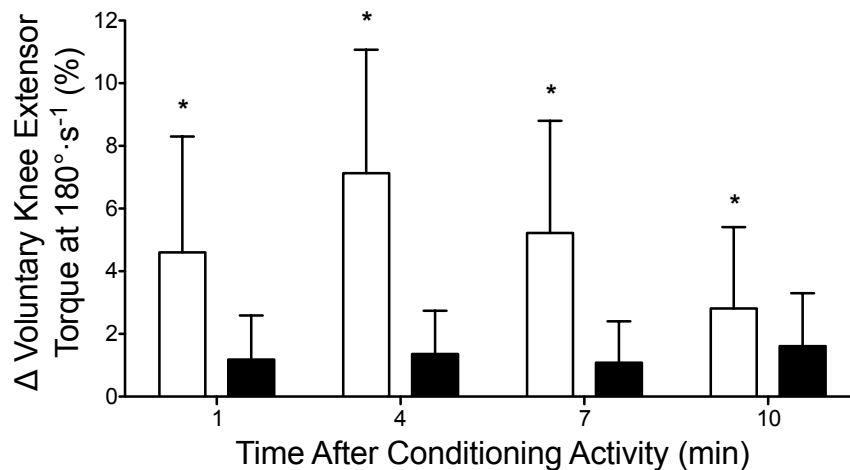
#### 4.2.6. Statistical analyses

Separate two-way repeated measures ANOVAs were performed to compare all variables (20-Hz and catch-inducing trains, 20:80 and the force-augmenting effect of the catch-inducing train) between conditions (CA vs. control) over time (before and 1, 4, 7 and 10 min after). Pairwise comparisons with Bonferroni corrections were performed when significant interaction effects were detected. Pearson's correlation coefficients were computed

to quantify the relationships between changes in voluntary torque and changes in all variables. The strength of relationships was assessed using the following criteria (Cohen, 1988): 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 ( $r \geq 0.9$ ). For all statistical analyses, the level of significance was set at an  $\alpha$ -level of 0.05.

### 4.3. Results

There was a significant interaction (time  $\times$  condition) effect for voluntary PAP (Figure 4.2 and Table 4.1). Post-hoc analyses revealed statistically significant increases in voluntary torque at 1, 4, 7 and 10 min after the CA.



**Figure 4.2.** Time course of peak voluntary torques at  $180^\circ \cdot s^{-1}$  after the conditioning activity (white columns) and the control condition (black columns). \*significantly different from baseline ( $p < 0.05$ ).

There was a significant interaction effect for the torques produced during the 20-Hz and catch-inducing trains (Table 4.1). Post-hoc analyses revealed statistically significant increases in peak torque elicited by 20-Hz at 1 min ( $10.2 \pm 6.3\%$ ;  $p = 0.002$ ), 4 min ( $13.0 \pm 7.0\%$ ;  $p = 0.001$ ) and 7 min ( $6.3 \pm 4.0\%$ ;  $p = 0.002$ ) after the CA. Similarly, peak torque elicited by the catch-inducing train was statistically increased at 1 min ( $5.6 \pm 3.8\%$ ;  $p = 0.002$ ), 4 min ( $5.7 \pm 3.9\%$ ;  $p = 0.001$ ) and 7 min ( $2.9 \pm 2.1\%$ ;  $p = 0.002$ ) after the CA.

There was a significant interaction effect for the 20:80 Hz ratio (Table 4.1). Post-hoc analyses revealed statistically significant increases in 20:80 at 1 min ( $7.9 \pm 6.7\%$ ;  $p=0.007$ ), 4 min ( $13.8 \pm 8.8\%$ ;  $p=0.000$ ) and 7 min ( $5.6 \pm 4.5\%$ ;  $p=0.01$ ) after the CA. The force-augmenting effects of the catch-inducing train diminished as the magnitude of PAP increased and then increased as the magnitude of voluntary PAP diminished in the recovery period after the CA (Table 4.2). The reduced force-augmenting effects of catch-inducing train in the presence of voluntary PAP were taken to indicate that these two phenomena may be associated. No significant interaction effect was found at 10 min after the CA or at any time after the control condition ( $p > 0.05$ ) (Table 4.1 and 4.2).

**Table 4.1** Torque data before and after the conditioning activity and the control condition.

	Before	1 min	4 min	7 min	10 min
Experimental					
T <sub>peak</sub> , Nm	147.9 ± 23.8	155.7* ± 30.2	159.1* ± 30.2	156.2* ± 29.4	152.4* ± 26.3
20-Hz, Nm	68.8 ± 12.5	76.4* ± 17.3	78.3* ± 17.9	73.5* ± 15.5	69.8 ± 14.6
CIT, Nm	76.8 ± 13.3	81.4* ± 16.4	81.5* ± 16.4	79.3* ± 15.2	77.6 ± 14.2
80-Hz, Nm	100.1 ± 17.9	102.7 ± 20.7	99.3 ± 17.0	101.2 ± 19.8	101.5 ± 18.6
20:80	0.69 ± 0.03	0.74* ± 0.05	0.78* ± 0.06	0.73* ± 0.05	0.69 ± 0.04
Control					
T <sub>peak</sub> , Nm	150.1 ± 23.9	151.7 ± 23.0	152.1 ± 24.3	151.5 ± 22.7	152.6 ± 25.0
20-Hz, Nm	69.1 ± 13.4	69.6 ± 12.7	70.8 ± 14.5	70.3 ± 13.9	70.0 ± 13.4
CIT, Nm	76.6 ± 14.4	77.6 ± 15.7	77.7 ± 14.0	77.8 ± 15.1	77.3 ± 14.4
80-Hz, Nm	101.5 ± 14.9	99.8 ± 15.6	102.2 ± 17.0	101.9 ± 16.8	101.2 ± 18.2
20:80	0.68 ± 0.06	0.70 ± 0.05	0.69 ± 0.06	0.69 ± 0.05	0.69 ± 0.04

T<sub>peak</sub> = peak voluntary torque at 180°·s<sup>-1</sup>, CIT = catch-inducing train, 20:80 = ratio of the torque produced by 20- and 80-Hz trains.

**Table 4.2.** Voluntary PAP magnitude and force-augmenting effects of the CIT.

	Before	1 min	4 min	7 min	10 min
Experimental					
Voluntary PAP (%)	-	4.8 ± 3.9	7.1 ± 3.9	5.0 ± 3.6	2.8 ± 2.6
Force-augmenting effects of CIT (%)	11.9 ± 3.9	7.3 ± 4.9	4.7 ± 4.5	7.3 ± 5.3	11.4 ± 4.7
Control					
Voluntary PAP (%)	-	1.2 ± 1.4	1.4 ± 1.4	1.1 ± 1.3	1.6 ± 1.7
Force-augmenting effects of CIT (%)	10.9 ± 4.6	11.2 ± 7.8	10.4 ± 5.3	10.9 ± 6.0	10.6 ± 6.9

PAP = post-activation potentiation, CIT = catch-inducing train

In addition, statistically significant correlations were observed between changes in voluntary torque production (i.e. voluntary PAP) and changes in torque produced by 20-Hz and catch-inducing trains, 20:80, and force-augmenting effects of the catch-inducing train at 1, 4 and 7 min after the CA (Table 4.3).

**Table 4.3.** Relationships between changes in voluntary torque (voluntary PAP) and changes in torque produced during the 20-Hz and catch-inducing trains, 20:80 and force-augmenting effects of the catch-inducing train.

	Time after conditioning activity			
	1 min	4 min	7 min	10 min
20-Hz/PAP	0.75*	0.76*	0.72*	0.45
80-Hz/PAP	0.31	-0.28	0.27	0.18
CIT/PAP	0.60*	0.63*	0.57*	0.47
20:80/PAP	0.57*	0.74*	0.54*	0.18
Force-augmenting effects of CIT/PAP	-0.50*	-0.81*	-0.65*	-0.23

PAP = post-activation potentiation, CIT = catch-inducing train, 20:80 = ratio of the torques produced by 20- and 80 Hz trains.

#### 4.4. Discussion

The aim of the present research was to provide a more detailed investigation of the potential role of changes in the E-C coupling process to the voluntary PAP response induced by a voluntary CA in human skeletal muscle. Thus, torques produced during voluntary knee extensions as well as those elicited by 20 Hz and catch-inducing trains (i.e. with a doublet at short interval pulse interval preceding a constant frequency train of stimuli) were examined. Torques elicited by 20- vs. 80-Hz trains (20:80) as well as the force-augmenting effects of the catch-inducing train were also compared. The results indicate that the E-C coupling process was significantly altered after the CA, as indicated by statistically increases in 20-Hz and catch-inducing torques, increases in the 20:80 ratio, and decreases in the force-augmenting effects of the catch-inducing train. Moreover, these changes were correlated with changes in voluntary knee extension torque production (i.e. voluntary PAP; Table 4.3), particularly when the peak voluntary PAP effect was most strongly observed (i.e. at 4 min post-CA). Consistent

with some research, the present data demonstrate that voluntary knee extension torque production was increased in response to an isokinetic CA (for reviews, see Hodgson et al., 2005; Tillin & Bishop, 2009) with the present changes being of similar magnitude to those reported in Studies 1 and 2 using similar contraction velocities for both the voluntary CA (i.e.  $60^{\circ}\cdot\text{s}^{-1}$ ) and voluntary knee extension test (i.e.  $180^{\circ}\cdot\text{s}^{-1}$ ).

In the present investigation torques produced by the 20-Hz train were statistically increased after the performance of the CA (Table 4.1). While similar increases have been reported after an electrically-evoked CA (Ding et al., 2003), to the best of our knowledge the present study is the first to demonstrate that such increases occur after a voluntary CA and that these increases are correlated with the changes in voluntary peak torque (i.e. voluntary PAP) (Table 4.3). Torques produced by 20- vs. 80-Hz trains (20:80) before and after the CA were taken as an indicator of changes in  $\text{Ca}^{2+}$  release from the sarcoplasmic reticulum and/or  $\text{Ca}^{2+}$  sensitivity (Keeton & Binder-Macleod, 2006; MacIntosh & Rassier, 2002). While increases in the ratio of the forces produced at 60- vs. 200 Hz frequency trains have been observed following a PAP-inducing CA in rat muscle (Rijkkelijkhuizen et al., 2005), the present study is the first to report increases in the 20:80 Hz ratio after a PAP-inducing CA in human skeletal muscle. Importantly, strong correlations were observed between these increases and the changes in voluntary peak torque (Table 4.3). Thus, greater increases in 20:80 were observed in individuals with the greatest voluntary PAP response. The present data suggest that increases in voluntary torque production after a voluntary CA in human skeletal muscle might be influenced by changes in  $\text{Ca}^{2+}$  release and/or sensitivity from sarcoplasmic reticulum. One possible explanation is that the CA may have improved the interaction between voltage-sensitive dihydropyridine receptors and calcium-release ryanodine receptors (MacIntosh & Rassier, 2002), resulting in greater  $\text{Ca}^{2+}$  binding to troponin, and therefore an increase in muscle tension (MacIntosh & Rassier, 2002).



Changes in the efficacy of the muscle's catchlike property were also taken as an indicator of changes in the E-C coupling efficiency (i.e. increased sarcoplasmic  $\text{Ca}^{2+}$  release and/or increased  $\text{Ca}^{2+}$  sensitivity to actin-myosin interaction) via the analysis of the force-augmenting effects of the catch-inducing train. These force-augmenting effects represent the extra force produced during a catch-inducing train (i.e. 20-Hz train preceded by a doublet) in comparison to a 20-Hz constant frequency train without a doublet. The results indicate that the force-augmenting effects of the catch-inducing train were reduced as the magnitude of PAP increased and then increased as the magnitude of PAP diminished during the 10-min period after the CA (Table 4.1). Moreover, these changes were strongly correlated with changes in voluntary torque production (Table 4.3). These observations are in line with the findings of others who used electrically-evoked CAs to induce PAP (Ding et al., 2003). For example, Ding et al. (2003) observed in human skeletal muscle that a catch-inducing train produced similar torques to a 14-Hz train in the presence of PAP yet approximately 10% more torque in a non-potentiated state. In the present study, the catch-inducing train elicited 11.9% more torque than the 20-Hz constant frequency train before the CA, whereas it elicited 4.7 and 11.4% more torque than the 20-Hz train at time points where voluntary PAP was maximum (i.e., 4 min) and minimum (i.e. 10 min), respectively (Table 4.1). Furthermore, these changes were strongly correlated with changes in the voluntary torque production. The present data and those of Ding et al. (2003) indicate that a saturation process (i.e. when  $\text{Ca}^{2+}$  release and/or sensitivity are at their maximal level) may have limited the force-augmenting effects of the catch-inducing train when the muscles were in a highly potentiated state. It could therefore be argued that similar mechanisms might be shared by these phenomena. Indeed, if increased  $\text{Ca}^{2+}$  sensitivity to actin-myosin interaction is an important mechanism of both PAP (Grange et al., 1993; Palmer & Moore, 1989; Vandenboom et al., 1995) and the muscle's catchlike property (Abbate et al., 2002; Binder-Macleod & Barrish, 1992; Duchateau & Hainaut, 1986), little force advantage may be provided by the catch-inducing

train after a PAP-inducing CA because  $\text{Ca}^{2+}$  sensitivity has already been increased by the CA. Conversely, before the CA the sensitivity of actin-myosin to  $\text{Ca}^{2+}$  may have been lower and therefore a greater advantage may be gained by the use of the catch-inducing train (i.e. with a doublet) by enhancing  $\text{Ca}^{2+}$  sensitivity and thus augmenting muscular force. It is interesting to note that the maximum voluntary PAP magnitude was more strongly correlated with the force-augmenting effects of the catch-inducing train ( $r=-0.81$ ) than the 20:80 Hz ratio ( $r=0.74$ ) (Table 4.3). This result may indicate that the maximum voluntary PAP magnitude in human skeletal muscle may be more clearly underpinned by increases in myosin  $\text{Ca}^{2+}$  sensitivity rather than increased  $\text{Ca}^{2+}$  release, although this requires explicit examination. Such a finding corroborates findings in the animal model indicating that PAP is underpinned by increases in myosin RLC phosphorylation in response to a PAP-inducing CA (Houston et al., 1985; Klug et al., 1982; Moore & Stull, 1984; Palmer & Moore, 1989), which renders actin and myosin more sensitive to  $\text{Ca}^{2+}$ .

A low level of voluntary PAP persisted 10 min after the CA when no statistical increase in any of these variables remained (Table 4.1). Thus, the possibility exists that other factors might also influence the increase in voluntary torque after a voluntary CA. Importantly, dephosphorylation of the myosin RLC typically occurs within 5-6 min of the cessation of muscle activity (MacIntosh et al., 2012), suggesting that changes in the E-C coupling efficiency may have had minimal influence on voluntary PAP by 10 min. Increases in both the H-reflex (Folland et al., 2008; Gullich & Schmidtbleicher, 1996; Trimble & Harp, 1998) and EMG (Hough et al., 2009) amplitudes have been observed during voluntary PAP suggesting that an improved central (neural) drive and thus increase in motor unit recruitment might influence the PAP response. However, some methodological limitations associated with these techniques might prevent strong conclusions being drawn regarding the direct influence of changes in central drive, and recent studies have found no evidence of changes in the EMG:M ratio in the presence of voluntary PAP (Study 1; Fukutani et al., 2013). Thus

other methodologies (e.g. cortical, cortico-medullary or peripheral nerve stimulation) methodologies could be used in future experiments to more clearly quantify potential changes at the central level.

In conclusion, the present data suggest that increases in voluntary torque production following a voluntary CA in human skeletal muscle (i.e. voluntary PAP) are associated with changes in peripheral function, specifically changes in the E-C coupling process. This conclusion is based on 1) the statistically significant increases in torque produced by 20-Hz and catch-inducing trains as well as increases in the 20:80 Hz ratio; 2) decreases in the force-augmenting effects of the catch-inducing train after the performance the CA; and 3) the moderate-to-strong correlations observed between these variables and the change in voluntary torque production although future research using larger participant cohorts may be required to confirm these findings.

. The greater correlation between voluntary PAP and the force-augmenting effects of the catch-inducing train than the 20:80 Hz ratio indicates that changes in  $\text{Ca}^{2+}$  sensitivity may have played a greater role than an increase in  $\text{Ca}^{2+}$  release from the sarcoplasmic reticulum, although explicit testing of this hypothesis is required in future research. It is important to note that others mechanisms may have contributed to the observed voluntary PAP effect, particularly at 10 min after the CA when no statistical increase in any of these variables remained at this time point.

## CHAPTER 5

### Overall Discussion and Conclusion

The aims of the present thesis were to: 1) determine whether the voluntary PAP is a consequence of the performance of a CA, or whether it simply reflects warm-up and/or familiarisation effects; 2) clarify the influence of the characteristics of the CA (e.g. velocity, duration and total work on voluntary PAP effects; 3) determine the factors allowing stronger individuals to express higher levels of voluntary PAP; and 4) determine the peripheral and central mechanisms of voluntary PAP. This thesis was divided into three studies; the first study was designed to clarify whether the voluntary PAP phenomenon commonly observed after a voluntary CA is a consequence of acute neuromuscular alterations relating to the CA itself, or whether it simply reflects warm-up and/or familiarisation effects. This study also examined the influence of the contraction velocity, duration and total work of voluntary CAs on voluntary PAP, and the influence of peripheral and central mechanisms on voluntary PAP. The second study was designed to examine the factors within individuals that may influence their PAP response. Based on the results of Studies 1 and 2, a third study was designed to examine potential changes in peripheral function, including changes in the E-C coupling process, following the performance of a PAP-inducing voluntary conditioning activity.

#### 5.1. Post-activation potentiation after continued knee extensor task-specific practice

The main finding of the first experimental study (Study 1, Chapter 2) was that even after maximal voluntary contractile capacity was achieved after completing a complete warm-up, the imposition of CAs of different force-time characteristics (but with a longer total contraction duration (6 s) and a minimum total work of ~750-900 J) elicited significant increases in both voluntary (for 7 min) and twitch (for 4 min) torques (i.e., voluntary and

twitch PAP, respectively) regardless of the CA velocity. Despite this, no changes in EMG:M or M-wave amplitude were detected after any conditioning activity. These findings suggest that 1) the voluntary PAP phenomenon may result specifically from acute physiological changes in response to the CA rather than being either a warm-up or familiarisation effect; 2) a minimum CA contraction duration and total work appear important to trigger the mechanisms of voluntary PAP, although movement velocity appears relatively unimportant; and 3) changes in central drive may not be a major factor influencing the voluntary PAP response whereas there is some evidence, albeit inconclusive, that changes in peripheral function may have contributed to the observed voluntary PAP under the present testing conditions. Future research is therefore required to more clearly determine the influence of changes in peripheral function to voluntary PAP.

These research findings are important for several reasons. First, previous PAP studies have used different warm-up activity strategies, and others did not appear to impose any warm-up before the baseline testing. The findings from such studies are important from a practical standpoint because they show that voluntary PAP can be elicited after a voluntary CA, particularly when extensive warm-ups are not possible (or feasible). However, they do not allow speculation as to whether improvements in performance might be possible in exercise, sporting and some clinical contexts where extensive pre-exercise routines are completed before exercise. It is also not possible to determine whether the increase in muscular performance observed after a PAP-inducing CA is a consequence of acute neuromuscular alterations relating to the CA itself or whether it simply reflects warm-up and/or familiarisation effects. In the present study, a complete warm-up including extensive task-specific practice to the point where no further improvement in voluntary torque production could be achieved with repeated test practice was imposed before baseline testing. The results indicate that the performance of dynamic CAs could contribute to improved subsequent muscular performance under these specific conditions. From a practical

standpoint, the present findings demonstrate that even if maximal voluntary contractile capacity (i.e. peak torque) has previously been achieved during a warm-up, the performance of voluntary CAs may further increase muscular performance under specific conditions.

A second important point is that the characteristics of dynamic CAs influence the PAP magnitude. Indeed, CAs of longer contraction duration elicited significant increases in voluntary torque production regardless of the movement velocity and total work done during a CA, although a minimum of work appears to have been required for the PAP effect to be elicited. These findings have important practical implications because they demonstrate that a minimum contraction duration or total work must be reached during a dynamic CA to impact subsequent muscular performance. Thus, consideration should be given to the choice of CA characteristics, and further research is required to find optimal CA regimes for different exercise, sporting and clinical contexts. Despite providing important practical implications, one limitation of this study is the use of isokinetic exercises as CA and test protocol. It would be interesting to determine what would happen with traditional resistance exercises as they are commonly used in the practical environment to induce and measure PAP.

A final important finding is that PAP appears to be underpinned by peripheral (muscular) rather than central (neural) mechanisms. Increases in twitch torque were evident without changes in the M-wave amplitude, suggesting that changes in peripheral function may have been influenced by the CAs, yet the EMG:M amplitude remained unaltered. Collectively, these results suggest that changes in central drive were not a significant factor influencing the PAP response. However, one limitation of this study is that only two measures were taken as indicators of changes in peripheral and neural functions influencing PAP. More specific methodologies should be employed in future studies to look for potential small changes in and peripheral and neural function. Also, more detailed investigation of muscle function is required to determine the precise mechanisms of the PAP effect as observed under

the present experimental conditions. This may then provide information as to how best elicit PAP in individuals who do not seem to respond to a CA.

## **5.2. The relationships between muscular strength level, muscle size and myosin heavy chain isoform composition and voluntary post-activation potentiation**

It is clear from the literature and the results of Studies 1 and 2 that there is a substantial inter-individual variability in the voluntary PAP response. Indeed, some individuals appear to express PAP after a voluntary CA while others do not. One explanation might be that the voluntary PAP response is influenced by an individual's neuromuscular characteristics. For example, correlations have been reported between muscle strength and voluntary PAP (Ruben et al., 2010; Seitz et al., 2014a; Seitz et al., 2014b). Theoretically, this might be explained by stronger individuals having a larger muscle mass, which might amplify any PAP effect elicited at the fibre level. Nonetheless, the possibility exists that the relationships between voluntary PAP and both strength and muscle size might be explained by these individuals having a greater mass of type II fibres. Therefore, the second study (Chapter 3) was designed to examine the relationships between voluntary PAP and maximal voluntary knee extensor torque, quadriceps and VL CSA and volume, and type II MHC isoform percentage. A main finding was that large to very large correlations were found between voluntary PAP magnitude and maximal voluntary knee extensor torque ( $r=0.62$ ), quadriceps ( $r=0.68$ ) and VL ( $r=0.62$ ) CSA, and quadriceps ( $r=0.63$ ) and VL ( $r=0.65$ ) volume. However, these relationships were no longer statistically significant after adjusting for the influence of type II MHC isoform percentage using partial correlation analysis. By contrast, the correlation between voluntary PAP and type II MHC percentage was the largest, and it remained significant after adjusting for the other variables. This suggests that the voluntary PAP magnitude is mostly clearly associated with the type II myosin isoform percentage in the human quadriceps femoris. It was considered possible, therefore, that myosin RLC

phosphorylation is an important mechanism influencing the PAP response since this process is greater in type II fibres in animal studies (Klug et al., 1982; Manning & Stull, 1982; Moore & Stull, 1984). However, it is important to note that only 13 participants were tested in the present study and future research using larger participant cohorts may be required to confirm the present finding. Nevertheless, this result has important practical implications because it suggests that interventions leading to an increase in type II MHC isoform content might also allow greater voluntary PAP magnitudes to be achieved. Cumulatively, the results of Studies 1 and 2 indicate that changes at the peripheral level may play an important role in the observed voluntary PAP process.

### **5.3. Associations between changes in the excitation-contraction coupling process and voluntary post-activation potentiation**

While studies indicate that changes in the E-C coupling process may influence PAP under electrically-evoked conditions in human skeletal muscle, it has yet to be determined whether this occurs under voluntary conditions. Therefore, the third study (Chapter 4) was designed to provide a more detailed investigation of the potential role of changes in the E-C coupling process to the voluntary PAP response induced by a voluntary CA in human skeletal muscle. 20-Hz and catch-inducing trains elicited by transcutaneous electrical stimulation, the ratio of the torques produced during 20- and 80-Hz trains (20:80) as well as the force-augmenting effects of the catch-inducing train were used to quantify alterations in the E-C coupling process. The main findings were that: 1) increases in voluntary torque, torques elicited by 20-Hz and catch-inducing trains, and 20:80 were observed 1, 4 and 7 min after the CA; 2) the force-augmenting effects of the catch-inducing train diminished as the magnitude of PAP increased and then increased as the magnitude of PAP diminished; and 3) large to very large correlations ( $r=0.50-0.81$ ) were observed between the changes in voluntary torque production (i.e. voluntary PAP) and the changes in these variables. These results suggest that



increases in voluntary PAP following a voluntary CA are strongly associated with changes in peripheral function, and most probably changes in the E-C coupling efficiency, although explicit testing of this hypothesis is required in future research. However, other mechanisms may have contributed to the observed voluntary PAP, particularly at 10 min after the CA when no statistical increase in any of these variables remained at this time point despite the presence of a small voluntary PAP. Future studies are therefore required to more clearly examine the mechanisms of voluntary PAP later in time (e.g. 10 min) after completing a voluntary CA. In addition, future research using larger participant cohorts may be required to confirm the present findings.

#### **5.4. Conclusion**

In summary, the findings of the present thesis indicate that a voluntary CA can induce voluntary and twitch PAP after a complete warm-up including extensive task practice. Such a PAP response would result specifically from acute physiological changes in response to the CA rather than being either a warm-up or familiarisation effect. Therefore, CAs may be used even in conditions where a complete warm-up is performed, such as in exercise and sporting environments, in order to further improve muscular performance. Moreover, the contraction duration and amount of total work of the CA appear to influence voluntary and twitch PAP, whereas the velocity of the CA may not be an important factor. However, there is a notable inter-individual difference in the PAP response. This variability may be strongly related with an individual's strength level and muscle size, but is in fact most clearly associated with its type II myosin isoform percentage, at least in the knee extensors. Also, there is substantive evidence that voluntary PAP induced by a voluntary CA is strongly associated with changes in the E-C coupling process.

The present study has provided novel information regarding the influence of CA characteristics as well as the importance of muscle fibre type (over muscle strength and size) on voluntary PAP. Ultimately, the effect appears to be traceable to an alteration in the E-C coupling process, probably in type II fibres. However, future studies using more direct measures of cortico-spinal drive (e.g. cortico-electrical/magnetic stimulation or V- and F-wave measurements, or H-reflex amplitudes measured in contracting muscle) are required to assess changes at the central level.

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# APPENDICES

## Appendix 1: Ethics Approval

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**From:** Research Ethics  
**Sent:** Wednesday, 21 March 2012 1:01 PM  
**To:** Laurent SEITZ  
**Cc:** Tony BLAZEVIICH; Greg HAFF; Research Assessments  
**Subject:** 7818 SEITZ ethics approval

Dear Laurent

**Project Number:** 7818 SEITZ  
**Project Name:** Mechanisms Underpinning Postactivation Potentiation Following Voluntary Isokinetic Knee Extensions

**Student Number:** 10167687

The ECU Human Research Ethics Committee (HREC) has reviewed your application and has granted ethics approval for your research project. In granting approval, the HREC has determined that the research project meets the requirements of the *National Statement on Ethical Conduct in Human Research*.

The approval period is from 21 March 2012 to 21 February 2014.

The Research Assessments Team has been informed and they will issue formal notification of approval. Please note that the submission and approval of your research proposal is a separate process to obtaining ethics approval and that no recruitment of participants and/or data collection can commence until formal notification of both ethics approval and approval of your research proposal has been received.

All research projects are approved subject to general conditions of approval. Please see the attached document for details of these conditions, which include monitoring requirements, changes to the project and extension of ethics approval.

Please feel free to contact me if you require any further information.

Regards  
Kim

**Kim Gifkins, Research Ethics Officer**, Office of Research & Innovation, Edith Cowan University, 270 Joondalup Drive, Joondalup, WA 6027 [research.ethics@ecu.edu.au](mailto:research.ethics@ecu.edu.au) Tel: +61 08 6304 2170 | Mobile: 0428 035 397 | Fax: +61 08 6304 5044 | CRICOS IPC 00279B



## **Appendix 1: Information Letter to Participants (Studies 1 and 2)**



### **Information Letter to Participants**

Thank you for expressing your interest in this research; the purpose of this document is to explain the study that you may choose to participate in as a subject. Please read this documents carefully, and do not hesitate to ask any questions.

#### **Project Title**

Mechanisms Underpinning Postactivation Potentiation Following Voluntary Isokinetic Knee Extensions.

#### **Researchers**

This research project is being undertaken as part of the requirements of a PhD candidature (Sport and Exercise Sciences) at Edith Cowan University (ECU).

PhD Candidate: Laurent Seitz ([l.seitz@ecu.edu.au](mailto:l.seitz@ecu.edu.au)) 6304 5819

Supervisor: A/Prof. Anthony Blazeovich ([a.blazeovich@ecu.edu.au](mailto:a.blazeovich@ecu.edu.au)) 6304 5472

Co-supervisor: Dr Gregory Haff ([g.haff@ecu.edu.au](mailto:g.haff@ecu.edu.au)) 6304 5416

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

#### **Purpose of the study**

The purpose of this study is to examine quadriceps cross sectional area, muscle fibre type, force production, muscle activity and the ability to activate muscle maximally after knee extensions at different speeds.

## **Research Outline**

In order to participate in this study, you will be asked to complete a medical questionnaire and to refrain from performing sports or hard exercise training for one day prior to the experimental day. You are also required to abstain from taking any stimulants or depressants, including caffeine for at least 6 hours or alcohol for at least 24 hours prior testing. You are also asked refrain from taking any anticoagulant medications, aspirin, or other medications that affect blood clotting, for two days prior to procedure.

If you participate in this study, you will be asked to report to the Exercise Physiology Lab (Building 19, Room 19.150) on five days each separated by one week (scheduling is flexible) at the same time of the day. Each day, before the measurements start, you will be asked to do a 5-minute warm-up on a stationary bicycle and to complete submaximal and maximal isokinetic knee extensions. On the first day you will be acquainted with all testing procedures such as: muscle cross sectional area measurement, maximal voluntary knee extensions and electrical nerve stimulation techniques. Electrical stimulation procedures require a small electrical current to be applied to the femoral nerve using self-adhesive surface electrodes. The stimulation will be started at very low intensities and progressively increased until your muscle is maximally activated or you feel discomfort; at maximal intensities the electrical stimulation might be slightly uncomfortable.

On visits 2-5 you will complete the knee extension test and, the electrical stimulation protocols, before and 1, 4, 7, 10, 13, 16 and 19 minutes after eight knee extensions protocols that will be randomised over four sessions (i.e there will be two test sequences performed in each session). Small self-adhesive skin-mounted electrodes will be used to record the

electrical signals emanating from your quadriceps and hamstrings muscles during contractions (these sit passively on the skin and there is no discomfort). The skin under the electrodes will be gently abraded and cleaned with alcohol (the alcohol minimises the risk of skin infection).

You will then be asked to report to the Exercise Physiology Lab on one more occasion at a time of your choice to undertake a muscle microbiopsy procedure. A single biopsy sample will be obtained from your vastus lateralis muscle (i.e. quadriceps muscle).

First day measurements will take about 2.5 hours, second, third, fourth and fifth days will take about 2 hours, and the sixth (muscle biopsy) day will take about 45 minutes. You are free to complete the study without undertaking the biopsy procedure.

### **Eligibility**

You will be eligible for this study if:

You are between 18 and 35 years old

You have no neuromuscular injuries

You have been engaged in a lower body resistance training for power and/or muscular strength for at least 6 months

### **Risks**

As with all tests of maximal muscle force production, there is the chance for muscle or tendon strain. This risk is low given that proper warm-up and familiarisation will be performed, the tests will be conducted by a researcher who is experienced in the procedures, and isokinetic (i.e. constant speed) muscle actions carry a relatively low risk of injury.

Electrical stimulation procedures can be uncomfortable, but SHOULD NOT be painful; the researcher will ask for continuous feedback from you.

The light skin abrasion performed immediately prior to the attachment of skin-based electrodes can increase the chance of skin infections. To further reduce this small risk, alcohol wipes will be applied to the skin after abrasion as well as after removal of the electrodes.

As for the muscle biopsies, despite the slight invasive nature of this procedure, complications are rarely noted when performed in the quadriceps muscle. However, you may experience mild pain or discomfort during muscle biopsy procedure. In order to minimise this, the biopsy site will be anaesthetised prior to the procedures.

Biopsy procedures may also incur bleeding. However, this will be managed by allowing any bleeding to stop on its own to minimise blood accumulation in the muscle, before a compression bandage and ice pack is applied.

Muscle soreness, mild swelling and pain is rare but may persist for 1-2 days following the biopsy procedure, although in exceptional circumstances when there is some bleeding into the muscle during the procedure the pain may last for several weeks. Of course, if you experience severe pain, swelling or bleeding, or suspect the onset of infection, you should immediately contact the research investigator or supervisors (listed below). You will be brought to the university's medical practitioner for further treatment.

All biopsies will be performed under strict sterile conditions, by a university academic staff trained in this procedure. Nevertheless, the procedure may result in minor scarring and there is a small risk of infection.

## **Benefits**

You will have a unique opportunity to learn about the neuromuscular system and see high level data acquisition techniques.

You will learn about research strategies and research design, and have the opportunity to ask questions about research or any aspect of sports science.

You will get free quadriceps cross sectional area and knee extension strength assessments.

We will tell you your fast-twitch to slow-twitch muscle fibre type ratio if you complete the muscle microbiopsy procedure, (this data may take up to a year to be available)

### **Confidentiality of Information**

Your anonymity is ensured as much as possible during the investigation by assigning number codes to your data by the investigator. All information provided by you will be treated with full confidentiality. Your contact information will only be accessible by the chief researcher during the period of the study and only the researcher and supervisors will have access to the raw information for this study. The information and data gathered from you during the study will be used to answer the research question of this study. Data will be stored in a password-protected computer and is only available to the researchers. Hard copy data will only be kept in the researcher's office and locked in a specific drawer/filing cabinet. All data will be stored according to ECU policy and regulations following the completion of the study.

### **Results of the Research Study**

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

### **Muscle biopsy procedure**

You will be asked to report to the Exercise Physiology Lab on one occasion to undertake the muscle microbiopsy procedure. Detailed information about this procedure can be found in the document untitled “Microbiopsy Muscle Sampling Technique”. Your participation in this extra visit is voluntary and no explanation or justification is needed if you choose to not participate. Please tick a box below:

-I have decided to participate in the microbiopsy procedure ☐

-I have decided to not participate in the microbiopsy procedure ☐

-I don't know if I will participate in the microbiopsy procedure and authorise the researcher to contact me at a later date ☐

### **Invitation for Study 2**

You will be asked to report to the Exercise Physiology Lab at a later date to undertake the Study 2 of this PhD project. Your participation in this study is voluntary and no explanation or justification is needed if you choose to not participate. Detailed information about Study2 can be found in the ‘information letter to participants-Study 2’. Please tick a box below:

- I have decided to participate in Study 2 ☐

- I have decided to not participate in Study 2 ☐

- I don't know if I will participate in Study 2 and authorise the researcher to contact me at a later date ☐

### **Voluntary Participation**

Your participation in this study is voluntary. No monetary reward will be provided. No explanation or justification is needed if you choose to not participate. Your decision if you not want to participate or continue to participate will not disadvantage you or involve any penalty.

## **Withdrawing Consent to Participate**

You are free to withdraw your consent to further involvement in this project at any time. You also have the right to withdraw any personal information that has been collected during the research.

## **Questions and/or Further Information**

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

Laurent Seitz (PhD Student – Researcher)

Office 19.384

School of Exercise and Health Sciences, Edith Cowan University

270 Joondalup Drive, Joondalup, WA 6027, Australia

Ph: (+61 8) 6304 5819

E-mail: [l.seitz@ecu.edu.au](mailto:l.seitz@ecu.edu.au)

If you have any concerns or complaints about the research project and wish to talk to an independent person, you may contact:

Research Ethics Officer

Edith Cowan University

270 Joondalup Drive

JOONDALUP WA 6027

Phone: (08) 6304 2170

Email: [research.ethics@ecu.edu.au](mailto:research.ethics@ecu.edu.au)

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

\_\_\_\_\_  
Signature

\_\_\_\_\_  
Name

\_\_\_\_\_  
Date



## **Appendix 2: Information Letter to Participants (Study 2)**



### **Information Letter to Participants**

Thank you for expressing your interest in this research. The purpose of this document is to explain the study that you may choose to participate in as a subject. Please read this document carefully, and do not hesitate to ask any questions.

#### **Project Title**

Mechanisms Underpinning Postactivation Potentiation Following Voluntary Isokinetic Knee Extensions

#### **Researchers**

This research project is being undertaken as part of the requirements of a PhD candidature (Sport and Exercise Sciences) at Edith Cowan University (ECU).

PhD Candidate: Laurent Seitz ([l.seitz@ecu.edu.au](mailto:l.seitz@ecu.edu.au)) 6304 5819

Supervisor: A/Prof. Anthony Blazeovich ([a.blazeovich@ecu.edu.au](mailto:a.blazeovich@ecu.edu.au)) 6304 5472

Co-supervisor: Dr Gregory Haff ([g.haff@ecu.edu.au](mailto:g.haff@ecu.edu.au)) 6304 5416

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

#### **Purpose of the study**

The purpose of this study is to examine, force production, muscle activity and ability to activate muscle maximally after the conditioning activity that maximises postactivation potentiation response.

## **Research Outline**

In order to participate in this study, you will be asked to complete a medical questionnaire and to refrain from performing sports or hard exercise training for one day prior to the experimental day. You are also required to abstain from taking any stimulants or depressants including caffeine for at least 6 hours or alcohol for at least 24 hours prior testing.

If you participate in this study, you will be asked to report to the Exercise Physiology Lab (Building 19, Room 19.150) on three days separated by one week (scheduling is flexible) at the same time of the day. Each day, before the measurements start, you will be asked to do a 5-minute warm-up on a stationary bicycle and to complete submaximal and maximal isokinetic knee extensions. On the first day you will be acquainted with all testing procedures such as: maximal voluntary knee extensions, electrical and muscle nerve stimulation techniques. Electrical stimulation procedures require a small electrical current to be applied to the femoral nerve or the quadriceps (thigh) muscle using self-adhesive surface electrodes. The stimulation will be started at very low intensities and progressively increased until your muscle is maximally activated or you feel discomfort; at maximal intensities the electrical stimulation might be slightly uncomfortable.

On visits 2-3, you will complete the knee extension test and, the electrical stimulation protocols, before and 1, 4, 7, 10, 13, 16 and 19 minutes after one set of knee extensions. Small self-adhesive skin-mounted electrodes will be used to record the small electrical signals emanating from your quadriceps muscle during contractions (these sit passively on the skin and there is no discomfort). The skin under the electrodes will be gently abraded and cleaned

with alcohol (the alcohol minimises the risk of skin infection). First day measurements will take about 1.5 hours and second, third and fourth days will take about 1 hour.

### **Eligibility**

You will be eligible for this study if:

You are between 18 and 35 years old

You have no neuromuscular injuries

You have been engaged in a lower body resistance training for power and/or muscular strength for at least 6 months

### **Risks**

As with all tests of maximal muscle force production, there is the chance for muscle or tendon strain. This risk is low given that proper warm-up and familiarisation will be performed, the tests will be conducted by a researcher who is experienced in the procedures, and isokinetic muscle actions carry a relatively low risk of injury.

Electrical stimulation procedures can be uncomfortable, but SHOULD NOT be painful; the researcher will ask for continuous feedback from you.

The light skin abrasion performed immediately prior to the attachment of skin-based electromyogram electrodes can increase the chance of skin infections. To further reduce this small risk, alcohol wipes will be applied to the skin after abrasion as well as after removal of the electrodes.

### **Benefits**

You will have a unique opportunity to learn about the neuromuscular system and see high-level data acquisition techniques.

You will learn about research strategies and research design, and have the opportunity to ask questions about research or any aspect of sports science.

You will get free knee extension strength assessments.

### **Confidentiality of Information**

Your anonymity is ensured as much as possible during the investigation by assigning number codes to your data by the investigator. All information provided by you will be treated with full confidentiality. Your contact information will only be accessible by the chief researcher during the period of the study and only the researcher and supervisors will have access to the raw information for this study. The information and data gathered from you during the study will be used to answer the research question of this study. Data will be stored in a password-protected computer and is only available to the researchers. Hard copy data will only be kept in the researcher's office and locked in a specific drawer/filing cabinet. All data will be stored according to ECU policy and regulations following the completion of the study.

### **Results of the Research Study**

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

### **Invitation for Study 3**

You will be asked to report to the Exercise Physiology Lab at a later date to undertake the Study 3 of this PhD project. Your participation in this study is voluntary and no

explanation or justification is needed if you choose to not participate. Detailed information about Study 3 can be found in the ‘information letter to participants-Study 3’. Please tick a box below:

- I have decided to participate in Study 3 ☐
- I have decided to not participate in Study 3 ☐
- I don’t know if I will participate in Study 3 and authorise the researcher to contact me at a later date ☐

### **Voluntary Participation**

Your participation in this study is voluntary. No monetary reward will be provided. No explanation or justification is needed if you choose to not participate. Your decision if you not want to participate or continue to participate will not disadvantage you or involve any penalty.

### **Withdrawing Consent to Participate**

You are free to withdraw your consent to further involvement in this project at any time. You also have the right to withdraw any personal information that has been collected during the research.

### **Questions and/or Further Information**

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

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If you have any concerns or complaints about the research project and wish to talk to an independent person, you may contact:

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Email: [research.ethics@ecu.edu.au](mailto:research.ethics@ecu.edu.au)

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

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Signature

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Name

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Date

## **Appendix 3: Information letter to Participants – MRI**



### **Information Letter to Participants – Follow-up Study**

Thank you for expressing your interest in this research; the purpose of this document is to explain the follow-up study that you may choose to participate in. Please read this document carefully, and do not hesitate to ask any questions.

#### **Project Title**

Mechanisms Underpinning Post-activation Potentiation Following Voluntary Isokinetic Knee Extensions.

#### **Researchers**

This research project is being undertaken as part of the requirements of a PhD candidature (Sport and Exercise Sciences) at Edith Cowan University (ECU).

PhD Candidate: Laurent Seitz ([l.seitz@ecu.edu.au](mailto:l.seitz@ecu.edu.au)) 6304 5819

Supervisor: A/Prof. Anthony Blazeovich ([a.blazeovich@ecu.edu.au](mailto:a.blazeovich@ecu.edu.au)) 6304 5472

Co-supervisor: Dr Gregory Haff ([g.haff@ecu.edu.au](mailto:g.haff@ecu.edu.au)) 6304 5416

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

## **Purpose of the study**

The purpose of the follow-up study is to examine quadriceps volume using magnetic resonance imaging (MRI) technique.

## **Research Outline**

In order to participate in this study, you will be asked to complete a medical questionnaire and to refrain from performing sports or hard exercise training for one day prior to the experimental day. You are also required to abstain from taking any stimulants or depressants, including caffeine for at least 6 hours or alcohol for at least 24 hours prior testing.

If you participate in this follow-up study, you will be asked to report to Insight Medical Imaging (Joondalup) on one occasion to undertake an MRI procedure. The MRI procedure days will take about 45 minutes.

## **Eligibility**

You will be eligible for this follow-up session if:

You have participated in all aspects of the previous study

You are between 18 and 35 years old

You have no neuromuscular injuries

You have been engaged in a lower body resistance training for power and/or muscular strength for at least 6 months

You do not have artificial limbs, pacemakers or other implanted (metal containing) medical devices

You will be asked to remove all metal objects from your person.

## **Risks**



There are no known side effects of undertaking an MRI scan in men. However, the magnet may cause artificial limbs, pacemakers or other implanted (metal containing) medical devices to malfunction or heat up during the exam. You will be asked to remove all metallic objects before entering the MRI room. The MRI staff will be available during the entire scanning procedure. You will be allowed to communicate with the staff during scanning using a buzzer and microphone and you can stop the scan any time.

### **Benefits**

You will have a unique opportunity to learn about the neuromuscular system and see high level data acquisition techniques.

You will learn about research strategies and research design, and have the opportunity to ask questions about research or any aspect of sports science.

You will get free assessment of your quadriceps cross sectional area and volume.

### **Confidentiality of Information**

Your anonymity is ensured as much as possible during the investigation by assigning number codes to your data by the investigator. All information provided by you will be treated with full confidentiality. Your contact information will only be accessible by the chief researcher during the period of the study and only the researcher and supervisors will have access to the raw information for this study. The information and data gathered from you during the study will be used to answer the research question of this study. Data will be stored in a password-protected computer and is only available to the researchers. Hard copy data will only be kept in the researcher's office and locked in a specific drawer/filing cabinet. All data will be stored according to ECU policy and regulations following the completion of the study.

### **Results of the Research Study**

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

### **MRI procedure**

You will be asked to complete one more session to undertake the MRI procedure. Your participation in this extra visit is voluntary and no explanation or justification is needed if you choose to not participate. Please tick a box below:

-I have decided to participate in the MRI procedure ☐

-I have decided to not participate in the MRI procedure ☐

-I don't know if I will participate in the MRI procedure and authorise the researcher to contact me at a later date ☐

### **Voluntary Participation**

Your participation in this study is voluntary. No monetary reward will be provided. No explanation or justification is needed if you choose to not participate. Your decision if you not want to participate or continue to participate will not disadvantage you or involve any penalty.

### **Withdrawing Consent to Participate**

You are free to withdraw your consent to further involvement in this project at any time. You also have the right to withdraw any personal information that has been collected during the research.

## Questions and/or Further Information

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

Laurent Seitz (PhD Student – Researcher)

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Phone: (08) 6304 2170

Email: [research.ethics@ecu.edu.au](mailto:research.ethics@ecu.edu.au)

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

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Signature

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Name

---

Date

## Appendix 4: Informed Consent Appendix: Muscle Biopsy Questionnaire



Muscle Biopsy Questionnaire - Edith Cowan University

School of Exercise and Health Sciences

Name:

### Muscle Biopsy Information

1. Have you or your family suffered from any tendency to bleed excessively? (e.g. Haemophilia) or bruise easily?

Yes                  No                  Don't know

If yes, please elaborate \_\_\_\_\_  
\_\_\_\_\_

2. Are you allergic to local anaesthetic?

Yes                  No                  Don't know

If yes, please elaborate \_\_\_\_\_  
\_\_\_\_\_

3. Do you have any skin allergies?

Yes                  No                  Don't know

If yes, please elaborate \_\_\_\_\_  
\_\_\_\_\_

4. Have you any allergies that should be made known?

Yes                      No                      Don't know

If yes, please elaborate \_\_\_\_\_

5. Are you currently on any medication?

Yes                      No                      Don't know

If yes, please elaborate \_\_\_\_\_

6. Do you take Aspirin, non-steroidal analgesics or Warfarin?

Yes                      No                      Don't know

If yes, please elaborate \_\_\_\_\_

7. Do you have any other medical problem that should be made known?

Yes                      No                      Don't know

If yes, please elaborate \_\_\_\_\_

8. Do you suffer from low blood pressure or postural hypotension?

Yes                      No

9. Have you ever had problems when donating blood?

Yes                      No

**I believe that the information that I have supplied is true and correct.**

\_\_\_\_\_

Signature

\_\_\_\_\_

Name

\_\_\_\_\_

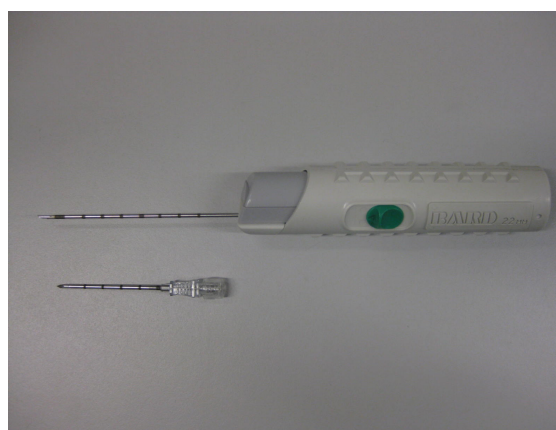
Date



### Microbiopsy Muscle Sampling Techniques

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Muscle biopsies have been used for many years in medicine to allow the sampling of tissues to help diagnose many issues with the nervous, vascular and musculoskeletal systems, and connective tissues and visceral organs. Individuals with myopathies resulting in low muscle mass and strength (i.e. problems with



**Microbiopsy device**

the muscle itself) and neuropathies (problems with the nerves innervating the muscle) have been diagnosed with the use of muscle biopsies in recent years.

In sports science research, muscle biopsies have enabled the study of fibre types and the concentrations of a number of different chemicals, proteins and enzymes that the muscle produces as a response to exercise. In more recent times muscle biopsies have been a useful tool in medicine and in sport science research as a tool in assessing the genetic make-up of a muscle. In medicine, interest lies in predicting of the likelihood of someone developing any one of a number of diseases. In sports science, muscle biopsies can be useful in assessing the different genes that are related to different aspects of the physiological response to training, such as hypertrophy.

Muscle biopsies are done either by inserting a needle into the muscle to remove a small part of the tissue, or alternatively through a surgical incision. Both of the procedures are considered invasive, however the former is less invasive and therefore more comfortable for the patient. In the former technique (microbiopsy) a needle is inserted into the muscle of choice, commonly the vastus lateralis, to remove a small sample of tissue. Usually 20 mg of muscle tissue will be taken, as opposed to the 150 mg taken during the traditional biopsy methods. The needle that is inserted into the muscle is a 14-gauge needle, the size is only slightly larger than that which is commonly used to give a tetanus injection.

It is common practice to also use an anaesthetic cream to numb the skin before the muscle biopsy is done, and so subjects will often only feel slight pressure in the muscle, which has been described as 2-3 on a scale of 1-10.

The risks associated with this microbiopsy procedure are almost nil (even more so than a traditional biopsy as there is no wound management required and so reducing the chance of infection). Both the traditional and new microbiopsy techniques are safe when performed by an appropriately trained PhD qualified researcher, as will be done in the present study.

Typically, the only side effects of the microbiopsy procedure are slight bruising at the site, and slight muscle stiffness, but no more than that of an average vaccination from a local general practitioner. However, in rare instances the accumulation of blood within the biopsy area can cause more significant soreness that can last for up to 2 weeks and may impair walking/running performance. You should be aware of this possibility before volunteering for the procedure.

If you have any questions, would like to learn more about the procedure, or would like to see a procedure performed before volunteering, please contact the lead researcher of the study.



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## Appendix 6: Information Letter to Participants (Study 3)



### Information Letter to Participants

Thank you for expressing your interest in this research. The purpose of this document is to explain the study that you may choose to participate in as a subject. Please read this document carefully, and do not hesitate to ask any questions.

#### Project Title

Mechanisms Underpinning Postactivation Potentiation Following Voluntary Isokinetic Knee Extensions.

#### Researchers

This research project is being undertaken as part of the requirements of a PhD candidature (Sport and Exercise Sciences) at Edith Cowan University (ECU).

PhD Candidate: Laurent Seitz ([l.seitz@ecu.edu.au](mailto:l.seitz@ecu.edu.au)) 6304 5819

Supervisor: A/Prof. Anthony Blazeovich ([a.blazeovich@ecu.edu.au](mailto:a.blazeovich@ecu.edu.au)) 6304 5472

Co-supervisor: Dr Gregory Haff ([g.haff@ecu.edu.au](mailto:g.haff@ecu.edu.au)) 6304 5416

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

## **Purpose of the study**

The purpose of this study is to examine quadriceps cross sectional area, force production, muscle activity and ability to activate muscle maximally after knee extensions at different speeds.

## **Research Outline**

In order to participate in this study, you will be asked to complete a medical questionnaire and to refrain from performing sports or hard exercise training for one day prior to the experimental day. You are also required to abstain from taking any stimulants or depressants including caffeine for at least 6 hours or alcohol for at least 24 hours prior testing.

If you participate in this study, you will be asked to report to the Exercise Physiology Lab (Building 19, Room 19.150) on three days separated by one week (scheduling is flexible) at the same time of the day. Each day, before the measurements start, you will be asked to do a 5-minute warm-up on a stationary bicycle and to complete submaximal and maximal isokinetic knee extensions. On the first day you will be acquainted with all testing procedures such as: maximal voluntary knee extensions and electrical stimulation techniques. Electrical stimulation procedures require a small electrical current to be applied to the quadriceps (thigh) muscle using self-adhesive surface electrodes. The stimulation will be started at very low intensities and progressively increased until your muscle is maximally activated or you feel discomfort; at maximal intensities the electrical stimulation might be slightly uncomfortable.

On the second, and third visits you will complete the following experimental procedure: performance of voluntary knee extensions and electrical stimulation protocols randomised over two sessions which will be completed before and 1, 4, 7, 10, 13, 16 and 19 min after one set of voluntary knee extensions. Small self-adhesive skin-mounted electrodes

will be used to record the small electrical signals emanating from your quadriceps muscle during contractions (these sit passively on the skin and there is no discomfort). The skin under the electrodes will be gently abraded and cleaned with alcohol (the alcohol minimises the risk of skin infection). First day measurements will take about 1.5 hours and second, third and fourth days will take about 1 hour.

### **Eligibility**

You will be eligible for this study if:

You are between 18 and 35 years old

You have no neuromuscular injuries

You have been engaged in a lower body resistance training for power and/or muscular strength for at least 6 months

### **Risks**

As with all tests of maximal muscle force production, there is the chance for muscle or tendon strain. This risk is low given that proper warm-up and familiarisation will be performed, the tests will be conducted by a researcher who is experienced in the procedures, and isokinetic muscle actions carry a relatively low risk of injury.

Electrical stimulation procedures can be uncomfortable, but SHOULD NOT be painful; the researcher will ask for continuous feedback from you.

The light skin abrasion performed immediately prior to the attachment of skin-based electromyogram electrodes can increase the chance of skin infections. To further reduce this small risk, alcohol wipes will be applied to the skin after abrasion as well as after removal of the electrodes.

### **Benefits**

You will have a unique opportunity to learn about the neuromuscular system and see high-level data acquisition techniques.

You will learn about research strategies and research design, and have the opportunity to ask questions about research or any aspect of sports science.

You will get free knee extension strength assessments.

### **Confidentiality of Information**

Your anonymity is ensured as much as it is possible during the investigation by assigning number codes to your data by the investigator. All information provided by you will be treated with full confidentiality. Your contact information will only be accessible by the chief researcher during the period of the study and only the researcher and supervisors will have access to the raw information for this study. The information and data gathered from you during the study will be used to answer the research question of this study. Data will be stored in a password-protected computer and is only available to the researchers. Hard copy data will only be kept in the researcher's office and locked in a specific drawer/filing cabinet. All data will be stored according to ECU policy and regulations following the completion of the study.

### **Results of the Research Study**

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

### **Voluntary Participation**

Your participation in this study is voluntary. No monetary reward will be provided. No explanation or justification is needed if you choose to not participate. Your decision if you not want to participate or continue to participate will not disadvantage you or involve any penalty.

### **Withdrawing Consent to Participate**

You are free to withdraw your consent to further involvement in this project at any time. You also have the right to withdraw any personal information that has been collected during the research.

### **Questions and/or Further Information**

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

Laurent Seitz (PhD Student – Researcher)

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Email: [research.ethics@ecu.edu.au](mailto:research.ethics@ecu.edu.au)

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

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Signature

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Name

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Date

## Appendix 7: Informed Consent (Studies 1, 2 and 3)



### 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 without 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.

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Signature

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Name

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Date



## Appendix 8: Study 1 (Publication – In press)

Page 1 of 36

1

### Title page

#### **Post-activation potentiation during voluntary contractions after continued knee extensor task-specific practice**

Laurent B. Seitz<sup>1</sup>, Gabriel S. Trajano<sup>1</sup>, Fabien Dal Maso<sup>2</sup>, G. Gregory Haff<sup>1</sup>, Anthony  
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