Post-activation Potentiation

- Effects on performance, tensile, and contractile properties of the plantar flexor muscles

Anja Zoellner

THE SWEDISH SCHOOL OF SPORT
AND HEALTH SCIENCES
Master Degree Project: 29:2016
Master Program in Sport Science: 2014-2016
Supervisor: Paulo Gago
Examiner: Mikael Mattsson

Abstract

Aim

The present thesis aims to provide scientific based evidence that might expand the limited information available regarding the post-activation potentiation (PAP) effects on the rate of torque development (RTD) and muscle activity during repeated isokinetic concentric maximal voluntary contractions (MVC). Additionally, we aim to provide new insights related to the possible effects that such repeated maximal contractions might have on the muscle tendon unit (MTU) stiffness.

Methods

Nine active participants were tested in two separate sessions. Participants performed plantar flexor supra-maximal twitches evoked via electrical stimulation of the tibial nerve and concentric MVCs at 60°/s. These were evoked/performed before and from 5s to 15 minutes after a 6s plantar flexion maximal voluntary isometric contraction (MVIC). Twitch RTD and time to peak torque (TTP) were measured. Concentric MVC peak torque (PT), soleus, medial and lateral gastrocnemius muscle activation, as well as RTD during different portions of the rising torque curve (e.g. 50-100ms) were assessed. Passive stiffness index of the MTU was calculated using the torque-angle relation attained during the 5°/s passive ankle angle rotation that followed each maximal concentric contraction.

Results

Twitch RTD significantly increased immediately after the conditioning contraction (CC) and remained enhanced for 5s-8min, with increases of 59.7%-6.0%. Twitch TTP significantly decreased from 5s-1min post CC by 9.9%-2.0%. TTP then increased by 4.6%-2.2% from 3-8min. In the maximal voluntary concentric contractions, there was a significant increase of 5.7, 6.0 and 5.9% at 1.5, 3, and 5min respectively in the PT. Voluntary RTD showed significant increases during the 100-200ms, 50-200ms and 0-200ms phases. These increases were seen at 3min (7.3%), 1.5-5min (8.0-6.9%), and 1.5-5min (8.6-9.5%) respectively. Stiffness showed no significant changes and any changes in EMG do not appear to be due to PAP effects.

Conclusions

The results from the current study show that PAP affects voluntary performance at a lower angular velocity than previously reported and in a time frame where twitch contractile properties were also potentiated. The lack of stiffness changes suggests that the acute effects might be mainly related to mechanisms within the muscle and that similar testing protocols should expect low interference from factors related to passive MTU stiffness changes.

Contents

Tables

Figures

Abbreviations

ANOVA	Analysis of variance
Ca^{2+}	Calcium
CC	Conditioning contraction
CMJ	Countermovement jump
CV	Coefficient of variance
EMG	Electromyography
EMG_{RMS}	Root-mean-square of the electromyography signal
$EMG_{\%MVIC}$	Electromyography signal as a percentage of that during the maximal voluntary isometric contraction
$ICC_{2,k}$	Intraclass correlation
LG	Laterl gastrocnemius
LSD	Least significant difference
MG	Medial gastrocnemius
MLC	Myosin regulatory light chain
MLCK	Myosin light chain kinase
MTU	Muscle tendon unit
MVC	Maximal voluntary contraction
MVIC	Maximal voluntary isometric contraction
Nm	Newton meter
PAP	Post-activation Potentiation
PSI	Passive stiffness index
PT	Peak torque
RM	Repetitions maximum
RMS	Root-mean-square
RTD	Rate of torque development
sEMG	Surface electromyography
SOL	Soleus
TA	Tibialis anterior
TTP	Time to peak torque
TS	Triceps surae
°/s	Degrees per second

1 Introduction

1.1 Post-activation potentiation

Post-activation potentiation (PAP) can be defined as an acute enhancement of muscle performance as a response to previous activation, or a conditioning contraction (CC) (Reardon et al., 2014, Tillin and Bishop, 2009). The mechanisms thought to be responsible for this phenomenon include enhanced recruitment of high order motor units (Tillin and Bishop, 2009), mechanisms that result in changes in Ca^{2+} sensitivity (e.g. myosin regulatory light chain phosphorylation, decreases in the distance between actin and myosin) (Iglesias-Soler et al., 2011, Tillin and Bishop, 2009, Zhi et al., 2005), and changes to the muscle tendon unit (MTU) properties and architecture (e.g. decreased pennation angle) (Reardon et al., 2014, Tillin and Bishop, 2009). PAP has gained substantial interest in the past few years mainly due to the significant enhancements reported in explosive tasks, such as jumping and sprinting, and the possibility that these might be able to be applied by athletes to improve performance. Most of these tasks are greatly associated with force development efficiency, as well as power and rate of force production, which are known to be enhanced after a PAP induction protocol (Hodgson et al., 2005, Sale, 2002, Seitz and Haff, 2015, Tillin and Bishop, 2009, Wilson et al., 2013). According to the review by Wilson et al. (2013), acute enhancements after a medium to maximal CC have their greatest effect in voluntary explosive performances after 7 to 12 minutes. However, PAP effects and behaviour in twitch and tetanus properties are normally significant immediately and up to 5-10 minutes after CC. In this sense, it seems that a disagreement exists between PAP effects on voluntary explosive tasks and on evoked contractions. Different subjects and CC characteristics and, much less explored, different effects of a CC on the architecture and properties of the MTU (i.e. pennation angle and stiffness respectively) could point to a possible explanation for the disagreements between studies. This suggests there is further research to be conducted in order to identify possible explanations for the differences in PAP behaviour between controlled laboratory setups and more applied settings (Iglesias-Soler et al., 2011, Sale, 2002, Tillin and Bishop, 2009). A multidimensional approach (e.g. including evoked, voluntary, and tensile assessments) to PAP research is needed in order to have a wider perspective of the PAP phenomenon and better evaluation and understanding of PAP behaviour and applications.

1.1.1 Background

1.1.1.1 Mechanism

The full description of the proposed mechanisms involved in PAP goes beyond the scope of the present thesis, however the main mechanisms will be briefly described. Some studies suggest that one mechanism associated with PAP might be related to an increase in excitability and recruitment of Type II muscle fibres, caused by increased recruitment of high order motor units (Tillin and Bishop, 2009). However, it should be mentioned that recent studies have shown, via stronger methodological approaches, a lack of association between neuromuscular excitability and PAP (Iglesias-Soler et al., 2011, Xenofondos et al., 2015). Decreased pennation angle could increase the force transfer efficiency, however the effects of several PAP CC protocols have shown only small to insignificant decreases in pennation angle (Reardon et al., 2014, Tillin and Bishop, 2009). This would translate into very small enhancements in force and power production (Reardon et al., 2014, Tillin and Bishop, 2009). The fact that the contributions of these mechanisms have been shown to be only small to insignificant suggests that another mechanism may be more prominent. The most prominent and accepted mechanism underlying PAP seems to be myosin regulatory light chain (MLC) phosphorylation.

1.1.1.2 Myosin regulatory light chain phosphorylation.

MLC phosphorylation is a process that occurs naturally in our bodies, so it is not a special process induced by a maximal CC or by a PAP induction protocol. However, it has been shown that an intense muscle contraction (such as a CC) could lead to greater MLC phosphorylation and that this increase is associated with twitch and tetanus enhancement post CC (Stull et al., 2011). Probably the most relevant studies related to PAP mechanisms are the ones performed by Szczesna et al. (2002) and Zhi et al. (2005). In brief, Szczesna et al. (2002) found that skinned muscle fibres reconstructed with phosphorylated myosin had greater force development than fibres with no phosphorylated myosin at a given calcium concentration. This was interpreted as an increased calcium sensitivity caused by MLC phosphorylation. Zhi et al. (2005) found that genetically modified mice with no myosin light chain kinase (MLCK) presented almost insignificant MLC phosphorylation levels as well as very low twitch and tetanus potentiation when compared to non-genetically modified mice.

Previous studies suggest that MLC phosphorylation causes the release of the myosin heads from their blocked site resulting in a decrease in distance between the myosin heads and actin (Stull et al., 2011, Szczesna et al., 2002, Zhi et al., 2005). This would increase the rate of interaction between actin and myosin, and thus, increased calcium sensitivity and enhanced force production. Despite controversial results from human experiments (Stuart et al., 1988), it is generally accepted that enhanced MLC phosphorylation is in fact the main biochemical mechanism responsible for increase in calcium sensitivity and the enhancements in force development at submaximal calcium concentrations that characterise the PAP phenomenon (Stull et al., 2011).

1.1.2 How can we test PAP?

PAP can be assessed by performing several voluntary tasks and/or evoked muscle responses before and after a specific CC. The methods that are most commonly used in the literature are:

Functional performance tests: Theoretically functional performance tests should simulate an athlete's natural activity, in a controlled environment. These types of assessments involve activities such as counter movement jumps (CMJ), drop jumps, and cycle sprints. These were previously used before and after a PAP induction protocol to measure the CC effects on performance (French et al., 2003, Fukutani et al., 2014b, Kilduff et al., 2007). However, in this situation enhancements or lack of enhancements between pre and post CC values could be related to factors such as mood swings, small technique shifts, changes in muscle architecture and MTU stiffness, fatigue, or MLC phosphorylation. Since controlling and measuring all of the mentioned factors in a single study is extremely difficult, it is important to take extra caution when interpreting results and providing possible explanatory mechanisms in these situations.

Isokinetic performance testing: With help of commercial isokinetic dynamometers, it is easier to standardise movements, as it is possible to have full control of movement speed and range of motion over a single joint, as has been done in a number of previous studies (Baudry and Duchateau, 2007, Fukutani et al., 2013, Gago et al., 2014b). Force, velocity and angular position data are directly monitored and essential for reliable calculation of parameters such as power, rate of torque development (RTD), force ratios between flexors and extensors, and

stiffness. However, since isokinetic assessments are most commonly performed in movements over a single joint (flexion and extension or adduction and abduction) there is a loss in terms of freedom of movement and thus it is less correlated to sports performance. Furthermore, it is still voluntary and thus dependent on individual's mood and inability to fully activate the muscle.

Supra-maximal electro stimulation: This is one of the most reliable and non-invasive ways to test the presence of PAP, as it ensures non-voluntary full activation of the muscle/s involved. This means that the individual's mood, or inability to voluntarily activate a muscle do not affect results. It has been mentioned by Baudry and Duchateau (2007) that without supra-maximal twitches, PAP cannot be assessed in a consistent and reliable way. The main downfall with this method is that as it is much less applied, the results might not correlate as well to sports performance as the functional performance tests.

1.1.3 What is a supra-maximal twitch and what do PAP related studies use it for?

A twitch is an evoked muscle contraction, usually achieved by electrically stimulating the nerve supplying that muscle. This method, when performed correctly, allows contractions to be more standardised than what voluntary contractions are, as the level of activation can be set and remain constant between and during trials. Many studies involving twitches use supra-maximal stimuli, at 10-20% above the stimulus intensity which showed maximal muscle recruitment (Baudry and Duchateau, 2007, Gago et al., 2014a, Hamada et al., 2003). This ensures that the entire muscle, including all motor units, is recruited. This is important when studying PAP, to ensure that changes after the CC are due to modulations occurring within the muscle, not as a result of additional muscle recruitment.

Twitch parameters (e.g. RTD) could be interpreted as indicators of muscle performance. From these parameters it is possible to determine a fair amount of information about the muscle's activity, including fatigue levels, as well as indirect information related to the prevalence of muscle fibre type. In PAP related studies twitch peak torque (PT) has been used to provide information related to fatigue resistance and potentiation levels between endurance and power athletes (Morana and Perrey, 2009). On the other hand, Hamada et al. (2000), used time to

peak (TTP) to provide some indications related to fibre type distribution. However, supra-maximal twitches are most commonly used to provide acceptable and reliable evidence that an acute enhancement of muscle contractile properties was achieved after a CC (i.e. PAP). This, in turn, could be related to mechanisms such as MLC phosphorylation, since a correlation has been found between MLC phosphorylation levels and both twitch PT and RTD (Vandenboom et al., 1995, Zhi et al., 2005).

1.1.4 Overview of PAP effects on performance and factors that affect its behaviour

Between-study variations in PAP behaviour could be related to a number of factors, all of which are known to affect PAP. In brief, PAP is known to depend on:

- Muscle fibre distribution (greater in individuals with a higher prevalence of type II fibres) (Hamada et al., 2000)
- Strength levels (greater in individuals with greater strength levels) (Kilduff et al., 2007, Tillin and Bishop, 2009)
- Fatigue levels post CC (fatigue is increased after CC and will counteract PAP effects) (Morana and Perrey, 2009, Rassier, 2000))
- Muscle length, direction and speed (greater at short muscle length and during fast shortening contractions) (Babault et al., 2008, Gago et al., 2014b, Miyamoto et al., 2011)
- Training status and background (greater in individuals with power training background if CC is maximal (Seitz and Haff, 2015, Wilson et al., 2013) and greater in individuals with endurance training background if the CC is high volume and submaximal (Morana and Perrey, 2009))
- Muscle group and the intensity of CC (Fukutani et al., 2014a)
- MTU architectural and property changes (e.g. pennation angle and stiffness) (Tillin and Bishop, 2009)

Knowing that the vast majority of these factors cannot be completely and simultaneously controlled, especially in applied scenarios, the risk of conflicting results is quite high. These conflicting results hinder the full acceptance of PAP induction implementation by athletes and trainers. For this reason, it would be beneficial to have a wider approach to research

concerning the effects of different CC and their variations in different settings. Only then can a fully informed decision be made regarding what type of PAP induction protocol should be used to maximise an athlete's performance. In the last 5 years, reviews carried out with a meta-analysis approach have proposed several recommendations for PAP induction protocols.

The review by Wilson et al. (2013), suggests that the overall results of PAP induction studies point towards:

- No differences between gender
- Rest intervals of 7 to 12min, between CC and PAP effects
- PAP is greater in trained individuals
- Greater PAP following a moderate intensity (60-84% 1RM) CC than high intensity (≥85% 1RM)
- Greater PAP after multiple sets than single sets in untrained individuals, however greater PAP following single set CC in resistance trained individuals

Recently, Seitz and Haff (2015) put forward additional recommendations in a review:

- Use a plyometric activity as CC, rather than traditional moderate or maximal intensity exercises.
- Shorter recovery following plyometric CC (0.3-4min) than traditional moderate to high intensity CC (≥5min)
- Greater PAP after shorter intervals in stronger individuals (5-7min), and longer periods in weaker individuals (≥8min)
- Single set, 1RM CC in stronger individuals, multiple set, sub maximal CC in weaker individuals
- If using squats as CC, use shallow squats

Although these recommendations could be extremely helpful for trainers, athletes, and future research studies, it should be noted that these reviews excluded studies involving evoked conditioning contractions (i.e. supra-maximal twitch assessments) even if combined with voluntary explosive performance tests. In Table 1 we provide a small example of previous studies investigating PAP using twitches, functional performance testing, and a combination of both. This introduces some factors known to modulate PAP effects (e.g. training background, type of CC and age). It could also highlight the previously mentioned

disagreement that exists in the PAP field by highlighting differences in the timing for maximal PAP effects on performance. These can be seen within the functional performance test investigations sector, but becomes less evident in the investigations using twitches or combined voluntary performance testing with evoked twitches. The reason for greater agreement between results of twitches and the combined method might be due to the direct monitoring of CC effects on muscle contractile properties. This allows timing of post CC voluntary tasks to be fine-tuned and the effects in these more reliable types of voluntary tasks (e.g. explosive isokinetic concentric plantar flexion) can be better investigated. Combined studies usually show a PAP response in both evoked and voluntary explosive tasks immediately and up to 5-10 minutes (Fukutani et al., 2014b, Miyamoto et al., 2011). This could mean that the time frame proposed by recent reviews might be excluding, as well as limiting, further research in a potentially important additional potentiation phase. Furthermore, the recommendations to use stretch shortening cycle based CC would result in a stretch shortening induced facilitation leading to a performance increase. This increase might not be related to PAP mechanisms such as MLC phosphorylation however, it might lead to tensile shifts of the MTU components. Therefore, it is important to investigate the effects of a plyometric type of CC on both contractile and tensile properties of the MTU, both immediately and in later time phases following conditioning. In this way it will be possible to provide additional information related to possible mechanisms underlying acute performance enhancement and possible risk factors associated with the different types of CC.

Table 1: Summary of previous studies investigating PAP using twitches, applied settings, and a combination of twitch and applied.

Study	Subjects	PAP assessment method	Analysed parameters	Analysis timing	Conditioning contraction (type /volume/intensity)	Results
Functional Performance Assessments						
(Kilduff et al., 2007)	23 male rugby player (3 years of regular RT)	CMJ, BBT	PPO	15s, 4, 8, 12, 16 and 20min after CC	BS, BP 3 RM	15s: CMJ (2.9%↓) and BBT (4.7%↓) 4min: ↔ 8-12 min: CMJ (6.8/ 8.0 %↑), BBT (2.8/5.3 %↑) 16-20min: CMJ (↔), BBT (0.8↑/↔)
(Seitz et al., 2016)	14 rugby league players	SBJ	SBJ distance	90s between CC and Jump test.	PAP group: warm up plus 2 PBS (84% 1RM) Control: warm up plus SBJ	Four sets 1.5min after CC: PAP group: SBJ (4.0-5.7%↑) Control group: SBJ (↔) Strong ↑ than weaker
(Weber et al., 2008)	12 division 1 male track and field athletes	SJ	Mean and peak JH, peak GRF	3min rest between CC and jump test.	BS (85% 1RM), 5 reps SJ (body weight), 5 reps	3 min after CC: BS: mean JH (↑5.8%), peak JH (↑4.7%), peak GRF (↑4.6%) SJ: mean JH (↓2.7%), peak JH (↓4.0%), peak GRF (↓1.3%)
Twitch Studies						
(Morana and Perrey, 2009)	8 END 7 POW All males	Supra-maximal twitches	Twitch PT, EMG_{RMS}	Every 10 seconds, between conditioning	5s rest/5s knee extension at 50% MVIC during 10 min	1 min (52% PT↑) END and POW 1-10 min (PAP PT↓) in POW 1-10 min (PAP PT↔) in END END maintain PAP in fatiguing tasks
(Gago et al., 2014b)	11 highly trained male athletes	Supra-maximal twitches in fast and slow lengthening and shortening, isometric	PT, RTD, RTR, RT, HRT	5, 30, 60, 120, 180, 240, 300, 600s after CC	6s MVIC	PAP effects immediately after and up to 5 min. PT, RTD, RTR ↑ in all modes RT ↓ in lengthening, ↑ in shortening HRT ↓ in all modes except fast lengthening
(Baudry et al., 2005)	10 young (31.8yr) 10 old	Supra-maximal twitches	PT, RTD, HRT, RTR	5s, every min for 10 min, every 5min	6s MVC	Young: PT (↑148%), RTD (↑124%), RTR (↑185%) Old: PT (↑87%), RTD (↑64%), RTR (↑63%)

	(79.2yr)			between 10-20min post CC		
Combined studies (Twitch and voluntary explosive tasks)						
(Baudry and Duchateau, 2007)	10 healthy, mixed gender	Supra-maximal twitch, isometric MVC	Twitch RTD, voluntary RTD	5s, every min for 10 min post CC	6s MVC	Twitch RTD ↑ 5s-5min, biggest increase immediately post CC Voluntary RTD ↑ 5s-2min, biggest increase at 1min post CC
(Miyamoto et al., 2011)	9 recreationally active men	Supra-maximal twitch, concentric MVC	Twitch PT, concentric PT, EMG_{RMS}	5s, 1, 2, 3, 4, 5, 10min post CC	10s MVC	Twitch PT ↑ 5s-5min Concentric PT ↑1min-3min EMG_{RMS} MG ↓ 5s only
(Fukutani et al., 2013)	12 healthy males	Supra-maximal twitch, concentric MVC	MVC PT at 30°/s (slow) and 180°/s (fast), Twitch PT	5s, 1min, 5min	6s MVIC	Twitch PT ↑ at 5s, 1min, 5min MVC PT 30°/s ↔ MVC PT 180°/s ↑ at 5s

Abbreviations: resistance training (RT), back squat (BS), bench press (BP), repetitions maximum (RM), repititions (reps), countermovement jump (CMJ), ballistic bench throw (BBT), peak power output (PPO), standing broad jump (SBJ), paused box squats (PBS), squat jumps (SJ), jump height (JH), ground reaction force (GRF), endurance (END), power (POW), peak torque (PT), rate of torque development (RTD), rate of torque relaxation (RTR), rising time (RT), half relaxation time (HRT), heavy intensity (HI), moderate intensity (MI)
↑: significant increase, ↓: significant decrease, ↔: no significant change

1.1.4.1 Electromyography

As can be seen in Table 1, another measure that is often included in PAP studies is electromyography (EMG). EMG is a measure of the muscle activation, which is another factor that may be affected, both in terms of improved muscular performance due to PAP and in terms of measuring fatigue or neuromuscular efficiency. It has been suggested that enhacing and depressing mechanisms (e.g. MLC and fatigue related factors respectively) coexist after a given CC. Thus mechanical measurements of PAP are in fact a reflection of the relationship between these two mechanisms (Stull et al., 2011). Along with torque curve behaviour, additional amplitude and frequency analysis of the EMG signals might help to distinguish signs of fatigue (De Luca, 1983). As an example, an increase in muscle activation, thus EMG amplitude, with no change in muscular performance factors (e.g. PT) could imply that more motor units are required to produce the same PT, suggesting effects associated with fatigue. On the other hand, if muscle activation decreases and muscular performance factors increase, or stay constant, this suggests muscle efficiency has increased, as fewer motor units are producing the same or greater PT, suggesting PAP effects are greater than fatigue. Morana and Perrey (2009) is a perfect example of this relationship. They compared PAP effects with EMG amplitude (normalised RMS) in both endurance and power athletes, and while the PAP effects were similarly enhaced immediately after a sub-maximal CC, PAP dropped quicker in the power athletes, while remaining potentiated for longer in the endurance athletes. Conversely, fatigue increased more rapidly in power athletes than in endurance athletes, as seen by increased EMG signals and decreasd PT. In this sense EMG analysis could provide information related to fatigue and neuromuscular efficiency. Furthermore, EMG is vital when establishing supra-maximal stimulation intensities (i.e. full muscle recruitment). Careful analysis of the muscle compound response (i.e. M-wave) is required to ensure that changes in muscle contractile properties are due to changes from mechanisms within the muscle rather than a results of further muscle recruitment.

1.1.5 What is lacking in the literature?

As previously mentioned, there is a lack of studies that use a multidimensional approach to PAP evaluations. Ideally, a PAP related study should investigate PAP behaviour via both evoked and voluntary assessments. The lack of information and results from combined studies leads to less exposure of the possible outcomes and implications, which might be interpreted

as less relevant for future reviews. Therefore it is important to increase the number of investigations using a combined approach. Furthermore, no information seems to exist from the analysis of RTD behaviour in separate phases of the maximal isokinetic concentric plantar flexion rising curve following a PAP induction protocol. This seems interesting, as RTD has for some years been considered an important parameter both for performance and clinical purposes (Maffiuletti et al., 2016). Additionally, twitch RTD is often related to the rate of cross-bridge entering in a force production state and has been previously correlated to MLC phosphorylation levels (Lewis et al., 1986, Vandenboom et al., 1995). However, as far as we know, simultaneous twitch RTD and overall peak RTD of explosive tasks following a PAP induction protocol has only been previously approached by Baudry and Duchateau (2007). Here they found that twitch RTD of the thumb adductor muscle was immediately enhanced after a 6s MVIC or tetanus. Additionally, it seems PAP dependency on angular velocity of explosive voluntary concentric contractions is poorly investigated. Fukutani et al. (2013), investigated the effects of a PAP conditioning protocol (6s MVIC) on both twitch and maximal voluntary plantar flexion at 30 and 180°/s. Concentric maximal voluntary contraction (MVC) PT potentiation only occurred immediately after CC (i.e. 5s) if the task was performed at high angular velocity (i.e. 180°/s), not at low angular velocities (i.e. 30°/s). However, Gago et al. (2014b) found a significant increase in contractile properties during twitch and voluntary muscle shortening at 60°/s. This begs the question of whether there are also significant PAP effects in voluntary contractions at lower angular velocities than 180°/s. Also overlooked are the effects of both the CC and the repeated maximal voluntary tasks on the MTU components. A given CC can induce both PAP and also stiffness and architecture changes in the MTU. A number of studies have shown that both the volume and the intensity of the CC affect stiffness, with some suggesting that the use of MVICs may lead to a decrease in tendon stiffness (Kay and Blazevich, 2009, Kay and Blazevich, 2010, Obst et al., 2013). Stiffness of the passive components of the MTU can affect both force production and muscular performance, especially during explosive activities. The elasticity of MTU components has been suggested to be a significant factor in muscle efficiency, as increasing stiffness of the MTU leads to more efficient and quicker force transfer, allowing greater force and power to be produced (Fletcher, 2010, Obst et al., 2013). This idea is also supported by Kay and Blazevich (2009), who suggest that a significant correlation exists between stiffness and a number of performance factors, including the RTD and maximal isometric force production. Due to the importance of stiffness and changes in stiffness for muscle function

and efficiency (Fletcher, 2010, Kay and Blazevich, 2009, Obst et al., 2013), it seems a relevant parameter to be measured pre and post PAP conditioning protocol. According to the existing literature, a single MVIC leads to PAP without affecting Achilles tendon stiffness (Gago et al., 2014a) or passive stiffness index (Gago, 2016). However, as far as the author knows, there is no PAP related study assessing what happens to MTU stiffness if repeated concentric MVCs are used to measure PAP. This begs the question of whether PAP or lack of PAP effects in such studies could partially be explained by shifts in stiffness caused by the PAP assessment method itself.

1.2 Aim, hypothesis and research questions

The general aim of this thesis was to present a brief and updated overview of PAP conditioning contraction effects on both voluntary and involuntary contractions while expanding the existing results with new insights on the topic.

Based on previous research, PAP following a conditioning MVIC would only be significant if the angular velocity was sufficiently high (i.e. 180°/s for the plantar flexors). We hypothesised that no significant changes would occur at 60°/s. Furthermore, the MVIC itself should not cause any significant changes in the MTU stiffness. However, it is possible that the repeated concentric MVCs could induce sufficient stress and strain capable of modulating the MTU stiffness.

The research questions were:

- Does PAP affect subsequent explosive concentric contractions (PT, RTD, and EMG) when performed at low angular velocity (i.e. 60°/s)?
- Is there a simultaneous acute enhancement of twitch properties and explosive voluntary concentric contractions after a single 6s MVIC?
- Could the PAP effects be related to acute changes in the MTU stiffness induced by the experimental tests, the explosive concentric contractions, themselves?

2 Method

The current thesis was conducted as part of a bigger study, which was carried out and analysed as part of a team and funded by The Swedish National Centre for Research in Sports (CIF) in 2015.

2.1 Subjects

This study consisted of nine subjects; three females and six males (age 24.0±2.7years; height 176.0±10.8cm; mass 75.6±12.5kg; BMI 24.2±1.5kg/m^2). Subjects were students at The Swedish School of Sport and Health Sciences, recruited by advertising at the university and on social media. All subjects were free from previous injury of the right ankle. Subjects were informed of the objectives of the study and signed an informed consent form. The regional ethics committee in Stockholm approved the study and all procedures adhered to the declaration of Helsinki.

2.2 Overview of study design acquired data and analysed variables.

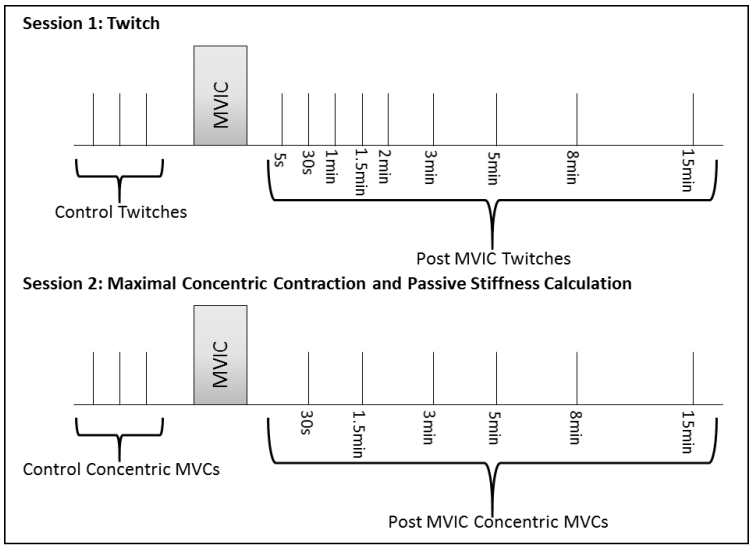

Figure 1: Outline of the two sessions, performed on different days (adapted from Gago et al., 2014a).

The study included in the present thesis involved two sessions, no more than 1 week apart. Each participant took part in both sessions. The sessions consisted of 3 control trials, a conditioning contraction and subsequently several other assessment trials from 5s to 15mins (Figure 1). Batterham and Hopkins (2005) state under research designs, that it is possible to measure statistical significance with a relatively low sample size (approximately 10 subjects), when using this design with a single group and trials in time series.

2.3 Materials and acquisition

2.3.1 Torque, angle, electrical stimulation and electromyography

Torque data during plantar flexion was assessed using an isokinetic dynamometer (Isomed 2000, D&R Ferstl Gmbh, Henau, Germany) with a custom-made footplate. A single rectangular pulse (1ms) was applied to the posterior tibial nerve by an electrical current delivered by a constant current stimulator (Digitimer, model DS7A, Hertfordshire, UK).

Surface EMG (sEMG) signals were recorded using circular 7mm diameter Ag-AgCl electrodes (Ambu Blue Sensor, Medicotest, Denmark). Before applying the electrodes, the skin over the involved muscle bellies was shaved and cleaned, in order to minimise impedance. The electrodes were placed along the belly of the tibialis anterior (TA) muscle, and in a belly-tendon configuration on the soleus (SOL) muscle. Electrode pairs were also placed over the bellies of the medial gastrocnemius (MG) and lateral gastrocnemius (LG). Two ground electrodes were applied, one on the skin covering the head of the fibula and the other on the skin covering the medial femoral head. An amplification of 1000 (MG, LG, and TA) and 200 (SOL) times (NL 824, Digitimer, UK) was used on the sEMG signal, as well as a band-pass filter (30 Hz–1 kHz with a 50 Hz notch filter) (NL 125, Digitimer, UK).

A sampling rate of 5kHz was used, and data was collected using a 16-bit Power 1401, monitored and recorded via Spike2 software (version 7.0, CED, Cambridge, England). Custom written software linked to the Spike2 software control panel was used to control the dynamometer shaft rotation and the time of stimulation.

2.4 Experimental procedure

2.4.1 Experimental setup and procedures for twitch assessment in the isometric mode

The supra-maximal twitch assessment session lasted approximately 1.5 hours. At the start of the session, subjects carried out a 10 minute warm up, involving submaximal cycling on a cycle ergometer. Following the warm up, the subjects had some time (approximately 10 minutes) to rest while electrodes were applied (see section 2.3.1) and they were subsequently aligned on the isokinetic dynamometer (Isomed 2000, D&R Ferstl Gmbh, Henau, Germany),

in the prone position with arms and hands beside their body. The right foot was securely strapped onto the custom-made footplate, while the rest of their body was fixed in place by the shoulders, hips and legs. The joint axis of the ankle and the rotational centre of the dynamometer shaft were aligned. Built in software was used to adjust the dynamometer. Manufacturer's guidelines were used to perform foot alignment, the range of motion and gravity correction on each subject. The knee was kept fully extended with the ankle joint at 90˚.

Twitches in the plantar flexors were evoked by electrically stimulating the tibial nerve. A single rectangular pulse (1ms) was used by a constant current stimulator (Digitimer, model DS7A, Hertfordshire, UK) to deliver electrical current to the posterior tibial nerve. A small cathode electrode was placed on the popliteal fossa as stimulator. The best stimulation zone on the popliteal fossa was found by manual location in the area by using a custom made stimulating pen. The anode was placed on the anterior surface of the knee, proximal to the patella, then taped in place. Subjects were familiarised with submaximal electrical stimuli by gradually increasing the current intensity in 5milliampere increments. The intensity of the stimulus was increased until there were no further increases in either twitch force or compound muscle action potential (i.e. SOL M-wave) with increasing stimulus intensity. After this the stimulation intensity was increased by a further 20%, as is common in PAP related studies (Fukutani et al., 2013, Gago et al., 2014a), in order to ensure muscle fibre activation remained maximal throughout the experiment. In order to avoid the effects of any remaining potentiation from the familiarisation, which could affect results, this was followed by a 10 minute rest. The ankle remained in neutral position throughout the stimulation protocol. The protocol involved 3 supra-maximal stimulations, followed by a 6s MVIC conditioning phase, then, 9 subsequent stimulations over a 15 minute recovery period (Figure 1, Session 1). These subsequent stimulations occurred at 5s, 30s, 1min, 1.5min, 2min, 3min, 5min, 8min, and 15min after the conditioning MVIC.

2.4.2 Experimental setup and procedures for maximal voluntary concentric contraction

As in the twitch assessment session, subjects carried out a 10 minute warm up at submaximal intensity on a cycle ergometer at the start of the session. This was then followed by a break of

approximately 10 minutes, while sEMG electrodes were applied (see section 2.3.1) and the subject was aligned on the isokinetic dynamometer as in the twitch assessments (see section 2.4.1). Manufacturer's guidelines were, once again, used to perform the foot alignment and gravity correction. Baseline passive torque was measured individually for each subject throughout the whole range of motion. The isokinetic dynamometer was set to move at a velocity of 60˚/s during concentric plantar flexor movement. This movement was triggered by a 5Nm increase in force on the foot plate above the individual baseline passive torque. After each concentric contraction the foot plate was brought back to the starting position at a velocity of 5˚/s. Subjects started with 3 concentric MVCs, which were used as controls. Timing of the start and finish of the contractions was controlled using a signal from a custom made sound file. After the 3 control contractions, subjects performed a 6s MVIC CC, after which another 6 concentric MVCs were performed. These contractions occurred at 30s, 1.5min, 3min, 5min, 8min, and 15min after the conditioning MVIC and were used to investigate possible PAP effects.

2.4.3 Stiffness index

Passive stiffness index was calculated by the torque-angle data acquired during passive ankle rotation, from 0 to 18 degrees into plantarflexion, as was done by Nordez et al. (2006). Passive ankle rotation was imposed by the isokinetic dynamometer after each maximal concentric contraction at 5°/s.

2.5 Analysed variables

2.5.1 Analysis of twitch properties

Scripts for Spike2 software were custom written for previous studies in our laboratory (Gago et al., 2014a, Gago et al., 2014b). These scripts were used to analyse twitch parameters. As suggested by Rassier in MacIntosh (2010) and Baudry and Duchateau (2007) the best method to investigate PAP is through supra-maximal evoked twitches. The present study investigates maximal RTD and TTP. RTD was calculated as the peak of the first derivative of the torque development (dF/dt), as previously performed in Gago et al. (2014a) and Baudry and Duchateau (2007). TTP was measured as the time from onset of contraction to the peak of the twitch torque curve.

2.5.2 Analysis of maximum torque and voluntary rate of torque development

The mean torque of the MVIC was measured for 3 seconds, from 1.5 seconds before to 1.5 seconds after the maximum torque value of the MVIC. Spike2 software was used to create a custom script, to place cursors during the concentric MVCs. These cursors were placed at 0, 30, 50, 100, and 200ms of the rising portion of the torque curve. Visual inspection was used to determine onset of torque production, as it has been suggested that the use of automated procedures may lead to inaccurate placement of the torque onset cursor (Tillin et al., 2013). RTD was calculated as the slope of the torque-time curve in each phase (i.e. from 30-100ms, 50-100ms, 50-200ms, 100-200ms, and 0-200ms). The first 30ms of the curve were not included as it was difficult to distinguish between noise signal and actual meaningful data (Maffiuletti et al., 2016).

2.5.3 Analysis of passive stiffness

The subject's foot was moved passively, by the footplate of the isokinetic dynamometer, from 20º plantar flexion to 20º dorsiflexion at an angular velocity of 5º/s. Throughout the full range of motion, torque and joint angles were continuously recorded. From these, torque values could be obtained, using custom written software, in 3º increments from 0 to 18º dorsiflexion.

The passive torque-angle relationship was used to calculate passive stiffness. Torque–angle data was exported to Origin software (Version 9.0, OriginLab, Northampton, MA), thereafter data was subjected to curve-fitting, using a second-order polynomial function as has been previously performed (Nordez et al., 2006). Experimental constants a, b and c for each passive dorsiflexion movement (i.e. 3 pre and 7 posts MVIC) were retrieved

$$T(\theta) = a\theta^2 + b\theta + c$$

The slope of the torque-angle relationship curve was taken as the passive stiffness index

$$PSI = 2a$$

Nordez et al. (2006) suggest that, instead of using other stiffness measurement models, this model of curve fitting and measuring passive stiffness index (PSI) is a good and valid alternative, as stiffness information can be calculated for the whole range of motion and torque levels.

2.5.4 Muscle activity and co-activation

In order to analyse muscle activity, root-mean-square (RMS) values were calculated from the sEMG amplitudes (EMG_{RMS}) in different phases of the torque rising curve during the concentric MVC (i.e. 0-50ms, 0-100ms, 0-200ms and 0-peak). All raw EMG_{RMS} values were normalised to a percentage of maximal values ($EMG_{\%MVIC}$). These maximal values were calculated from the mean EMG_{RMS} value over 3 seconds during the MVIC (1.5 seconds before and 1.5 seconds after the maximum value). The normalised values for the SOL, the MG and the LG were analysed individually and also combined to represent overall plantar flexor, triceps surae (TS), muscle activity. To get the combined values, the normalised values of the SOL, MG, and LG were averaged. This approach has previously been used by Kay and Blazevich (2009). Any co-activation signs related to TA muscle activity or activation of TS muscles were carefully inspected when analysing torque-angle data to calculate posterior PSI.

2.5.5 Consistency between sessions

Spike2 software (Spike2, version 7.0, CED, Cambridge, England) was used to digitally control the time of stimulation. In concentric movement, a torque greater than the threshold, 5Nm above baseline (passive torque), triggered the 60º/s shaft rotation of the isokinetic dynamometer. The movement was digitally controlled by Spike2 software, and following the concentric contraction was brought back to the starting position at 5º/s. The MVIC, beginning and ending of the concentric MVC (to give the duration), as well as the timing between sessions was signalled by a sound. This was used to give feedback to participants and to standardise both between subjects and between sessions.

2.6 Statistical analysis

Data was analysed using IBM SPSS Statistics 22 (Version 22. Armonk, NY: IBM Corp). Distribution of data was checked using Shapiro-Wilks tests. Non-normal data was log-

transformed to reduce skewness. Intra-class correlation coefficients ($ICC_{2,k}$) were used to analyse the consistency of the control trials for all parameters. Mean values for torque and duration of the MVICs conducted in both the twitch and voluntary trials were compared using a paired student t-test. All other parameters were analysed using a one-way repeated measures ANOVA, using absolute values, to compare values at different times post MVIC to the mean of the three control trials. Any significant results in the ANOVA were further assessed using LSD post hoc analyses (Nordez et al., 2009). Significance level was set at $p \leq 0.05$ for a significant difference. $ICC_{2,k}$ values of 0.6-0.8 were considered as having good consistency, values of 0.8-1 were considered as having excellent consistency. All values are reported as mean ± standard deviation.

3 Results

3.1 Maximal voluntary isometric contraction

The twitch trials and voluntary trials had mean MVIC torque values of 194.3±55.0Nm and 201.0±61.5Nm respectively. No significant difference was observed between these values (T=-1.45; p=0.19). Additionally, the mean MVIC duration values for the twitch trials (6.44±0.3s) and the voluntary trails (6.36±0.2s) showed no significant difference either (T=-0.64; p=0.54).

3.2 Twitch

The $ICC_{2,k}$ value indicated excellent consistency between control trials for the RTD (0.98) and TTP (0.99).

3.2.1 RTD

A significant main effect of time was found for twitch RTD ($F_{9,72}$=52.007, p<0.01). Post hoc test showed that compared to control values (252.51±69.6Nm/s), the twitch RTD were significantly enhanced immediately after a 6s MVIC at 5s (404.39±120.3Nm/s; p<0.01), 30s (363.06±108.0Nm/s; p<0.01), 1min (309.39±90.1Nm/s; p<0.01), 1.5min (287.55±82.3Nm/s; p<0.01), 2mins (281.55±77.2Nm/s; p<0.01), 3mins (273.42±75.1Nm/s; p<0.01), 5mins (273.32±73.8Nm/s; p<0.01), and 8mins (267.19±71.5Nm/s; p<0.01). This corresponds to a

significant increase from 59.7% down to 6.0% (Figure 2), significantly greater than the CV of 1.9%.

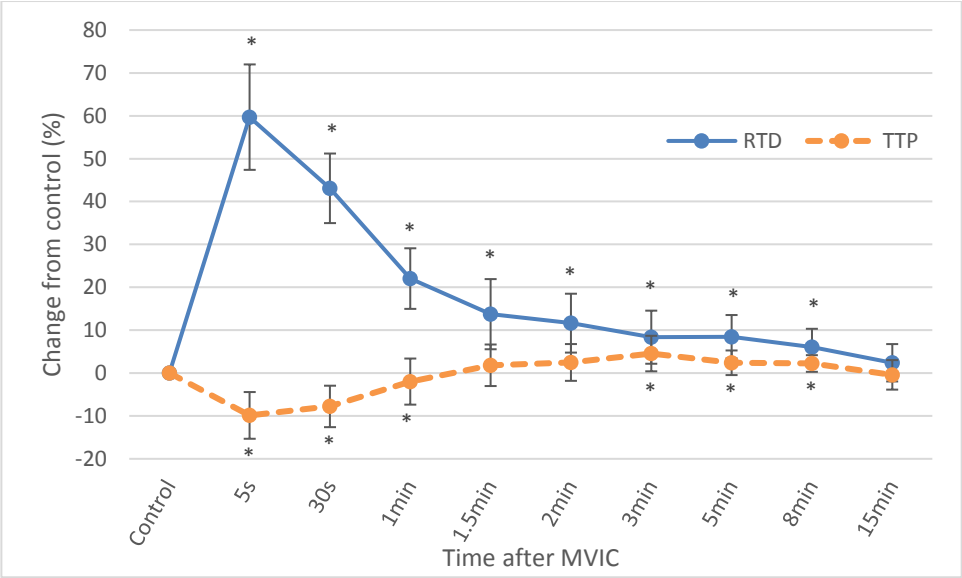

Figure 2: Results of the twitch RTD and TTP, during the control and for 15mins after a 6s MVIC

**Significant difference ($p<0.05$) from control.*

3.2.2 Time to peak

A significant main effect of time was found for the twitch TTP ($F_{9,72}=25.162$, $p<0.01$). Post hoc analysis showed that compared to control values (0.148±0.013s), twitch TTP was significantly decreased after a 6s MVIC at 5s (0.133±0.005s; $p<0.01$), 30s (0.132±0.006s; $p<0.01$), and 1min (0.141±0.005s; $p=0.02$), which corresponds to a significant decrease of 9.9% to 2.0%. Following this there was also a significant increase in twitch TTP after the 6s MVIC at 3mins (0.154±0.014s; $p=0.02$), 5mins (0.152±0.014s; $p=0.03$), and 8mins (0.152±0.015s; $p=0.01$), which corresponds to a significant increase of 4.6% to 2.2% (Figure 2). All changes are considerably greater than the CV of 1.4%.

3.3 Concentric maximal contraction

The $ICC_{2,k}$ value indicated excellent consistency between control trials for the PT (0.99). The $ICC_{2,k}$ in the different phases (i.e. 30-100, 50-100, 50-200, 100-200, 0-200ms) of voluntary RTD also indicated excellent consistency and ranged from 0.96-0.99.

3.3.1 Peak torque

A significant main effect of time was found for PT ($F_{6,48}$ =2.458, p=0.04). Post hoc test showed that compared to control values (99.78±27.6Nm), the maximal value in the concentric MVC was significantly enhanced immediately after the 6s MVIC. PT at 1.5min (105.47±32.6Nm; p=0.04), 3min (105.81±33.6Nm, p=0.03) and 5min (105.62±33.8Nm, p=0.03) increased by 5.7; 6.0 and 5.9 % respectively, while the CV was 4.9%.

3.3.2 Voluntary rate of torque development

A significant main effect of time was found in RTD for the 100-200ms ($F_{6.48}$=2.981, p=0.02), 50-200ms ($F_{3.21}$=4.648, p=0.014), and 0-200ms ($F_{3.22}$=5.208, p=0.01) phases of the concentric MVC. No significant changes were found in the 50-100ms phase ($F_{2.17}$=2.651, p= 0.10) or the 30-100ms phase ($F_{2.18}$=2.848, p= 0.080) (Table 2). Post-hoc testing of the 100-200ms revealed a significant increase in RTD from pre MVIC values (435.30±127.8Nm/s) at 3min (467.05±216.5Nm/s; p=0.02) post MVIC, before returning to baseline values by 5min (p=0.16) (Table 2). This corresponds to an increase of 7.3% from the control values, this increase is lower than the CV of 7.7%. In the 50-200ms interval a significant increase from pre MVIC values (460.83±138.5Nm/s) was seen at 1.5min (491.01±208.9Nm/s; p<0.01), 3min (492.59±222.1Nm/s; p=0.01) and 5min (492.24±218.5Nm/s; p=0.02) (Table 2), corresponding to 8.1, 6.9, and 6.8% increases in relation to baseline values respectively. All of these increases at all time points, are greater than the CV of 5.9%. Post-hoc testing in the 0-200ms phase showed a significant increase in RTD from pre MVIC values (393.6±113.1Nm/s) at 1.5min (431.05±112.9Nm/s; p<0.01), 3min (427.51±134.1Nm/s; p=0.01) and 5min (430.43±129.2Nm/s; p=0.01). This corresponds to increases of 9.5%, 8.6%, and 9.4% respectively. All of these increases are greater than the CV of 4.8%.

Table 2: Results for voluntary RTD at different phases of the torque curve during control contractions and for 15mins following a 6s MVIC

**Significant difference (p<0.05) from control.*

RTD (Nm/s)	Control	30sec	1.5min	3min	5min	8min	15min
30-100ms	457.1 ±185	480.4 ±198	513.7 ±193	494.6 ±213	517.6 ±204	412.0 ±227	479.6 ±173
50-100ms	511.9 ±198	533.5 ±193	569.7 ±200	543.7 ±216	566.3 ±209	449.3 ±229	527.4 ±177
100-200ms	435.3 ±128	460.4 ±226	462.2 ±211	467.1* ±216	455.2 ±213	448.7 ±204	425.4 ±195
50-200ms	460.8 ±139	484.8 ±207	498.0* ±209	492.6* ±222	492.2* ±218	448.9 ±183	459.4 ±200
0-200ms	393.6 ±113	416.2 ±119	431.0* ±113	427.5* ±134	430.4* ±129	387.1 ±107	398.5 ±113

3.4 Stiffness

The $ICC_{2,k}$ value indicated excellent consistency between control trials (0.99). The ANOVA showed that there was no significant effect of time ($F_{3,26}$=2.637, p=0.07) (Figure 3) between the control values (0.0854±0.05Nm/°) and the post MVIC values.

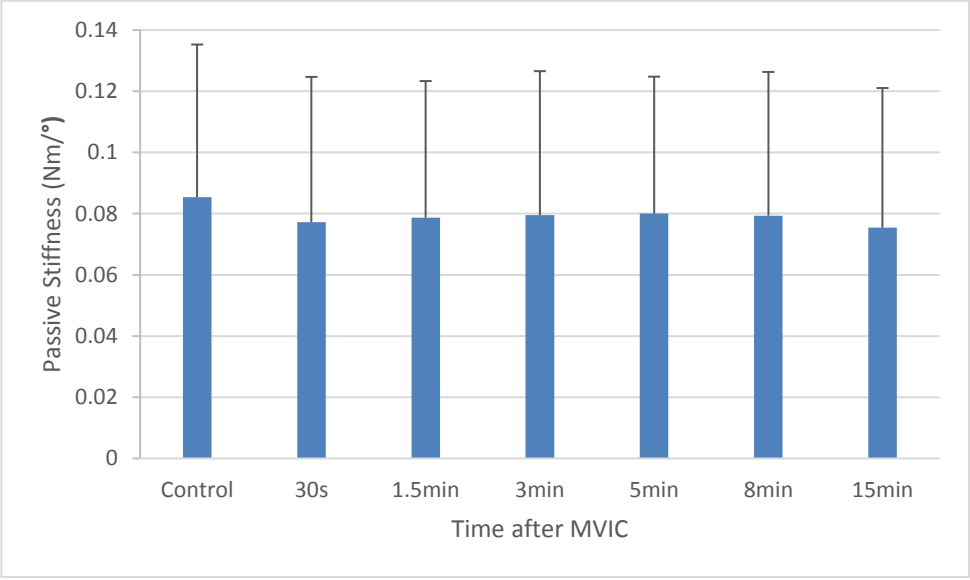

Figure 3: Passive Stiffness Index of the plantar flexor MTU during control contractions and for 15mins following a 6s MVIC

3.5 EMG

The $ICC_{2,k}$ values indicate excellent consistency between the control trials in the different phases (i.e. 0-50ms, 0-100ms, 0-200ms and 0-peak) of the combined EMG, ranging from 0.94-0.99. The normalised EMG SOL also showed excellent consistency in all phases, ranging from 0.89-0.97. Normalised EMG MG showed mostly excellent consistency, with values from most phases (0-100ms, 0-200ms, and 0-peak) ranging from 0.86-0.94. The 0-50ms phase showed good consistency with a value of 0.78. The normalised EMG LG showed excellent consistency in all phases with values ranging from 0.89-0.97.

3.5.1 Combined EMG

ANOVA of the combined EMG results of the plantar flexor group showed no significant effect of time at 0-50ms ($F_{3,22}$=1.751, p=0.19), or in the 0-200ms phase ($F_{6,48}$=1.654, p=0.15). However, a significant main effect of time was detected at 0-100ms ($F_{6,48}$=3.146, p=0.01) and 0-peak ($F_{6,48}$=2.328, p=0.05). Post hoc analysis at 0-100ms revealed a significant decrease in $EMG_{\%MVIC}$ of the TS from the control (113.06±26.4%) at 3min (102.61±27.4%; p=0.01) and 8min (100.01±31.3%; p=0.01) after MVIC (Figure 4). This corresponds to a 9.3% and 11.6% decrease respectively in relation to control values, compared with the CV of 9.4%. Post hoc analysis at 0-peak showed a significant decrease in $EMG_{\%MVIC}$ from the control (120.11±21.5%) at 1.5min (113.59±16.5%; p=0.04) and 8min (108.42±17.6%; p=0.01) after MVIC (Figure 4). This corresponds to a 5.4% and 9.7% decrease respectively in relation to control values, compared with the CV of 5.6%.

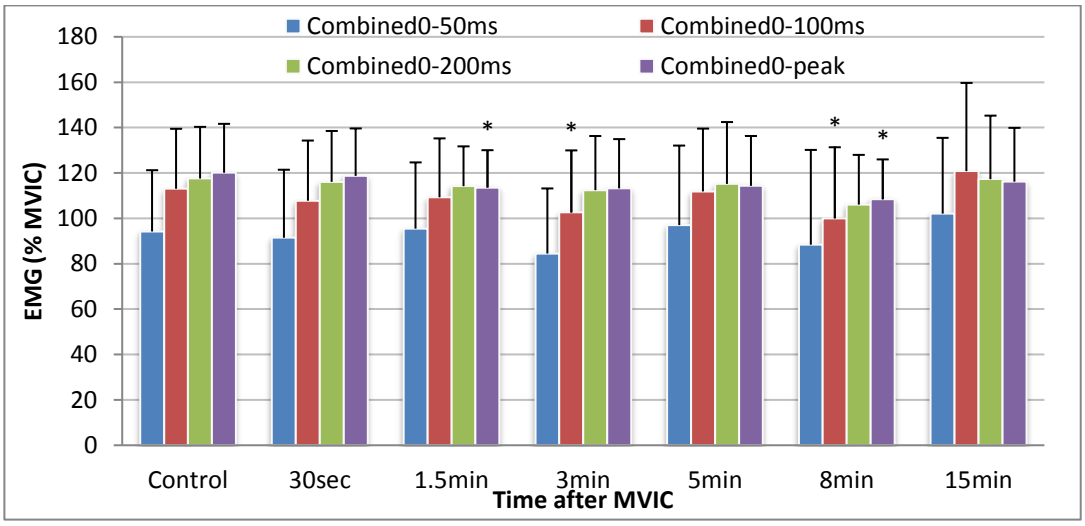

Figure 4: Plantar flexor activation during several phases of maximal voluntary contractions, during control cntractions and for 15mins following a conditioning MVIC

**Significant difference ($p<0.05$) from control*

3.5.2 Soleus EMG

Analysis of the individual normalised values for EMG of the SOL showed no significant effect of time in the ANOVA at 0-50ms ($F_{3.23}$=1.179, p=0.34), 0-100ms ($F_{6,48}$=1.692, p=0.14), 0-200ms ($F_{6,48}$=1.114, p=0.37), or during the 0-peak phase ($F_{6,48}$=2.053, p=0.08).

3.5.3 Medial gastrocnemius EMG

Analysis of the individual normalised values for EMG of the MG showed no significant effect of time in the ANOVA at the 0-200ms ($F_{6,48}$=1.581, p=0.17) or during the 0-peak phase ($F_{6,48}$=1.779, p=0.12). However, there was a significant effect of time at 0-50ms ($F_{6,48}$=2.920, p=0.02), and 0-100ms ($F_{6,48}$=3.141, p=0.01). Post hoc analysis at 0-50ms showed a significant decrease in $EMG_{\%MVIC}$ from the control values (82.75±20.0%) to the values at 3min (70.39±23.5%; p=0.03) and 8min (67.01±25.5%; p=0.03) post MVIC. This corresponds to a decrease of 14.9 and 19.0% respectively, while the CV is 22.8%. Post hoc analysis at 100-200ms also showed a significant decrease from the $EMG_{\%MVIC}$ control values (100.05±24.1%) to the values at 8min (78.28±18.3%; p<0.01) post MVIC. This corresponds to a decrease of 21.8%, while the CV is 13.9%.

3.5.4 Lateral gastrocnemius EMG

Analysis of the individual normalised values for EMG of the LG showed no significant effect of time in the ANOVA at 0-200ms ($F_{6,48}$=1.996, p=0.09) or during the 0-peak phase ($F_{6,48}$=1.552, p=0.18). There was, however a significant effect of time at both 0-50ms ($F_{6,48}$=2.502, p=0.04) and 0-100ms ($F_{6,48}$=3.983, p<0.01). Post hoc analysis at 0-50ms showed a significant decrease from the $EMG_{\%MVIC}$ control values (80.20±27.4%) to the values at 30sec (69.88±24.6%; p=0.02) post MVIC. This corresponds to a decrease of 12.9%, with a CV of 24.4%. Post hoc analysis at 100-200ms showed a significant decrease in $EMG_{\%MVIC}$ from control (98.50±26.6%) at 30sec (81.69±19.9%; p<0.01), 3min (87.69±24.1%; p=0.02) and 8min (90.40±27.0%; p=0.05) post MVIC. This corresponds to a decrease of 17.1, 11.0, and 8.2% respectively when compared to the control value, with a CV of 15.6%.

4 Discussion

The main finding from this study was the presence of PAP in a similar time frame in both evoked twitch contractions and concentric MVCs. Acute enhancements in voluntary explosive contractions were significant up to 5 minutes post CC and visible at lower angular velocities than has been previously reported. Additionally, it seems that repeated maximal concentric contractions together with a maximal isometric conditioning contraction have no effect on plantar flexor passive stiffness.

4.1 Twitch

The results from the evoked twitch contractions show very clear PAP effects in RTD with large increases (59.7%) seen immediately following the CC. Acute enhancements were significant up to 8 minutes and returned to baseline levels at 15 minutes post CC (Figure 1). These enhancements could be related to increased efficiency of the cross-bridge cycle, since twitch RTD has been previously mentioned as a proxy of the rate of cross-bridges entering in a force production state (Vandenboom et al., 1995, Lewis et al., 1986). MVIC effects on twitch PAP (i.e. peak effects immediately after and progressive effect decay) seem to be fairly consistent across similar literature (Babault et al., 2008, Baudry and Duchateau, 2007, Hamada et al., 2000).

TTP was significantly decreased (i.e. faster) by 9.9% immediately after the CC. However, the PAP effects on TTP were less durable than the ones found in RTD (i.e. 1min vs 8min post CC respectively). A significant TTP increase (4.6-2.2%) was also identified 3-8 minutes post CC. The increase in TTP might be related to the relationship in PAP effects between twitch PT and RTD. Twitch PT was not measured in the current study, however, PAP effects on RTD are known to be greater than PT immediately after MVIC, however they also dissipate faster than in PT (Gago et al., 2014b). As TTP is dependent on the ratio between the PAP effects on PT and RTD, it is therefore understandable that the time it takes to achieve a given PT (i.e. TTP) will decrease immediately following the CC and increase later, if RTD is less potentiated than PT due to PAP effects dissipating faster. From this it could be hypothesised that the increased TTP seen from 3-8 minutes might be due to a greater dissipation of PAP effects in RTD in relation to PT.

4.2 Concentric maximal contraction

During explosive plantar flexion both PT and RTD were significantly increased (5.7-6.0% and 6.8-9.5% respectively) post CC. These enhancements occurred during the time frame when twitch RTD was also significantly potentiated. Similar results have been reported for peak twitch and peak concentric plantar flexion at 180º/s, from 1-3 minutes post CC (Miyamoto et al., 2011). The focus of the present study was on twitch and voluntary RTD enhancements at several phases of the rising torque curve, rather than PT enhancements only. This was done with the idea that RTD might be a better representation of possible muscle performance enhancements in a more applied scenario, than PT (Maffiuletti et al., 2016). As far as the author knows, twitch RTD and peak RTD of explosive tasks has only been previously approached by Baudry and Duchateau (2007), who investigate the thumb adductor muscle. Twitch RTD enhancements were seen immediately and up to 5 minutes following both a 6s MVIC and a tetanic contraction. Peak RTD during loaded ballistic voluntary contractions was also immediately significantly enhanced, but the enhancements lasted only 2 minutes post CC. Results from the present study expand some of these previous findings to the plantar flexors, as well as providing more extensive information related to the RTD behaviour by including separate phases of the rising torque curve. The 0-200ms phase of RTD is promising in this sense, with the changes seen unlikely to be due to changes in the first 100ms, since no significant changes occurred in the 30-100 or 50-100ms phases. The first 25-

75ms of the voluntary RTD have been linked to neuromuscular factors (Maffiuletti et al., 2016), so these results suggest neuromuscular changes may not be as relevant as other factors. A better explanation of the acute enhancements in RTD seen in the present study may be changes in MLC phosphorylation and/or stiffness. Fukutani et al. (2013) found that twitch PT was maximally enhanced immediately and up to 5 minutes after the CC, while maximal voluntary concentric PT PAP only occurred immediately after CC (i.e. 5s), and only during high angular velocity contractions (i.e. 180°/s). No significant differences were found at low angular velocities (i.e. 30°/s). The present results clearly show that PAP effects after a single 6s MVIC can enhance concentric MVC PT from 1.5 to 5 minutes at a lower angular velocity (i.e. 60°/s) than previously reported. Although our increase in PT (5.7-6.0%) is lower than that reported by Fukutani et al. (2013) (7%), we have to consider the ICC and CV reported by Fukutani and colleagues at 180°/s was 0.78 and 5.6% respectively while we found an ICC of 0.99 and CV of 4.9%. This, along with the ICC (0.92) and CV (3.8%) reported by Fukutani et al. (2013), for 30°/s suggests that testing PAP effects at lower angular velocities might be more reliable. From this we can hypothesise that lower angular velocities (e.g. 60°/s) may yield more reliable results than those at higher velocities, even if PAP effects seem to be greater at higher velocities (Fukutani et al., 2013, Gago et al., 2014b). Additionally, lower angular velocities than the ones attained in explosive tasks such as CMJ are used in power or hypertrophy based resistance training. In that sense, it is possible that PAP would also optimise gains in that scenario.

4.3 Stiffness

It was hypothesised that the repeated concentric MVCs in addition to a 6s conditioning MVIC could have caused shifts in MTU stiffness which could modulate force transfer. A stiffness index proposed by Nordez et al. (2006) was used as an initial approach for the investigation of MTU passive stiffness changes caused by the testing methods. No changes were found in the PSI, suggesting that the acute enhancements in concentric PT and RTD post CC might have been mainly related to a biochemical factor associated with PAP (i.e. MLC phosphorylation) rather than shifts in the tensile properties of the MTU. It has been previously shown that a single 6s MVIC does not have a significant impact on Achilles tendon stiffness (Gago et al., 2014a) or pennation angle (Rodriguez-Falces et al., 2015). However, to our knowledge, there are currently no studies investigating possible stiffness shifts caused by testing setups

involving repeated concentric contractions following a conditioning MVIC. For this reason, it would be interesting to further investigate possible shifts in the active stiffness of the MTU using other methods. Additionally, it would be interesting to investigate stiffness of specific passive components of the plantar flexors, in order to determine possible effects of repeated contractions on stiffness of individual passive components.

4.4 EMG

There were a number of significant decreases in EMG, both in the combined TS and in some of the individual muscles (specifically the MG and LG). However, there were also quite high CV values, in many cases these were higher than the actual changes in EMG were. This raises the question of whether the recorded changes really were due to changes in muscle activation or whether they were the result of random error. This affects the majority of the significant differences in the EMG, leaving just the changes at 8 minutes post CC in the combined TS as well as the MG, and those at 30s post CC in the LG. All of these changes occur outside of the time frame when PAP is seen in both voluntary RTD and PT, so it is unlikely that these are related to the effects of PAP.

4.5 Limitations

There are a number of limitations that may have affected the results obtained in this study. These include a small sample size, the method of calculation of stiffness and the measured phases in the EMG. The study design used has been shown to be reliable even with a small sample size of approximately 10 subjects (Batterham and Hopkins, 2005). The current study had 9 subjects with a reasonably high ICC and low CV values for the majority of the analysed parameters. In that sense we believe that further increases in the number of subjects would not affect the final outcomes in a significant way. Stiffness index provides a global measure of MTU stiffness, including the TS muscle and other plantar flexor muscles, ligaments and structures stabilising the ankle joint and has been suggested and tested by Nordez et al. (2006). Since the stiffness calculated was performed passively using torque-angle data, it only provides stiffness information of the passive portion of the MTU overall stiffness. This means additional research should be conducted to measure the remaining portions of MTU stiffness (i.e. active portion) possibly via ultrasound based techniques. The fact that EMG was measured in slightly different time phases than RTD makes it difficult to compare the data

between the two, however, we have EMG information for the phase where RTD was the most potentiated (0-200ms). We believe that it is unlikely that alteration of the time phases within the ranges that were currently presented would result in a drastic change in our final findings. However, in order to address this issue, additional efforts were made to include EMG analysis in the same time frames in the manuscript that follows this thesis. No relevant differences were found between the results in this thesis and the manuscript, in terms of muscle activity.

5 Conclusions

The results from the current study clearly show that PAP effects are present in both evoked twitches and in concentric MVCs at similar time frames (i.e. 1.5 to 5min post MVIC). Furthermore, it seems clear that PAP affects explosive voluntary performance at a lower angular velocity (60°/s) than previously reported in the literature (180°/s). This suggests that PAP effects could possibly be useful for athletes to improve their explosive performance immediately after an MVIC, not only after 8 to 12 minutes post CC as previously suggested by the literature. However, before this can be reliably implemented, further research is required to determine the best method of including MVICs and a PAP induction protocol in a warm up. Additionally, PAP could improve acute performance in lower angular velocities, as would be used during power or hypertrophy based resistance training. This acute enhancement, if induced consistently every training, could result in a chronic adaptation and thus, optimised training efficiency. Further research should be conducted to test this hypothesis. Passive stiffness did not show any significant changes, so it would appear that there is no effect of multiple concentric MVCs following a 6s conditioning MVIC on passive stiffness of the MTU. It would, however, be interesting to see if this is the same for active stiffness since this might have some important implications for future PAP related investigations.

6 Acknowledgments

Data used for this thesis was extracted from experiments performed under Maria Ekblom and Paulo Gago's post activation potentiation related project, which was financed by The Swedish National Centre for Research in Sports (CIF) in 2015.

I would like to thank my family for their ongoing belief and support throughout my studies. I would also like to express my sincere gratitude towards my supervisor, Dr Paulo Gago for his many hours of time, help and effort that went into helping me with this thesis. I would also like to thank him for the passion he showed both when helping me with my thesis and for the work in parallel related to the manuscript which accompanies this thesis. Additionally, I would like to thank the rest of the team members; Associate Professor Maria Ekblom, Julio Cézar, and Tiago Jacques; for the time and effort they have contributed towards data collection, analysis and writing the manuscript. Finally, of course, thank you to all the participants, without whom this study would not have been possible.

References

Babault, N., Maffiuletti, N. A. & Pousson, M. 2008. Postactivation potentiation in human knee extensors during dynamic passive movements. *Medicine and science in sports and exercise,* 40**,** 735.

Batterham, A. M. & Hopkins, W. G. 2005. *A Decision Tree for Controlled Trials* [Online]. Sportscience 9, 33-39. Available: sportsci.org/jour/05/wghamb.htm [Accessed].

Baudry, S. & Duchateau, J. 2007. Postactivation potentiation in a human muscle: effect on the rate of torque development of tetanic and voluntary isometric contractions. *Journal of Applied Physiology,* 102**,** 1394-1401.

Baudry, S., Klass, M. & Duchateau, J. 2005. Postactivation potentiation influences differently the nonlinear summation of contractions in young and elderly adults. *Journal of Applied Physiology,* 98**,** 1243-1250.

De Luca, C. J. 1983. Myoelectrical manifestations of localized muscular fatigue in humans. *Critical reviews in biomedical engineering,* 11**,** 251-279.

Fletcher, I. M. 2010. The effect of different dynamic stretch velocities on jump performance. *European journal of applied physiology,* 109**,** 491-498.

French, D. N., Kraemer, W. J. & Cooke, C. B. 2003. Changes in dynamic exercise performance following a sequence of preconditioning isometric muscle actions. *The Journal of Strength & Conditioning Research,* 17**,** 678-685.

Fukutani, A., Hirata, K., Miyamoto, N., Kanehisa, H., Yanai, T. & Kawakami, Y. 2014a. Effect of conditioning contraction intensity on postactivation potentiation is muscle dependent. *Journal of Electromyography and Kinesiology,* 24**,** 240-245.

Fukutani, A., Miyamoto, N., Kanehisa, H., Yanai, T. & Kawakami, Y. 2013. Potentiation of isokinetic torque is velocity-dependent following an isometric conditioning contraction. *SpringerPlus,* 2**,** 1-10.

Fukutani, A., Takei, S., Hirata, K., Miyamoto, N., Kanehisa, H. & Kawakami, Y. 2014b. Influence of the intensity of squat exercises on the subsequent jump performance. *The Journal of Strength & Conditioning Research,* 28**,** 2236-2243.

Gago, P. 2016. Post activation potentiation: Modulating factors and mechanisms for muscle performance.

Gago, P., Arndt, A., Tarassova, O. & Ekblom, M. M. 2014a. Post activation potentiation can be induced without impairing tendon stiffness. *European journal of applied physiology,* 114**,** 2299-2308.

Gago, P., Marques, M. C., Marinho, D. A. & Ekblom, M. M. 2014b. Passive muscle length changes affect twitch potentiation in power athletes. *Medicine & Science in Sports & Exercise,* 46**,** 1334-1342.

Hamada, T., Sale, D., MacDougall, J. & Tarnopolsky, M. 2003. Interaction of fibre type, potentiation and fatigue in human knee extensor muscles. *Acta Physiologica Scandinavica,* 178**,** 165-173.

Hamada, T., Sale, D. G., MacDougall, J. D. & Tarnopolsky, M. A. 2000. Postactivation potentiation, fiber type, and twitch contraction time in human knee extensor muscles. *Journal of Applied Physiology,* 88**,** 2131-2137.

Hodgson, M., Docherty, D. & Robbins, D. 2005. Post-activation potentiation. *Sports Medicine,* 35**,** 585-595.

Iglesias-Soler, E., Paredes, X., Carballeira, E., Márquez, G. & Fernández-Del-Olmo, M. 2011. Effect of intensity and duration of conditioning protocol on post-activation potentiation and changes in H-reflex. *European Journal of Sport Science,* 11**,** 33-38.

Kay, A. D. & Blazevich, A. J. 2009. Isometric contractions reduce plantar flexor moment, Achilles tendon stiffness, and neuromuscular activity but remove the subsequent effects of stretch. *Journal of Applied Physiology,* 107**,** 1181-1189.

Kay, A. D. & Blazevich, A. J. 2010. Concentric muscle contractions before static stretching minimize, but do not remove, stretch-induced force deficits. *Journal of Applied Physiology,* 108**,** 637-645.

Kilduff, L. P., Bevan, H. R., Kingsley, M. I., Owen, N. J., Bennett, M. A., Bunce, P. J., Hore, A. M., Maw, J. R. & Cunningham, D. J. 2007. Postactivation potentiation in professional rugby players: optimal recovery. *The Journal of Strength & Conditioning Research,* 21**,** 1134-1138.

Lewis, D., Al-Amood, W. & Rosendorff, C. 1986. Stimulation of denervated muscle: what do isometric and isotonic recordings tell us? *Electrical stimulation and Neuromuscular disorders.* Springer.

MacIntosh, B. R. 2010. Cellular and whole muscle studies of activity dependent potentiation. *Muscle Biophysics.* Springer.

Maffiuletti, N. A., Aagaard, P., Blazevich, A. J., Folland, J., Tillin, N. & Duchateau, J. 2016. Rate of force development: physiological and methodological considerations. *European journal of applied physiology***,** 1-26.

Miyamoto, N., Kanehisa, H., Fukunaga, T. & Kawakami, Y. 2011. Effect of postactivation potentiation on the maximal voluntary isokinetic concentric torque in humans. *The Journal of Strength & Conditioning Research,* 25**,** 186-192.

Morana, C. & Perrey, S. 2009. Time course of postactivation potentiation during intermittent submaximal fatiguing contractions in endurance-and power-trained athletes. *The Journal of Strength & Conditioning Research,* 23**,** 1456-1464.

Nordez, A., Cornu, C. & McNair, P. 2006. Acute effects of static stretching on passive stiffness of the hamstring muscles calculated using different mathematical models. *Clinical Biomechanics,* 21**,** 755-760.

Nordez, A., Guével, A., Casari, P., Catheline, S. & Cornu, C. 2009. Assessment of muscle hardness changes induced by a submaximal fatiguing isometric contraction. *Journal of Electromyography and Kinesiology,* 19**,** 484-491.

Obst, S. J., Barrett, R. S. & Newsham-West, R. 2013. Immediate effect of exercise on achilles tendon properties: systematic review. *Medicine and science in sports and exercise,* 45**,** 1534-1544.

Rassier, D. E. 2000. The effects of length on fatigue and twitch potentiation in human skeletal muscle. *Clinical Physiology,* 20**,** 474-482.

Reardon, D., Hoffman, J. R., Mangine, G. T., Wells, A. J., Gonzalez, A. M., Jajtner, A. R., Townsend, J. R., McCormack, W. P., Stout, J. R. & Fragala, M. S. 2014. Do changes in muscle architecture affect post-activation potentiation? *Journal of sports science & medicine,* 13**,** 483.

Rodriguez-Falces, J., Duchateau, J., Muraoka, Y. & Baudry, S. 2015. M-wave potentiation after voluntary contractions of different durations and intensities in the tibialis anterior. *Journal of Applied Physiology,* 118**,** 953-964.

Sale, D. G. 2002. Postactivation potentiation: role in human performance. *Exercise and sport sciences reviews,* 30**,** 138-143.

Seitz, L. B. & Haff, G. G. 2015. Factors Modulating Post-Activation Potentiation of Jump, Sprint, Throw, and Upper-Body Ballistic Performances: A Systematic Review with Meta-Analysis. *Sports Medicine***,** 1-10.

Seitz, L. B., Mina, M. A. & Haff, G. G. 2016. Post-activation potentiation of horizontal jump performance across multiple sets of a contrast protocol. *The Journal of Strength & Conditioning Research,* Publish Ahead of Print.

Stuart, D., Lingley, M., Grange, R. & Houston, M. 1988. Myosin light chain phosphorylation and contractile performance of human skeletal muscle. *Canadian journal of physiology and pharmacology,* 66**,** 49-54.

Stull, J. T., Kamm, K. E. & Vandenboom, R. 2011. Myosin light chain kinase and the role of myosin light chain phosphorylation in skeletal muscle. *Archives of biochemistry and biophysics,* 510**,** 120-128.

Szczesna, D., Zhao, J., Jones, M., Zhi, G., Stull, J. & Potter, J. D. 2002. Phosphorylation of the regulatory light chains of myosin affects Ca2+ sensitivity of skeletal muscle contraction. *Journal of Applied Physiology,* 92**,** 1661-1670.

Tillin, M. N. A. & Bishop, D. 2009. Factors modulating post-activation potentiation and its effect on performance of subsequent explosive activities. *Sports medicine,* 39**,** 147-166.

Tillin, N. A., Pain, M. T. & Folland, J. P. 2013. Identification of contraction onset during explosive contractions. Response to Thompson et al."Consistency of rapid muscle force characteristics: influence of muscle contraction onset detection methodology"[J Electromyogr Kinesiol 2012; 22 (6): 893–900]. *Journal of Electromyography and Kinesiology,* 23**,** 991-994.

Vandenboom, R., Grange, R. & Houston, M. 1995. Myosin phosphorylation enhances rate of force development in fast-twitch skeletal muscle. *American Journal of Physiology-Cell Physiology,* 268**,** C596-C603.

Weber, K. R., Brown, L. E., Coburn, J. W. & Zinder, S. M. 2008. Acute effects of heavy-load squats on consecutive squat jump performance. *The Journal of Strength & Conditioning Research,* 22**,** 726-730.

Wilson, J. M., Duncan, N. M., Marin, P. J., Brown, L. E., Loenneke, J. P., Wilson, S. M., Jo, E., Lowery, R. P. & Ugrinowitsch, C. 2013. Meta-analysis of postactivation potentiation and power: effects of conditioning activity, volume, gender, rest periods, and training status. *The Journal of Strength & Conditioning Research,* 27**,** 854-859.

Xenofondos, A., Patikas, D., Koceja, D. M., Behdad, T., Bassa, E., Kellis, E. & Kotzamanidis, C. 2015. Post-activation potentiation: The neural effects of post—activation depression. *Muscle & nerve,* 52**,** 252-259.

Zhi, G., Ryder, J. W., Huang, J., Ding, P., Chen, Y., Zhao, Y., Kamm, K. E. & Stull, J. T. 2005. Myosin light chain kinase and myosin phosphorylation effect frequency-dependent potentiation of skeletal muscle contraction. *Proceedings of the National Academy of Sciences of the United States of America,* 102**,** 17519-17524.

Appendix 1 Literature Search

Syfte och frågeställningar:

The general aim of this thesis was to present a brief and updated overview of PAP conditioning contraction effects on both voluntary and involuntary contractions while expanding the existing results with new insights on the topic.

The research questions were:

- Does PAP affect subsequent explosive concentric contractions (PT, RTD, and EMG) when performed at low angular velocity (i.e. 60°/s)?
- Is there a simultaneous acute enhancement of twitch properties and explosive voluntary concentric contractions after a single 6s MVIC?
- Could the PAP effects be related to acute changes in the MTU stiffness induced by the experimental tests, the explosive concentric contractions, themselves?

Vilka sökord har du använt?

Post activation potentiation, stiffness and post activation potentiation, postactivation potentiation, twitch potentiation, factors affecting post activation potentiation, factors affecting stiffness

Var har du sökt?

GIH:s bibliotekskatalog, PubMed, Ebsco, Google Scholar

Sökningar som gav relevant resultat

All combinations of searches gave results

Kommentarer

Most references were found by reading through the reference lists of relevant articles to find more articles that may be relevant.

Appendix 2 Informed consent form

Information till dig som deltar i studie om muskelpotentiering hos idrottare

Tidigare forskning har visat att upprepade maximala muskelkontraktioner förbättrar muskelns förmåga att utveckla kraft under ett fönster på ca 15 min efter kontraktionen. Dessa studier har framför allt utförts på djurmodeller eller i specifika idrottsgrenar där det är svårt att utesluta att psykologiska faktorer påverkat prestationen. I denna studie vill vi undersöka om idrottare kan använda dessa rutiner för att förbättra vadmusklernas kontraktila egenskaper. Studien består av 3 testtillfällen. Ett där vi mäter hopp och maximal kontraktion prestation i en sledge och isokinetisk dynamometer. Vid det andra tillfället vi el-stimulerar tibialisnerven i knävecket för att utlösa muskelsammandragningar genom vilka vi kan studera musklernas kontraktila egenskaper och hur de påverkas av potentiering. Elstimuleringen består av 0,5s långa tåg av elpulser som vardera är 0,5 ms långa. Stimuleringen är ofarlig men kan medföra visst övergående obehag. Vid det tredje tillfället tar vi vävnadsprover från vadmusklerna för att via muskelanalyser undersöka mekanismerna för potentieringen före och vid 3 tillfällen under återhämtningen efter upprepade maximala muskelkontraktioner. Vid provtagningen bedövas huden efter rengöring först med 2-3 ml xylocain. Därefter görs ett 5-7 mm snitt i huden och underliggande vävnad. Därefter används en tång för att ta ett prov på 50-75 mg (stor som ärta). Du ska inte känna smärta vid prov. Bedövningen sitter sedan i under 2-5 timmar. Därefter blir området ömt i några timmar till någon dag. Du skall inte bada eller väta förbandet upp till 2 dygn efteråt. Det sår som blir på huden, bleknar och är borta efter något år eller så. Styrketesten kan medföra viss träningsvärk liknande den efter styrketräning. Varje tillfälle tar ca 1,5 h.

Studien äger rum i Laboratoriet för Biomekanik och Motorisk Kontroll och Åstrandslaboratoriet vid Gymnastik och Idrottshögskolan på Lidingövägen 1.

Deltagande ersätts med 1200 kr efter de tre testtillfällena.

Du har rätt att när som helst avsluta ditt deltagande i studien utan att behöva förklara varför.

Har du frågor om studien och/eller ditt deltagande, kontakta ansvarig forskare Maria Ekblom på 08-4022240 eller 070-5660051.