NEURAL CONTRIBUTION TO POSTACTIVATION POTENTIATION

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ABSTRACT

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The current study was designed to investigate the contribution of neural factors to postactivation potentiation (PAP). Neuromuscular function (i.e. twitches, H-reflexes, motor evoked potentials, and voluntary isometric ballistic contractions) of 8 power-trained (POW) and 8 endurance-trained (END) athletes was recorded before and after a 8-second maximal isometric conditioning contraction (CC) to induce PAP, to elucidate discriminating neural factors in exploiting PAP that might arise from the former training background compared to the latter.

After CC, twitch peak force and rate of force development were significantly increased, with higher potentiation in POW (29 ± 11% and 64 ± 24%) than END (8 ± 12% and 34 ± 20%). Among evoked potentials, only motor evoked potentials were short-term facilitated (127 ± 111% and 93 ± 89%, for END and POW respectively), similarly between groups. No differences were reported in ballistic performance ($P \geq 0.069$), whose neural drive was significantly depressed in POW at 1 and 2 minutes post-CC, respectively compared to END and to baseline (19 ± 11%).

PAP was characterized from enhanced muscle contractile characteristics as well as short-term facilitation of corticospinal excitability, whose individual contribution to performance enhancement could not be quantified. However, when POW were compared to END, the former group benefited from PAP in triceps surae muscles only in terms of higher potentiation of muscular contractile characteristics, as neural pathways were affected likewise from CC. In addition, neural drive of ballistic performance, if affected, might be depressed in POW rather than enhanced, presumably due to neural fatigue from CC. Differences between groups in exploiting PAP in ballistic actions might be therefore primarily related to muscular potentiating mechanisms, although only non-significant potentiation (p-value close to the significance threshold) was found in ballistic performance in our experiments.

ABBREVIATIONS

CaM	Calmodulin
CC	Conditioning contraction
CH	Conditioned H-reflexes
CI	Confidence intervals
CM	Conditioned MEPs
CMS	Cervicomedullary stimulation
CNS	Central nervous system
CT	Contraction time
CTw	Conditioned twitches
CV	Coefficient of variation
EC	Excitation-contraction
EEC	Electrically-evoked contractions
ELC	Essential light chain
EMG	Electromyography / Electromyographic
END	Endurance-trained group
FF	Fast contracting – fast to fatigue
FR	Fast contracting – fatigue resistant
Hmax	Maximal H-wave
H-reflex	Hoffmann reflex
HRT	Half-relaxation time
ICC	Intraclass correlation coefficient
LG	Lateral gastrocnemius
MEP	Motor evoked potentials
MLCK	Myosin light chain kinase
Mmax	Maximal M-wave
Msubmax	M-wave preceding the H-wave
MVC	Maximal voluntary contraction
MVIC	Maximal voluntary isometric contraction
MU	Motor unit

NMJ	Neuromuscular junction
PAP	Postactivation potentiation
pBRFD	Peak rate of force development of the ballistic contraction
pRFD	Peak rate of force development
PF	Peak force
PNS	Peripheral nervous system
POW	Power-trained group
P-P	Peak-to-peak amplitude
RFD	Rate of force development
RLC	Regulatory light chain
RLC-P	Regulatory light chain phosphorylation
RMS EMG	Root mean square of emg from its onset to the peak force of the ballistic contraction
rMT	Resting motor threshold
S	Slow contracting – fatigue resistant
SOL	Soleus
SR	Sarcoplasmic reticulum
TA	Tibialis anterior
TES	Transcranial electrical stimulation
TM	Tropomyosin
TMS	Transcranial magnetic stimulation
Tn	Troponin

CONTENTS

1 INTRODUCTION

At any point in time, the performance of a skeletal muscle is affected by its contractile history. The most obvious effect is neuromuscular fatigue, which impairs the performance (Sale, 2002). However, muscles can positively respond to previous activity with improvements in their capabilities (Vandervoort et al., 1983). This phenomenon is referred to as postactivation potentiation (Sale, 2002).

Postactivation potentiation (PAP) is induced by a voluntary conditioning contraction (CC) performed at a maximal or near-maximal intensity (Fukutani et al., 2014). Subsequent muscle actions are facilitated through occurred intramuscular changes, namely, phosphorylation of regulatory light chains of the myosin heads (Stull et al., 2011). Following CC, the time frame of the potentiation lasts up to 15 minutes (Baudry & Duchateau, 2004), whose magnitude is measured through improvements in twitches mechanical responses of a muscle group (Hodgson et al., 2005).

Parallelly, enhancements in the muscle contractile machinery functioning within the potentiation time course lead to improvements in power performances, through increased rate of force development (RFD) of ballistic actions (Sale, 2002). This behaviour has been described as an upward and rightward shift of the force-velocity curve. (Sale, 2002).

Albeit muscular mechanisms are deemed to be the principal cause of RFD enhancements, neural mechanisms might also play a role. CC might lead to changes in neural motor pathways excitability (Gandevia et al., 1999). Being spinal and supraspinal components integral in the performance of voluntary tasks, alteration of neural excitability might result in modulation of RFD of ballistic actions. To date, this phenomenon has been neither denied nor confirmed.

The purpose of the present study is to investigate the contribution of neural factors to ballistic performance enhancement during PAP time course in plantar flexors.

The report of the investigation itself will be preceded from the review of the literature. The latter has been divided in three parts. Firstly, the motor system and the interaction

of its components for voluntary muscle actions will be described. Focus will be directed toward the anatomical components involved in PAP and the measuring techniques used in the present study. The second part will regard PAP phenomenon in its underlying muscular mechanism, quantification, affecting factors and enhancement of power performance. Indications from previous studies of possible neural motor pathways implication in ballistic performance enhancement in PAP will be also reported, introducing the last part, namely, the purpose of the present study.

2 THE MOTOR SYSTEM

The motor system consists of the parts of the nervous system and the skeletal muscle that are responsible for movement (Enoka, 2008, 171) (fig. 1). From a comprehensive standpoint, activation signals are generated from neural components, whereas muscular ones exert forces to produce movement.

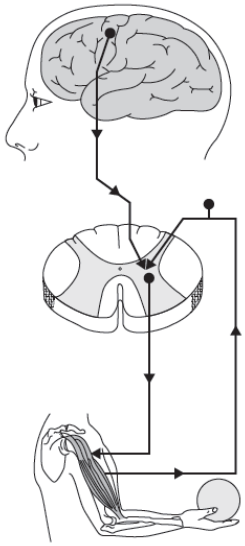

FIGURE 1. Control of muscle by the nervous system. The central nervous system (CNS) consists of four parts, namely, the brain, the brain stem, the cerebellum and the spinal cord (only brain and one section of the spinal cord is illustrated). The peripheral nervous system (PNS) is composed of afferent and efferent neurons, conveying respectively sensory information from the surroundings to the spinal cord, and output signals oppositely to the muscle. (From Komi, 2011).

The nervous system is composed of a central part and a peripheral part, respectively, central nervous system (CNS) and peripheral nervous system (PNS) (fig.1). Within the motor system, the CNS consists of four main parts, namely, the brain, the brain stem, the cerebellum, and the spinal cord (Enoka, 2008, 288). The PNS is represented from afferent and efferent neurons outside the CNS, conveying respectively sensory information from the surroundings to the spinal cord, and output signals oppositely to the effector organ (fig.1). In the motor system, the effectors are the skeletal muscles,

whose innervating (efferent) neurons are referred to as motor neurons (Enoka, 2008, 250).

The skeletal muscle characteristic of exerting force resides in its properties, that is, the ability of responding to stimuli deriving from motor neurons (irritability), propagating action potential (conductivity), and modifying its length (contractility) (Enoka, 2008, 205). The particular organization and variety of proteins of muscle fibres allow executing these functions (MacIntosh et al., 2006, 6).

The next sections will present more in depth how the above-mentioned parts of the motor system are integrated for movement generation. Firstly, starting from the periphery, the structure of the skeletal muscle and the mechanisms responsible for muscle contractions will be described. Secondly, motor neurons and their organization with muscle fibres in motor units will be depicted. Focus will be directed upon their recruitment, activation frequency, and the twitch as a measure of motor units and muscle group contractile properties. Lastly, the organization of the CNS will be illustrated, including the main methods for measuring neural pathways excitability.

2.1 Structure of the skeletal muscle and muscle contraction

2.1.1 The gross skeletal muscle structure

To achieve the function that they serve, skeletal muscles are arranged in fibres embedded in a collagenous connective tissue, hierarchically organized in three levels, the epimysium (insheathing the muscle belly), perimysium (bundling muscle fibres into fascicles) and endomysium (surrounding the individual muscle fibers) (MacIntosh et al., 2006, 8) (fig. 2).

Muscle fibres are the polynucleated cells of the muscle tissue, encircled from an excitable cell membrane, the sarcolemma. Its function, other than a structural support, is to transmit action potentials deriving from the motor neurons to the contractile components within the muscle fibres, allowing muscle contraction (Enoka, 2008, 205).

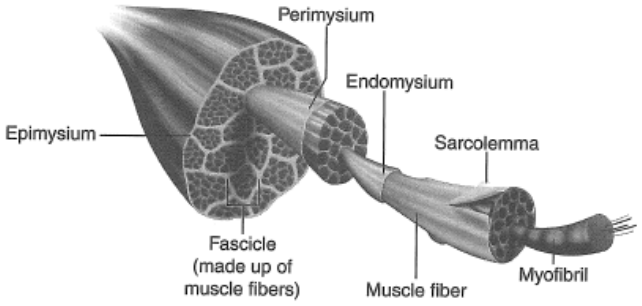

FIGURE 2. The gross structural elements of a skeletal muscle (Zatsiorsky and Prilutsky, 2012, 5).

A muscle fibre consists of several myofibrils in parallel. Each myofibril is a series of sarcomeres connected end to end, the latter ones representing the anatomo-functional units of the muscle tissue, responsible for its contraction and relaxation through thick and thin filaments sliding one past the other (fig. 3) (Enoka, 2008, 207).

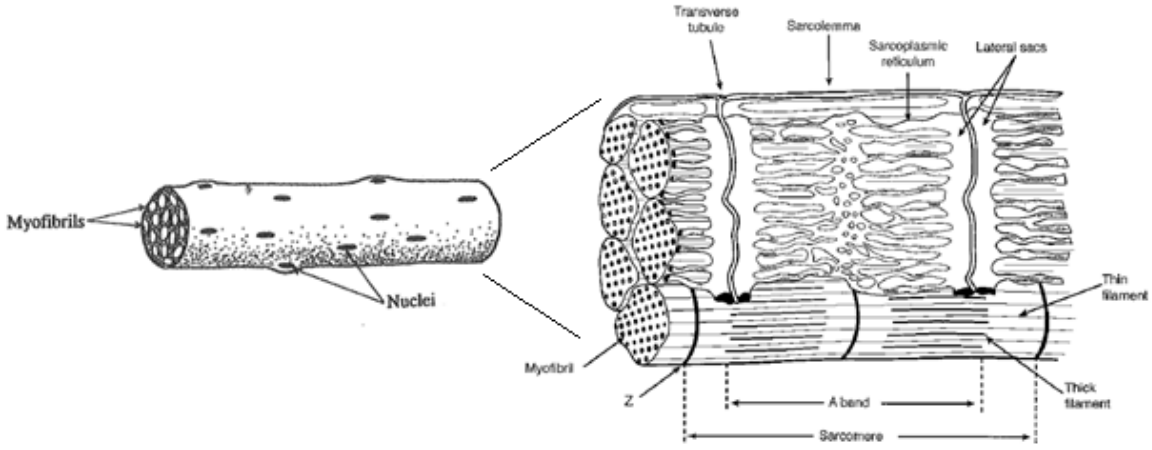

FIGURE 3. Structure of the polynucleated muscle fibres and myofibrils (adapted from Enoka, 2008, 206; MacIntosh et al., 2006, 18).

2.1.2 Tubular system within the muscle fibres

The transition between sarcolemma action potentials and muscle fibre shortening is guaranteed from the release of calcium by the extensive tubular system within the sarcoplasma. This tubular system comprises two parts: the sarcoplasmic reticulum (SR) and the transverse tubules (T-tubules) (fig.3).

The SR can be defined as a meshwork of channels, surrounding individual myofibrils (MacIntosh et al., 2006, 16). These channels are divided in longitudinal reticulum, running along the myofibrils, and in terminal cisternae (or lateral sacs), anchoring the network to the T-tubules. The SR is the calcium ions (Ca^{2+}) storage of the skeletal muscle. The function of the SR is to release Ca^{2+} into the cytosol around the myofibrils, allowing contraction to take place, and to pump Ca^{2+} back inside, allowing the contraction to terminate. The former role is guaranted from Ca^{2+} channels mainly at the lateral sacs membrane level, while the latter one is accomplished by Ca^{2+} ATPase pumps, primarily associated with longitudinal reticulum membrane. (MacIntosh et al., 2006, 17).

The T-tubules are branched invaginations of the sarcolemma (Enoka, 2008, 207). Their membrane is continuous with the plasmalemma, they encircle the myofibrils and are closely apposed to the lateral sacs of the SR at regular intervals (MacIntosh et al., 2006, 17). In light of their anatomical structure, the function of the T-tubules is to conduct action potentials from the surface to the interior of the muscle fibre, triggering Ca^{2+} release from the SR, therefore muscle fibre shortening (Enoka, 2008, 207).

2.1.3 The sarcomeres and the myofilaments

As previously mentioned, the sarcomeres are the anatomo-functional units of the muscle tissue, responsible for its contraction and relaxation through thick and thin filaments sliding one past the other (fig. 3) (Enoka, 2008, 207).

From an exterior standpoint, the overlapping of interdigitated thick and thin myofilaments within sarcomeres gives the striated appearance to the muscle fibers (fig. 4) (MacIntosh et al., 2006, 17).

Sarcomere. The sarcomere is anatomically recognized as the zone of myofibril between two Z-lines, whereas connections between thin filaments take place (Fig. 4B). Thick filaments attach to the M-line, placed at the centre of each sarcomere (Enoka, 2008, 207). The A-band corresponds to the presence of thick filaments, while the I-band contains thin filaments. However, the latter ones extend also within the A-band, as the filaments overlap to some extent. The central region without filaments overlap constitute the H-band (MacIntosh et al., 2006, 14).

Myofilaments. The myofibrils mainly contain thick and thin filaments, which are referred to as myofilaments (fig.4A). Although myofibrils are made up of several other proteins (e.g. titin and desmin) (Billeter & Hoppeler, 2003, 54), necessary for the build-up and maintenance of the sarcomere, the description will be restricted to the molecules taking part of thin and thick filaments and protagonist in muscle contraction (i.e. actin, troponin, tropomyosin, myosin) (Billeter & Hoppeler, 2003, 54).

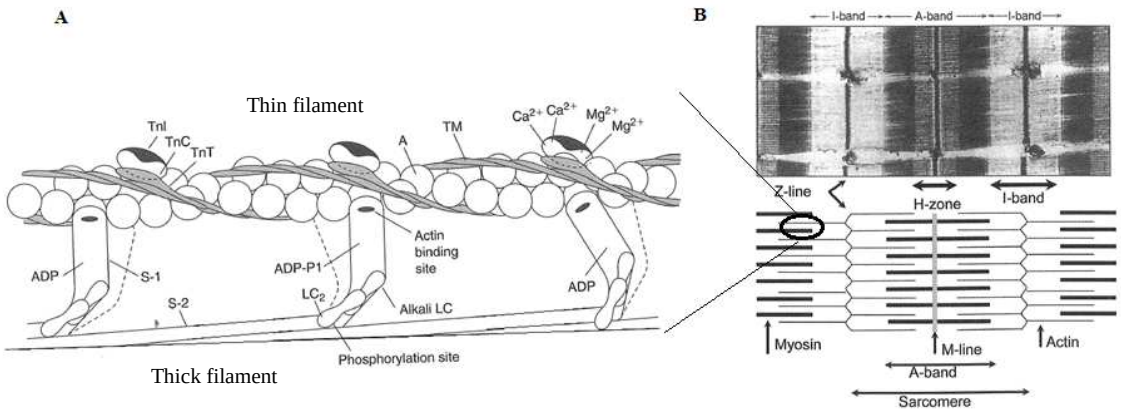

FIGURE 4. A) Schematic of interaction between thick and thin filaments. A = actin; TM = Tropomyosin; Tn = Troponin; LC = light chain. B) On the top: characteristic striation of a sarcomere. On the bottom: overlapping arrangement of thick and thin filaments responsible for the striated appearance (adapted from Gardiner, 2001, 24; MacIntosh et al., 2006, 14).

The thin filaments are mainly composed by actin but also include proteins such as troponin and tropomyosin for regulating the interaction between actin and myosin (regulatory proteins) (Enoka, 2008, 207; MacIntosh, 2003, 158). The backbone of thin filaments is a double-helical strand of individual actin molecules. Two coiled coils of tropomyosin (TM) are located in the grooves formed from the actin molecules, covering the myosin-binding sites on the thin filaments. Lastly, a troponin (Tn) complex (made of three subunits, TnI, TnT, and TnC) is tied to both actin and tropomyosin. TnC has two binding sites for Ca^{2+} and two for either Ca^{2+} or Mg^{2+} (fig. 4A) (Enoka, 2008, 208). When Ca^{2+} concentration rises in the sarcoplasma (see "Excitation-contraction coupling"), four calcium ions bind to TnC, allowing the Tn complex changing

conformation and moving the TM away from the myosin-binding site, therefore initializing the cross-bridges interaction (MacIntosh, 2003, 159).

The thick filaments are composed mainly of myosin molecules (fig. 4A). Although several other proteins within the thick filaments regulate the myosin-actin interaction (Schiaffino & Reggiani, 1996), the description will be restricted to the myosin molecule, which plays a fundamental role in PAP phenomenon (Vandervoort et al., 1983; Grange et al., 1993; Tillin & Bishop, 2009). A single myosin molecule consists of two heavy chains with long intertwined tails connected to elongated heads, with two light chains bound to each neck region (Billeter & Hoppeler, 2003, 51) (Fig. 5A). The hinge region allows the globular heads to stand out from the myosin tails, enabling interaction with actin. Thereafter, the myosin molecule is divided into two fragments, S1 and S2, the former including the two globular heads, the force-generating sites in muscle (Fig. 5A). Three components have been identified within each globular head, with molecular weights of 50 kD, 25 kD, 20kD (MacIntosh, 2003, 157) (Fig. 5B). The 50 kD segment contains a pocket for binding and hydrolyzing ATP (through the ATPase enzyme), as well as a cleft for attachment to actin, whereas the 20 kD part includes two light chains (MacIntosh, 2003, 157), termed regulatory and essential (respectively, RLC and ELC), and having an important modulatory role in the cross-bridge cycle (Grange et al., 1993; Gardiner, 2001, 18; MacIntosh, 2003, 158) (see “Role of the RLCs in muscle contraction”).

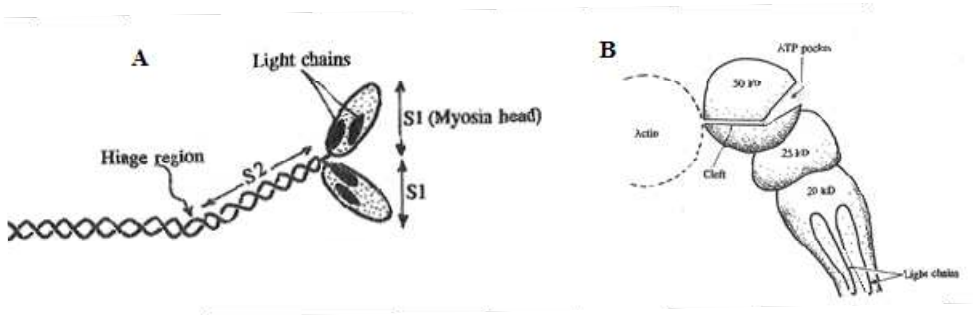

FIGURE 5. A) The myosin molecule and its different components. The intertwined tails extend after the hinge region (S2 fragment), terminating with two globular heads (S1 fragment) whereas the two light chains are attached. B) Enlargement of one globular head, showing the three different segments. The light chains are included in the lightest component (20 kD) of the myosin head. The terminal segment (50 kD) includes a pocket for binding and hydrolyzing ATP as well as a cleft for attachment to actin (adapted from MacIntosh et al., 2006, 157).

2.1.4 Excitation-contraction coupling

As previously mentioned, in the motor system skeletal muscles are innervated from motor neurons triggering muscle fibres contraction, thus muscle force production. Once motor neurons are depolarized, action potentials travel along the axons reaching the synapses with muscle fibres, whereas nerve electrical impulses are transferred to the muscle fibres' membranes. The site of this transduction, due to the linkage between two biologically different tissues, namely, nervous and muscular, has been named neuromuscular junction (NMJ). The transduction of the signal is referred to as neuromuscular propagation (Enoka, 2008, 210).

The complex of processes from nerve depolarization to muscle contraction is known as excitation-contraction (EC) coupling (Payne & Delbono, 2004). EC coupling begins with the transmission of the action potential down the axon of a motor neuron, neuromuscular propagation, propagation of the action potential along the muscle fibre, propagation of the action potential down the T-tubules, coupling of the action potential to the change in Ca^{2+} conductance of the SR, release of Ca^{2+} from the SR, reuptake of the Ca^{2+} into the SR, Ca^{2+} binding to Tn, and interaction of the contractile proteins. The last step corresponds to the cross-bridge cycle, whereas the others from the neuromuscular propagation, have been referred to as Ca^{2+} disinhibition (Enoka, 2008, 210). These events will be briefly explained below. Focus will be directed to those processes determining the onset of PAP and directly involved in it.

Ca^{2+} Disinhibition. The release of Ca^{2+} from the SR and the bonds of calcium ions with TnC allow moving away TM from myosin binding sites and initializing cross-bridges interaction (Enoka, 2008, 210).

Once the action potential reaches the muscle fibre through neuromuscular propagation, it is diffused along the sarcolemma and down the T-tubules (fig. 3). This signal is transmitted to the SR through voltage sensors. Thereby, Ca^{2+} channels open and calcium ions passively efflux into the sarcoplasma, following the concentration gradient. When Ca^{2+} is above a concentration threshold level (Enoka, 2008, 210), Ca^{2+} binds to TnC. This interaction between Ca^{2+} and TnC causes a rotation in the TM-Tn regulatory complex, uncovering the myosin-binding site for thin and thick filaments interaction. In

light of this mechanisms, sensitivity of TnC to Ca^{2+} can be considered as fundamental for cross-bridges interaction and muscle fibre force production (Gardiner, 2001, 25), mainly determined from the type of TM and TN isoforms within the thin filaments (Schaffino & Reggiani, 1996; Gordon et al., 2000; Gardiner, 2001, 26). When the action potential has decayed, Ca^{2+} is actively returned to the SR by Ca^{2+} ATPase pumps, allowing Ca^{2+} concentration returning to its resting level (Enoka, 2008, 211).

Cross-bridge cycle. The repeated actin-myosin interaction linked to the breakdown of ATP is referred to as cross-bridge cycle (Billeter & Hoppeler, 2003, 53). The ensemble of the cross-bridge cycles from each myosin head allows muscle force generation.

Once calcium ions have bound with TnC and myosin binding sites have been uncovered, the cross-bridge cycle takes place. According to Billeter & Hoppeler (2003, 52), it can be divided in 4 different states, related to the position of the myosin head (fig. 6).

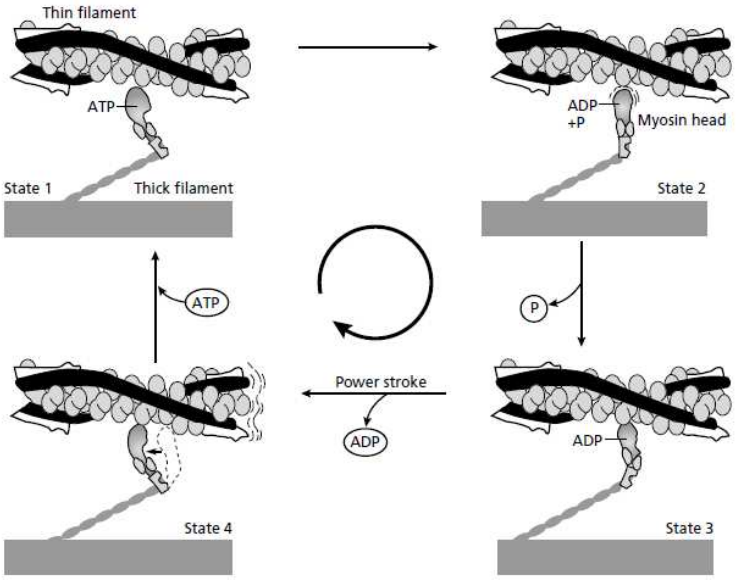

FIGURE 6. Model of the cross-bridge cycle. Myosin is bound to ATP, the globular head is detached from actin (state 1). ATP is cleft in ADP and P_i, which causes a weak bound between the myosin head and the actin site (state 2). P_i is released, strengthening the bond (state 3). The subsequent release of ADP leads to a swing of the myosin head lever arm (power stroke), shortening the sarcomere (state 4). ATP is taken up to detach actin and myosin and restart the cycle (adapted from Billeter & Hoppeler, 2003, 53).

In state 1, the myosin head is not bound to actin and has ATP in its respective pocket. In state 2, the ATP is cleft in ADP plus phosphate (P_i). This reaction allows the lever arm of the globular head moving, assuming the proper position for the stroke of the cycle. P_i is then released and the myosin head binds strongly to actin (state 3). This release, followed by the ADP one, is accompanied by a twist of the lever arm of the globular head, pushing the thin filament toward the middle of the sarcomere, i.e. shortening the muscle fibre (state 4). This phase has been referred to as "power stroke" of the cycle (Billeter & Hoppeler, 2003, 53). Subsequently, ATP binds to the myosin head causing detachment from actin, and making the complex ready to start another cycle. Although cross-bridge cycle dynamics is important for muscle fibre and muscle force production, the main regulatory step of the powerstroke is linked to the availability of the binding site for the myosin head (Billeter & Hoppeler, 2003, 53). This underlines (again) the importance of the Tn-TM complex (uncovering the myosin-binding site), and TnC sensitivity to calcium ions in muscle fibre contraction (Schiaffino & Reggiani, 1996; Gordon et al., 2000; Gardiner, 2001, 26).

2.1.5 Role of the RLCs in muscle contraction

As earlier mentioned (see "The sarcomeres and the myofilaments"), the myosin head contains a 20 kD portion, which include two small protein-subunits, the ELC and RLC, which play an important role in modulating actin-myosin interactions (Gardiner, 2001, 19). In the following section, focus has been directed toward the RLCs, due to its direct involvement in muscle contraction and PAP, facilitating mobility of myosin heads, thus cross-bridges formation (Grange et al., 1993; Sweeney et al., 1993; Stull et al., 2011).

The primary role of RLCs is altering cross-bridge function through phosphorylation (Gardiner et al., 2001, 22; Stull et al, 2011). When Ca^{2+} is released from the SR, it binds with TnC to allow uncovering the myosin-binding site. In addition, Ca^{2+} combines with calmodulin (CaM, a sarcoplasma calcium binding protein), which requires four Ca^{2+} to form a calcium-calmodulin complex (Stull et al., 2011). The complex activates then the myosin light chain kinase (MLCK), the enzyme responsible for RLC phosphorylation (RLC-P). From the cleft of one ATP molecule in ADP and P_i, the RLC is phosphorylated (Stull et al., 2011).

Being activation of MLCK Ca^{2+}-dependent, RLC-P is regulated from the same calcium signal modulating muscle contractions (Stull et al., 2011). Thus, RLC-P is enabled with the onset of muscle contraction, during prolonged, or repetitive activity (Sweeney et al., 1993; Stull et al., 2011).

The importance of myosin RLC-P resides in the alteration of myosin motor function, enhancing basic properties in actin-myosin interactions (Grange et al., 1993; Sweeney et al., 1993; Stull et al., 2011). Firstly, RLC-P results in the myosin head moving away from the thick filament toward the binding site on the thin filament (Stull et al., 2011), facilitating their interaction. Secondly, an increase in sensitivity of TnC to Ca^{2+} is mediated by RLC-P, leading to a higher number of active cross-bridges for the same sarcoplasma Ca^{2+} concentration (Grange et al., 1993; Sweeney et al., 1993; Stull et al., 2011).

Although RLC-P facilitates cross-bridges interaction, RLC-P has been reported to have scarce effects at high sarcoplasma Ca^{2+} concentration level, as well as little effects on maximal shortening velocity of the muscle fibre (Stull et al., 2011). When Ca^{2+} concentration is high, the fraction of cross-bridges in the force-generating state is already high, therefore contractile proteins do not benefit from an increase in Ca^{2+} sensitivity (Tillin & Bishop, 2009; Stull et al., 2011). Additionally, RLC-P does not influence the kinematic properties of the myosin head, resulting in a little ability to influence the maximal shortening velocity of the muscle fibre (Stull et al., 2011).

Overall, RLC-P is linked to single, prolonged or repetitive muscle contractions. The above mentioned aspects advocate RLC-P as fundamental for facilitating myosin mobility in cross-bridges formation. However, this phenomenon might modulate muscle fibre shortening neither when maximal velocity nor maximal force is required (i.e. at the extremes of the muscle fibre force-velocity curve), leading to cross-bridge interaction facilitation at muscle fibre sub-maximal force and velocity production (Grange et al., 1993; Sweeney et al., 1993; Stull et al., 2011). Enlarging the focus from the muscle fibre to the muscle function, muscle contraction has been found to benefit from phosphorylation of RLCs (Sale, 2002; Tillin & Bishop, 2009; Stull et al., 2011) (see “Muscle mechanics” and “Post-activation potentiation”).

2.1.6 Muscle mechanics

The contractile function of skeletal muscles resides in its complex structure, organization and composition (Enoka, 2008, 231). Indeed, the force a muscle exerts depends on both contractile (active process of cross-bridge cycling and filament overlap) and structural (connective tissue and cytoskeleton) properties (Enoka, 2008, 234). Because of this interaction, the force output is function of muscle length and speed of contraction. The ensemble of these relationships is referred to as "muscle mechanics" (Enoka, 2008, 234).

Force-length relationship. When a muscle is maximally activated, the isometric force developed depends on the length at which the muscle is held (Rassier et al., 1999) (fig. 7). The contribution of active and passive components to total muscle force vary over the range from the minimal contraction length to the maximal stretched length (Enoka, 2008, 235). The total force (open connected circles in fig. 7) is the sum of both components.

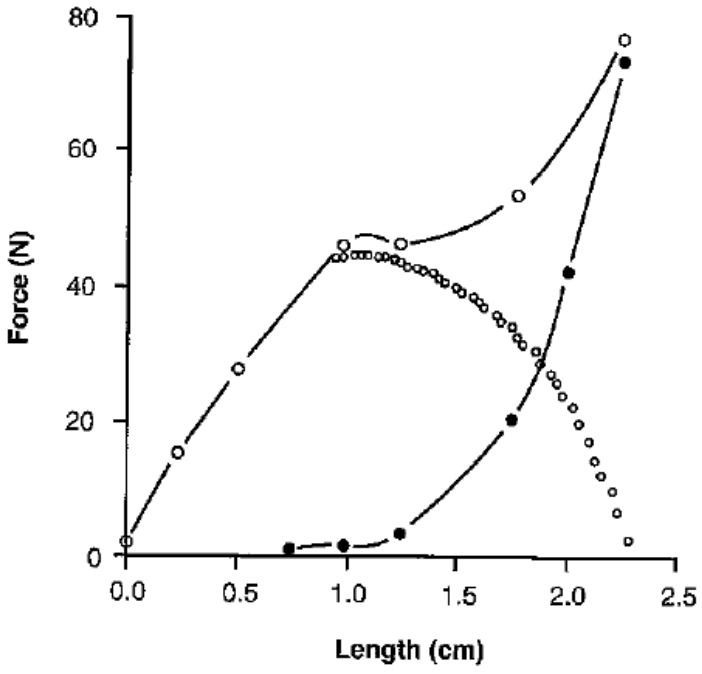

FIGURE 7. Contribution of active (open unconnected circles, parabolic function) and passive (filled circles) elements to the overall (open connected circles) force-length relationship of the whole muscle. (From Enoka, 2008, 235).

The active component (open unconnected circles in fig. 7) represents the force deriving from the contractile elements (MacIntosh, 2003, 169). It has an ascending limb, a plateau and a descending limb. The plateau corresponds to the muscle length at which overlap of the myofilaments within the sarcomeres is optimal (MacIntosh, 2003, 169). Previous to, and beyond the optimal muscle length, the active force decreases, as less number of cross-bridges are formed (non-optimal actin and myosin filaments overlap) (Rassier et al., 1999).

When muscle length is beyond optimal (plateau region) for the active component, albeit the force exerted merely from cross-bridges interaction decreases, the overall force is enhanced due to the contribution of the passive component (filled circles in fig. 7). This portion of the curve is accompanied from the elastic force derived by the elongation of tendinuous and intramuscular connective tissue structures (Enoka, 2008, 235).

Although the force-length relationship generally follows the explained pattern in skeletal muscles, the contribution between active and passive force at a certain point in length might considerably differ between muscles, due to their different operating length in movement (Rassier et al., 1999).

Nevertheless, increased muscle length affects the twitch a muscle can exert, enhancing its response (Rack & Westbury, 1969). This phenomenon is due to contribution to the force output of passive structures (Rack & Westbury, 1969), and partially to geometrical changes at the fibre level, involving actin filaments and myosin heads brought closer from muscle elongation (Endo, 1973).

Overall, force-length properties are influential when evaluating muscle function. Comparability between measurements will benefit from assuring a constant muscle length at each point in time whereas muscle function is evaluated. At the joint level, the stable muscle length can be at best ensured maintaining a fixed joint angle (Rassier et al., 1999)

Force-velocity relationship. The force produced by the muscle-tendon complex depends on the speed of contraction (Enoka, 2008, 236). This concept was introduced from the pioneer work of Hill (1938), whereas the force-velocity relationship was described from isolated muscle preparations. It took several decades to validly confirm and expand the

fundamental properties of the skeletal muscle extracted from this first experiment to human skeletal muscles in vivo (Komi, 2011) (fig. 8).

In concentric contractions the force has been shown to become increasingly higher as the shortening speed is made slower, whereas in eccentric contractions, augmented lengthening velocity corresponds to increased force output (Komi, 2011) (fig. 8). With regard to the power that a muscle can produce, which is the product of the force it exerts and the speed at which it shortens, the peak occurs at about one third of the maximum shortening speed in concentric actions, and it increases as a function of lengthening velocity in eccentric actions (Komi, 2011) (fig.8).

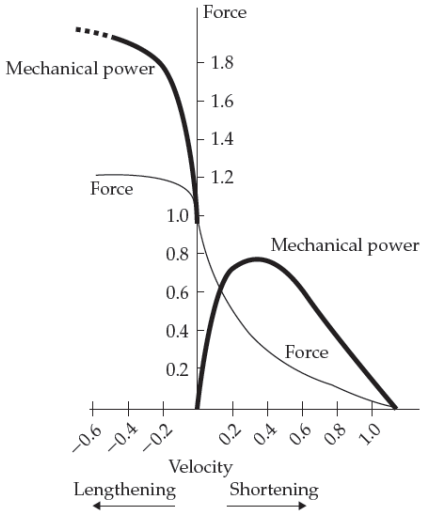

FIGURE 8. Force-velocity and power-velocity curves in concentric (right) and eccentric (left) actions. In concentric contractions, the force becomes increasingly higher as the shortening speed is made slower, whereas in eccentric contractions, augmented lengthening velocity corresponds to increased force output. The peak power is at a velocity of about one third of the maximal shortening speed for concentric actions, whereas it increases in the eccentric phase as a function of lengthening velocity. (From Komi, 2011)

The force output as a function of speed of contraction is dependent on muscle fibre type composition and muscle design (Enoka, 2008, 236). For the aim of the present investigation, two points of the force-velocity relationship are particularly noteworthy to be analyzed, that is, when the maximum shortening velocity, and the maximal isometric force are attained (the two extremes of the force-velocity curve within the concentric

action). Understanding the respective contributing mechanisms, in terms of fibre type composition and biochemical events within the muscle fibres, will help to understand the effects of PAP on muscle performance (see “Benefit from PAP in power performance”).

Maximum muscle shortening velocity is mainly affected from contractile proteins isoforms (i.e. fibre type distribution) and from the number of sarcomeres and muscle fibres in series within the muscle (Schiaffino & Reggiani, 1996; Gordon et al., 2000; Enoka, 2008, 236). The former variable determines the rate of cross-bridges formation (higher with contractile proteins isoforms belonging to type II fibres), whereas an increase in the latter morphological characteristic enhances the amount of muscle shortening per unit of time, resulting in a higher maximal contraction velocity (Schiaffino & Reggiani, 1996; Gordon et al., 2000; Enoka, 2008, 236).

When the speed of shortening is zero, that is, the muscle is maximally and isometrically activated, sarcoplasma calcium ions concentration is almost saturated, and the maximal exerted force depends on the number of active cross-bridges (MacIntosh, 2003, 171; Stull et al, 2011).

Overall, the force-velocity relationship of a muscle is determined from its composition (histochemical properties of fibres), intramuscular milieu, and design. Alterations in one of these characteristics affect the contractile machinery functioning, therefore muscle mechanics. As an instance, RLC-P has been found to increase the contractile response of concentric actions, enhancing the force output for a given shortening speed due to facilitation in cross-bridges interactions, with little or no effects when maximal shortening velocity or isometric force is required (Sale, 2002). This phenomenon will be later illustrated (see “Benefit from PAP in power performance”)

2.2 The efferent pathway of the PNS

2.2.1 Motor neuron

The motor neuron is a part of the PNS and represents the “final common pathway” by which the commands from the CNS are sent to the muscle (Enoka, 2008, 215). The cell body of the motor neuron is located in the anterior horn of the spinal cord (fig.9). The

axon of each motor neuron exits the spinal cord through the ventral root and runs in a peripheral nerve to the muscle. When the axon reaches the muscle, it branches and innervates from a few to several thousand muscle fibres, which ensemble is known as motor unit (MU) (fig. 9) (Enoka & Pearson, 2013, 768).

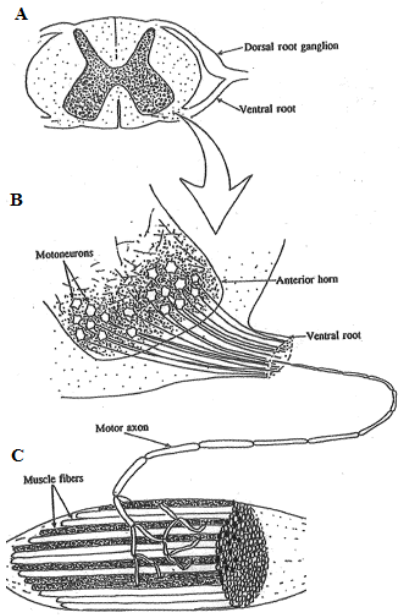

FIGURE 9. The anatomy of a motor unit. A) Cross section of the spinal cord, representing grey and white matters, dorsal and ventral roots. B) Enlargement of the anterior horn with the cell bodies of the motor neurons, each one sending its axon to the muscle underneath. C) Muscle fibres supplied by the branches of a single axon (darkened). This population of fibres, together with the motor neuron (cell body and motor axon), constitutes a single motor unit. (From MacIntosh, 2003, 157).

2.2.2 Motor unit

The MU (a motor neuron and the muscle fibres it innervates, fig.9) is the basic functional unit by which the nervous system controls muscle force, therefore human movement (Enoka & Pearson, 2013, 768). Most skeletal muscles comprise a few hundreds of MUs, from about 10 to 1500, respectively, for small and large muscles (Enoka, 2008, 215). Moreover, the number of muscle fibres within a MU (i.e. innervated by a motor neuron) varies from a few to several thousands, depending on the muscle (Enoka & Pearson, 2013, 770).

2.2.3 Twitch and tetanus as measures of a MU contractile properties

The properties of a MU are referred to as contraction speed, maximal force and fatigability. They can be assessed by examining the force exerted by the MU in response to a single action potential, namely the twitch contraction, or to a series of superimposed twitches, known as tetanus (fig.10) (Enoka & Pearson, 2013, 770).

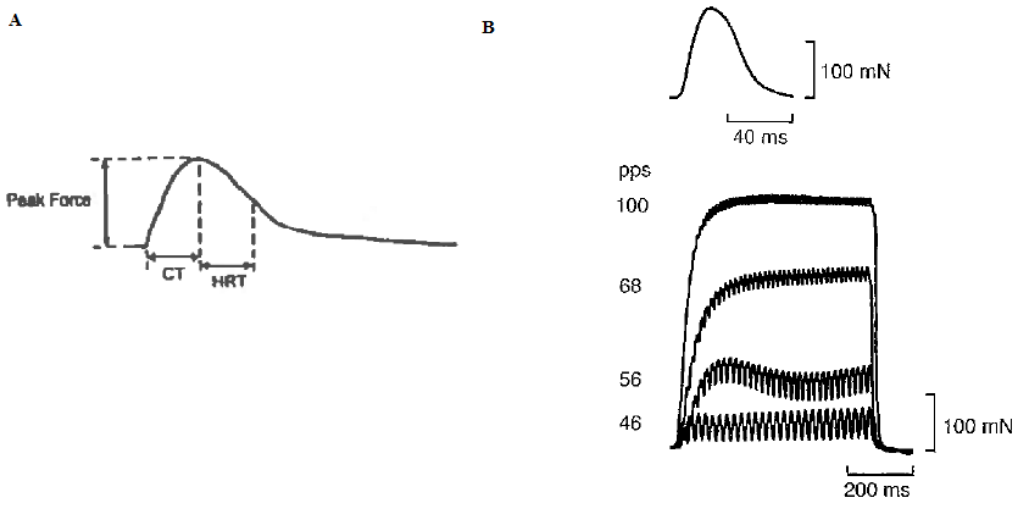

FIGURE 10. A) Twitch response of a MU. CT, contraction time; HRT, half-relaxation time. B) Twitch (at the top) and tetanic forces (at the bottom) for a MU. Four tetani with different rate of stimulations are shown. At 100 pulses per second (pps) stimulation rate, the MU exhibited a fused tetanus (adapted from Enoka, 2008, 220).

The time a twitch takes to reach its maximal force value (peak force) is represented from the contraction time (CT) (fig. 10A). In addition, the twitch response is characterized from the half-relaxation time (HRT) (fig. 10A), i.e. the time taken by the force to decline to one-half of its peak value. CT and HRT are used as measures of contractile speed and relaxation of the muscle fibres included in the MU and they mainly depend respectively from the rate of Ca^{2+} release and its reuptake in the SR. Additionally, sensitivity of the contractile machinery to calcium ions plays a role in their magnitude (Enoka, 2008, 220).

The tetanic contraction (fig. 10B) is characterized from the peak force, and the rate at which the peak force decreases over time (Enoka, 2008, 221), the latter estimating the fatigability of the MU. The peak force depends on the extent to which the twitches overlap and summate (rate of stimulation) (Enoka & Pearson, 2013, 771).

When assessing the maximal force of a MU, fused tetani (fig. 10B) are preferred to single twitches because they better reflect the nature of a MU activation (Enoka, 2008, 220). During a muscle contraction, MUs are rarely activated to produce individual twitches. Indeed, they receive a number of action potentials that result in overlapping twitches, which summate to produce the characteristic force profile of a tetanus (Enoka, 2008, 220).

2.2.4 MU and fibre type classification

Three types of MUs have been described, namely, slow contracting-fatigue resistant (S), fast contracting- fatigue resistant (FR), fast contracting –fast to fatigue (FF) (MacIntosh, 2003, 190), relating to their contractile properties (table 1; fig. 11). However, even though contractile properties tend to be associated within a MU, there are considerable overlaps between different MUs in twitch speed and force (MacIntosh, 2003, 190). The contractile properties of a MU depend on both the features of the motor neuron and the muscle fibres it innervates (fig. 11).

Several classification schemes, based on histochemical, biochemical, and molecular properties, have been used to identify different properties of the muscle fibres. One commonly used approach is to assay for the enzyme ATPase activity, index of shortening velocity of the muscle fibre (Enoka, 2008, 222). Type I fibres are referred as slow twitch fibre, and type II as fast-twitch fibres. Type II fibres can be further divided in type IIa and type IIb, reporting the type IIb fibres the fastest twitch (Enoka, 2008, 223). Referring to the histochemical and molecular properties, differences between type I and type II fibres involve the isoforms of myosin heavy chains, TM, Tn, ELC, RLC (Schiaffino & Reggiani, 1996; Gordon et al., 2000). Type II fibres exhibit a higher degree of RLC-P than type I fibres (Moore & Stull, 1984; Grange et al., 1993; Sweeney et al., 1993; Stull et al., 2011). Physiological differences between muscle fibres are strongly associated with variation in contractile properties between MUs (Table 1), so

that type I, IIa and IIb fibres generally belong respectively to type S, FR and FF MUs (MacIntosh, 2003, 191).

Numerous features of the motor neurons themselves are correlated with the muscle fibres they innervate, and respectively, with the MUs they belong (MacIntosh, 2003, 192). Motor neurons of type S MUs have smaller diameter, lower conduction velocity and innervate smaller number of muscle fibres per MU than motor neurons of type FR and FF MUs (fig. 11A) (MacIntosh, 2003, 192). Besides these characteristics being influential on the contractile properties of the MUs, they are determinants and discriminants in the recruitment of the MUs (see "MUs activation") (MacIntosh et al, 2006, p. 199).

TABLE 1. Motor unit and fibre type classification with respective contractile properties. FF, fast contracting -fast to fatigue; FR, fast contracting –fatigue resistant; S, slow contracting – fatigue resistant. Fibre types belonging to the respective MUs are reported in brackets. (Adapted from MacIntosh et al., 2006, 191)

Property	**Motor unit type**		
	S	**FR**	**FF**
	(I)	**(IIa)**	**(IIb)**
Twitch speed	Slow	Fast	Fast
Tetanic force	Small	Intermediate	Large
Fatigability	Low	Low	High

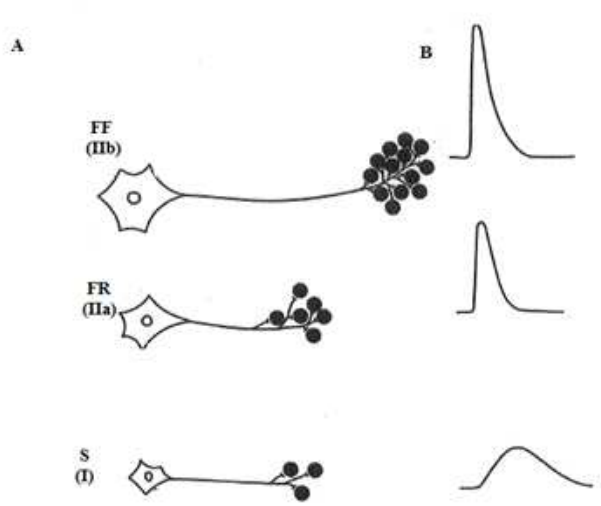

FIGURE 11. Summary of the MU properties. MU types: FF, fast contracting - fast to fatigue; FR, fast contracting - fatigue resistant; S, slow contracting - fatigue resistant. The fibre type belonging to the MUs is reported in brackets. A) Organization characteristics of MUs. B) Respective twitch responses. Note the increasingly higher number of muscle fibres innervated by the motor neurons from MUs type S to FF, and the corresponding increasing peak force of twitch responses. (Adapted from MacIntosh et al., 2006, 190)

2.2.5 Twitch as a measure of a muscle group contractile properties

Twitches have not exclusively been used for the assessment of individual MUs. Enlarging the focus at the muscular level, twitches can be evoked for the evaluation of a muscle group contractile properties (Pääsuke et al., 1999; Millet et al., 2011).

When a percutaneous stimulus is applied over the nerve trunk innervating a muscle group, motor axons are excited, resulting in an action potential within the respective innervated muscle fibres. If the stimulation is applied by a supramaximal current intensity (i.e. all the motor axons within the nerve trunk are recruited, see “H-reflexes” in “Measuring spinal excitability”), an action potential is evoked in all the MUs of the innervated muscles, being therefore fully activated. The resulting force exerted by the muscle group is known as supramaximal twitch, which represents its contractile properties (Pääsuke et al., 1999).

Likewise MUs contractile properties assessment, muscle supramaximal twitches are characterized from peak force, CT and HRT (Pääsuke et al., 1999; Hamada et al., 2000). Variations in these parameters have been widely used to detect modifications within the fibres of the stimulated muscles (Hamada et al., 2000; Baudry and Duchateau, 2004; Fukutani et al., 2014). For instance, changes in supramaximal muscle twitches peak force have been used to detect whether maximal voluntary contractions cause a subsequent potentiating effect on muscles contracting machinery (Hodgson et al., 2005) (see "Quantification and underlying mechanism of PAP").

2.2.6 M-wave as a measure of neuromuscular propagation integrity

From muscle twitches, the integrity of muscle neuromuscular propagation can be also estimated (Fuglevand et al., 1993). For this purpose, surface electromyography (EMG) is used, obtaining electromyogram responses called maximal M-wave (Mmax; Enoka, 2008, 258). Comparisons between Mmax amplitudes allow inferring whether changes in neuromuscular propagation has occurred (Fuglevand et al., 1993)

If surface EMG recording electrodes are placed over a muscle belonging to the stimulated muscular group, the sum of the electrical activity of the respective muscle fibres beneath the electrodes can be detected (i.e. electromyogram) (Enoka & Pearson, 2013, 769). When the stimulus evoking the supramaximal twitch is released, an action potential is generated in all the motor axons within the nerve trunk, propagated toward the NMJ and the muscle fibres membrane. The surface EMG response of this neuromuscular propagation is called Mmax (fig. 12), because it represents the simultaneous electrical activity of all the muscle fibres within the muscle (the entire muscle motor neuron pool is activated) (Enoka, 2008, 258). One of the most common used methods to quantify Mmax magnitude is the peak-to-peak amplitude (Zehr et al., 2002), namely, the difference between the highest and the lowest peak of the Mmax electromyogram trace.

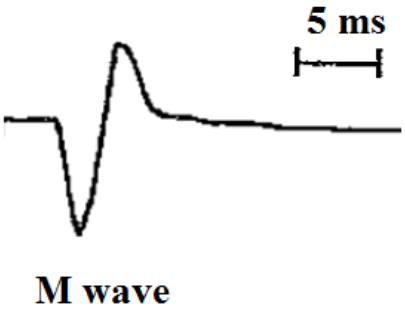

FIGURE 12. Corresponding surface EMG muscle response to a supramaximal twitch (maximal M wave). The peak-to-peak amplitude is characterized from the difference between the highest and the lowest peak of the EMG trace (Adapted from Enoka, 2008, 258).

The Mmax has been widely used to probe the integrity of the pathway between the site of the stimulus (motor nerve) and the recording site (muscle fibre membrane). That is, Mmax tests the integrity of the neuromuscular propagation (transduction of the action potentials) (Enoka, 2008, 258; Fuglevand et al., 1993). Due to the chief involvement of NMJ and muscle fibres' membrane in neuromuscular propagation, changes in Mmax peak-to-peak amplitude mainly represent alterations in either the failure of the neuromuscular transmission (at the neuromuscular junction level), or the amplitude of the muscle fibres action potentials, or both (Fuglevand et al., 1993).

2.2.7 MUs activation

The CNS controls muscles force by varying both the number of activated MUs (MU recruitment) and their activation frequency (rate coding) (Enoka, 2008, 223).

MU recruitment. MUs are always recruited in order of increasing size, as revealed from the pioneer work of Henneman et al. (1965). Since this principle was proposed, named "the size principle", which was based upon results from cat motor neurons, several studies have presented strong evidence of the observance of this pattern of MUs recruitment in human muscle action (Desmedt & Godaux, 1978; Feiereisen et al., 1997; Ivanova et al., 1997).

During the execution of a voluntary movement, motor neurons receive inputs from spinal and supra-spinal centres. For a similar amount of current input (i.e. post-synaptic

current), the change in voltage (variation in membrane potential) that can be expected from small motor neurons is higher than from larger ones due to the higher input resistance, mainly caused from their lower cross-sectional areas (Henneman et al., 1965). Thus, action potential thresholds (resulting from changes in membrane potential) are easier to be evoked in smaller motor neurons, namely, smaller motor neurons have lower recruitment thresholds. MUs are hence recruited following the size principle, that is, fast-twitch MUs recruitment is preceded from slow-twitch MUs one (Henneman et al., 1965).

The recruitment order of MUs, although some variability for MUs with similar recruitment thresholds (Feiereisen et al., 1997), is respected for isometric and dynamic voluntary contractions (Duchateau and Enoka, 2008), and during rapid isometric or shortening actions (Desmedt & Godaux, 1978; Ivanova et al., 1997). Nevertheless, in rapid contractions, the recruitment threshold of MUs is reduced, allowing their earlier activation, facilitating the performance of fast movements (Desmedt & Godaux, 1978).

MUs rate coding. MUs can increase their exerted force through increases in discharge rate (Van Cutsem et al., 1997). The minimal discharge rate is lower for small, compared to large, MUs (Van Cutsem et al., 1997; Enoka, 2008, 225). Lastly, MUs peak discharge rates achieved during rapid (isometric or dynamic) contractions are much greater than those recorded during slow contractions (Ivanova et al., 1997; Van Cutsem & Duchateau, 2005).

Overall, the organization of the PNS in MUs allows transmitting action potentials to muscle fibres efficiently and regulating muscle force through modulation of MUs recruitment and rate coding (Enoka, 2008, 223). Changes in the responses to percutaneous supramaximal stimulation (twitch and Mmax) can be used to detect modification in the neuromuscular propagation integrity of a muscle, or at the level of the contractile machinery in a muscle group (Fuglevand et al., 1993; Pääsuke et al., 1999).

2.3 CNS

2.3.1 Structure of the CNS

As mentioned earlier, the CNS consists of four main parts, namely, the brain, the brain stem, the cerebellum, and the spinal cord (fig. 13) (Enoka, 2008, 288). In this complex structure, brain stem, cerebellum and spinal cord mediate reflexes and automatic behaviours, whereas cortical motor centres initiate and control voluntary actions (Enoka, 2008, 288), mainly through the brain primary motor cortex. To do so, the latter projects neural pathways to the spinal cord directed to synapse with contra lateral spinal motor neurons (Avela & Gruber, 2011, 118). Their ensemble is known as corticospinal pathways (Enoka, 2008, 289).

Once spinal motor neurons are excited, they discharge to the muscles, allowing their contraction, and movement. Therefore, from a simplified standpoint, the motor output depends not exclusively on the activity of the neurons located in the motor cortex (i.e. whereas corticospinal pathways descend from), but also on the excitability of the spinal motor neuron pool (Gandevia et al., 1999). This hierarchical organization of the motor system is represented in figure 14.

Corticospinal pathways for motor neurons innervation of a certain area of the body have a defined origin, due to the somatotropic organization of the primary motor cortex (Enoka, 2008, 209). Over the latter, following this structure, each body part is represented from a specific region. This motor representation is orderly arranged in an inverted fashion, i.e. the top of the cerebral hemisphere contains the leg and foot, whereas the bottom has the face, tongue and mouth representation (fig. 15) (Enoka, 2008, 289).

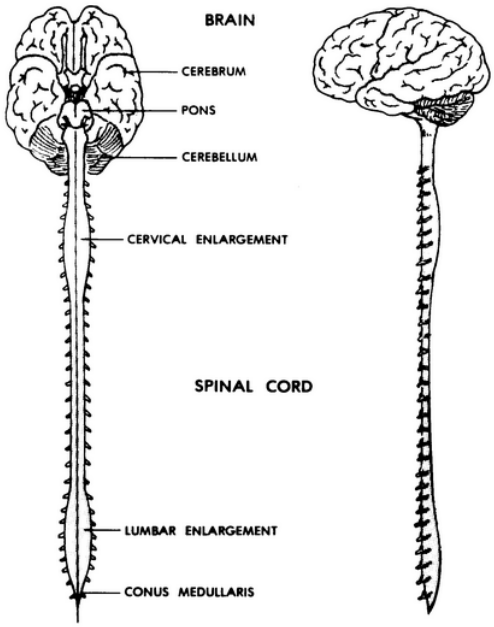

FIGURE 13. Structure of the CNS. The brain stem is composed from mesencephalon (or midbrain), pons, and medulla, the former and the latter placed respectively above and below the pons and not shown in this picture. The spinal cord extends from the medulla (just below the pons) to the conus medullaris. (From http://www.medtrng.com/anatomy%20lesson/bhp13.htm)

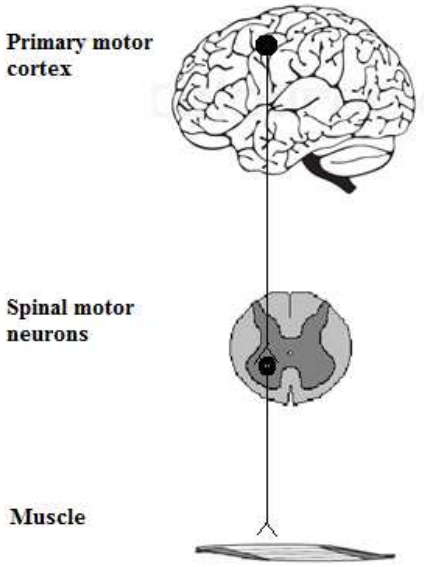

FIGURE 14. Schematic and simplified hierarchical structure of the motor pathways originated from the primary motor cortex. The motor output depends on both the activity of the neurons located in the motor cortex and the excitability of the spinal motor neuron pool (adapted from Avela & Gruber, 2011, 119; Petersen et al., 2003)

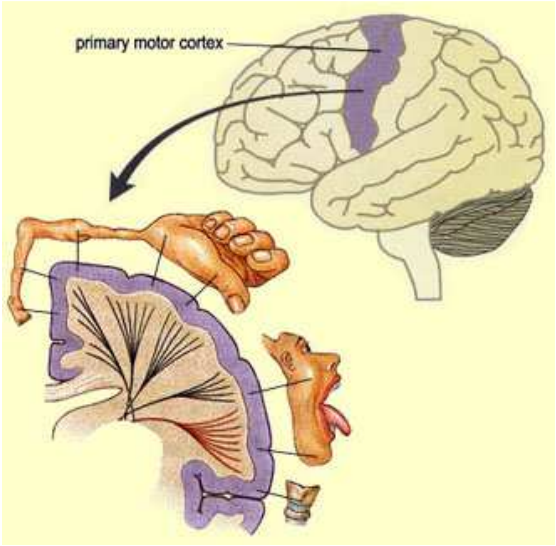

FIGURE 15. The primary motor cortex and its somatotropic organization. The top (medial) part contains the leg and foot, whereas the bottom (lateral part) has the face, tongue and mouth representation. (From http://editthis.info/psy3242/Primary_motor_cortex).

2.3.2 Measuring corticospinal pathways excitability: transcranial magnetic stimulation

Transcranial magnetic stimulation (TMS) refers to the activation of the corticospinal pathways by a magnetic field applied over the motor cortex (Avela & Gruber, 2011). Since Barker et al. (1985) discovered and introduced this technique, it has been widely used in human subjects (Taylor & Gandevia, 2001).

The principle behind the functioning of TMS resides in a rapid current pulse change within the coil placed over the motor cortex, that leads to the onset of a magnetic field. The rate of the emerging magnetic field induces an electric current through the scalp, depolarizing cortical neurons membrane (Avela & Gruber, 2011).

Corticospinal pathways are indirectly stimulated by TMS (Di Lazzaro et al., 2001) (fig. 16). Being the electric current evoked from TMS rather superficial, it does not result in direct activation of the corticospinal axons (Avela & Gruber, 2011). That is, corticospinal pathways are mainly activated from corticocortical neurons (Di Lazzaro et al., 2001; Avela & Gruber, 2011). Therefore, the stimulation output have an intra-

cortical component, besides a considerable impact of motor neurons excitability (McNeil et al., 2013).

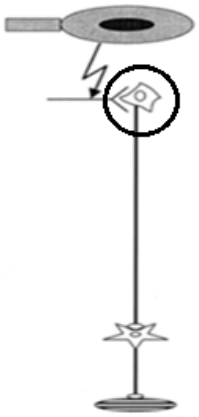

FIGURE 16. Stimulation of corticospinal pathways through TMS. The soma within the circle represents the cell body of one pyramidal neuron, within the primary motor cortex. Note that, in TMS, corticospinal pathways are activated indirectly by corticocortical neurons (adapted from Avela & Gruber, 2011)

Due to the previously mentioned somatotropic organization of the primary motor cortex, corticospinal pathways directing to a motor neuron pool of a specific body area can be stimulated (Avela & Gruber, 2011). If surface electrodes are place on the skin over the muscle included in the stimulated area, electromyogram responses can be recorded. These responses are referred to as motor-evoked potentials (MEPs), and they are used as indication of the global excitability of the corticospinal pathway (Avela & Gruber, 2011).

Despite the complex nature of the corticospinal volleys generated by TMS, they are deemed to produce an orderly recruitment similar to that found for voluntary activation, following the size principle (Bawa & Lemon, 1993).

As previously mentioned, the motor output is considerably influenced from the excitability of the spinal motor neuron pool (McNeil et al., 2013). That is, MEPs represent the overall responsiveness of cortical neurons and spinal motor neurons (Avela & Gruber, 2011). Therefore, for distinguishing between cortical and spinal

influence on corticospinal excitability, measurements of spinal segmental excitability have been performed and compared with MEPs (Gandevia et al., 1999; McNeil et al., 2013). The next part will briefly describe the techniques used to test spinal excitability in humans, focusing on the H-reflex technique, which was used in the present investigation.

2.3.3 Measuring spinal excitability

From a functional perspective, spinal excitability has a primary role in muscle contraction (McNeil et al., 2013). Two main pathways converge at the spinal segmental level, namely the corticospinal and spinal reflex pathways (see fig. 1 for a schematic representation), both extensively regulating human movement (Gollhofer, 2003, 331; McNeil et al., 2013). As both tracts project to motor neurons, the potentials evoked from direct current stimulation of these pathways have been used as a measure of motor neurons pool excitability. Having access to the evaluation of motor neurons excitability is a great advantage in neural studies. For instance, a higher excitability of the motor neuron pool might bring to a greater number of recruited MUs, or to discharge at higher frequencies, in muscle contractions (McNeil et al, 2013). Three methods have been mainly used for testing spinal excitability, and for comparison with MEPs evoked by TMS to infer of central or spinal adaptations, namely, transcranial electrical stimulation (TES), cervicomedullary stimulation (CMS), and Hoffmann reflexes (H-reflexes) (Avela & Gruber, 2011; McNeil et al., 2013). The functioning of these methodologies is briefly explained below, with their main advantages and drawbacks in comparing the resulting stimulation output with corticospinal excitability measurement.

TES and CMS. Both TES and CMS directly activate corticospinal descending pathways (Rothwell et al., 1994; Ugawa et al., 1991). TES consists in electrical stimuli applied to the motor cortex (Rothwell et al., 1994), whereas CMS is applied between the mastoids, at the level of the brainstem (pyramidal decussation, medulla) (Ugawa et al., 1991).

Comparisons of MEPs evoked by TES and CMS, to those from TMS, allow inferring whether changes occurred mainly at the cortical or spinal level (Gandevia et al., 1999; Avela & Gruber, 2011). The reasons behind this use are mainly two. Firstly, TES and CMS output is not affected from corticocortical mechanisms as in TMS (Avela &

Gruber, 2011). Secondly, corticospinal pathways appear to lack presynaptic inhibition (Nielsen & Petersen 1994), and thus TES and CMS responses are likely to reflect changes in the corticospinal axonal path and motor neurons (Gandevia et al., 1999).

Despite the aforementioned advantages, TES and CMS induce a considerable discomfort in the subjects. Moreover, CMS might not allow to record responses of sufficient size in some muscles (McNeil et al., 2013).

H-reflexes. This technique is based upon reflex responses evoked from the stimulation of a peripheral nerve. H-reflexes were termed so because firstly described in the soleus muscle by Hoffmann (1918). The H-reflex reflects the response of the motor neurons pool to a volley from muscle spindle afferents (McNeil et al., 2013) (fig. 17), involving therefore the spinal reflex pathway.

Likewise Mmax, which results from a muscle twitch, H-reflexes are recorded from surface EMG by placing the recording electrodes on the skin over the muscle of interest. The electromyographic outputs are known as H-waves. H-reflexes are quantified through the peak-to-peak amplitude of the respective evoked H-waves (fig. 18) (Zehr et al., 2002).

When a series of progressively stronger stimuli are applied over the nerve trunk innervating a muscle group, the Ia afferents that innervate muscle spindles sensory receptors, because of their large diameter, will be excited (Zehr, 2002). The synaptic Ia input will recruit motor neurons in an orderly fashion, following the size principle (Buchthal & Schmalbruch, 1970). This mechanism results in a recorded H-wave deriving from the membrane action potential of the recruited muscle fibres within the muscle of interest.

The H-reflex is recorded if stimulation intensity is above the threshold for activation of Ia afferents (Zehr, 2002). Once the threshold has been reached, the higher the stimulation intensity, the greater the sensory volley, and the higher the number of MUs recruited through the reflex loop (Zehr, 2002). The H-wave amplitude response increases as a consequence of the latter factors, reaching the highest value known as Hmax (Zehr, 2002) (Fig. 19A).

However, increases of stimulation intensity also cause direct depolarization in the motor neurons, resulting in an additional, earlier than the H-wave, onset of muscle fibres

action potentials, referred to as M-wave (Zehr, 2002)(Fig. 19B). Further increases in stimulus intensities contribute to the growing M-wave, due to the higher number of activated motor axons, and to decreases in H-waves amplitudes, owing to the consequent raising antidromic volley (McNeil et al., 2013) (Fig. 19A). There is a stimulus intensity at which all the motor axons are directly activated, and the antidromic volley collides and overcome MUs action potentials evoked through the reflex loop (Zehr, 2002). The resulting M-wave has the maximum value due to the full activation of the muscle. This stimulation intensity is known as “maximal” and the M-wave is referred to as Mmax (Zehr, 2002) (Fig. 19A). Current intensities above this value and the evoked mechanical responses are referred to as respectively “supramaximal”, and “supramaximal muscle twitches” (see “Twitch as a measure of a muscle group contractile properties”).

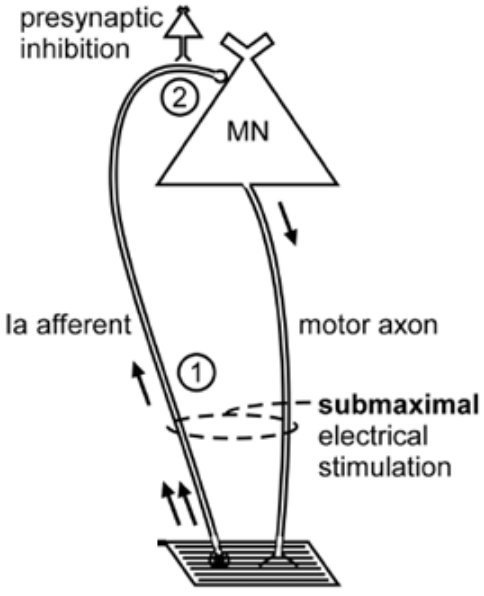

FIGURE 17. Schematic representation of the volley and pathway involved in the production of the H-reflex. 1) The sensory volley is evoked from the submaximal electrical stimulus over the nerve trunk, which recruits MUs within the motor neurons pool according to the size principle. 2) Presynaptic inhibition can influence afferent input to the motor neurons (MN). (Adapted from McNeil et al., 2013).

FIGURE 18. Corresponding surface EMG muscle response to an evoked H-reflex (H wave). The peak-to-peak amplitude is characterized from the difference between the highest and the lowest peak of the EMG trace (Adapted from Enoka, 2008, 257).

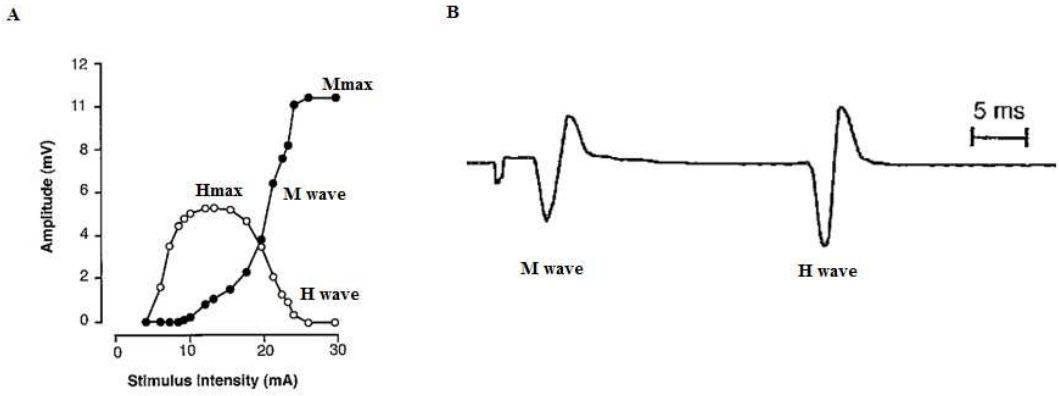

FIGURE 19. A) Example of recruitment curve of H-reflexes. Note the variations of the H-wave and M-wave with increasing stimulus intensities. B) EMG response to a stimulation intensity evoking both M-wave and H-wave. (Adapted from Enoka, 2008, 258).

Direct comparisons of the H-reflex evoked potentials with MEPs by TMS to differentiate between cortical and spinal (motor neuronal) mechanisms is improper (Petersen et al., 2003). Although H-reflexes converge to motor neurons, the evoked volley crosses a different pathway and synapse than in the corticospinal tract, respectively the spinal reflex pathway and the Ia-α motor neuron synapse. This makes the evoked volley susceptible to a number of mechanisms which alter the size of H-

reflexes independently of motor neurons, thus adding uncertainty in interpreting responses as indicators of motor neurons pool excitability. The major mechanism contributing to this incongruity is presynaptic inhibition of Ia terminals, which extensively controls and fine-tunes the afferent signal to motor neurons (Zehr et al., 2002; McNeil et al.,2013) (fig.17). Indeed, for the same peripheral stimulus intensity and motor neurons pool excitability, an increase and decrease in presynaptic inhibiton lead respectively to suppressed and enhanced recorded H-wave, without real changes at the motor neurons pool level (Zehr, 2002). Other factors, to be considered when interpreting H-reflexes after previous activation and which curtail their outcome, are postactivation (homosynaptic) depression (i.e. decrease in transmitter release from the Ia afferent terminals due to ongoing or previous repetitive activation of the Ia - motor neuron synapse), and axonal hyperpolarization of Ia afferents (Hultborn & Nielsen, 1998; Pierrot-Deseilligny & Burke, 2012, 85). Finally, contribution of oligosynaptic pathways to the reflex response is another mechanism that can selectively influence the size of the H-reflex regardless of changes in motor neurons excitability (Pierrot-Deseilligny & Burke, 2012, 11). In light of these considerations, it seems reasonable that H-reflexes should not be used to infer changes in excitability at the motor neuronal level, and especially they should not be compared to MEPs evoked by TMS for discriminate between changes at the spinal or supraspinal compartment. If caution is taken to control for constant experimental conditions, H-reflexes represent a valid mean for measuring the efficacy of the spinal reflex pathway, which has a considerable contribution in force development in movements (Gollhofer, 2003, 339).

Overall, the CNS controls the motor output through complex interactions. Voluntary actions are extensively controlled through descending corticospinal pathways from the primary motor cortex. Presenting the latter a somatotropic organization, corticospinal pathways excitability of the neural circuitry directed to a specific body area can be detected through TMS. Due to the dependence of the resulting MEPs on the excitability of the motor neurons pool, attempts have been made to distinguish between cortical and spinal mechanisms or site of adaptations using converging stimulation techniques, namely TES, CMS, H-reflexes, each of these having advantages and drawbacks. As reported, albeit the volley evoked with H-reflexes involves the motor neurons, several factors at the Ia-α motor neuron presynaptic level affect the resulting evoked potentials.

Thus, H-reflexes should be interpreted as changes in the efficacy of the spinal reflex pathway rather than altered motor neurons excitability.

3 POST-ACTIVATION POTENTIATION

The performance of a skeletal muscle is affected by its contractile history (Sale, 2002). The most obvious example is neuromuscular fatigue, which can be defined as any exercise-induced decrease in maximal voluntary force or power produced by a muscle or muscle group (Taylor & Gandevia, 2008). Conversely to this impairment of muscular function, studies have supported the hypothesis that contractile history may also facilitate the production of force (Vandervoort et al., 1983; Güllich & Schmidtbleicher, 1996; Baudry & Duchateau, 2006). This phenomenon has been named post-activation potentiation (PAP) (Sale, 2002). PAP is induced from a voluntary contraction performed at a maximal or near-maximal intensity (Fukutani et al., 2014), named conditioning contraction (CC) (Sale, 2002). The results are subsequent enhanced muscle contractile properties, and power performances through increased RFD (Sale, 2002).

The next sections will examine the aforementioned phenomenon and the resulting improvements in power performances. Firstly, the physiological mechanism underlying PAP, and the prevalent measure for its quantification will be described. Secondly, the dependency of PAP on CC parameters (i.e. type, duration and intensity) and target muscle fibre composition will be depicted. In the last two sections, the reasoning behind increases in RFD and power output of rapid actions concurrently with PAP, and indications from previous studies on possible neural contribution will be illustrated.

3.1 Quantification and underlying mechanism of PAP

The supramaximal muscle twitch represents the prevalently used method for quantification of PAP (Hodgson et al., 2005). In presence of the latter, the twitch contraction shows an increased peak force and RFD (Sale, 2002) (fig. 20A). To date, RLC-P has been proposed as the main responsible mechanism of PAP (Hamada et al., 2000a; Sale, 2002; Baudry & Duchateau, 2004; Stull et al., 2011).

The force and RFD of a twitch contraction are enhanced following a sustained maximal or near-maximal voluntary contraction, an evoked tetanic contraction, or during sequential twitch contractions (Hodgson et al., 2005). The former event refers to PAP

(Sale, 2002), while the second and third ones refer respectively to post-tetanic potentiation (PTP), and staircase (MacIntosh, 2003, 158).

In PAP, twitch potentiation might last up to 9-15 minutes after the conditioning contraction (Baudry & Duchateau, 2004). Muscle twitches are usually evoked at regular intervals during the time course of PAP to evaluate its magnitude and decay (fig. 20B). Twitch potentiation is measured expressing the absolute peak force and RFD changes values as percentages of a non-potentiated (control) twitch (% change from pre-CC value) (fig. 20B) (Hamada et al., 2000a). The control twitch is evoked and recorded before CC and used as a reference value. The extent of twitch peak force and RFD potentiation is used to describe and quantify PAP (Hodgson et al., 2005).

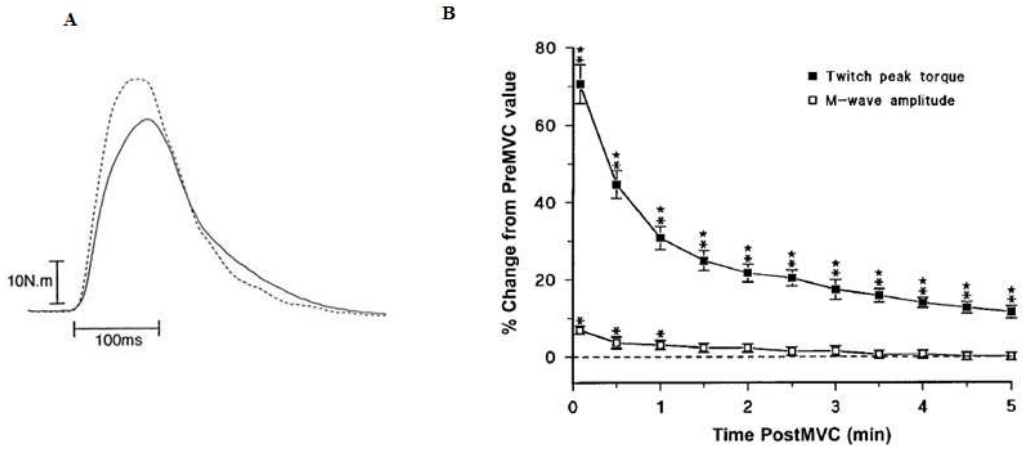

FIGURE 20. Example of potentiated twitches in knee extensors after a 10-sec maximal voluntary isometric contraction (MVIC), obtained from stimulation of the femoral nerve. A) Pre- (solid line) and immediate (5 s) post-MVIC (dashed line) twitch recording from one subject. Note the potentiated peak torque and rate of torque development of the post-MVIC twitch, relative to the pre-MVIC ones. B) Potentiated twitches peak torques and respective M-waves, recorded from the vastus medialis. Values are expressed as % changes from pre-MVIC values. * and ** indicate significant differences with pre-MVIC values. (Adapted from Hamada et al., 2000a)

The conclusion of RLC-P as principal cause of PAP comes from direct and indirect evidences in animals and humans muscles (Moore & Stull, 1984; Stuart et al., 1988; Hamada et al., 2000a).

Moore & Stull (1984) directly investigated the effect of RLC-P on twitch potentiation. Although the experiment was conducted on animal muscles (rats gastrocnemius) and CC was a 10-second electrically evoked tetanus, subsequent twitches peak torque potentiation was strongly correlated to the extent of RLC-P (fig. 21). In addition, four years later (Stuart et al., 1988), the relation between RLC-P and twitch peak torque potentiation was almost confirmed directly in humans (with a high but non-significant correlation between the two variables) using a 10-second maximal voluntary isometric contraction (MVIC) involving knee extensors.

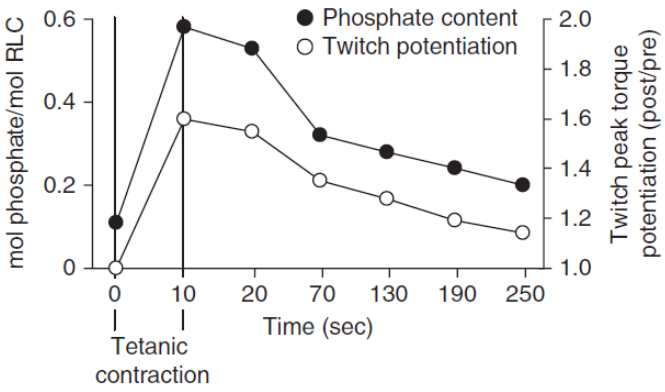

FIGURE 21. The time course of RLC-P and twitch peak torque potentiation, following a 10-second tetanic contraction. Twitch potentiation is represented as the ratio of its peak torque value on a specific time after the tetanic contraction, to its pre-tetanus value. These results indicate a strong correlation between RLC-P and twitch tension potentiation. (From Moore & Stull, 1984).

Indirect evidence on human subjects also confirmed the dependence between RLC-P and PAP (Hamada et al., 2000a). Hamada et al. (2000a) used a 10-second MVIC of knee extensors to induce twitch potentiation, and took from the sample size the four subjects with the highest and lowest PAP. Following vastus lateralis biopses, the authors reported a strong correlation between type II muscle fibres and PAP, having the

group with the highest PAP the greatest amount of fast-twitch fibres. Due to the higher degree of RLC-P exhibited from type II fibres (see “MU and fibre type classification”) (Grange et al., 1993; Sweeney et al., 1993; Stull et al., 2011), the results of Hamada et al (2000a), albeit indirectly, supported the dependency of PAP on RLC-P.

Lastly, the hypothesis of RLC-P as the main responsible mechanism of PAP is also supported from a theoretical standpoint (Grange et al., 1993; Sweeney et al., 1993; Vandenboom et al., 1995; Stull et al., 2011). As previously mentioned (see “Role of the RLCs in muscle contraction”), RLC-P allows both structural and biochemical changes within the contractile proteins (Stull et al., 2011). Structurally, it facilitates cross-bridges interactions moving the myosin heads toward the thin filaments, whereas chemically, it increases sensitivity of the TM-Tn regulatory complex to Ca^{2+} (Grange et al., 1993; Sweeney et al., 1993; Stull et al., 2011). When a twitch is evoked through supramaximal nerve trunk stimulation, the action potential travels from the stimulated motor axons to muscle fibres SR, the latter releasing Ca^{2+}. If RLCs are phosphorylated, for the same amount of released Ca^{2+}, a greater number of cross bridges will be activated, and faster, due to the increased sensitivity to calcium ions, enhancing twitch RFD and peak force (Grange et al., 1993; Sweeney et al., 1993; Vandenboom et al., 1995; Stull et al., 2011).

3.2 CC duration and PAP decay trend

The duration of CC can affect PAP magnitude. In addition, for a CC (MVIC) length between 5 and 10 seconds (i.e. optimal duration) (Vandervoort et al., 1983; Rassier, 2000), PAP is characterized from a typical exponential decay trend over time (Vandervoort et al., 1983; Sale, 2002; Hodgson, 2005; Tillin & Bishop, 2009).

Several CC durations have been used in different studies (Tillin & Bishop, 2009). However, as a general rule, the length of the MVIC for inducing maximal PAP should be between 5 and 10 seconds (Vandervoort et al., 1983; Rassier, 2000). The reason underlying this principle is PAP as the net result of a coexistence between potentiation and fatigue resulting from CC (Rassier & MacIntosh, 2000). While RLC-P increases the sensitivity of the contractile proteins to Ca^{2+} after voluntary contractions, calcium ions release is depressed during the recovery from fatigue (Rassier & MacIntosh, 2000).

These mechanisms have opposing effects and coexist during PAP. Hence, CC (MVIC) duration between 5 and 10 seconds have been suggested to allow the maximum PAP and lower fatigue to occur (Vandervoort et al., 1983; Rassier, 2000).

As cited previously (see “Quantification and underlying mechanism of PAP”), the effect of PAP might last up to 9-15 minutes post-CC (Baudry & Duchateau, 2004). The trend of the twitch potentiation is characterized from its peak immediately after CC. Thereafter, both peak force and RFD decline exponentially, returning to control values within 9-15 minutes (Baudry & Duchateau, 2004; Tillin & Bishop, 2009) (see the trend of the twitch peak torque potentiation curve in fig. 20B).

3.3 Other factors affecting the extent of PAP

PAP can be influenced by a combination of factors (Tillin & Bishop, 2009). These include mainly CC type (Baudry & Duchateau, 2004; Jubeau et al., 2010; Miyamoto et al., 2011), intensity (Miyamoto et al., 2011; Fukutani et al., 2014), and fibre type distribution of the muscles involved in CC (Moore and Stull, 1984; Hamada et al., 2000a).

CC type. Different CC types exhibit similar amount of PAP (Baudry & Duchateau, 2004). This finding comes from direct twitch potentiation comparisons after isometric, eccentric, and concentric maximal voluntary contractions (MVCs) of ankle dorsiflexors. CC duration was set to 6 seconds, and eccentric and concentric contractions were performend at a movement speed of 5°/s over a 30° range of motion. Both twitch peak force and RFD showed similar potentiation between the protocols, leading to the conclusion that PAP is not related to the type of maximal CC (Baudry & Duchateau, 2004). However, it is possible that variation in the range and velocity of movement in dynamic CCs might affect twitch potentiation (Baudry & Duchateau, 2004).

Electrically-evoked contractions (EECs) have also been used to cause PAP (Jubeau et al., 2010; Miyamoto et al., 2011). However, when comparing voluntary isometric and EECs in quadriceps femoris at an intensity higher than 40% MVC, the former ones induced higher twitch potentiation than the latter throughout the post-CC time course. This discrepancy is due to the lower number of activated MUs and higher induced muscle fatigue in EECs compared to voluntary contractions for corresponding

intensities, revealing these factors as drawbacks in using electrical stimulation to induce PAP (Jubeau et al., 2010; Miyamoto et al., 2011).

CC intensity. The extent of twitch potentiation has been reported to be related with the intensity of CC: the higher CC intensity, the more the muscle was potentiated (Vandervoort et al., 1983; Sasaki et al., 2012). However, recent evidence has supported the hypothesis that the threshold of CC intensity inducing maximal PAP is different among different muscles (Fukutani et al., 2014). Indeed, the magnitude of PAP is saturated after a CC intensity close to the complete recruitment of the MUs within a muscle (Fukutani et al., 2014) (fig. 22). These results suggest that the whole muscle motor neuron pool has to be recruited for exhibiting the highest twitch potentiation. Thus, for some muscles (e.g. adductor pollicis muscle), sub-maximal contraction intensities that would allow to fulfill the complete MUs activation might be enough to evoke the maximal PAP (fig. 22). This strategy would cause a reduction in fatigue subsequent to CC, an attenuating factor of the potentiation effect (Fukutani et al., 2014).

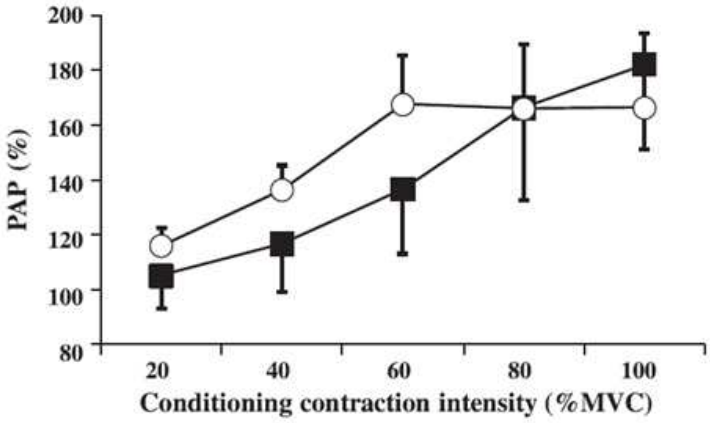

FIGURE 22. The extent of PAP at various CC intensities in thumb adductor (open circles) and plantar flexors (closed squares), quantified through twitches evoked respectively from the ulnar and posterior tibial nerves, 10 seconds after the end of CC. Plantar flexion was performed with a knee angle of 90°, therefore emphasizing the role of the soleus muscle. The maximum PAP was reached at 60 % MVC for thumb adduction and at 100% MVC for plantar flexion, whereas all the MUs (muscle fibres) are recruited at 40% and 90% MVC respectively in adductor pollicis and soleus (Fukutani et al., 2014). (Adapted from Fukutani et al., 2014).

Fibre type distribution of the muscles involved in CC. The higher the fibre type II percentage within a muscle, the higher the extent of its potentiation (Moore & Stull, 1984; Hamada et al., 2000a). Moore & Stull (1984) compared rats gastrocnemius type I and type II fibres twitch potentiation in response to a tetanic contraction. Type II fibres exhibited higher twitch potentiation in both peak torque and RFD. The authors suggested the higher and lower amount of respectively MLCK (enzyme responsible of RLC-P) and myosin light chain phosphatase (enzyme responsible for the removal of the phosphate from the RLC) in fast-twitch fibres related to slow-twitch fibres as the mechanism responsible for a higher degree of RLC-P, therefore potentiation, in the former fibre type. Hamada et al. (2000a) later confirmed this finding, revealing a higher magnitude of PAP in subjects with higher fibre type II composition in the vastus lateralis.

Overall, numerous aspects might affect the extent of PAP. Studies have mainly focussed on CC duration, type, intensity, and fibre type distribution of the muscles involved in CC (Moore and Stull, 1984; Hamada et al., 2000a; Rassier et al., 2000; Baudry & Duchateau, 2004; Jubeau et al., 2010; Miyamoto et al., 2011; Fukutani et al., 2014). These factors must be considered to correctly interpret twitch potentiation magnitude subsequent to a CC. Moreover, paragons of the extent of PAP between studies can be made only if the aforementioned factors are comparable.

3.4 Benefit from PAP in power performance

As the name suggests, power performances are largely determined by mechanical power. Mechanical power can be defined as the rate of force developed over a range of motion, or as force multiplied by velocity (Tillin & Bishop, 2009). Decreasing the time over which a specific force is developed, without altering the range of motion, will increase mechanical power. PAP can increase mechanical power through an increased RFD, resulting the concentric portion of the force-velocity curve in an upward and rightward shift (Sale, 2002; Tillin & Bishop, 2009) (fig. 23A).

PAP has little effect on the endpoints of the force-velocity curve, namely, the maximal isometric force and shortening velocity (Sale, 2002; Tillin & Bishop, 2009) (fig. 23A). The reason resides on the mechanism inducing PAP, that is, RLC-P (Grange et al.,

1993; Sweeney et al., 1993; Stull et al., 2011). Increased sensitivity of the actin-myosin complex to Ca^{2+} from RLC-P has meagre or no effects in conditions of sarcoplasma Ca^{2+} saturation, such as those caused by maximal isometric efforts or tetanic contractions, resulting in lack of changes in the exerted peak force (Vandenboom et al., 1993; Abbate et al., 2000; Sale, 2002). Parallelly, RLC-P does not affect the kinematic properties of the myosin head, thus having little effect on the maximal shortening velocity, if any, only due to actin and myosin filaments brought closer (Stuart et al., 1988; Stull et al., 2011).

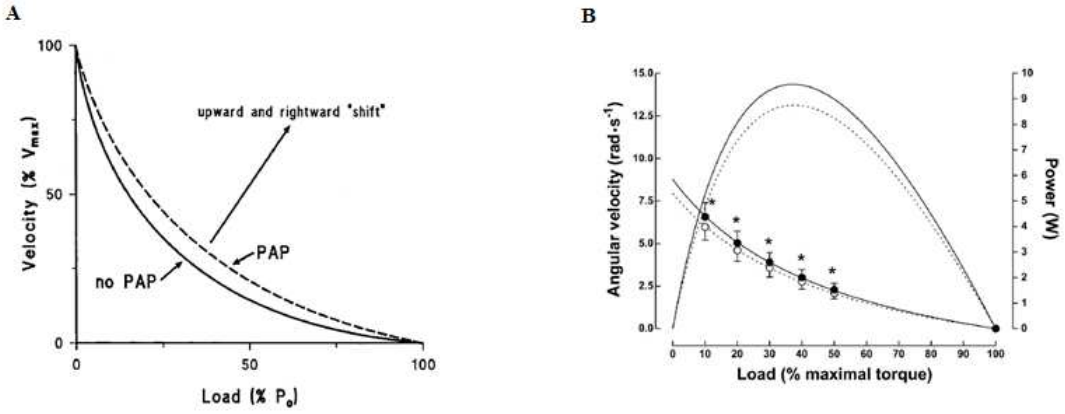

FIGURE 23. A) Hypothesized effect of PAP on the force-velocity relationship. PAP has little or no effect on the maximum isometric force or maximum shortening velocity. In contrast, PAP can increase RFD, thus shifting upward and rightward the force-velocity curve. The relation becomes less concave. B) Load-velocity relationship resulting from ballistic contractions before (open circles) and 1 minute after (filled circles) a 6-s MVIC of the thumb adductor muscles. The relations are fitted by Hill's equation (dashed line = before MVC; continuous line = 1 minute after MVC). An upward shift of the load-velocity curve leads to increases in power performance, as indicated by the load-power relationship (Adapted from Sale, 2002; Baudry & Duchateau, 2007).

Although PAP has been found to have scarce effects at the extremes of the force-velocity curve, increases in rate of torque development have been shown with loads between zero (i.e. maximal shortening velocity), and the peak isometric torque (Baudry & Duchateau, 2007). The higher rate of torque development would cause an increase in the attained movement acceleration and velocity, hence enhancing power output and

shifting the force-velocity and power-velocity curves (Sale, 2002; Baudry & Duchateau, 2007) (torque- angular velocity and power-angular velocity in fig. 23B).

Increased rate of torque development in isometric rapid contractions due to PAP effect, is comparable with that of concentric explosive actions (Baudry & Duchateau, 2007). Indeed, two experiments conducted from Baudry & Duchateau (2006; 2007) on the same muscular group (thumb adductor muscles) showed an analogous enhancement of the rate of torque development in the two aforementioned types of action (fig. 24). Rapid isometric contractions have been used in several other studies to quantify the time course of the changes in RFD within a potentiated muscle state (Güllich & Schmidtbleicher, 1996; Hodgson et al., 2008; Smith et al., 2014).

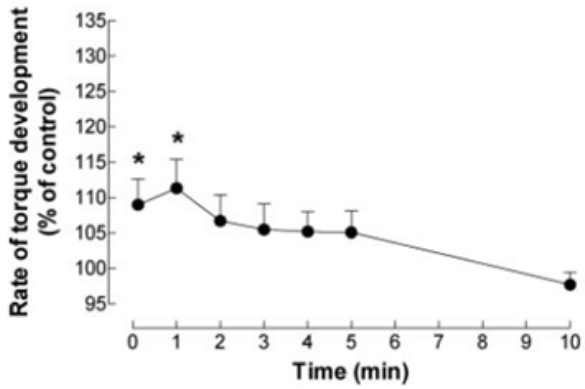

FIGURE 24. A) Effect of a 6-s MVIC of thumb adductor muscles on the rate of torque development of rapid isometric contractions, expressed as percentage of the control contraction (i.e. pre-MVIC) (Adapted from Baudry & Duchateau, 2004)

Although evidence has been reported for enhanced power performances in a PAP state (Tillin & Bishop, 2009), the high inter-subjects variability on the extent of RFD increases is worth to be mentioned (Tillin & Bishop, 2009). Several studies have reported level of muscular strength, training state and background, specificity of the subsequent activity, and gender as influential factors of such potentiation (see for review Tillin & Bishop, 2009). However, to date, the role and influence of these variables have still to be determined (Sale, 2002).

3.5 Does PAP also have neural contribution?

Increases in RFD of rapid actions in a potentiated state might be related to changes in excitability of the neural motor pathways. This assertion was first presented in a study conducted by Güllich and Schmidtbleicher (1996), whereas 5 sets of 5-s MVIC, plantar flexions, were used to induce PAP. In the potentiated state, a correlation between the times of the highest expression of the H-reflex amplitude in gastrocnemius lateralis and RFD in isometric maximal plantar flexions resulted in a r value of 0.89. In addition, the mean correlation coefficient of the time course of the two variables, was 0.9 and 0.75 for respectively H-reflexes evoked in the gastrocnemius lateralis and soleus (fig. 25). This high correspondence was however found only in power-trained athletes. The authors conjectured that these athletes, differently from the endurance-trained counterpart, exploited PAP as an increased excitability of the motor neurons pool (reported from H-reflexes measurement), which allowed easier and higher order MUs recruitment during rapid contractions, and RFD (Güllich & Schmidtbleicher, 1996). This was the first experimental evidence of neural contribution to PAP, selectively in power-trained athletes.

Although the striking correlations were found, the study presented two major limitations, namely, H-reflexes were not normalized to the M-wave, and twitch responses were not recorded as a measure of actual muscular PAP. In addition, H-reflexes were interpreted merely as changes in the motor neurons pool excitability. This interpetation must be cautious, as it is now known that H-reflex measurement is affected by several factors, and it should be interpreted as changes in the spinal reflex pathway (Pierrot-Deseilligny & Burke, 2012, 21) (see “Measuring spinal excitability”)

Due to the methodological limitations in the above-mentioned study, and to corroborate the finding, the investigation was repeated from Hodgson et al. (2008), targeting power-trained athletes only. From H-reflexes recorded in soleus, no relationship was found between their amplitude and increases in RFD of ballistic actions. However, it is worth to be mentioned that drawing correlations between RFD and H-reflexes without measuring the latter in the gastrocnemius muscles might be inappropriate. Indeed, evidence has been reported that the excitability of motor neurons pool might be differently modulated between soleus and gastrocnemius muscles when high RFDs are

required in rapid activities, showing a decrease in the “slow” soleus and an increase in the relatively “fast” gastrocnemius (Moritani et al., 1990). Concurrently, another study (Folland et al., 2008) used the H-reflexes technique to relate reflex potentiation following a MVIC with isokinetic and explosive knee extension strength, finding no relationship.

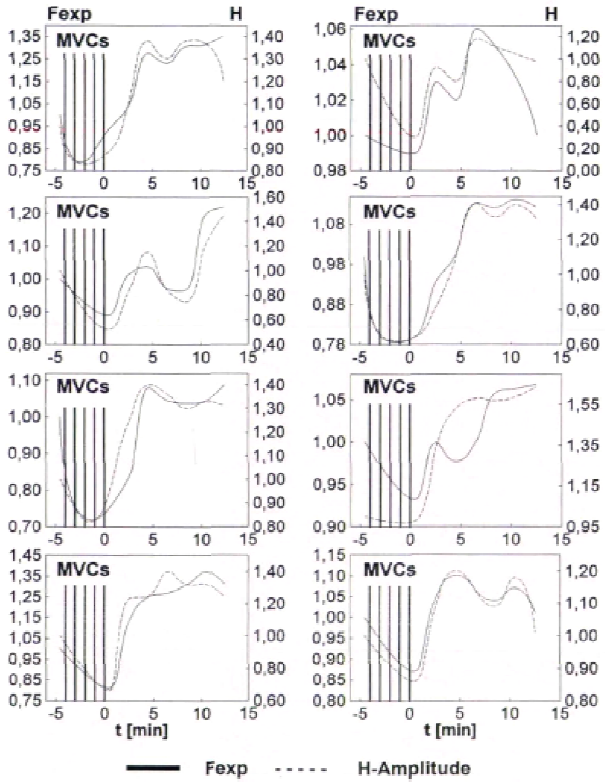

FIGURE 25. Correspondence of the time courses of RFD of voluntary isometric plantar flexions (Fexp, continuous line) and H-reflexes amplitude of the lateral gastrocnemius muscle (H, dashed line) before and after 5*5-s MVIC. (From Güllich & Schmidtbleicher, 1996).

It is known that reflexes contribute to a substantial fraction of force generated during human movement (Stein & Thompson, 2006), and that enhanced reflex contribution improves the capability for explosive force (Gollhofer, 2003, 339). Based on these knowledge, and on the original, not corroborated, findings from the study of Güllich and Schmidtbleicher (1996), the functional role of conditioned H-reflex in PAP is still under debate, especially for the hypothesis that power-trained athletes might present neural contribution to PAP compared to the endurance-trained counterpart.

Moving upstream to the spinal reflex pathway, it has not been over 20 years when altered excitability in the corticospinal pathways after conditioning activity has been experimentally determined. Research has shown that excitability of the corticospinal pathways and motor neurons pool (measured by means of evoked potentials) are modulated after evoked tetani or MVCs (Samii et al., 1996; Gandevia et al., 1999; Balbi et al., 2002; Petersen et al., 2003) (fig. 26), resulting in altered neural drive in subsequent submaximal voluntary contractions (Petersen et al., 2003).

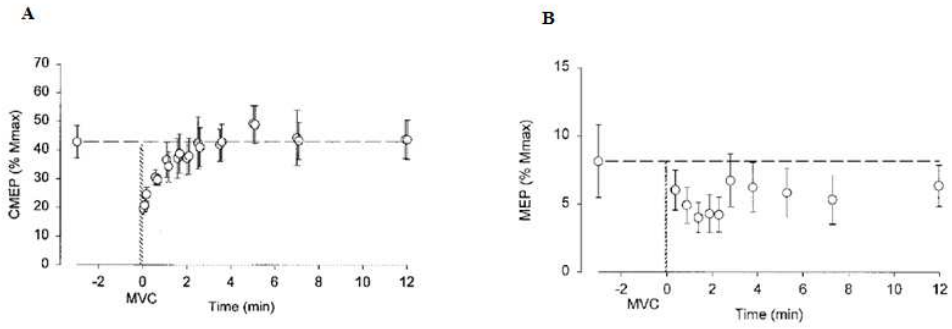

FIGURE 26. Normalized responses (to Mmax) of MEPs evoked in biceps brachii from CMS (A) and TMS (B) in 4 subjects after a 5-s MVIC of elbow flexors. The shaded area denotes the duration of the MVIC in each panel. The amplitude of the normalized MEPs represent in A) and B), respectively, the motor neurons pool excitability and the overall corticospinal pathways excitability. Note that the latter responses are dependent on the former ones. (Adapted from Gandevia et al., 1999).

Extending this concept to PAP, maximal voluntary activity might induce short-term plasticity of corticospinal pathways, which in turn might modulate neural drive in subsequent ballistic performance. To date, there are no studies directly either confirming or denying this hypothesis. For instance, an increase in neural drive as a consequence of either increased corticospinal or motor neurons pool excitability might be beneficial for recruiting higher order MUs in ballistic contractions, allowing enhanced RFD (Tillin & Bishop, 2009).

In conclusion, the nature of the neural mechanisms contributing to performance enhancement, if any, in PAP is not clear yet. Moreover, the question whether power-

trained athletes might exploit short-term plasticity of neural pathways for performance enhancement, compared to the endurance-trained counterpart, is still persisting. Being spinal and supra-spinal components primarily involved in the performance of voluntary tasks, a possible role of short-term facilitation of neural excitability in ballistic contractions in PAP needs to be investigated.

4 PURPOSE OF THE STUDY

MVCs cause PAP, an increase in the twitch mechanical response of a muscle group (Sale, 2002). Parallelly, RFD of ballistic voluntary actions is enhanced (Sale, 2002).

From a neural standpoint, MVCs might concurrently affect the excitability of the spinal reflex (Güllich & Schmidtbleicher, 1996) and corticospinal motor pathways (Gandevia et al., 1999). PAP might be therefore characterized from a coexistence of increased muscle mechanical responses and altered corticospinal and spinal reflex excitability. Due to the role of spinal and supra-spinal components in voluntary tasks, it is plausible that performance enhancement in PAP would be affected from muscular as well as neural factors.

As mentioned in the previous section, there is no direct evidence so far for the latter conjecture. In addition, evidence has been presented that power-trained athletes might report neural potentiation and exploit it in subsequent performance, differently from the endurance-trained counterpart (Güllich & Schmidtbleicher, 1996). This evidence however has been neither confirmed nor enlarged from spinal reflex to corticospinal pathways.

Thus, the purpose of the present investigation was to search for direct evidence of neural contribution to PAP, and its transferability to subsequent performance enhancements. Muscular PAP was recorded through supramaximal twitch contractile characteristics, whereas neural factors were represented by evoked potentials through the spinal reflex and corticospinal pathways, namely, H-reflexes and MEPs. Force output and neural drive of ballistic performances were also monitored during PAP, and interpreted in regard to muscular and neural responses. Moreover, the sample was composed of power-trained and endurance-trained athletes, to check for discriminating factors coming from the training background in exploiting neural contribution to PAP (Güllich & Schmidtbleicher, 1996).

PAP will be evoked in plantar flexor muscles. This muscle group has been chosen mainly for three reasons. Firstly, comparisons of our findings with previous studies (Güllich & Schmidtbleicher, 1996; Hodgson et al., 2008) involving the same muscles

can be made. Secondly, measurements of corticospinal and spinal reflex excitability on soleus and gastrocnemius muscles are relatively easy to be performed and detected, compared to other muscles. Lastly, being the activity of the aforementioned muscle group intensively modulated during daily (e.g. walking) and ballistic activities from CNS components (Moritani et al., 1990; Dietz, 2003), studying the implications of the nervous system in PAP would reflect a functional purpose.

A 8-second MVIC was chosen as CC to avoid development of fatigue (Vandervoort et al., 1983) and to allow inducing the maximal PAP in plantarflexor muscles (Sasaki et al., 2012; Fukutani et al., 2014).

We hypothesized that enhanced ballistic performance after CC would be a result of both enhanced muscular contractile characteristics and neural pathways excitability, and that power-trained would exhibit higher performance potentiation than endurance-trained athletes due to both amplified mechanisms.

5 METHODS

5.1 Participants

Two groups of 8 participants were tested. The first group was composed of endurance-trained athletes (henceforth END, 7 males and 1 female, mean ± SD: 27.0 ± 2.4 years old, 75.7 ± 10.3 kg weight, 1.78 ± 0.06 m height). Four participants practiced long-distance running, 1 cross-country skiing, 1 orienteering, 1 long-distance cycling, and 1 long-distance rowing. The second group was composed of 8 power-trained athletes (henceforth POW, 7 males and 1 female, mean ± SD: 24.4 ± 3.2 years old, 80.3 ± 12.3 kg weight, 1.81 ± 0.09 m height), consisting of 4 volleyball players, 2 weightlifters, and 2 explosive-trained subjects, whereas lower body explosive exercises represented a sizable part of their training. This classification allowed discriminating between performances requiring plantar flexions in a repeated endurance and powerful manner, respectively for END and POW. Participants were defined trained as performing regular training in their respective discipline (≥ 3 sessions per week) for ≥ 2 years, according to previous studies (Hodgson et al., 2008; Tillin et al., 2010). Seven subjects belonging to END trained for competitions at national and international level, while the 4 volleyball players belonging to POW trained for competitions at the national level. None of the participants presented any injury within 12 months prior to measurements, and had any history of neuromuscular disease. All subjects were volunteers, fully informed about the procedures and risks involved in the study, and they provided their written informed consent (Appendix 1) prior to measurements. In addition, they were screened for contraindications to TMS (Rossi et al., 2009, Appendix 2). Participants were instructed to refrain from any heavy leg exercise 24h before testing. The methods were approved by the local Ethics Committee and performed in accordance with the Helsinki Declaration.

5.2 Experimental procedure

Participants visited the laboratory in two sessions, separated by at least 48 hours. In both sessions, PAP in plantarflexors was induced through a 8-sec MVC (conditioning

contraction, CC). This duration was chosen according to previous evidence reporting that MVCs between 5 and 10 seconds yielded maximum PAP, as the best trade-off between resulting potentiation and fatigue (Vandervoort et al., 1983; Rassier, 2000). A schematic view of the protocol for both sessions is pictured in figure 27.

In the first visit, the effect of PAP on single supramaximal twitches (conditioned twitches, CTw), H-reflexes (conditioned H-reflexes, CH) and corticospinal excitability (conditioned MEPs, CM) recorded from LG was measured. After determination of the respective stimulation intensities, participants performed 5 times the PAP protocol. Each protocol was composed of control measurements, one CC (8-sec MVC), and post-CC measurements of either CTw, CH, or CM temporal profiles across a 10-min time frame (fig. 27A). CTw were recorded in 1 trial, whereas CH and CM were measured in 2 trials each. The order of the trials was randomized. Each CC was separated by 20 minutes, determined in pilot measurements as the optimum time lapse allowing full recovery of both mechanical and neural values. This was also in accordance with previous studies, which showed that twitch contractile characteristics and evoked potentials might be altered up to 15-20 minutes after CC (Gandevia et al., 1999; Baudry & Duchateau, 2004). In control conditions, 3 stimuli were elicited for supramaximal twitches, whereas H-reflexes and MEPs were evoked 5 times. Inter-stimulus interval was 10 seconds. Before each trail involving CH, H-reflexes were checked to be on the ascending limb of the curve. This was done ensuring that a 1-mA increase in stimulating current intensity led to higher H-reflex responses in LG. During all CCs, strong verbal encouragement was given to the participants. For conditioned values, CTw were evoked 5 seconds after CC, and every minute between the 1st and the 10th minute post-CC. For CH and CM, the same time-sequence was used. However, each time point between the 1st and the 10th minute was represented by two stimuli, evoked 5 seconds before, and 5 seconds after the minute, respectively (i.e. inter-stimulus interval of 10 seconds). This approach allowed increasing the number of collected evoked potentials from LG, without compromising timing requirements. During all stimulation procedures (i.e. determination of H-reflexes, supra-maximal twitches and TMS stimulation intensities, and the 5 PAP trials), participants were presented an attentive task. The latter consisted of landscape pictures (without human action) displayed on a tv screen, in front of the subjects. Images and videos without human agents have been commonly used as a method to keep level of attention high, minimizing changes in both spinal and

corticospinal excitability from extraneous factors that might arise (Zehr, 2002; Bassolino et al., 2014). Participants were observed during the recording time to ensure compliance to the attentive task. In addition, suffused light and quiet surroundings were maintained.

In the second session, the effect of PAP on ballistic voluntary contractions was explored. The latter actions consisted in rapid isometric plantarflexions, whereas participants were instructed to exert force as fast and as hard as possible on the clue of one of the investigators. At the beginning of the session, a number of trials between 3 and 5, depending on the familiarity of the subjects with the ballistic task, was allowed for accustoming to the contractions. Each familiarizing trial was separated by 3 minutes, determined in pilot measurements as the optimum time lapse allowing mechanical and neural values to fully recover. The control condition consisted of 1 ballistic voluntary contraction, recorded 3 minutes after the last familiarization trial. Thereafter, each trial consisted of one CC and one post-CC ballistic contraction. The latter was executed either 5 seconds, 1 minute, 2 minutes post-CC, or at arbitrarily considered optimal time points determined from individual CTw, CH, and CM temporal profiles (see “Data Analysis”). Ballistic performance was assessed once at a single time point for each trial, to avoid the confounding effect that repeated muscle activity could have on the influence from CC. This approach has been previously used in PAP studies (Folland et al., 2008). Trials were randomized. As for the first session, each CC was separated by 20 minutes, and the same attentive task was presented to the participants, to standardize the recording conditions. Trials were repeated if countermovement in the ballistic performances was noticed, either visually, or from the obtained force-time curve, as a clear depression in force before its rising phase. The number of trials in the second session ranged between 4 and 7.

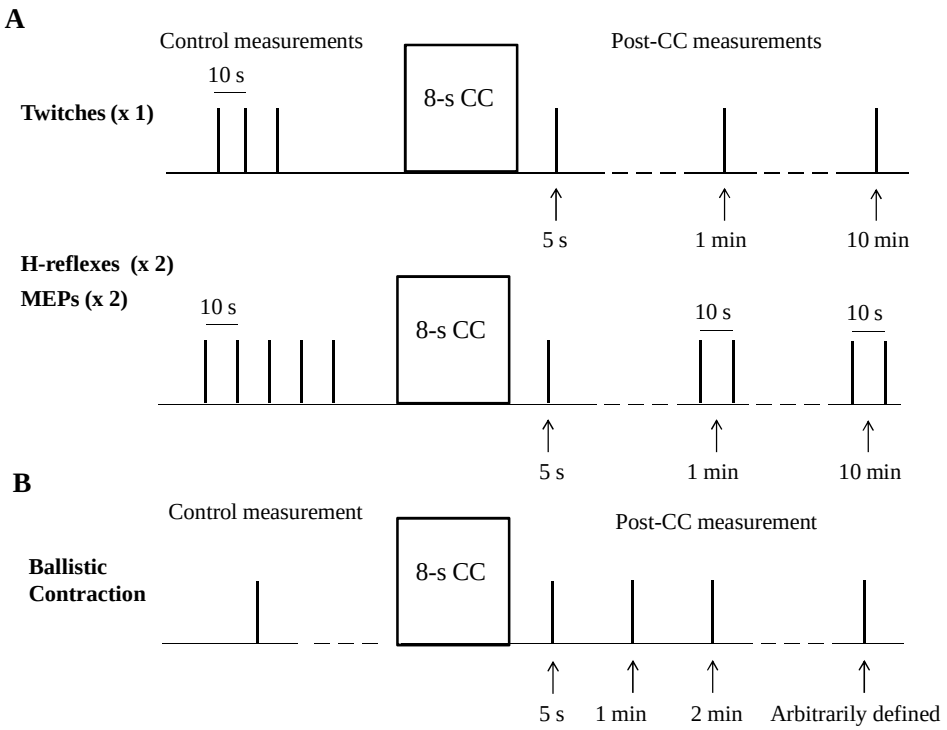

FIGURE 27. Schematic view of the protocol for the first (A) and second (B) sessions. 8-s CC corresponds to the 8-second conditioning contraction. A) The effect of PAP on supramaximal twitches (CTw), H-reflexes (CH) and MEPs (CM) was measured. CTw were recorded in 1 trial. CH and CM were measured in 2 trials each. For CH and CM, each time point between the 1st and the 10th minute was represented by two stimuli, evoked 5 seconds before, and 5 seconds after the minute, respectively. Inter-stimulus interval between consecutive stimuli was 10 seconds. B) The effect of PAP on ballistic contractions was measured. Control ballistic performance was recorded at the beginning of the second session. Note that, each trial consisted of one CC and one post-CC ballistic contraction, executed either 5-seconds, 1 and 2 minutes post-CC , or at arbitrarily considered optimal time points determined from CTw, CH, and CM temporal profiles (see "*Data Analysis*"). Each CC within each session was separated by 20 minutes rest.

5.3 Measurements of neuromuscular function

Force recording. Isometric force produced by the plantarflexors during voluntary and electrically-evoked contractions was measured through a custom-made ankle dynamometer (Neuromuscular Research Centre, University of Jyväskylä, Jyväskylä, Finland), consisting in a vertically mounted footplate, and an adjustable car seat, at the

opposite ends. The subjects seated with the hip, knee, and ankle joints respectively at 110°, full extension, and 90°. This reciprocal knee-ankle joint position was chosen to favour the neural recruitment (Cresswell et al., 1995; Kennedy et al., 2001) and anatomical features (Kawakami et al., 1998) of the two-joint gastrocnemii muscles in isometric plantarflexions. Being the latter muscles preferentially activated during explosive-type actions (Moritani et al., 1990), and having more type II muscle fibres than the soleus (Harridge et al., 1995), this set-up was fundamental for the fulfilment of the study purpose. The foot of the dominant lower limb was placed on the dynamometer footplate, which was determined by asking to the participants which lower extremity was preferred to use for leap take-off. This dominancy test was chosen among others for better complying with the tasks being measured. The line between the lateral malleolus and the head of the fibula, and the ankle centre of rotation were, respectively, orthogonally oriented and aligned with the axis of rotation of the dynamometer pedal. The seat was slid forward as far as to stabilize the hip and minimize any lift of the heel off the footplate during the isometric contractions. Straps across the instep, over the knee, and seat belts were used to further prevent movement during isometric plantarflexions. Force signals from the footpedal were sampled at 5000 Hz using a 16-bit A/D converter (CED 1401, Cambridge Electronics Design, Cambridge, UK), and recorded on a personal computer using Spike2 software (version 6.10, Cambridge Electronics Design).

Electromyography. Surface EMG was recorded from lateral gastrocnemius (LG) and soleus (SOL) using self-adhesive electrodes (Blue Sensor N, Ag/AgCl, 0.28 cm^2 , Ambu A/S, Ballerup, Denmark) arranged in a monopolar fashion. Monopolar was preferred to bipolar configuration as it allows recording evoked potentials of higher-quality and larger amplitude (Kamen & Gabriel, 2010, 66). Detectable MEPs could be therefore evoked with a greater ease, decreasing subjective resting motor threshold. The active electrodes were placed for LG over the muscle belly, and for SOL at 2/3 of the line between the medial condylis of the femur and the medial malleolus. The reference electrodes were placed on the anterior surface of the tibia for both muscles. Furthermore, bipolar electrodes (Blue Sensor N, Ag/AgCl, 0.28 cm^2 , Ambu A/S, Ballerup, Denmark) were placed on the TA over the muscle belly, set 2 cm apart, and oriented according to the muscle fibres direction. A general ground electrode (Blue Sensor N, Ag/AgCl, 0.28 cm^2, Ambu A/S, Ballerup, Denmark) was placed over the

head of the fibula. For all the aforementioned muscles, correct electrodes placement was checked by means of the clinical tests suggested by the surface electromyography for non invasive assessment of muscles (SENIAM) recommendations (Hermens et al., 2000). The skin underlying the electrodes was prepared by shaving, light abrasion and cleaning with alcohol to decrease recording resistance. For bipolar arrangement, interelectrode resistance resulted less than 5 kΩ. For monopolar settings on LG and SOL, the resting noise level was monitored to be below 50 μV. This ceiling value was chosen according to the criterium for determining the rMT in TMS. In our experiment, rMT was defined as the lowest stimulation intensity whereas a MEP of at least 50 μV peak-to-peak amplitude (P-P) was elicited in at least 3 of 5 consecutive trials (Rossini et al., 1994). Therefore, a 50-μV noise ceiling was the minimal requirement for allowing to distinguish real MEPs from noise contamination. The raw EMG signals were amplified and high-pass filtered (x100 for monopolar and x 1000 for bipolar configuration, 10 Hz cut-off frequency) by a preamplifier (NL824, Digitimer Ltd., Welwyn Garden City, Hertfordshire, UK), subsequently band-pass filtered (10-1000 Hz) by a custom-made differential hardware amplifier (Neuromuscular Research Centre, University of Jyväskylä, Jyväskylä, Finland). The signals were sampled and recorded at 5000 Hz using the same A/D converter and computer software that enabled synchronization with the force recordings.

Peripheral nerve stimulation. To evoke H-reflexes and M-wave responses in LG, single square electrical stimuli (1-ms pulse width, 400V) were delivered to the posterior tibial nerve, by means of a constant current stimulator (DS7AH, Digitimer Ltd., Welwyn Garden City, Hertfordshire, UK). The anode (5.08 cm x 10.16 cm oval electrode, V Trode, Mettler Electronics Corp., Anaheim, USA) and cathode (0.77 cm^2 circular area, Unilect 4535M, Unomedical Ltd., Stonehouse, UK) were placed just above the patella and over the popliteal fossa, respectively. The optimal position of the stimulating electrode was located by means of a custom-made hand-held cathode (about 1 cm in diameter) as the stimulation site providing the greatest amplitude of the evoked responses in LG with the minimal current intensity. Caution was taken to apply constant pressure over the popliteal fossa while moving the hand-held electrode during these preliminary recordings, as a higher pressure might have brought closer the peripheral nerve to the stimulating locus, thus increasing the recorded response. Thereafter, the cathode was firmly fixed with tape to the optimal site. The H-reflexes and M-waves

curves of LG were then detected. The current was increased by 1-mA increments from 0 until a plateau in the M-wave was obtained, indicating the attainment of its maximal value (Mmax). One stimulus was delivered at each intensity, with a 10-s inter-stimulus interval. The latter time lapse was chosen as to avoid post-activation depression within the Ia afferent pathway (Crone & Nielsen, 1989), that would alter H-reflexes measurements. During these recordings, participants were placed on the ankle dynamometer, assuring the same posture as in the experimental procedures, to minimize extraneous factors that can alter presynaptic inhibition (PSI) and, parallely, H-reflex amplitude (Zehr, 2002). If movement of the participants was noticed, due to the potential extensive modulation of H-reflexes that could result (Zehr, 2002), the stimulus was repeated. For supramaximal muscle twitch stimulation, to select a current intensity that assured complete activation of the plantarflexors, both a plateau in the compound muscle action potential (Mmax) and in the evoked isometric twitch torque was sought. Thereby, stimulation intensity was further increased by 50% (absolute current values reported in table 1). For H-reflex measurements, the current eliciting an H-reflex on the ascending limb of its curve, and with the magnitude of the preceding M-wave (Msubmax) included between 5-25% of M max, was selected. Albeit it has been suggested that a range of 15-25% can be better sensitive to conditioning (Zehr, 2002), the latter band was attainable only in 4 out of 16 participants. Absolute and relative stimulation intensities for H-reflex measurements for both groups are reported in table 2.

TABLE 2. Absolute and relative current intensities for H-reflex measurements and supramaximal twitches. Data are presented as mean (range in brackets).

		Measurements	
Groups	Current intensity	H-reflexes	Supramaximal twitches
END	Absolute (mA)	9.25 (6 - 14)	27 (21 - 38)
	Msubmax (% Mmax)	16.2 (5 – 24)	
POW	Absolute (mA)	7.71 (3 - 11)	29.57 (9 - 45)
	Msubmax (% Mmax)	10.8 (6 – 14)	

END, Endurance-trained Group; POW, Power-trained Group; Mmax, Maximal M-wave Amplitude; Msubmax, Amplitude of the M-wave preceding the H-wave.

Transcranial magnetic stimulation. TMS was performed on the contralateral motor cortex respective to the leg being tested, to preferentially elicit MEPs in the LG, using 2 connecting monophasic Magstim 200[2] magnetic stimulators (BiStim[2], Whitland, UK). Single pulses were delivered through a 9-cm double batwing coil (Magstim, Whitland, UK). The coil was oriented so that current was delivered posterior-to-anterior directed to the motor cortex. Participants were asked to wear a white tightly-fitted swimming cap (plain moulded silicone cap, Speedo©, UK), whereas optimal position of the coil was marked by means of non-permanent markers. This procedure facilitated re-positioning of the coil within the testing session, especially after MVCs were performed, since only 5 s were available from the end of the MVC to the onset of the first post-MVC stimulus (see "Experimental procedure"). Initially, the vertex of the scalp was located on the swimming cap. Probing of the optimum coil location began with the coil placed over the hemisphere of interest 1 cm lateral and 1 cm posterior to the vertex, previously described as a favourable location for plantarflexors activation (Kumpulainen et al., 2014). Each participant was initially familiarized with 10 stimuli (30% maximal stimulator output). Thereafter, the optimum coil placement was determined moving the coil in 1 cm steps (marked on the swimming cap) in both lateral-medial and anterior-posterior directions, until the stimulating spot eliciting the largest MEP in LG with a constant stimulation intensity was found. The site was then marked over the cap. A custom-made coil holder and a neck support were used for keeping the relative coil-head position constant, which was also continuously visually checked from one of the investigators throughout the stimulation procedures. In addition, the control from the latter researcher was fundamental for promptly repositioning the coil, if moved, over the optimal spot when MVCs terminated and before subsequent stimulation began 5 seconds thereafter (see "Experimental procedure"). Resting motor threshold (rMT) was determined as the minimum stimulation intensity whereas a MEP of at least 50 μV P-P was elicited in not less than 3 of 5 consecutive trials (Rossini et al., 1994) in LG, and visually clearly detectable from the computer interface. Stimuli were delivered with a 10-s inter-stimulus interval during all stimulation procedures. Stimulus intensity throughout the experiment was set at 120% of rMT. Expressed as percentage of maximal stimulator output, it resulted in 67.88 (range 48 – 99) and 71.14 (range 54 – 84), respectively in END and POW.

5.4 Data Analysis

Evoked potentials. During all recordings, M-waves, H-waves and MEPs P-P from LG were determined and displayed on-line by means of a custom Spike software script (Cambridge Electronics Design, Cambridge, UK). Values were then exported and analyzed off-line using Microsoft Office Excel software (version 2007, Microsoft Corp., Redmond, Washington, USA). To obtain conditioned values of the afore-mentioned evoked potentials of LG, firstly, H-waves and MEPs P-P were normalized, respectively to the Msubmax, and to the Mmax elicited at the closest time point, to obtain H/Msubmax (CH) and MEP/Mmax (CM). After that, for Msubmax, H/Msubmax and MEP/Mmax, stimuli representing the same time point within trials (i.e. control condition, and each minute between the 1^{st} and the 10^{th} minute post-CC), were averaged. Values were then expressed relative to control parameters. Finally, the resulting relative changes were averaged at a same time point between the 2 trials. As Mmax was recorded from LG in only 1 trial during the first session, no average was needed, and values were directly expressed relative to control parameters.

Force and other EMG measures. They were determined off-line using Igor Pro software (version 6.36, WaveMetrics, Portland, OR, USA). Prior to analysis, a finite impulse response filter with a low-pass cutoff of 50 Hz, and 555 coefficients was applied to force data, whereas EMG data were band-pass filtered between 6 and 500 Hz using a fourth-order zero-lag Butterworth filter, and fully-wave rectified.

The maximum voluntary force (MVF) during each CC was determined. To have group-representative (i.e. END and POW) MVF of CC performances of each session, MVFs across different trials within a session were averaged first for each participant, and then as group values.

For electrically evoked supramaximal twitches, peak force (PF), contraction time (CT), and half-relaxation time (HRT) were measured. The peak rate of force development (pRFD) was obtained as the maximal value of the first derivative (1 ms time resolution) of the mechanogram. Control condition for each parameter was represented as the average across the 3 control twitches. In addition, the twitch PF relative to MVF (PF/MVF) was computed first individually, as the ratio between control twitch PF and

the mean of the MVF of each CC within the 1st session, and then averaged as group-representative values.

The resulting temporal profiles of CTw, CH, and CM obtained from the 1st session allowed choosing individually the timing of performance of post-CC ballistic contractions, respectively, as the time points whereas the highest potentiation of supramaximal twitches peak force, H/Msubmax and MEP/Mmax was individually reported. Peak rate of force development was retained as a measure of performance from ballistic contractions (pBRFD), measured as the maximal value of the first derivative of the force with respect to time (1 ms time resolution). Neural drive in ballistic contractions was estimated from the LG through the root mean square of the EMG (RMS EMG), analyzed from its onset to the time at which the peak force occurred. Values were normalized for each individual to the average of the RMSs from LG of 500 ms epochs around MVF (250 ms either side) of CCs in the 2nd session.

All above-mentioned off-line analyses (except for MVF) were conducted by means of two custom Igor Pro software scripts (WaveMetrics, Portland, OR, USA). An interactive graphic method (Walter, 1984) was used for determination of signal onsets for both force and EMG data. This approach allowed benefitting of the quickness of a computer-based method without compromising the higher accuracy of manual-based gold-standard detections (Hodges and Bui, 1996; Tillin et al., 2013), therefore combining the best attributes of both (Kamen & Gabriel, 2010, 114). A graphical user interface allowed defining the time interval of interest from the force and EMG traces (fig. 28 A and 29 A). The baseline signal was determined across the 200 ms window preceding the selection. One script was used for determination of contractile characteristics of supramaximal twitches (i.e. PF, CT, HRT and pRFD). The force trace was enlarged in the selected interval, and force onset was determined as 5 SDs of signal from baseline (fig. 28 B). By means of the second script, ballistic performance indicators (i.e. pBRFD and RMS EMG) were calculated. The fully-rectified EMG trace was enlarged in the selected time interval. EMG onset was determined as 3 SDs of signal from baseline (fig. 29 B). Once force and EMG waveforms were represented with their respective automatically-determined onset points, a visually-based analysis allowed to accurately identify signal onset. This procedure was fundamental as multiple automatically-based onset points resulted, especially when a fluctuating signal such as

EMG was analyzed (fig 29). Once the onsets were manually established, twitch contractile characteristics and ballistic performance indicators were automatically calculated.

Group-representative values in control condition and at each time point for each parameter were calculated as the average of individuals' absolute values within each group, and session. To represent the effect of CCs on the recorded parameters, data are expressed as relative to control values. For the sake of clarity, group-representative absolute values for each measure at each time point are reported in Appendix 3.

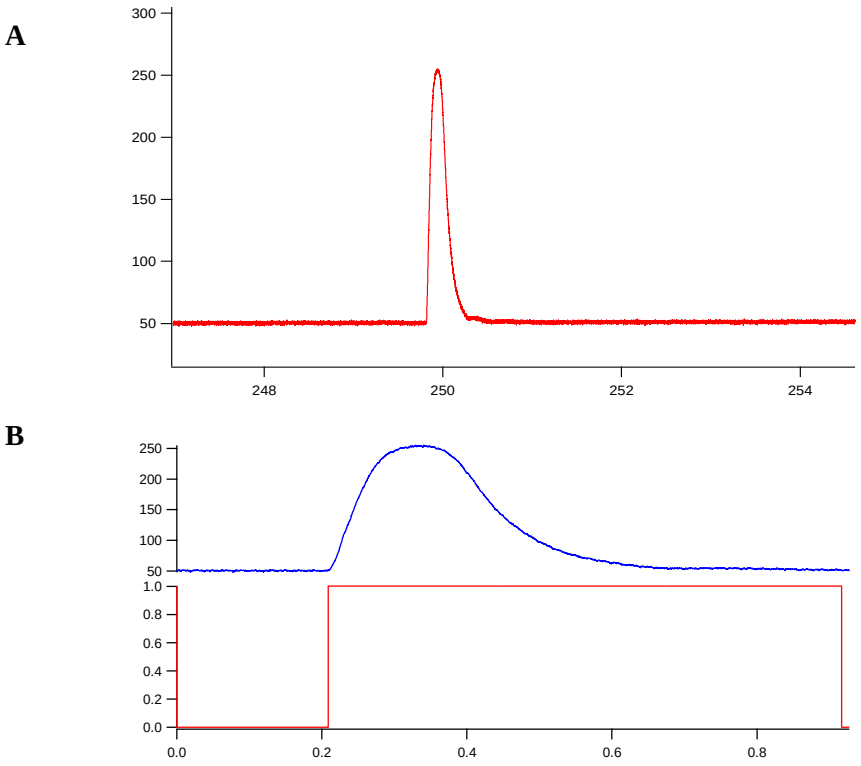

FIGURE 28. Determination of contractile characteristics of supramaximal twitches. A) The twitch force trace was first visualized, and the graphic user interface allowed to select the time interval of interest. B) Enlarged view of a selected time interval around the twitch. The upper trace represents the force, while the lower trace indicates the signal onset (5 SDs of the signal from the baseline). If the automatically determined onset of force was deemed correct after visual inspection, twitch PF, CT, HRT and pRFD could be calculated. If not, either the procedure could be repeated or the onset could be manually selected through visual inspection of the signal.

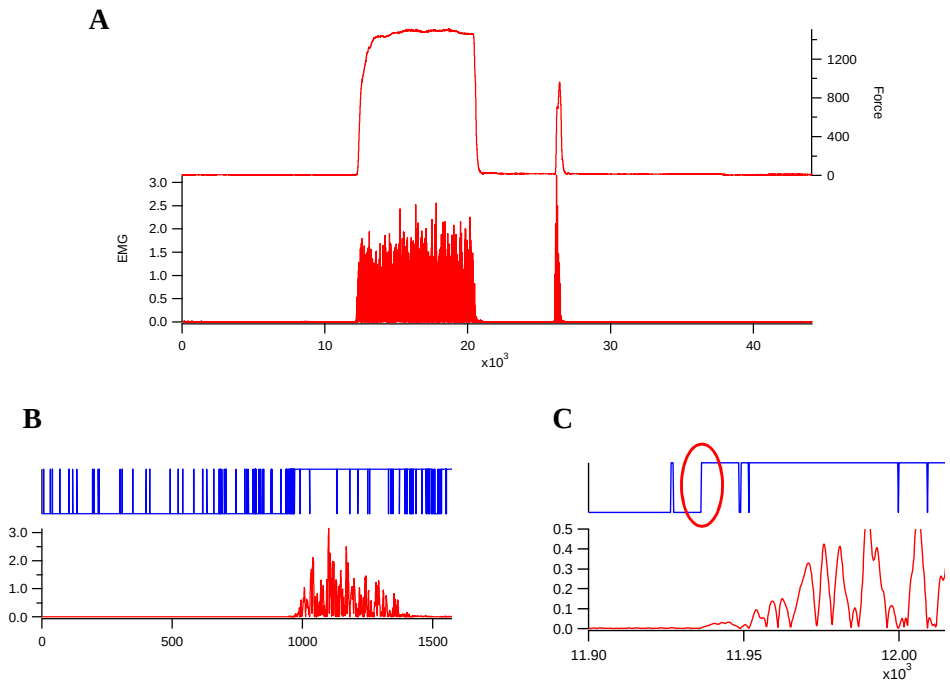

FIGURE 29. Determination of ballistic performance indicators. A) The fully-rectified EMG was first synchronized and represented below the force trace, in the image representing CC immediately followed from a ballistic contraction. The graphic user interface allowed to select the time interval of interest. B) Enlarged view of a selected time interval around the ballistic contraction. The lower trace represents the fully-rectified EMG, whereas the upper trace indicates the EMG signal onset (3 SDs of the signal from the baseline). Note that, being the EMG a fluctuating signal, multiple close onsets were automatically determined. Visual inspection was therefore necessary in all cases. When the signal was further zoomed (C), signal onset was clearly distinguishable (red oval), and when selected, pBRFD and RMS EMG were automatically calculated.

5.5 Statistical Analysis

Data are presented as group mean values ± SD. All statistical analyses were conducted using SPSS software (IBM SPSS Statistics, version 20, Inc., Chicago, IL, USA). Due to the non-normal distribution of some parameters of the data set, non-parametric statistics was used when necessary. To assess between-group differences in baseline values, group-representative MVF of CCs for each session, control twitch (i.e. PF, pRFD, CT, HRT, PF/MVF) and control ballistic (i.e. pBRFD, RMS EMG) contraction

characteristics were compared using Mann-Whitney U-test. For each group, to assess the possibility of fatigue development within each session, a dependent t-test was used to compare MVF of the first and last CC. To check for consistency of CCs, within-session reliability of the respective MVFs was analysed for each group using intraclass correlation coefficient ($ICC_{1,1}$) (Rankin & Stokes, 1998) and coefficient of variation (CV) (O'Leary et al., 2015). The ICC 95% confidence intervals (CI) were also calculated. An ICC $\geq$ 0.8 was considered as good reliability (O'Leary et al., 2015). The CV was calculated as: SD/ mean x 100 for each participant, and then averaged (O'Leary et al., 2015). To better represent measurements of absolute reliability for all individuals, SD of CV was also reported in addition to group mean (Atkinson & Nevill, 1998). Within-session reliability was also analysed for ballistic contractions, whereas pBRFD and RMS EMG of the last familiarization and the first control trials were compared, using the same indexes reported above. To investigate for within- and between-group differences in PAP phenomenon, relative changes post-CC from control values were considered. Within-group differences for each parameter between control and post-CC values were assessed by using Wilcoxon signed-rank test. Between-group differences at each post-CC time point were analyzed by means of Mann-Whitney U test. The level of statistical significance was set at $P < 0.05$ for all analyses.

6 RESULTS

Baseline and potentiated absolute values, and MVF. Group-representative MVFs were 1420 ± 461 and 1458 ± 480 N for END, 1554 ± 390 and 1602 ± 228 N for POW, in the 1st and 2nd session respectively. No significant differences (independent t-test) were reported between groups for both sessions. Control twitch and ballistic contraction characteristics, as well as evoked potentials are presented in table 3. END reported greater twitch PF (216.19 ± 34.10 N vs. 167.11 ± 21.07 N, $P < 0.01$) and PF/MVF (0.162 ± 0.036 vs. 0.112 ± 0.024, $P < 0.01$) than POW. Twitch pRFD, CT, HRT, as well as Mmax, MEP/Mmax and ballistic performance indicators were similar among groups. Msubmax, H-wave and H/Msubmax could not be compared between groups owing to the different relative stimulation intensities that were used in terms of Msubmax relative to Mmax (see table 2).

Consistency of CCs and assessment of development of fatigue. Within-session ICCs and CVs of MVFs are reported in table 4. All values showed good reliability in both groups and sessions (ICC ≥ 0.903, CV ≤ 0.084), indicating high consistency of CCs. Group-mean MVFs attained for each session during the first and last CCs are depicted in table 5. No statistical differences were reported for each group within sessions, indicating no development of fatigue.

Reliability of ballistic performance indicators. ICCs and CVs of pBRFD and RMS EMG are presented in table 6. Good reliability was reported for pBRFD for both groups (ICC ≥ 0.864, CV ≤ 0.086). Albeit ICCs of RMS EMG demonstrated good reliability (ICC ≥ 0.829), 95% CI, magnitude and distribution of CVs indicate substantial variability in both groups.

TABLE 3. Baseline twitch contractile characteristics, ballistic performance indicators, and evoked potentials P-P recorded from LG. Data are mean ± SD.

	Groups	
Variables	END	POW
Twitch		
PF (N)	216.19 ± 34.10**	167.11 ± 21.07
pRFD (N/ms)	3.66 ± 0.36	3.28 ± 0.62
CT (ms)	123.14 ± 11.95	109.83 ± 23.22
HRT (ms)	114.84 ± 29.85	115.92 ± 28.86
PF/MVF	0.162 ± 0.036**	0.112 ± 0.024
Ballistic contraction		
pBRFD (N/ms)	7.90 ± 2.99	8.66 ± 2.23
RMS EMG	1.23 ± 0.5	1.09 ± 0.55
Evoked potentials		
Mmax (mV)	14.12 ± 6.88	13.99 ± 4.00
Msubmax (mV)	2.29 ± 1.91	1.56 ± 0.77
H-wave (mV)	3.48 ± 2.43	2.75 ± 2.49
H/M submax	1.79 ± 0.53	2.40 ± 2.36
MEP (mV)	0.48 ± 0.34	0.42 ± 0.24
MEP/Mmax	0.04 ± 0.02	0.03 ± 0.02

P-P, peak-to-peak amplitude; END, Endurance-trained Group; POW, Power-trained Group; PF, Peak Force; pRFD, Peak Rate of Force Development; CT, Contraction Time; HRT, Half-Relaxation Time; PF/MVF, Ratio between Twitch Peak Force and Maximal Voluntary Force; pBRFD, Peak Rate of Force Development of the Ballistic Contraction; RMS EMG, Normalized Root Mean Square of EMG from its Onset to the Peak Force of the Ballistic Contraction; Mmax, Maximal M-wave; Msubmax, M-wave preceding the H-wave; MEP, Motor Evoked Potential. ** Significant difference between groups ($P < 0.01$)

TABLE 4. Within-session reliability of MVF of CCs. CV is reported as mean ± SD.

		Reliability Indices	
Groups	Sessions	$ICC_{1,1}$ (95% CI)	CV (%)
END	1st	0.947 (0.866-0.987)	8.4 ± 4.0
	2nd	0.929 (0.793-0.984)	7.9 ± 4.8
POW	1st	0.903 (0.754-0.980)	6.9 ± 5.3
	2nd	0.928 (0.789-0.983)	4.9 ± 2.7

MVF, Maximal Voluntary Force; CC, Conditioning Contraction; CV, Coefficient of Variation; $ICC_{1,1}$, Intraclass Correlation Coefficient, Equation 1,1; CI, Confidence Intervals; END, Endurance-trained Group; POW, Power-trained Group.

TABLE 5. Group-mean MVF for the first and last CCs in each session. Data are mean ± SD

		Groups	
Sessions	CC	END	POW
1st	1st	1384 ± 460 N	1556 ± 475 N
	Last	1424 ± 505 N	1576 ± 343 N
2nd	1st	1466 ± 344 N	1659 ± 276 N
	Last	1484 ± 569 N	1572 ± 333 N

MVF, Maximal Voluntary Force; CC, Conditioning Contraction; END, Endurance-trained Group; POW, Power-trained Group. No significant differences were reported for each group within sessions.

TABLE 6. Within-session reliability of ballistic performance indicators. CV is reported as mean ± SD.

		Reliability Indices	
Variables	Groups	$ICC_{1,1}$ (95% CI)	CV (%)
pBRFD	END	0.943 (0.766-0.988)	8.6 ± 7.4
	POW	0.864 (0.503-0.970)	7.2 ± 6.7
RMS EMG	END	0.844 (0.397-0.971)	13.0 ± 12.4
	POW	0.829 (0.353-0.968)	13.6 ± 14.5

CV, Coefficient of Variation; $ICC_{1,1}$, Intraclass Correlation Coefficient, Equation 1,1; CI, Confidence Intervals; pBRFD, Peak Rate of Force Development of the Ballistic Contraction; RMS EMG, Normalized Root Mean Square of EMG from its Onset to the Peak Force of the Ballistic Contraction; END, Endurance-trained Group; POW, Power-trained Group.

Post-CC twitch temporal profile. Post-CC contractile characteristics (i.e. PF, pRFD, CT, HRT) are reported in fig. 30. Right after CC, PF was potentiated by 1.08 ± 0.13, and 1.30 ± 0.11 from control values, for END and POW, respectively ($P < 0.05$). While PF returned to control value in 1 min after CC for END (1.03 ± 0.07, $P > 0.05$), enhanced PF persisted up to the 5^{th} minute post-CC in POW (1.06 ± 0.05, $P < 0.05$). A significantly greater increase of PF was observed in POW compared with END, at 5 seconds ($P < 0.01$), 1 and 2 minutes ($P < 0.05$) after CC. Twitch pRFD was significantly elevated up to the 6^{th} minute in both groups ($P < 0.05$). The potentiation of pRFD was markedly higher in POW compared to END at 5 seconds post-CC ($P < 0.05$). Twitch CT was shortened in both groups immediately after CC (0.86 ± 0.11 and 0.76 ± 0.11, respectively for END and POW, $P < 0.05$), unaltered instead for the remaining recording span, similarly among groups. Twitch HRT was significantly lengthened ($P< 0.05$) for END at 1 minute post-CC compared to control values (1.08 ± 0.08), and to POW. In contrast, no changes were reported for POW throughout the 10-min period.

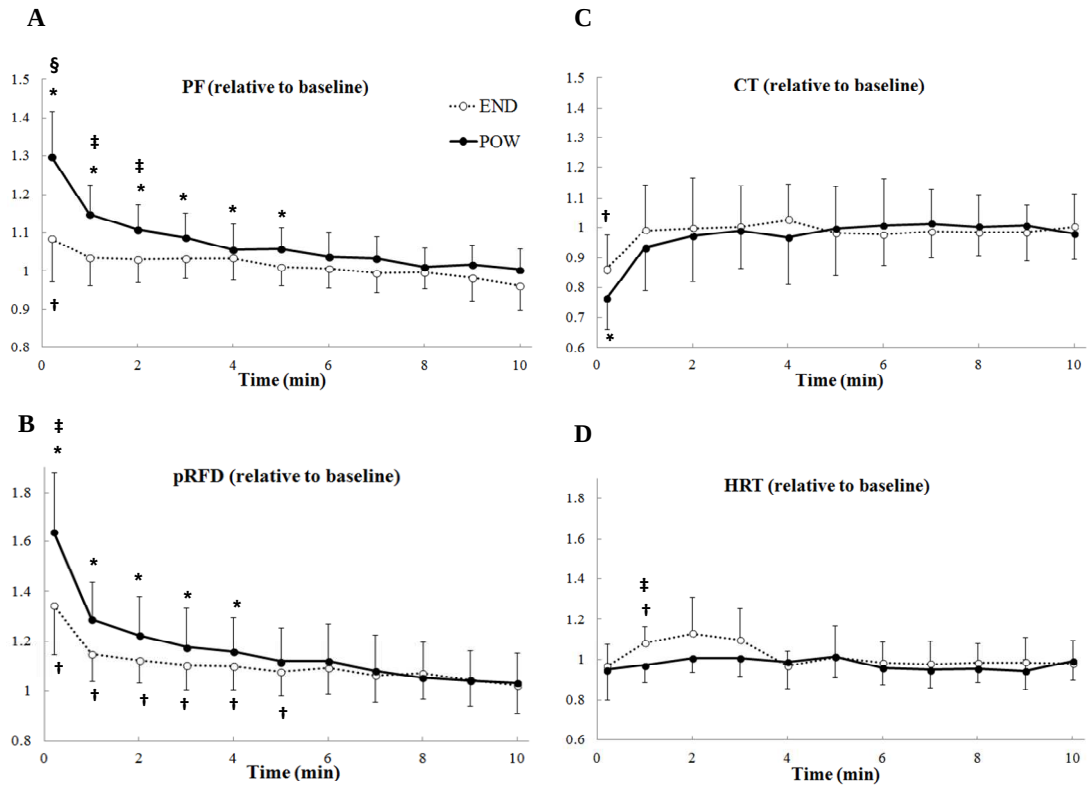

FIGURE 30. Effect of a 8-s CC on twitch peak force (PF; A), peak rate of force development (pRFD; B), contraction time (CT; C), and half-relaxation time (HRT; D), in endurance-trained (END; dashed line and empty circles) and power-trained (POW; continuous line and filled circles) athletes. All data are expressed relative to baseline as mean ± SD. *Significant difference within POW from pre-CC values ($P < 0.05$). †Significant difference within END from pre-CC values ($P < 0.05$). Significant difference between groups (‡$P < 0.05$; §$P < 0.01$)

Post-CC evoked potentials time course. The temporal profiles of Mmax, MEP and MEP/Mmax are depicted in fig. 31, whereas those of Msubmax, H-wave and H/Msubmax are presented in fig. 32. No significant changes were reported in Mmax as well as in Msubmax for both END and POW. No differences were reported in both measurements between groups, although the dissimilar changes in Msubmax closely approached significance immediately after CC ($P = 0.052$). No significant differences were found in the conditioned H-wave when compared both to baseline and between groups. H/Msubmax (CH) was similar ($P > 0.05$) throughout the 10-min post-CC both within- and between-groups. MEP, as well as MEP/Mmax (CM) were markedly increased ($P < 0.05$) at 5 seconds post-CC for both END (2.30 ± 1.12 and 2.27 ± 1.11)

and POW (1.95 ± 0.90 and 1.93 ± 0.89), without statistical group differences throughout the recording span.

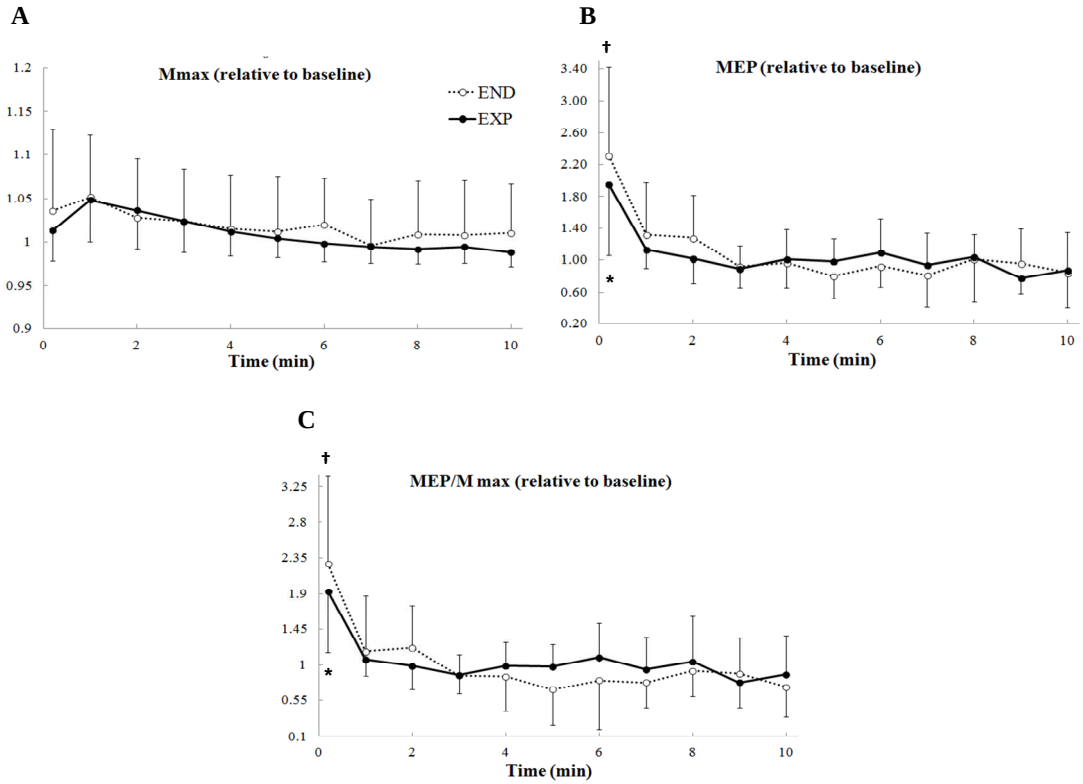

FIGURE 31. Effect of a 8-s CC on the amplitude of maximal M-wave (Mmax; A), motor evoked potential (MEP; B), and motor evoked potential normalized by the maximal M-wave (MEP/Mmax), in endurance-trained (END; dashed line and empty circles) and power-trained (POW; continuous line and filled circles) athletes. All data are expressed relative to baseline as mean ± SD. *Significant difference within POW from pre-CC values ($P < 0.05$). †Significant difference within END from pre-CC values ($P < 0.05$). No significant differences between groups were reported.

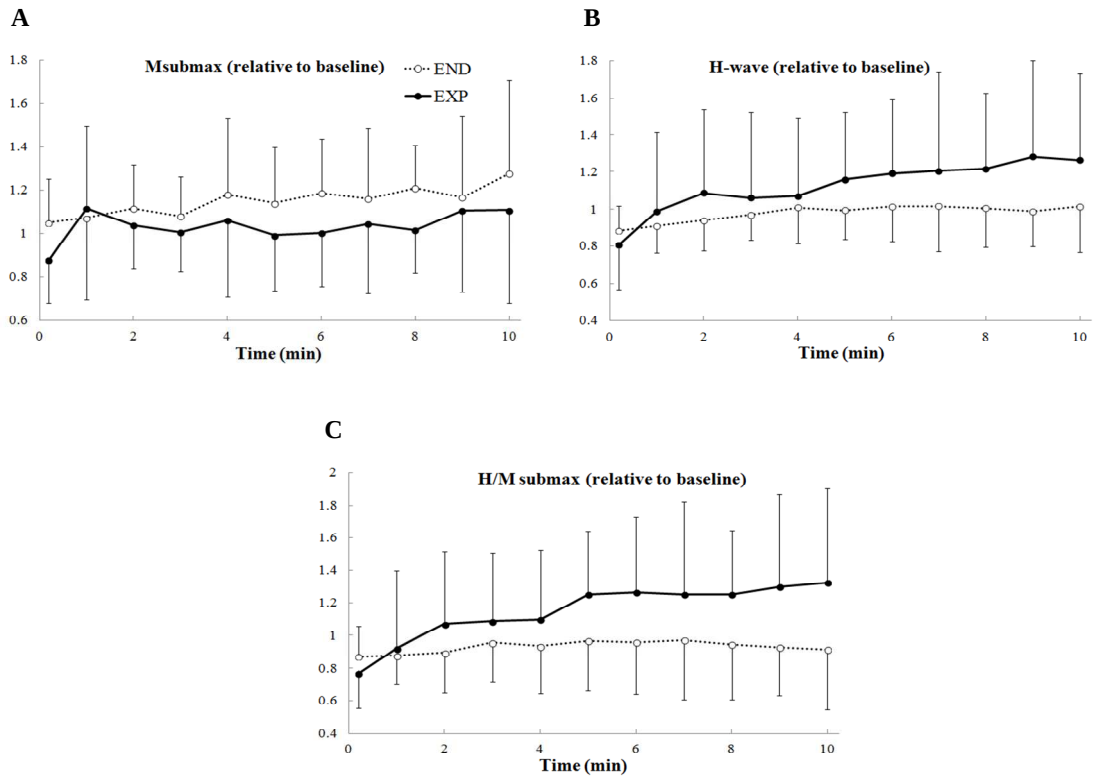

FIGURE 32. Effect of a 8-s CC on the amplitude of the M-wave preceding the H-wave (Msubmax; A), H-wave (B), and H-wave normalized to Msubmax (H/Msubmax; C), in endurance-trained (END; dashed line and empty circles) and power-trained (POW; continuous line and filled circles) athletes. All data are expressed relative to baseline as mean ± SD. No significant differences from baseline or between groups were reported.

Effect of CC on ballistic performance. Ballistic contractions were executed at 5 seconds, 1 minute, 2 minutes, and when CTw peak force, CH, and CM were individually most potentiated, after CC. Based on values obtained from the 1st session, CTw peak force attained the greatest enhancement immediately post-CC (5 seconds) in all participants. Similarly, CM was consistently most potentiated at 5 seconds post-CC within the sample, albeit 1 participant in each group reported the peak enhancement at delayed time points, i.e. 2 and 6 minutes in END and POW, respectively. In contrast to CTw and CM, CH showed high inter-subject variability among participants, as outlined from no clear observable trends from fig. 32. CH peak enhancement was attained in the range 5 seconds - 9 minutes and 3-10 minutes for END and POW, respectively (table 7).

TABLE 7. Range of time elapsed after the conditioning contraction whereas CTw, CM and CH attained their peak values.

Groups	Measurements		
	CTw	CM	CH
END	5 s	5 s - 2 m	5 s - 9 m
EXP	5 s	5 s - 6 m	3 m - 10 m

CTw, Conditioned Twitches; CM, Conditioned MEP/Mmax; CH, Conditioned H/Msubmax; END, Endurance-trained Group; POW, Power-trained Group. Note that, all participants attained the greatest twitch peak force potentiation 5 seconds after the conditioning activity.

TABLE 8. Performance indicators of ballistic contractions performed at arbitrarily considered optimal time points determined from CH and CM. All data are expressed relative to baseline as mean ± SD.

Measurements	Groups	Ballistic performance indicators	
		pBRFD	RMS EMG
CM	END	0.954 ± 0.103	1.116 ± 0.161
	EXP	1.187 ± 0.364	1.166 ± 0.371
CH	END	1.007 ± 0.067	1.143 ± 0.357
	EXP	1.011 ± 0.109	0.967 ± 0.177

CM, Conditioned MEP/Mmax; CH, Conditioned H/Msubmax; END, Endurance-trained Group; POW, Power-trained Group; pBRFD, Peak Rate of Force Development of the Ballistic Contraction; RMS EMG, Normalized Root Mean Square of EMG from its Onset to the Peak Force of the Ballistic Contraction. No significant differences from baseline or between groups were reported.

Changes in pBRFD and RMS EMG of ballistic contractions during the first 2 minutes of PAP time course are presented in fig. 33, whereas 5 seconds post-CC represents also the time point when CTw attained the greatest potentiation (see table 7). No significant differences were reported in END for both pBRFD and RMS EMG. Within POW, no statistical potentiation was found for pBRFD (although the p-value closely approached significance 1 minute post-CC, $P = 0.069$), whereas RMS EMG showed a significant depression at 2 minutes post-CC ($P < 0.05$). Between-group differences were significant

only for RMS EMG, as POW showed a significant depression compared to END at 1 minute after CC ($P < 0.05$). Notably, 5 seconds after CC, the potentiation of pBRFD of POW in relation to END attained a p-value of 0.093, lessening thereafter ($P \geq 0.208$). When contractions were performed at arbitrarily considered optimal time points determined from CH and CM, neither pBRFD nor RMS EMG reported any difference compared to baseline or between groups (table 8).

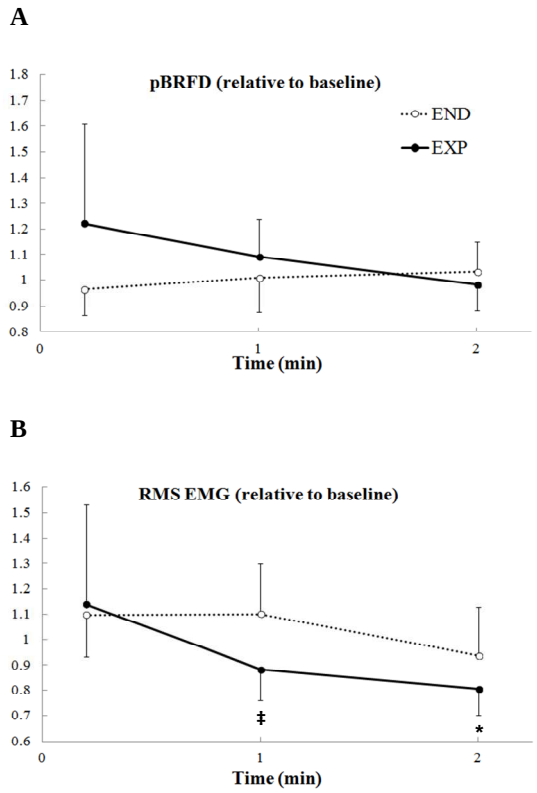

FIGURE 33. Effect of a 8-s CC on ballistic performance of the endurance-trained (END; dashed line and empty circles) and power-trained (POW; continuous line and filled circles) group. A) Peak rate of force development (pBRFD). B) Normalized root mean square of EMG from its onset to the peak force of the ballistic contraction (RMS EMG). All data are expressed relative to baseline as mean ± SD. *Significant difference within POW from pre-CC values ($P < 0.05$). ‡Significant difference between groups ($P < 0.05$). No significant differences within END from pre-CC values were found.

7 DISCUSSION

The purpose of the current study was to examine whether neural factors contribute to PAP, and to potentiation of voluntary ballistic performance. PAP was induced by a 8-sec maximal voluntary isometric plantarflexion, and the neuromuscular system was assessed thoroughly, enlarging our understanding from merely muscle level. To additionally fulfil the scope of our research, we compared endurance- and power-trained groups, the latter being speculated to benefit in ballistic performance from PAP due to neural mechanisms (Güllich & Schmidtbleicher, 1996). Only non-significant differences were found when comparing ballistic performance potentiation within and between groups, concurrent to a decrease in neural drive in such actions in POW. While markedly higher enhancement was reported in supramaximal twitches peak force in POW compared to END up to 2 minutes after CC, evoked potentials were similarly altered within the latter time period. In light of these results, we below discuss that PAP includes both enhanced muscle contractile characteristics and short-term facilitation within the corticospinal pathway, the former being the discriminating factor for POW being more incline to exploit PAP in ballistic performance than END.

7.1 Control measures and MVFs

In the current study, no significant differences in maximal voluntary strength and pBRFD of ballistic contractions were found among groups. Conversely, previous studies comparing plantar flexion maximal isometric strength reported higher values in power- compared to endurance-trained athletes (Kyröläinen & Komi, 1994; Pääsuke et al., 1999). In our results mean values were considerably higher in POW in both MVF and pBRFD, and the lack in statistical significance can be mainly attributed to the high standard deviation (i.e. heterogeneity) within the groups.

In addition, from our measurements END showed statistically higher twitch PF than POW. To our knowledge, this is the first investigation reporting this result. Indeed, previous research, exclusively focusing on neuromuscular differences in plantarflexor muscles contractile characteristics between the above-mentioned groups, showed a

converse outcome, that is twitch PF being higher in power-trained rather than endurance-trained athletes (Pääsuke et al., 1999; Maffiuletti et al., 2001; Lattier et al., 2003). Our result was unexpected, and deemed due to mainly two factors. Firstly, and most importantly in our opinion, a discrepancy between the level of the athletes of the two groups. More athletes within END trained for competitions, and at a higher level, compared to POW. Unfortunately, for the latter group we were not able to find high-level athletes whereas performance exclusively relied on explosive power output (i.e. sprinters, long and high jumpers), as previous studies did (Pääsuke et al., 1999; Maffiuletti et al., 2001; Lattier et al., 2003), bringing up a considerable limitation. Secondly, we measured muscular responses using a single supramaximal twitch. Single stimulus was chosen to better quantify changes in EC coupling across PAP timeline, without being affected from saturation processes (ceiling effects) of either Ca^{2+} or actin-myosin binding, which might arise using repetitive stimuli (Baudry & Duchateau, 2004; Baudry et al., 2005), and interfere with the quantification of muscular PAP. On the other hand, single muscle activation might be less indicative of muscular characteristics than repetitive muscle activation (e.g. doublet), mainly due to the slack in the series elastic components of the muscle, and to the scarce release of Ca^{2+} within the myoplasm (Parmiggiani & Stein, 1981; Duchateau & Hainaut, 1986; Baudry & Duchateau, 2004). Indeed, we are aware of one research (Garrandes et al., 2007) that reported no statistical differences between POW and END in any of the contractile parameters (i.e. PF, pRFD, CT and HRT) when single supramaximal twitches were elicited from knee extensors, yet, PF being significantly higher in POW when doublets were evoked. In light of this, we cannot rule out the slack in the series elastic components and the scarce release of Ca^{2+} as contributing to our results. It is noteworthy to mention that maximal Ca^{2+} release rate was shown to be substantially higher in resistance- compared to endurance-trained athletes (Li et al., 2002). This diversity was consistent with the higher percentage of Type II fibres in the former athletes, having Type II fibres a considerable higher Ca^{2+} release rate than Type I fibres (Ørtenblad et al., 2000, Li et al., 2002). Bearing in mind the steeper twitch PF potentiation in POW compared to END reported in our study, suggesting higher Type II fibre content in the former group (Moore & Stull, 1984; Hamada et al., 2000), the incomplete release of Ca^{2+} resulting from single twitches should have hindered END twitch PF to a higher extent than POW, contrary to what we found. Consequently, it seems likely that only the discrepancy between the level of the athletes of the two groups, and relegated to a minute extent the slack in the

series elastic components, might play a role in the group difference in twitch PF we found.

When PF/MVF is considered, lower values for POW compared to END were reported. This is in accordance with other studies regarding both plantar flexors (Pääsuke et al., 1999) and knee extensors (Hamada et al., 2000a; Pääsuke et al., 2007). The PF/MVF ratio was shown to be lower in fast compared to slow skeletal muscles of mammals (Close, 1967; Eken & Gundersen, 1987), and in human muscles with a greater percentage of type II fibres (Hamada et al., 2000a). Furthermore, a higher value of this ratio has been successfully reported in concomitance of loss of Type II fibres and slowing of the contractile properties in elderly compared to young adults (Baudry & Duchateau, 2005). Power-trained athletes exhibited markedly higher fast isomyosin composition of LG and SOL compared to the endurance-trained counterpart (Harridge et al., 1995), explaining why in our study and in Pääsuke et al. (1999), the PF/MVF relation was lower in POW compared to END.

Taken together, the greater mean of pBRFD, MVF, and the lower PF/MVF of POW than END exhibited in our study, suggest that the groups underwent respectively power- and endurance-training background-related adaptations.

7.2 Twitch postactivation potentiation

According to previous research (Vandervoort et al., 1983; Baudry & Duchateau, 2004; Hodgson et al., 2008), the current study shows potentiation of twitch kinetics after CC, and its characteristic exponential decline of PF and pRFD over time (Baudry & Duchateau, 2004). In addition, as reported from studies regarding both plantar flexors and knee extensors (Pääsuke et al., 1998; Pääsuke et al., 2007), power-trained athletes exhibited markedly higher enhancements in twitch PF and pRFD than endurance-trained athletes.

The degree of twitch kinetics potentiation in both groups in the current investigation is not directly comparable with other studies for mainly two reasons. Firstly, to the authors knowledge, only three studies examined dissimilarities in the extent of twitch PAP between groups with different chronic training background (Pääsuke et al., 1998; Hamada et al., 2000b; Pääsuke et al., 2007). However, these researches either did not

include a specifically power-trained group (Hamada et al., 2000b), took into consideration a different muscle group (i.e. knee extensors, Pääsuke et al., 2007), or presented differences in the protocol (Pääsuke et al., 1998). Secondly, investigations regarding PAP in plantar flexors have used either different ankle and knee joint configurations, number and duration of CCs, or time interval between the end of CC and the first supramaximal stimulus (e.g. Vandervoort et al., 1983; Pääsuke et al., 1998; Shima et al., 2005; Hodgson et al., 2008; Sasaki et al., 2011; Fukutani et al., 2014; Gago et al., 2014; Xenofondos et al., 2014). Both the molecular mechanisms of fatigue and potentiation (albeit distinct in nature), and the degree of their coexistence are dependent on contractile history and muscle length (Rassier, 2000; Rassier & MacIntosh, 2000). Hence, the extents of twitch PAP from previous studies is not directly comparable with ours.

An emerging observation of researches comparing male (Pääsuke et al., 1998) and female (Pääsuke et al., 2007) power-trained to endurance-trained athletes is the greater degree of twitch PF and pRFD potentiation resulting in association with the former training background compared to the latter. Our results abide by this finding, with twitch pRFD and PF enhancement being statistically higher in POW compared to END respectively at 5 seconds and up to 2 minutes post-CC. Although fibre type distribution in our sample was not directly measured, differences in baseline values between groups (see “Control measures and MVFs”) suggest a higher content or selective hypertrophy of type II fibres in POW than END, in accordance with chronic adaptations from strength and power training (Alway et al., 1988). Parallely, type II fibres present a higher and lower extent respectively of phosphorylation and dephosphorylation of RLC compared to type I fibres, ascribed to greater MLCK and lower myosin light chain phosphatase activities (Moore & Stull, 1984). Being RLC phosphorylation the primary accepted mechanism responsible of PAP (Moore & Stull, 1984; Stull et al., 2011), muscles with a higher percentage of type II fibres exhibit greater isometric twitch potentiation, experimentally verified in muscles of both small mammals and humans (Vandervoort & McComas, 1983; Moore & Stull, 1984; Westwood et al., 1984; Hamada et al., 2000a). Merging the afore-mentioned factors together, higher potentiation of twitch PF and pRFD after CC in POW compared to END, as in our study, can be explained.

In accordance with other reports, twitch CT was significantly reduced 5 seconds after CC (Hamada et al., 1999), with no significant differences between POW and END (Pääsuke et al., 1998). The decrease in CT might most likely be ascribed to the same molecular mechanisms involved in PAP (Hamada et al., 2000a; Stull et al., 2011).

Twitch HRT was slowed 1 minute after CC only for END, both related to control value and to POW. The relaxation phase mainly involves two mechanisms, namely cross-bridge detachment, and Ca^{2+} handling for re-uptake into the SR (Jones et al., 2006; Allen et al., 2008). Slowing of either processes leads to prolonged relaxation phase, whose consequentiality upon muscular fatigue has been commonly observed (Bigland-Ritchie &Woods, 1984, Fitts, 1994; Westerblad et al., 1997; Allen et al., 2008). The well-accepted effect or RLC-P in PAP is an increased fraction of cross-bridges in the force generating state (Sweeney & Stull, 1990; Stull et al., 2011), beneficial at sub-optimal Ca^{2+} activation for cross-bridge force generation. However, consequences of PAP on the relaxation phase are still unclear (Stull et al., 2011; Vandenboom et al., 2013). Being PAP a coexistence of potentiating and fatiguing mechanisms (Rassier & MacIntosh, 2000), slowed relaxation time of contractile responses would be expected to result from fatigue, as (to the authors knowledge) no investigations have reported a reasoning for modified HRT in supramaximal twitches from potentiating mechanisms alone. Yet, in our study, it seems unlikely that END was selectively fatigued. This assertion is supported from the following experimental evidence. As type I fibres exhibit lower fatigability than type II fibres (Gordon et al., 1990), and as endurance athletes are known to have higher percentage of type I fibre than the power counterpart (Gollnick et al., 1972; Harridge et al., 1995), higher resistance to fatigue in END compared to POW would be expected. This relation between fibre type distribution and fatigability has also been concluded in Hamada et al. (2003), whereas the quadriceps of participants with higher percentage of type I fibres exhibited concurrently both lower potentiation and fatigue compared to the quadriceps of those with higher type II fibres content. These evidences are in contrast to what we found, making very unlikely that END was selectively fatigued. We can give no explanation for the lengthened HRT only in END. However, we cannot rule out the possibility of contribution to PAP by factors other than or connected to RLC-P (Rassier et al., 1999; Rassier & MacIntosh, 2000), involved in the relaxation phase and that, similar to RLC-P, differentially acting on type

I and type II fibres. In this regard, more research is needed to explore secondary mechanisms to potentiation in muscles (Vanderboom et al., 2013).

Finally, it seems unlikely that changes in passive tensile components properties from CC have played a role in altering potentiated contractile responses. It has been recently reported that Achilles tendon stiffness was not modified after a MVC in plantar flexors to induce PAP (Gago et al., 2014). Although the latter research only included power athletes, as in the current study twitch PF, pRFD, and CT exhibited no between-group differences in the pattern of potentiation, it can be speculated that likewise POW, END underwent no changes in Achilles tendon stiffness across PAP timeline.

7.3 Modulation of evoked potentials

Following. the 8-s CC, both Mmax, Msubmax, H-wave and H/Msubmax reported no significant changes compared to control values. Only MEP and MEP/Mmax of both groups were potentiated, immediately after CC.

In our investigation, potentiation appeared not to alter neuromuscular propagation, as confirmed by no alterations from baseline in both Mmax and Msubmax. Our results are in disagreement with previous studies, which found significant increases in Mmax recorded from LG (Sasaki et al., 2012; Xenofondos et al., 2014). The exact reason of this dissimilarity is hardly identifiable, as different groups of participants and duration of contractions were used among protocols.

Similarly to Mmax and Msubmax, CC had neither potentiating nor depressing effects for both POW and END on H-wave and H/Msubmax. Previous studies have reported both changed (Güllich & Schmidtbleicher, 1996; Trimble & Harp, 1998; Folland et al., 2008; Xenofondos et al., 2014) and unchanged (Hodgson et al, 2008; Iglesias-Soler et al., 2011) post-exercise H-reflexes magnitude. However, either the muscle group under investigation or the conditioning stimulus for inducing PAP were substantially different than our experimental conditions. The closest comparable study to the current investigation, Xenofondos et al. (2014), reported a significant depression of H-reflexes recorded at rest in LG immediately after a 10-second CC in healthy subjects, whereas no changes occurred thereafter. The lack in H-reflexes depression from our results might be attributed to dissimilar participants' background and shorter CC duration.

When considering the immediate effect of previous activation on H-reflexes, great consideration must be given to postactivation depression, as it substantially affects the outcome (Crone & Nielsen, 1989; Hultborn & Nielsen, 1998; Pierrot-Deseilligny & Burke, 2012, 85). In this regard, measuring H-reflexes over a background contractile level (e.g. 15-20 % MVC) would greatly avoid the effect brought by postactivation depression (Stein et al., 2007). Indeed, the recent report of Xenofondos et al. (2014) underlined the necessity of a low-intensity background muscle activity when capturing H-reflexes directly after CC to reduce the effect of postactivation depression on outcome. When collecting H-reflexes across PAP timeline, a constant level of EMG activity was also maintained in the study conducted by Hodgson et al. (2008) to decrease variability of H-reflexes and increase control over the recording conditions. In our study, we opted for evoking H-reflexes at rest for mainly two reasons. Firstly, we avoided the influence that background activity could have on subsequent H-reflex recordings, as it would have modified the mere PAP effect from CC. This was an important consideration in our protocol as timing of ballistic contractions was chosen based upon the magnitude of the evoked potentials, and experimental conditions were to be kept as constant as possible between testing sessions. Secondly, it was unknown whether background activity, and the resulting mechanisms outweighing postactivation depression (Hultborn & Nielsen, 1998; Pierrot-Deseilligny & Burke, 2012), could affect differently POW and END. From our study we can anyway conclude, with reference to H-wave and H/Msubmax, that reflex transmission between Ia afferents and motor neurons was unchanged from the conditioning activity in both groups.

Differently to the afore-mentioned evoked potentials, MEP and MEP/Mmax were significantly increased directly after CC in both POW and END, with no between-group differences. MEP short-term facilitation after brief muscle activation found in our study is in agreement with previous reports (Samii et al., 1996; Nørgaard et al. 2000; Balbi et al., 2002). The primary mechanisms of this post-exercise MEP facilitation have been found to be located at the cortical level (Samii et al., 1996; Nørgaard et al. 2000; Lentz & Nielsen, 2002), manifesting as increased net excitatory output evoked from the motor cortex by the magnetic stimulus (Taylor et al., 2000a). In addition, enhanced corticospinal transmission immediately after brief MVCs, presumably at the corticospinal pathways-motor neuronal synapses (Giesebrecht et al., 2010), might play a role.

Yet, interpreting the MEP as an index of the responsiveness of the pathway from brain to muscle (i.e. subject to potential modulation at each point in the pathway) (Carroll et al., 2011), attempts to distinguish spinal and cortical contribution in MEP facilitation in POW and END, given our experimental approach, would be inappropriate for three reasons. Firstly, H-reflexes should not be used to represent changes in motor neurons pool excitability, due to oligosynaptic contribution, presynaptic inhibition and postactivation depression involved with the afferent volley (McNeil et al., 2013). Albeit participants position and recording conditions were carefully controlled, we might not exclude pre- versus post-CC changes in presynaptic inhibition and oligosynaptic contribution, and we are certain that postactivation depression shaped the H-reflex response after contraction (Xenofondos et al., 2014). These factors, all together, make difficult to draw conclusions about the excitability of plantarflexors motor neurons and their intrinsic membrane properties. Secondly, a magnetic stimulus traverses different synapses from the H-reflex. Thirdly, the fraction of motor neurons pool excited by peripheral and transcranial stimulation might be different, as deductible from the diversity in amplitude of H-waves and MEPs (see table 3). These considerations make MEP and H-reflex further incomparable.

In light of these considerations, using different stimulation techniques and stimulation paradigms to localize spinal or cortical mechanisms in PAP is advisable. For instances, CMS (Ugawa et al., 1991), which is known to be comparable with TMS because activating the same population of motor neurons (Taylor et al., 2002), or preferably the use of conditioned CMS by TMS (McNeil et al., 2009), would allow to account for changes in motor neurons responsiveness during PAP timeline. Therefore, further studies are needed to clearly divide the effect of PAP into spinal and supra-spinal compartments.

From our experiment, one main tract emerges though, i.e. facilitation of MEP is confined within the CNS, without any influence from altered neuromuscular propagation. Although peripheral stimulation (which has been used for M-wave measurements) might activate a different portion of motor neurons than MEP (as evident from the nature of the technique and the difference in amplitude of the evoked potentials, see table 3), the absence of changes in both Mmax and Msubmax, and the

persisted potentiation when MEP is normalized to Mmax, indicates the confinement of MEP short-term potentiation within the CNS.

Caution must be taken when interpreting MEP facilitation merely as enhancement of corticospinal pathways transmission, as the conditioning activity might lead to coexisting potentiating and fatiguing phenomena. Specifically, the considerable short-term MEP facilitation 5 seconds after CC might have obscured any manifestation of MEP depression resulting from fatigue. In this regard, it is difficult to claim a role of short-term increased excitability of corticospinal pathways in PAP, as additional measurements such as SP or paired TMS stimuli are needed in future studies to address coexistence of potentiation and fatigue (in form of cortical inhibition) from CC.

However, despite the unquantifiable role that inhibition might have played, no differences in MEP and MEP/Mmax facilitation were found between the two groups. Considering this, although MEP is the result of a sum of events at different cortical and spinal synapses (Petersen et al., 2003), it can be speculated that central pathways were affected likewise in POW and END from CC.

7.4 Postactivation potentiation and ballistic contractions

When ballistic performance was considered, CC had no statistical influence on pBRFD in both groups. However, RMS EMG of POW was significantly depressed at 1 minute post-CC, relative to END, and at 2 minutes post-CC, relative to control values.

The selective decrease of RMS EMG in POW might be explained from a greater susceptibility to neural fatigue from CC, of power- compared to endurance-trained athletes. From our data, lower PF/MVF, higher average MVF and twitch potentiation suggested higher type II fibre content in the plantar flexors in POW than END. According to previous studies (Komi & Tesch, 1979; Hamada et al., 2003), fatigue hinders neural drive and voluntary performance more rapidly in muscles composed of a higher proportion of type II fibres than in muscles composed of a higher proportion of type I fibres. It is therefore plausible that neural fatigue might have affected POW to a greater extent than END as after-effect from CC, impairing neural drive in the subsequent ballistic performance. The following observation though are worth to be mentioned. First, as depicted in table 6, RMS EMG reported substantial variability in

both groups, especially in absolute reliability (CV), whose consistency is fundamental when pre- and post-conditioning intervention values are compared, as in our protocol. This lack in good absolute reliability is a characteristic of EMG when used as a measure of neural drive in ballistic contractions (Buckthorpe et al., 2012), presumably due to the short window length available for EMG measurement and the intrinsic variability of the rapid actions. Second, EMG from only LG was included in our analysis, as evoked potentials were recorded from this muscle. However, EMG activity from solely LG might not be fully representative of a ballistic performance whereas force output comes from the whole triceps surae activity. Third, changes in signal amplitude from surface EMG might not exactly reflect altered levels of neural drive to the muscle. This incongruity resides on signal amplitude cancellation (Farina et al., 2004) that might arise. Given these observations, caution must be taken when interpreting lower RMS EMG as merely decreased or impaired neural drive to execute ballistic actions.

Previous investigations reported inconsistent results of the effect of PAP on ballistic performance, with increased (Baudry & Duchateau, 2007), unchanged (Folland et al., 2008), or decreased (Smith et al., 2014) pBRFD. When enhanced pBRFD was found (Baudry & Duchateau, 2007), as ballistic voluntary and electrically-induced contractions exhibited the same extent of potentiation, PAP was not related to neural mechanisms. When pBRFD was found to be depressed (Smith et al., 2014), central inhibition was ascribed as the primary cause, as EMG as a measure of neural drive in ballistic actions was decreased in parallel. In our study, pBRFD was statistically unchanged from CC. The lack of significance might ensue from the heterogeneity within groups, the fact that participants in POW were not high-level athletes, and the quite-low sample size included in the study. However, a trend toward significance was found when groups were compared 5 seconds after CC ($P = 0.093$), alluding to higher conditioned pBRFD in POW than END, and within POW 1 minute after CC ($P = 0.069$), suggesting a potentiating effect of the latter on ballistic performance exclusively in the power-trained group. In this regard, we are well-aware that non-significant p-values should not be used to infer causal relationships.

Notwithstanding, one particular trait emerges from our data. At the time points reported above, whereas neural values were either unchanged (Mmax, Msubmax, H-wave, H/Msubmax) or potentiated (MEP, MEP/Mmax) likewise in both groups, twitch PF and

pRFD were the only measures which resulted differentially potentiated, with greater enhancement in POW than in END. This might suggest a main role of muscular rather than neural factors in power athletes' capacity of exploiting PAP for ballistic performance enhancement. Considering this concept from another point of view with our data, impaired neural drive in POW in ballistic actions as a result of neural fatigue from CC, could have led to decreased pBRFD if considerable muscular potentiation had not occurred. Therefore, power-trained are more vulnerable than endurance-trained athletes to neural fatigue from CC, but if attention is paid to minimize such impairment, muscular factors might play a great role in potentiation of subsequent performance, which would not occur in endurance athletes. In addition, the lack of between-group differences found in ballistic performance indicators when CM and CH reached individually the maximum value, might suggest changes in excitability of spinal and corticospinal neural pathways not being functionally influential in POW compared to END for exploiting PAP. Our conjecture of the primary role of muscular potentiation is in accordance with earlier studies (Baudry & Duchateau, 2006; Baudry & Duchateau, 2007; Bergmann et al., 2013). Baudry & Duchateau (2006; 2007) indirectly excluded neural contribution to PAP, for conditioned ballistic voluntary and electrically-induced contractions exhibited the same extent of pBRFD potentiation. More recently, Bergmann et al. (2013) reported increased rebound height in drop jumps (conditioned with two-legged explosive hopping), significantly related with concomitant potentiated muscular twitches, with unchanged EMG activity.

Yet, inference based upon comparison of evoked potentials between POW and END, might present limitations. Indeed, evoked potentials measured at rest represent the default characteristics of the pathway under investigation (Carroll et al., 2011), and conjectures from them should be cautious, since they might be poorly linked to functional tasks (Taylor et al., 2000b; Carroll et al., 2011). However, the lower RMS EMG in POW reinforces the view of a primary role of muscular factors in performance enhancement in PAP. Other reports studying neural drive to muscles after a strong CC showed that less EMG is produced during subsequent ballistic (Smith et al., 2014) and submaximal (Petersen et al., 2003) efforts, the latter study however being the only one specifically addressing the effect of short-term neural plasticity on voluntary movement. Further investigations is therefore needed to explore causal relationships between

changes in neural pathways excitability from maximal conditioning activity, regulation of descending input to muscles, and ballistic performance output.

7.5 Limitations of the study

In our experimental approach, the nature of our study, time constraints and the techniques used reported some methodological drawbacks that must be taken into consideration when interpreting the results. First, as previously mentioned, we were not able to find high-level power-trained athletes. Their high type II fibre content would have additionally marked differences in both muscular potentiation and presumaby pBRFD enhancement between POW and END. Second, only one ballistic contraction was performed subsequent to each CC at each time point. Multiple contractions in one trial could confound the mere PAP effects. As 20 minutes of rest were necessary between CCs, and considering the trials disregarded for countermovements, only 4-5 conditioned ballistic contractions could be collected in the second session (i.e. 5 second, 1 and 2 minutes post-CC, at CTw, CM and CH). However, indices of reliability of ballistic performance (table 6) were similar to those previously reported (Buckthorpe et al., 2012), indicating the participants being acquainted with such actions. Third, different portions of the motor neurons pool have a functional role in evoked potentials and ballistic contractions, such that timing the latter on the basis of the former might be misleading. Both TMS and H-reflexes are known to recruit MUs in an orderly fashion (Bawa and Lemon, 1993; Buchthal & Schmalbruch, 1970), from slowest to fastest. This means that the output of evoked potentials involves to a major extent the neurophysiological characteristics of slow MUs. Differently, although being the size principle still followed in ballistic contractions (Desmedt & Godaux, 1978), such actions primarily rely on fast MUs for rapid force development. This might thus represent a drawback in using evoked potentials for timing the execution of ballistic performance, as different MUs might be targeted. In addition, still with reference to MEPs, we are aware that evoking them over a background activity could allow collecting measures of intracortical inhibition such as SP, useful to better define coexistence of neural potentiation and fatigue in PAP, the latter in form of intracortical inhibiton. However, we opted for evoking MEPs at rest for the same reason as H-reflexes, i.e. to avoid modifying the mere PAP effect from CC on subsequent MEP

recordings. The last, notable factor is the specificity of CC to the subsequent ballistic action. Closely matching the nature of both tasks (e.g. drop jumps conditioned by two-legged explosive hopping) is important for exploiting PAP in performance (Bergmann et al., 2013; Kümmel et al., 2014). We might not exclude that the qualitative difference between CC and the subsequent ballistic action in our study masked the transfer of the potentiating effect of the former on the latter. Indeed, considerable muscular potentiation is needed when MVCs are used to enhance subsequent ballistic actions (Baudry & Duchateau, 2006), and neural fatigue is more likely to occur.

7.6 Conclusions

In conclusion, the results from this study indicate that PAP was characterized from enhanced muscle contractile characteristics as well as short-term facilitation of corticospinal excitability. When power-trained were compared to endurance-trained athletes, the former group benefited from PAP in triceps surae muscles only in terms of higher potentiation of muscular contractile characteristics. Alterations in neural pathways do not seem to play a discriminating role in PAP, as potentials evoked from the Ia afferent - α motor neurons and corticospinal pathways were affected likewise in POW and END from the conditioned activity, and as neural drive, if affected, might be depressed in the power-trained group rather than enhanced, presumably due to neural fatigue from the conditioning activity. Although the individual contributions of increased corticospinal excitability and muscle contractile characteristics to performance enhancement could not be individually quantified in our study, differences between groups in exploiting PAP in ballistic actions might be primarily related to muscular potentiating mechanisms, although only non-significant potentiation was found in ballistic performance in our experiments. It is difficult to extend findings from carefully controlled lab-setting to practice, and based on the current knowledge neural factors cannot universally be excluded from playing a role in PAP. Further studies are needed to address this topic, possibly including high-level athletes, multi-joint exercises, conditioning contractions which match the nature of the performance to be enhanced, and using stimulation techniques in a functional context, whereas they might better explain the role of the nervous system in such situations. The fulfilment of these

criteria, although very challenging, would allow to be closer to whom might benefit from these investigations and to performance requirements.

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7 APPENDICES.

7.1 Appendix 1 – Informed consent

JYVÄSKYLÄN YLIOPISTO
UNIVERSITY OF JYVÄSKYLÄ

UNIVERSITY OF JYVÄSKYLÄ/ ETHICAL COMMITTEE

INFORMATION SHEET FOR RESEARCH SUBJECTS AND CONSENT TO PARTICIPATE IN RESEARCH

Name of the study:

IMPLICATION OF NEURAL MOTOR PATHWAYS EXCITABILITY IN RATE OF FORCE DEVELOPMENT OF BALLISTIC CONTRACTIONS DURING POSTACTIVATION POTENTIATION.

Contact information of researchers

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Patricio Andres Pincheira Miranda, Han Yu Wu, Jenni Vähämaa.

Research background information

You are being asked to volunteer for a research study because you are healthy, with either an explosive-type or endurance-type sport background. Please read the following paragraphs carefully. If you have any questions or concerns regarding participation in this study, you are encouraged to raise these concerns with the investigators.

The investigator in charge of this study is Luca Ruggiero. Luca Ruggiero is presently a student in the MSc in Biomechanics in the Department of Biology of Physical Activity in the University of Jyväskylä. This study has been devised to fulfil the attainment of the Master Degree.

Purpose, target and significance of the research

The purpose of this study is to determine how muscular characteristics and excitability of neural motor pathways affect rate of force development in rapid contractions during postactivation potentiation. Potentiation in muscular contractile characteristics will be induced through an 8-second maximal voluntary contraction (MVC). For quantification of muscular characteristics and neural excitability, two forms of stimulation will be employed, namely, peripheral nerve stimulation and transcranial magnetic stimulation (TMS). In the former, a weak electrical current will be induced into the peripheral nerve, whereas in the latter, a weak current will be induced in the brain area responsible for the active movement of the lower leg.

The current will activate the neural tract beneath the site of stimulation. Peripheral nerve stimulation and TMS will be presented prior to and after an 8-second MVC, to monitor changes in excitability in the different activated neural loci. The study consists of two

sessions, separated from at least 48 h. In the first session, muscular characteristics and neural motor pathways excitability will be measured both before and after the 8-second MVC, using peripheral nerve stimulation and TMS respectively in different trials. Responses will be monitored up to 10 minutes following the MVC. In the second session, rate of force development of rapid contractions both before and after the MVC will be measured. Data obtained through your participation may help to gain an insight about phenomena involved in performance output increases as a consequence of a potentiating method (e.g. MVC).

Purpose of use, handling and storage of research data

Research data will be used only and exclusively for the research investigation purpose. Data will be stored in the Hard drive of the computer inside the lab, and in a USB memory stick. After the completion of all the tests and data analysis, namely about 8 months, data will be removed from the afore mentioned computer. However, data will be saved in a protected folder for further analysis and considerations. Anonymity will be preserved in all the aforementioned passages.

Procedures targeted to the research subjects

Approximately 16 subjects will participate in the current study. Eligibility to participate has been chosen with the following criteria: i) be between the ages of 18 and 30, ii) having an explosive-type or endurance-type sport background, iii) be able to provide informed written consent, and iv) eligible from a TMS safety screening .

Participants in this study are recruited through emails within email lists, advertisements, and personal acquaintances.

The following paragraph relates to the description of the study procedures. If you agree to be in this study, the following will happen:

i) You will take part in 2 separate sessions, whose time and schedule will be agreed according to your availability. All the measurements will be conducted in the Biomechanics lab, Viveca building, Rautpohjankatu 8. Participants will meet in the main entrance of Viveca with Luca Ruggiero when agreed.

ii) **Session 1**: Participants will be asked to read and sign the informed consent. Additionally, a safety screening for TMS will be presented. All the following procedures will be explained during and throughout the measurement session by the investigators prior to their application. If you have any questions or concerns regarding the protocol, you are encouraged to raise these concerns with the investigators. Surface Electromyography electrodes will be placed on the posterior surface of

the lower leg on muscles gastrocnemius lateralis (GL) and soleus (SOL), and on the anterior surface of the lower leg on tibialis anterior (TA). Furthermore, electrodes for peripheral nerve stimulation will be positioned above the patella and in the popliteal fossa behind the knee, according to the procedures used in previous studies. Participants will be then asked to wear a swimming cap, for better placement of the TMS stimulating coil above the head. Stimulation parameters for both peripheral nerve stimulation and TMS will be then determined. Finally, the participants will be asked to perform 5 trials of an 8-second MVC, separated from 20 minutes each. Values of either muscular contractile characteristics or motor pathways excitability will be quantified through peripheral nerve stimulation or TMS applied prior to the MVC (control values), and during a 10-minute period following the MVC. Instructions will be given from the investigators before each trial. The duration of the first session will be between 2.5 and 3 hours.

iii) **Session 2**: Session 1 and Session 2 will be performed at least 48h apart. Surface Electromyography electrodes will be placed on the lower leg of the participants following the procedures in Session 1. However, in Session 2, peripheral nerve stimulation and TMS will not be applied. Participants will be asked to perform three ballistic contractions, separated from 2-minute intervals. Thereafter, 8-second MVC will be performed. Following the MVC, within a 10-minute period, participants will be asked to perform an additional ballistic contraction. Instructions will be given from the investigators about the timing and technique of ballistic contractions performances. The number of trials required will be notified at the beginning of Session 2. The estimated time for Session 2 will be between 1.5 and 3 hours, depending on the number of trials to be performed.

Benefits and potential risks to participants

Benefits: There is no prediction that participants will directly benefit from participation in this experiment. Although greater motor function (i.e. increase rate of force development of ballistic contractions) with plantar flexor muscles may result from the MVC, the effects are short-term, confined to the 10-minute periods post-MVC. However, collected data through participation will help to gain an insight on phenomena involved in performance output increases, allowing to develop efficacious conditioning methods for both sport-related and health-related issues (e.g. movement-related diseases).

Potential Risks:

Peripheral Nerve stimulation: Peripheral nerve stimulation induces a weak electric current within the aimed motor nerve. This allows motor fibres firing to the muscles of interest, therefore, muscle contraction. Peripheral nerve stimulation,

performed with the parameters applied in this investigation that has been established as "being safe" by previous studies, has been used safely in many experimental researches. Application of peripheral nerve stimulation may cause some discomfort, e.g. tingling when stimulation is triggered. However, effects are only temporary. Please report any adverse effects you may experience to the experimenters, so that they can monitor these symptoms

TMS: According to the guidelines released from the Safety of TMS Consensus Group in 2008, single sessions of TMS or do not carry the risk of significant magnetic field exposure since the total time is too short. Stimulation with TMS is generally well tolerated and experienced by most participants as painless. In some participants, it may result in a minor headache or discomfort at the site of stimulation. However, provided that participants fulfil TMS safety screening requirements, discomforts from TMS are harmless, and if any, they rapidly vanish. Please report any adverse effects you may experience to the experimenters, so that they can monitor these symptoms

Use of research results

As previously mentioned, data will be used for writing the Master Thesis. Moreover, results might be presented in seminars and congresses, and reported in international publications. The results will be anonymous at all time. If wanted, participants can be informed of their own results after measurements will be completed.

Rights of research subjects

Your participation in this research is completely voluntary. If you choose to participate in it, you have the right to withdraw from the study at any time without any consequences.

The organization and conduct of the research and the reporting of its findings will be done so that your identity is treated as confidential information. No personal information that is collected during the research will be disclosed to anyone else besides you and the research group. When the results of the research will be published, no information will be included that would reveal your identity. At any point, you will have the right to receive further information about the research from the members of the research group.

Insurance

The personnel and activities of the University of Jyväskylä are covered by insurance. This includes insurance for the treatment of injury, liability insurance and voluntary accident insurance.

During the study, the research subjects are insured against damages, accidents and injuries caused by an external cause. Accident insurance is valid during physical tests and journeys directly to the research site and back. However, insurance companies do not cover muscle or tendon sprains caused by sudden strain if no external cause is involved. In case of sudden injury or illness during physical testing, the research unit is prepared to provide immediate first aid. The laboratory has first aid equipment and the personnel are trained to use them. As insurance companies do not provide a complete insurance coverage for research projects, for example, in case of a sudden illness, it is recommended that the research subjects also have a personal accident/health insurance and a life insurance

Consent to participate in research

I have been informed of the purpose and content of the research, the use of its research materials, and the potential risks and problems it may cause to myself as a research subject, as well as of my rights and insurance protection. I hereby agree to participate in the study in accordance with the instructions given by the researchers. In case of illness – cold, fever, for example– , while recuperating from an illness, or if I'm not feeling well, I will not participate in physical tests that involve such measurements as blood tests or other sampling, or physical strain. I can withdraw from the research or refuse to participate in a test at any time. I give my consent to the use of my test results and the data collected on me in such a way that it is impossible to identify me as a person.

Date Name of the research participant (capital letters) and signature

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Date Name of the researcher (capital letters) and signature

7.2 Appendix 2 – TMS Safety screening

TMS PATIENT SCREENING FORM (Rossi et al., 2009)

This section has to be filled out by the PARTICIPANT.
Please answer to the questions:

1)	Do you have epilepsy or have you ever had a convulsion or a seizure?	YES	NO
2)	Have you ever had a fainting spell or syncope? If yes, please describe on which occasion(s) in the notes section on next page.	YES	NO
3)	Have you ever had a head trauma that was diagnosed as a concussion or was associated with loss of consciousness?	YES	NO
4)	Do you have any hearing problems or ringing in your ears?	YES	NO
5)	Do you have cochlear implants?	YES	NO
6)	Do you have metal in the brain, skull or elsewhere in your body (e.g., splinters, fragments, clips, etc.)? If so, please specify the type of metal in the next page.	YES	NO
7)	Do you have any implanted neurostimulator (DBS, epidural/subdural, VNS) ?	YES	NO
8)	Do you have a cardiac pacemaker or intracardiac lines?	YES	NO
9)	Do you have a medication infusion device?	YES	NO
10)	Are you taking any medications? If so, please list them in the next page	YES	NO
11)	Did you ever undergo TMS in the past? If so, please specify in the next page.	YES	NO

12) Did you ever undergo MRI in the past? If so, please specify in the next page. YES NO

Reference:

Rossi S, Hallett M, Rossini PM, Pascual-Leone A. The Safety of TMS Consensus Group. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation. Clin Neurophysiol, Dec 2009; 120(12): 2008-2039.

NOTES

_____

Date

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Name of the research subject (capital letters) and signature

_____

Name of the researcher (capital letters) and signature

7.3 Appendix 3 – Absolute values of measures.

TABLE 9. Group representative absolute values for each measure at each time point. Data are presented as mean (SD in brackets)

			Time points post-CC										
Groups	Variables	Baseline	5 s	1 m	2 m	3 m	4 m	5 m	6 m	7 m	8 m	9 m	10 m
END	**Twitch**												
	PF (N)	216.19 (34.10)	229.93 (40.03)	223.98 (39.59)	223.43 (41.01)	223.95 (41.37)	224.33 (42.39)	219.1 (39.66)	218.01 (39.40)	215.38 (38.05)	215.46 (36.12)	212.61 (37.78)	208.61 (38.34)
	pRFD (N/ms)	3.66 (0.36)	4.86 (0.81)	4.19 (0.46)	4.10 (0.43)	4.03 (0.45)	4.02 (0.46)	3.94 (0.46)	4.00 (0.49)	3.89 (0.53)	3.91 (0.50)	3.81 (0.54)	3.74 (0.57)
	CT (ms)	123.14 (11.95)	105.00 (15.00)	122.03 (21.39)	123.43 (25.56)	123.75 (20.63)	126.9 (21.05)	121.35 (22.54)	120.75 (19.69)	122.2 (19.53)	121.55 (16.63)	121.58 (18.03)	123.73 (17.27)
	HRT (ms)	114.84 (29.85)	119.5 (41.44)	123.60 (30.82)	129.73 (48.83)	126.34 (47.13)	110.37 (26.71)	114.93 (28.85)	114.73 (23.41)	110.95 (25.79)	111.82 (25.99)	111.69 (25.21)	111.04 (25.52)
	Evoked potentials												
	Mmax (mV)	14.12 (6.88)	13.79 (6.06)	14.40 (6.88)	14.24 (6.70)	14.20 (6.74)	14.02 (6.57)	13.96 (6.66)	13.99 (6.81)	13.89 (6.63)	13.91 (6.67)	13.86 (6.57)	13.85 (6.61)
	Msubmax (mV)	2.29 (1.91)	2.39 (1.72)	2.33 (1.66)	2.44 (1.61)	2.45 (1.22)	2.77 (1.69)	2.34 (1.06)	2.64 (1.44)	2.68 (1.51)	2.66 (1.46)	2.82 (1.69)	3.08 (2.15)
	H-wave (mV)	3.48 (2.43)	3.27 (1.69)	2.93 (2.03)	3.22 (2.43)	3.32 (2.43)	3.45 (2.34)	3.41 (2.38)	3.46 (2.35)	3.43 (2.28)	3.42 (2.30)	3.38 (2.21)	3.39 (2.10)
	H/Msubmax	1.79 (0.53)	1.91 (1.41)	1.45 (0.76)	1.52 (0.92)	1.61 (1.00)	1.67 (1.25)	1.80 (1.17)	1.75 (1.29)	1.81 (1.47)	1.76 (1.35)	1.69 (1.28)	1.73 (1.50)
	MEP (mV)	0.48 (0.34)	0.95 (0.39)	0.57 (0.56)	0.58 (0.23)	0.45 (0.36)	0.40 (0.41)	0.31 (0.26)	0.41 (0.30)	0.43 (0.38)	0.41 (0.39)	0.45 (0.42)	0.37 (0.43)
	MEP/ M max	0.04 (0.02)	0.08 (0.04)	0.04 (0.02)	0.05 (0.03)	0.04 (0.03)	0.03 (0.02)	0.02 (0.01)	0.03 (0.01)	0.03 (0.02)	0.03 (0.02)	0.03 (0.02)	0.03 (0.01)
	Ballistic contraction						**CM**	**CH**					
	pBRFD (N/ms)	7.90 (2.99)	7.88 (3.59)	7.92 (3.04)	8.15 (3.11)		7.73 (3.65)	8.17 (4.05)					
	RMS EMG	1.23 (0.50)	1.19 (0.64)	1.35 (0.55)	1.14 (0.42)		1.33 (0.59)	1.34 (0.43)					

TABLE 9. Contd

Groups	Variables	Baseline	Time points post-CC										
			5 s	1 m	2 m	3 m	4 m	5 m	6 m	7 m	8 m	9 m	10 m
POW	**Twitch**												
	PF (N)	167.11 (21.07)	217.05 (32.98)	191.95 (26.32)	184.91 (23.91)	181.51 (22.83)	176.09 (21.08)	176.57 (22.12)	173.22 (21.79)	172.32 (21.60)	169.00 (23.20)	169.94 (24.62)	167.85 (24.73)
	pRFD (N/ms)	3.28 (0.62)	5.40 (1.31)	4.23 (0.95)	4.02 (0.96)	3.86 (0.95)	3.80 (0.90)	3.68 (0.89)	3.69 (0.96)	3.56 (0.90)	3.46 (0.88)	3.42 (0.80)	3.39 (0.80)
	CT (ms)	109.83 (23.22)	83.73 (21.39)	102.48 (26.59)	107.63 (31.18)	109.50 (30.41)	106.55 (30.78)	110.03 (30.32)	110.95 (30.12)	111.58 (27.91)	110.40 (26.75)	110.90 (25.79)	107.70 (25.73)
	HRT (ms)	115.92 (28.86)	108.27 (23.89)	110.76 (20.25)	116.85 (29.14)	116.45 (30.08)	115.25 (34.70)	118.13 (33.53)	111.77 (33.03)	110.33 (30.34)	110.62 (30.15)	110.13 (31.51)	115.69 (33.34)
	Evoked potentials												
	Mmax (mV)	13.99 (4.00)	14.00 (4.01)	14.51 (4.34)	14.33 (4.23)	14.39 (4.29)	14.19 (4.04)	14.20 (4.23)	14.03 (3.98)	13.91 (3.99)	13.92 (4.08)	13.94 (4.15)	13.83 (4.00)
	Msubmax (mV)	1.56 (0.77)	1.47 (0.67)	2.03 (1.69)	1.80 (1.17)	1.77 (1.16)	2.01 (1.68)	1.64 (0.83)	1.80 (1.22)	1.96 (1.57)	1.80 (1.17)	2.07 (1.80)	2.06 (1.72)
	H-wave (mV)	2.75 (2.49)	2.33 (2.07)	3.01 (2.83)	3.25 (2.75)	3.26 (2.89)	3.23 (2.88)	3.33 (2.81)	3.27 (2.78)	3.36 (2.81)	3.33 (2.72)	3.45 (2.82)	3.37 (2.76)
	H/Msubmax	2.40 (2.36)	2.16 (2.00)	2.59 (2.72)	2.80 (2.76)	2.86 (2.82)	2.78 (2.82)	2.82 (2.76)	2.79 (2.70)	2.87 (2.77)	2.82 (2.57)	2.95 (2.80)	2.80 (2.71)
	MEP (mV)	0.42 (0.24)	0.87 (0.52)	0.46 (0.24)	0.35 (0.16)	0.33 (0.17)	0.38 (0.18)	0.37 (0.13)	0.41 (0.23)	0.32 (0.16)	0.34 (0.25)	0.28 (0.17)	0.30 (0.20)
	MEP/Mmax	0.03 (0.02)	0.06 (0.03)	0.03 (0.02)	0.03 (0.02)	0.02 (0.01)	0.03 (0.01)	0.03 (0.01)	0.03 (0.01)	0.02 (0.01)	0.02 (0.01)	0.02 (0.01)	0.02 (0.01)
	Ballistic contraction						**CM**	**CH**					
	pBRFD (N/ms)	8.66 (2.23)	10.22 (2.61)	9.52 (1.33)	8.77 (2.50)		10.03 (2.56)	8.07 (1.40)					
	RMS EMG	1.09 (0.55)	1.21 (0.65)	0.89 (0.59)	0.77 (0.40)		0.90 (0.52)	1.24 (0.63)					

CC, Conditioning Contraction; END, Endurance-trained Group; POW, Power-trained Group; PF, Peak Force; pRFD, Peak Rate of Force Development; CT, Contraction Time; HRT, Half-Relaxation Time; Mmax, Maximal M-wave; Msubmax, M-wave preceding the H-wave; MEP, Motor Evoked Potential; pBRFD, Peak Rate of Force Development of the Ballistic Contraction; RMS EMG, Normalized Root Mean Square of EMG from its Onset to the Peak Force of the Ballistic Contraction; CM, Conditioned MEP/Mmax; CH, Conditioned H/Msubmax.