EFFECTS OF MUSCLE ACTION TYPE ON CORTICOSPINAL EXCITABILITY AND TRICEPS SURAE MUSCLE-TENDON MECHANICS.

Pedro Frederico Valadão

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Department of Biology of Physical Activity
University of Jyväskylä
Supervisors: Janne Avela and Taija Finni

ABSTRACT

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The present study was designed to investigate corticospinal excitability modulation during maximum isometric and eccentric muscle actions with two different velocities. Moreover, the study aimed to clarify the effect of muscle action type on muscle-tendon mechanical behavior in order to shed light into the possible role of sensory information in modulating corticospinal excitability during different muscle actions. We compared motor evoked potentials (MEPs) to transcranial magnetic stimulation and Hoffman reflexes (H-reflex) in soleus muscle during isometric, slow eccentric (25 deg/s) and fast eccentric (100 deg/s) muscle actions. Concomitantly, ultrasonography was utilized to access soleus and medial gastrocnemius fascicle behavior, characterized by fascicle length, velocity and pennation angle.

The main finding was that soleus H-reflex was depressed ($P < 0.001$) during both fast and slow eccentric protocols as compared to isometric, while no differences in fascicle length ($P = 0.569$) and pennation angle ($P = 0.293$) were found among the three protocols. Furthermore, although the fast eccentric protocol had a greater fascicle velocity than slow eccentric ($P < 0.05$), there were no differences in H-reflex ($P > 0.05$). No differences in MEP sizes were found among the three protocols ($P = 0.750$), while absolute silent period was significantly shorter ($P = 0.009$) for both eccentric protocols as compared to isometric.

Taken together, the present results corroborates with the idea that the central nervous system has an unique activation strategy during eccentric muscle actions (Duchateau & Enoka 2008, Enoka 1996), and further refutes the hypothesis that sensory information plays an important role in modulating these actions.

Keywords: Motor control; Corticospinal excitability; Muscle-tendon dynamics.

ABBREVIATIONS

ACh	Acetylcholine
AL	Activation level
AP	Action potential
ATP	Adenosine triphosphate
CNS	Central nervous system
CMEP	Cervicomedullary motor-evoked potential
D wave	Direct wave
EMG	Electromyography/ Electromyographic
EPSP	Excitatory post-synaptic potential
GABA	Gamma-aminobutyric acid
H-reflex	Hoffman reflex
Hmax	Maximum H-reflex
I wave	Indirect wave
MEP	Motor evoked potential
MG	Medial gastrocnemius
Mmax	Maximum compound muscle action potential
MN	Motoneuron
MTC	Muscle-tendon complex
MU	Motor unit
MVC	Maximal voluntary contraction
M-wave	Muscle compound action potential
PSI	Presynaptic inhibition
RMS	Root mean square
RMT	Rest motor threshold
SEC	Series elastic component
SOL	Soleus muscle
SP	Silent period
TA	Tibialis anterior
TMS	Transcranial magnetic stimulation

CONTENTS

1 INTRODUCTION

The human nervous system has the vital task of monitoring and controlling the internal body environment and also interpreting and responding to external stimuli. An estimate of 86 billion neurons compose the human brain (Herculano-Houzel 2009). They form a complex neural network with trillions of synaptic connections, capable of interpreting and associating information coming from a wide range of sensory receptors, and comparing them with stored knowledge.

Human movement requires the successful coordination of sensory information (e.g. vision, proprioception), and an orchestration of motor commands to various muscles. Motor control can be defined as the information processing carried out by the central nervous system resulting in the activation of the musculoskeletal system, creating coordinated and purposeful movements. Motor control is achieved via the interconnected function of several brain regions. The interaction between these regions creates a signal that goes to the motoneuron pool of a determined muscle. Motoneurons are located at the spinal cord, they carry the command from the higher centers to the muscles, and also receive a large amount of information coming from within the body (i.e. proprioception and interoception) and from outside the body (i.e exteroception). All this information may modulate the commands from higher centers and generate a final response that will go to the muscle fibers, resulting in their activation and thus force production.

An important area of research in motor control is understanding the differences between in vivo and in vitro data regarding eccentric muscle actions (i.e. stretching of an active muscle). In vitro data, with isolated muscle fibers, shows that eccentric muscle actions are capable of generating 150 to 190 % of the maximum isometric force. However, human in vivo studies found that maximal eccentric muscle actions were not statistically greater than isometric. If the full mechanical potential of the eccentric actions cannot be achieved, neural limiting mechanisms must play a role in modulating the activation of the muscle

during these actions. The stimulation of neural pathways and the measurement of the evoked responses in the muscle have been utilized to test the excitability changes of different parts of the central nervous system, usually referred as supraspinal and spinal centers. Accessing the excitability changes of different neural structures during a muscle action can reveal their role in movement control. An important aspect that has not been examined is the mechanical configuration (i.e. fascicle length, pennation angle and tendon/aponeurosis displacement) of the muscle-tendon unit during these different types of muscle action. If at the same joint angle, the muscle presents differences in its mechanical configuration, it is reasonable to expect that different afferences coming from the muscle are capable of modulating motoneuron excitability and may have a role in limiting the force production during the eccentric muscle actions.

The purpose of this study is to verify the effects of isometric and eccentric maximal voluntary muscle actions on corticospinal excitability, while concomitantly measuring soleus and medial gastrocnemius muscles fascicle length, fascicle velocity and pennation angle. Better understanding about the muscle's mechanical setup during neural testing is of vital importance, since it is already clear that many afferent inputs (i.e. information coming from receptors in the muscle to the central nervous system) can modulate the corticospinal excitability and thus potentially be the reason for the reported differences between these two muscle actions. If the mechanical configuration is the same between the two types of muscle action, and yet there is an inhibition in the eccentric action, it will further consolidate the view that a unique motor program is utilized by the higher command centers during eccentric actions (Duchateau & Enoka 2008, Enoka 1996).

Better understanding motor control will result in major improvements in medicine, not only allowing the understanding of underlying physiological processes of many diseases, but also making possible bioengineering interventions, helping humankind to further adapt and evolve.

2 LITERATURE REVIEW

2.1 Human nervous system: organization and function

The human nervous system has the vital task of monitoring and controlling the internal body environment and also interpreting and responding to external stimuli. It is divided into central (Figure 1) and peripheral nervous system; the first is a bilateral and essentially symmetrical structure with seven main parts: spinal cord, medulla oblongata, pons, cerebellum, midbrain, diencephalon, and the cerebral hemispheres. The peripheral nervous system consists of cranial and spinal nerves, ganglia, sensory and motor nerve endings (for a review refer to Kandel et al. 2000, 8-10).

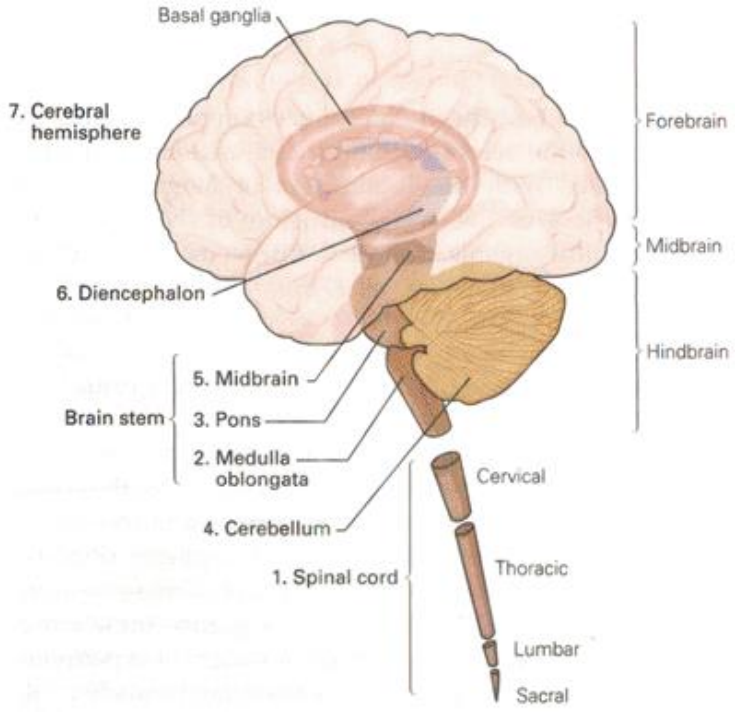

FIGURE 1 – The central nervous system. Source: Kandel et al. (2000).

“There are two main classes of cells in the nervous system: nerve cells (i.e. neurons) and glial cells (i.e. glia). A typical neuron has three morphologically defined regions: cell body,

dendrites and axon (Figure 2). The cell body (i.e. soma) is the metabolic center of the cell; it contains the nucleus, which stores the genes of the cell, as well as the endoplasmic reticulum, an extension of the nucleus where the cell's proteins are synthesized. The cell body usually gives rise to two kinds of processes: several short dendrites and one long, tubular axon. Dendrites branch out in tree-like fashion and are the main apparatus for receiving incoming signals from other nerve cells, although the soma may also receive inputs. In contrast, the axon extends away from the cell body and is the main conducting unit for carrying electrical signals to other neurons. The glial cells surround the neurons, and as far as is known, glia are not directly involved in information processing. They are thought to have several other vital roles such as providing physical support for the neurons, separating and insulating neuronal groups and synaptic connections from each other, debris removal after injury or neuronal death, among others". (Kandel et al. 2000, 20-23)

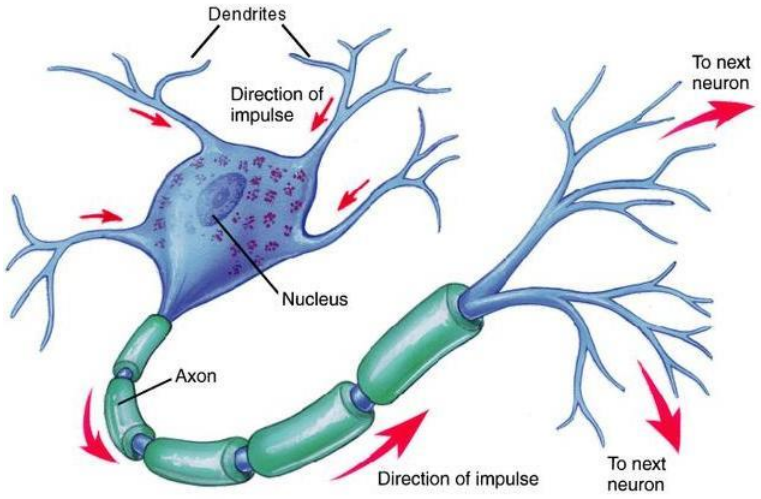

FIGURE 2 – Neuron morphology. Source: ygraph.com.

Neurons have a lipid bilayer membrane, which is virtually impermeable to ions, insulating two conducting solutions: the cytoplasm and the extracellular fluid. Ions can cross the lipid bilayer only by passing through ion channels (i.e. transmembrane proteins) in the cell membrane. At rest all cells, including neurons, maintain a difference in the electrical potential between the two sides of the cell membrane, called resting membrane potential. This voltage difference is a result of an unequal distribution of electrically charged ions,

accomplished by the membrane's selective permeability to different ions. (Squire et al. 2000, 85) The membrane potential can be significantly and quickly altered; this change serves as a signaling mechanism. The temporary combination of acetylcholine (ACh) with its receptor protein at the cell membrane is believed to cause a conformational change in it, which in turn allows ions to flow through an associated ion channel. During signal transmission a nerve cell releases sufficient amounts of ACh to cause the simultaneous opening of one to three hundred thousand channels. Minute currents briefly flow through each of these channels, and the sum of these currents passing across the membrane resistance causes a depolarizing voltage change which, if sufficiently large, gives rise to an action potential (AP). (Bevan et al. 1979) A reduction in membrane potential is called depolarization, and because it enhances the cell's ability to generate an AP, it is excitatory. In contrast, an increase in membrane potential is called hyperpolarization, which makes the cell less likely to generate an AP and is therefore inhibitory. The release of neurotransmitter serves as a neuron's output signal. The amount of neurotransmitter released is determined by the number and rate of APs arriving at the presynaptic terminals. After the neurotransmitter is released from the presynaptic neuron, it diffuses across the synaptic cleft to receptors in the membrane of the postsynaptic neuron. The binding of neurotransmitter to receptors causes the postsynaptic cell to generate a synaptic potential. Whether the synaptic potential has an excitatory or inhibitory effect will depend on the type of receptors in the postsynaptic cell, not on the particular neurotransmitter. The same neurotransmitter can have different effects on different types of receptors. (Kandel et al. 2000, 27-28)

"The action potentials are rapid, transient, all-or-none nerve impulses, with an amplitude and duration of about 100 mV and 1 ms, respectively. APs are initiated at a specialized trigger region at the origin of the axon called axon hillock; from there they are conducted along the axon without failure or distortion at rates of 1–100 m per second. The amplitude of an AP traveling along the axon remains constant because it is an all-or-none impulse that is regenerated at regular intervals throughout the axon. APs constitute the signals by which the brain receives, analyzes, and conveys information. These signals are highly stereotyped throughout the nervous system, even though they are initiated by a great variety of events in

the environment that impinge on our bodies: from light to mechanical contact, from odorants to pressure waves. Thus, the signals that convey information about vision are identical to those that carry information about odors. The information conveyed by an AP is determined not by the form of the signal but by the pathway that the signal travels in the brain. The brain analyzes and interprets patterns of incoming electrical signals and in this way creates our everyday sensations of sight, touch, taste, smell, and sound. To increase the speed by which APs are conducted, large axons are wrapped in a fatty, insulating sheath of myelin. The sheath is interrupted at regular intervals called nodes of Ranvier. It is at these uninsulated spots on the axon that the AP becomes regenerated". (Kandel et al. 2000, 20-23)

2.2 Motor control

The human motor control is accomplished via the interconnected function of several anatomical regions (Figure 3). They include the primary motor cortex, premotor cortex, supplementary motor cortex, basal ganglia, thalamus, cerebellum, brain stem, midbrain, reticular formation and spinal cord. (Kandel et al. 2000, 317-322) The primary motor cortex is located in the precentral gyrus, and it is organized in a somatotopic fashion: the legs and feet are represented most medially and the trunk, arm, neck and head are represented progressively more laterally (Kandel et al. 2000, 344).

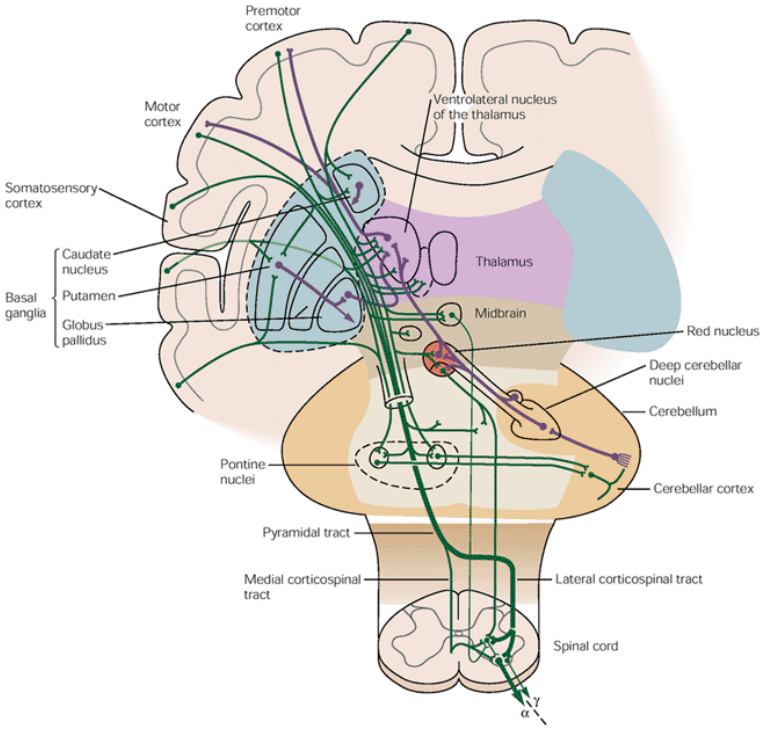

FIGURE 3 – Voluntary control requires the coordinated activity of all components of the motor system (see text). Green: Principal descending projections; Purple: feedback projections and local connections. Ultimately, all this processing converges on the motoneurons of the ventral horn of the spinal cord, which innervates muscle and elicits movements. Source: Kandel et al. (2000).

There are six distinct layers of neurons in the motor cortex. The main output cells are large pyramidal cells in the fifth layer, and smaller cells in the third layer. (Squire et al. 2000, 759-763) These cells are subjected to excitatory and inhibitory modulations from intracortical connections (Butler et al. 2007), and thus the cortical output will be the net effect of these interactions. The corticospinal pathway originates from the pyramidal cells, which project their axons to the contralateral side of the spinal cord (Figure 4). This is the only descending motor pathway known to make monosynaptic connections with the spinal motoneurons (MN; Bawa et al. 2002; Noordhout et al. 1999; Palmer & Ashby 1992) and being free from presynaptic inhibition (Nielsen & Petersen 1994). The observation that short-latency corticospinal facilitation increased prior to muscle action and was largest at the very onset of the action, suggests that the corticospinal projection is involved in bringing the motoneurons to threshold and thus initiate the movement (Nielsen & Petersen 1995).

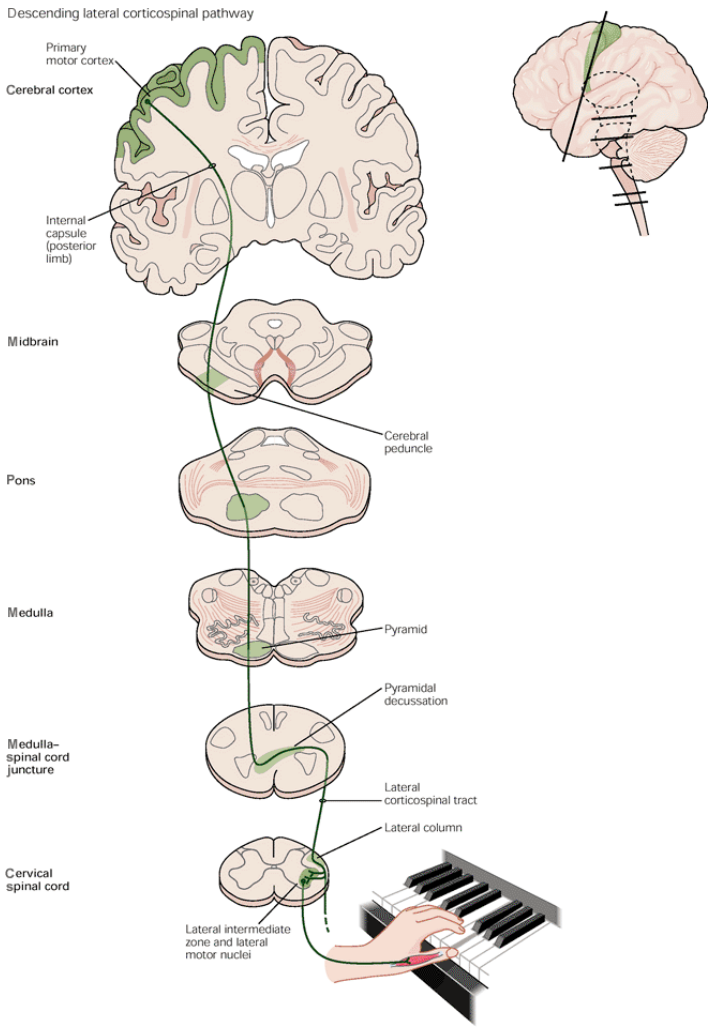

FIGURE 4 – Fibers that originate in the primary motor cortex and terminate in the ventral horn of the spinal cord constitute a significant part of the corticospinal tract. Source: Kandel et al. (2000).

At the spinal level, each cortical MN synapses with various spinal MNs and interneurons. Furthermore, each spinal MN receives inputs from many cortical MN and interneurons (Edgley 2001). One spinal motoneuron, usually called alpha motoneuron (αMN), innervates a variable number of muscle fibers (i.e. innervation ratio), and this functional unit is called motor unit (MU; Sale 1987). The spinal interneurons are responsible for integrating complex multisensory input from afferents of many different types and origins and they target multiple MN pools (Edgley 2001).

Afferent information from a wide array of sensory receptors are important for the generation of a correct pattern of muscle activity. Proprioceptors such as muscle spindles

(muscle length, rate of length change and limb position), Golgi tendon organs (muscle tension) and joint receptors (joint movement and end joint range, nociception) produce afferent inputs that modulate the MN pool of agonist, synergist and antagonist muscles. Reflexes are motor responses caused by sensory inputs, usually referred to as spinal reflexes, because the neural circuitry responsible for it is contained within the spinal cord. (Kandel et al. 2000, 713-724; Newton 1982) Supraspinal information processing utilizes this wide range of sensory information, integrating it to the stored knowledge in the prefrontal areas of the brain. Consequently, the αMNs are subjected to inhibitory and excitatory modulation of many different sensory inputs, from spinal interneuronal circuitry and also by the supraspinal centers. The supraspinal centers regulate the "information gain" of the afferences and spinal circuitry, allowing the reflexes to be smoothly incorporated into complex movements. (Butler et al. 2007; Faist et al. 1996; Nielsen et al. 1993; Robinson et al. 1982; Stein 1995)

Muscle spindles. Muscle spindle structure and function will be further detailed due to its important modulation upon αMN excitability during motor tasks. Muscle spindles are small, encapsulated sensory receptors that have a fusiform shape and are located within the fleshy part of the muscle. Their main function is to signal changes in the length of the muscle within which they reside. Changes in the length of muscles are closely associated with changes in the angles of the joints that the muscles cross. Thus, muscle spindles can be used by the CNS to sense relative positions of the body segments. (Winter et al. 2005) Each spindle has three main components: (1) a group of specialized intrafusal muscle fibers whose central regions are noncontractile; (2) large-diameter myelinated sensory endings that originate from the central regions of the intrafusal fibers; and (3) small-diameter myelinated motor endings that innervate the polar contractile regions of the intrafusal fibers (Figure 5). Because muscle spindles are arranged in parallel with the extrafusal muscle fibers that make up the main body of the muscle, the intrafusal fibers change in length as the whole muscle changes. Thus, when a muscle is stretched, the sensory endings are also stretched and increase their firing rate. When an inactive muscle shortens, the spindle is unloaded and the activity decreases. (Kandel et al. 2000, 720)

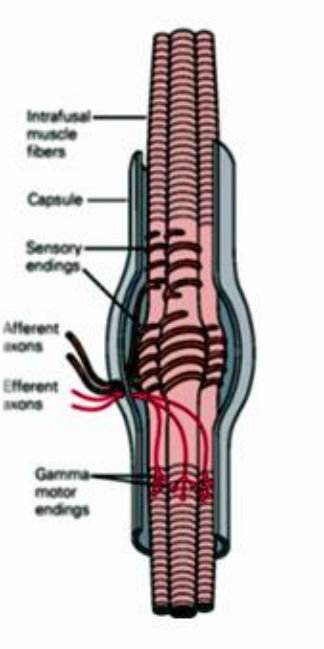

FIGURE 5 –The main components of the muscle spindle. Source: Kandel et al. (2000).

The motor innervation of the intrafusal muscle fibers comes from small-diameter MNs, called gamma MNs to distinguish them from the large-diameter alpha MNs that innervate the extrafusal muscle fibers. Activation of the intrafusal muscle fibers does not contribute to force generation during the muscle action. Rather, activation of gamma MNs causes shortening of the polar regions of the intrafusal fibers. This in turn stretches the noncontractile central region from both ends, leading to an increase in firing rate of the sensory endings or to a greater likelihood that stretch of the muscle will cause the sensory ending to fire. Thus, the gamma MNs provide a mechanism for adjusting the sensitivity of the muscle spindles. Muscle shortening causes slack in the muscle fibers (extrafusal and intrafusal), resulting in loss of spindle's sensitivity, making it unable to signal further length changes. To counter this problem, alpha MN activation is accompanied by concomitant gamma MN activation (i.e. alpha-gamma coactivation). Gamma MN activation maintains tension in the muscle spindle during muscle activity, thereby ensuring its responsiveness at different lengths. In addition to the axons of the gamma MNs, collaterals from alpha MNs also innervate the intrafusal fibers. These are referred to as skeletofusimotor, or beta, efferents. A significant, though still unquantified, amount of skeletofusimotor innervation has been found in spindles of both cats and humans. These

efferents provide the equivalent of alpha-gamma coactivation; when skeletofusimotor neurons are activated, unloading of the spindle by activation of extrafusal fibers is at least partially compensated by loading due to intrafusal contraction. (Gregory et al. 1998; Kandel et al. 2000, 725; Vallbo 1971; Wilson et al. 1997)

Stretch reflex. Mechanical deformation of the intrafusal muscle fibers generates APs by activating mechanically gated ion channels in the afferent axons coiled around the spindle. The APs travels towards the sensory neuron's soma located near the spinal cord, in a dorsal root ganglion. The sensory neuron forms a monosynaptic excitatory connection with the αMN in the ventral horn of the spinal cord that innervate the same (i.e. homonymous) muscle and, via local circuit neurons, inhibitory connections with the αMN of antagonistic (i.e. heteronymous) muscles (Figure 6). Henneman (1985) demonstrated that a single Ia afferent from a muscle spindle can have synapses with more than 80% of the homonymous motoneuron pool. The monosynaptic excitatory APs from the Ia axons onto the homonymous αMN provoke the stretch reflex, generating tension and thus resisting further stretching. The heteronymous inhibitory APs cause relaxation of the heteronymous muscles, preventing further stretching the homonymous muscle. Deviations from the desired length are detected by the muscle spindles, since increases or decreases in the stretch of the intrafusal fibers alter the level of activity in the sensory fibers that innervate them. These changes lead in turn to adjustments in the activity of the αMNs, returning the muscle to the desired length by activating the stretched muscle and relaxing the opposed muscle group, and by restoring the level of spindle activity to what it was before. (Crone et al. 1987; Jankowska et al. 1976; Kandel et al. 2000, 713-719; Petersen et al. 1998)

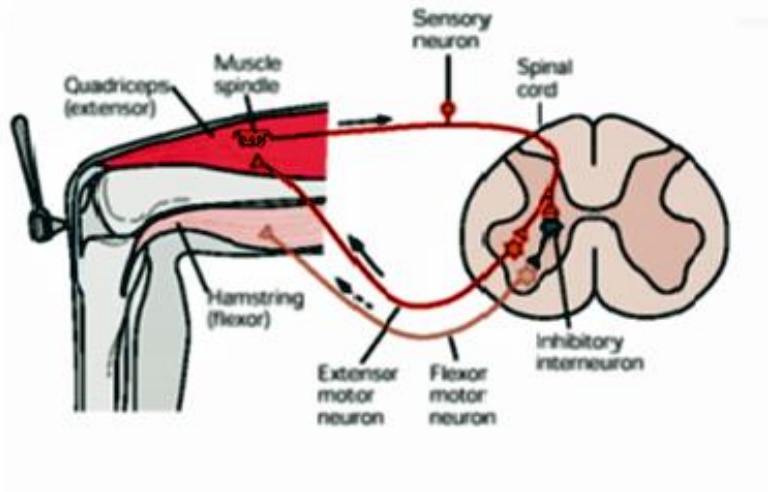

FIGURE 6 – Stretch reflex circuit for knee jerk. Source: Kandel et al. (2000)

MN excitability. The excitability of MNs is modulated by two mechanisms. The first has already been briefly explained in section 2.1, consisting of synaptic inputs (i.e. ionotropic inputs) that depolarize or hyperpolarize MNs by generating postsynaptic potentials. The ionotropic mechanism works via neurotransmitters that open ion channels in the cell membrane. In the second mechanism, neurotransmitters bind to receptors that activate intracellular signaling pathways. These pathways modulate the properties of voltage-sensitive channels that determine the intrinsic input–output properties of motoneurons. This neuromodulatory mechanism usually does not directly activate motoneurons but instead dramatically alters the neuron's response to ionotropic inputs. (Heckman et al. 2008; 2009) The combination of all ionotropic inputs upon the MN, and its actual intrinsic characteristics will cause a net effect, provoking or not its depolarization and subsequent muscle action (Morita et al. 2000).

2.3 Voluntary force production

Once an αMN (located at the ventral horn of the spinal cord) is depolarized, an AP travels along its axon towards the specialized innervation zone in the muscle membrane (i.e. neuromuscular junction; Figure 7). "At the region where the motor axon approaches the muscle fiber, the axon loses its myelin sheath and splits into several fine branches. The ends of the fine branches form multiple expansions or varicosities, called synaptic buttons,

from which the motoneuron releases neurotransmitter. Each button is positioned over a junctional fold, a deep depression in the surface of the postsynaptic muscle fiber that contains the neurotransmitter's receptors. The neurotransmitter released by the axon terminal is acetylcholine, and the receptor on the muscle membrane is the nicotinic type of ACh receptor". (Kandel et al. 2000, 188-189) The connection between ACh and its receptors allows an influx of ions through transmitter-gated channels that will progressively depolarize the postsynaptic membrane (excitatory post-synaptic potential, EPSP), resulting in the activation of voltage-gated channels that will further increase depolarization, until the membrane threshold is reached and an AP is propagated throughout the muscle cell. The ACh released from the presynaptic terminals is rapidly hydrolyzed by acetylcholinesterase, terminating the transmitter's action and leaving the muscle fiber ready to respond again in an all-or-none manner to the next AP. (Kandel et al. 2000, 188-192).

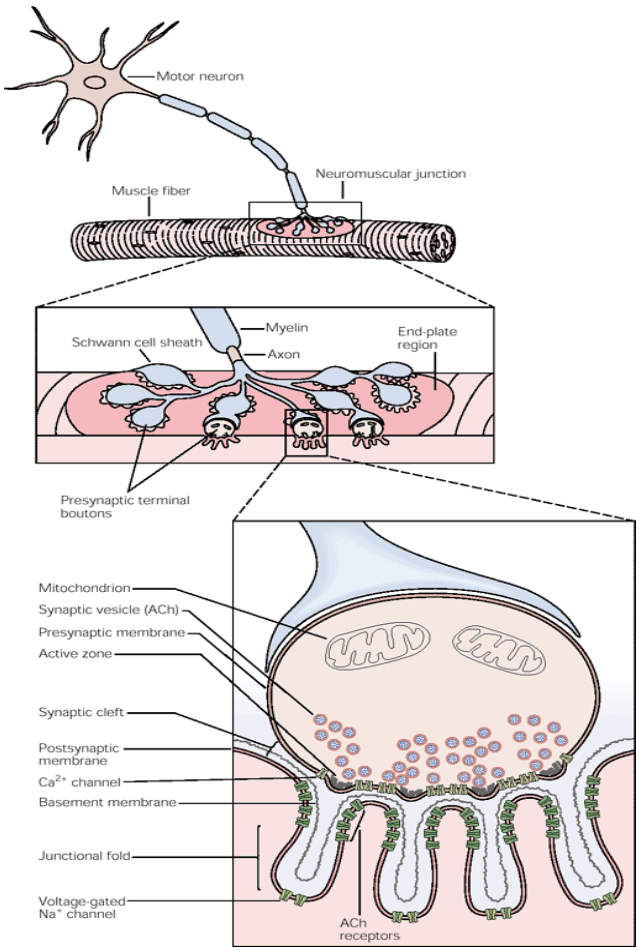

FIGURE 7 – The neuromuscular junction. Source: Kandel et al. (2000).

The AP propagates along the muscle's membrane (i.e. sarcolemma) and into the deep invaginations on the sarcolemma called T-tubule system. Inside the T-tubules, dihydropyridine receptors (i.e. voltage-mediated calcium channels) are activated by the APs, causing a conformational change in the ryanodine receptors, with which they are mechanically linked. The activation of the ryanodine receptors causes its channels to open and generate a flow of calcium ions from the lateral sacs of the sarcoplasmic reticulum into the cytoplasm. (Enoka 1994, 251–252) Calcium then binds to troponin, creating a conformational change in its structure that exposes a myosin binding site. Myosin's globular head then combine with actin and hydrolyzes an adenosine triphosphate molecule (ATP). The released energy changes the myosin shape, creating a movement often termed "power stroke", which results in sliding between the two filaments, and thus shortening the sarcomere. Another ATP molecule is necessary to disconnect the myosin head from the binding site, and the cycle can start again. (Kandel et al. 2000, 667–668) After the cessation of the neural drive, calcium is actively pumped back into the sarcoplasmic reticulum lateral sacs, and in the absence of it, the crossbridge mechanism will be inhibited and the muscle will relax (McArdle et al. 2010, 369).

2.4 Force modulation

The common drive concept states that in order to produce a determined force output in a given muscle, the central nervous system (CNS) regulates the net sum of excitatory and inhibitory inputs to the motoneuron pool. All motoneurons belonging to the pool receive the same net drive at any given time. Any differences that are displayed among the firing patterns of individual MUs are due to the organization of the pool's architecture with respect to the central and peripheral inputs along with differences in intrinsic characteristics (such as drive-firing rate relationships) of MUs. (De Luca & Erim 1994) Morphological differences between the MUs such as the size of the soma affects the depolarization process (Figure 8), as the smaller soma offers greater input resistance to the current and results in a higher voltage change (i.e. greater excitatory postsynaptic potential), according to Ohm's Law. Therefore, the common drive will first depolarize MUs with smaller soma and as the

firing frequency increases larger MUs will reach their thresholds and also depolarize. Hence, a common driving source can produce different activation patterns in different MUs. (Henneman 1957) The rank-ordered arrangement of the susceptibilities of the motoneurons relieves the CNS from the task of deciding which MUs to activate, and simplifies the circuitry that would have been necessary were the MU to be activated selectively. (De Luca & Erim 1994)

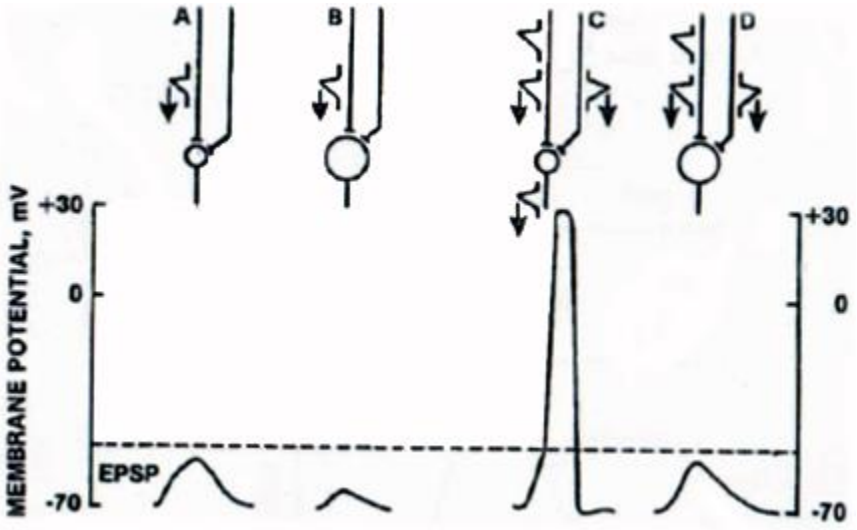

FIGURE 8 – Schematic illustration of how the size of the motoneuron's soma affects its response to excitatory synaptic inputs. The small soma (A) offers a greater input resistence to a given synaptic excitatory current than the large soma (B). A similar increase in excitatory current is sufficient to depolarize the small soma (C) to its threshold for firing, but only succeesds in producing a larger EPSP in the large soma (D). Source: Sale (1987).

According to Gandevia (2001), although the distinction between the various MU types may be more blurred in humans than experimental animals, the "size principle" of orderly recruitment appears to hold in humans, under most circumstances with isometric actions. Some exceptions appear to occur during dynamic actions, fatigue and for muscles with complex actions and different "task groups" of MUs (Duchateau & Enoka 2008; Nardone et al. 1989). It must be conceded that different inputs to motoneuron pools, be they reflex (e.g. group Ia inputs, reciprocal inhibition, cutaneous inputs) or central (e.g. corticospinal inputs, recurrent inhibition, reticulospinal inputs), do not change the firing frequency of all

motoneurons in the pool equally (Gandevia 2001). The recruitment threshold of the MUs can change depending of the situation, for example, during ballistic muscle actions the recruitment threshold is lower compared with slower muscle actions, and the initial discharge frequencies are higher (Desmedt & Godaux 1977).

In order to control the amount of force produced in a determined muscle, the CNS modulates the input rate to the target MN pool. Higher excitatory input rates will increase the firing rate of active MUs, resulting in greater depolarization of the sarcolemma of all its muscle fibers. This increased membrane depolarization will enhance the liberation of calcium ions by the sarcoplasmic reticulum, resulting in more cross-bridges and therefore greater force production (Figure 9A). Concomitantly, the increase in the excitatory input rate will also recruit new MUs by reaching their depolarization threshold, causing them to fire and thus contribute to the force production (Figure 9B). The relationship between these two phenomena is different for each muscle, for example in the deltoid muscle there is MU recruitment up to 80–90% of the maximum voluntary contraction (MVC), whereas the first dorsal interosseous reaches full MU recruitment at 50% MVC. (De Luca & Erim 1994; Sale 1987)

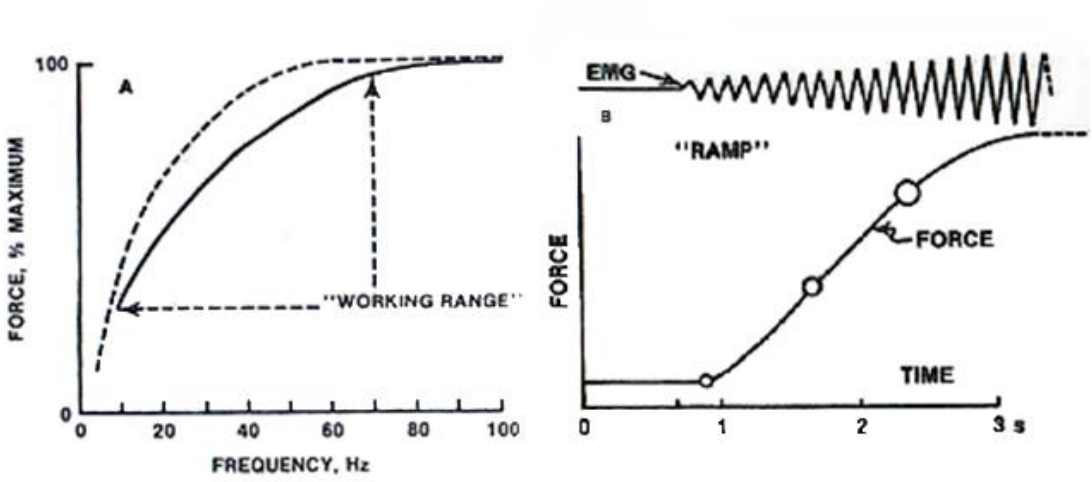

FIGURE 9 – Force modulation through increases of MU firing rate and MU recruitment. (A) The relationship between frequency of stimulation and force output takes the form of a force-frequency curve (solid-line curve). The curve has a relatively steep portion at the lower frequencies; thus, in this portion small changes in frequency cause large changes in force output. This “working range” (about 6 – 50 Hz) corresponds to the commonly observed range of MU firing rates in sustained

muscle actions. (B) Schematic illustration of surface EMG, contraction force and recruitment force thresholds of three MUs (circles) in a ramp muscle action. MUs are recruited according to the size principle. Increased MU recruitment and increased firing rates of already recruited MUs contribute to the increase in surface EMG. Source: Modified from Sale (1987).

2.5 Assessment of corticospinal excitability

A considerable array of methods and techniques has been developed to access corticospinal excitability. This review will focus on single-pulse TMS and H-reflex paradigms as they were combined in the present research. A brief description with references about other methodologies is provided.

2.5.1 Transcranial magnetic stimulation (TMS)

The magnetic stimulator consists of a coil of wire connected to a large electrical capacitance. When the capacitance is discharged through the coil, a large current pulse in the circuit is produced, generating a magnetic field oriented perpendicularly to the coil. According to Faraday's law of electromagnetic induction, this time-varying magnetic field induces an electric field with a magnitude proportional to the time rate of change of the magnetic field, which in the case of TMS is determined by the rate of change of the current in the coil. If the coil is held over a subject's head, the magnetic field penetrates the scalp and skull, and induces an electric field in the brain. The induced electric field causes ions to flow in the brain, without the need for current to flow across the skull and without charged particles being injected into the scalp (Figure 10). The flow of ions brought about by the electric field induced in the brain alters the electric charge stored on both sides of cell membranes, depolarizing or hyperpolarizing neurons. In order to stimulate an axon, there must be a difference in the electrical potential between two points along its length. The result of this is that an axon which runs parallel to an electric field will not be stimulated since the potential at all points along its length will be equal. Thus, a hypothetical circular axon placed concentrically under a circular magnetic stimulating coil will not be activated no matter how large the stimulus intensity. However, if the axon is bent out of the circle, it

will cut the electric field lines, resulting in different potentials along the axon near the bend, favoring stimulation at this point. (Rossi et al. 2009; Rothwell 1997)

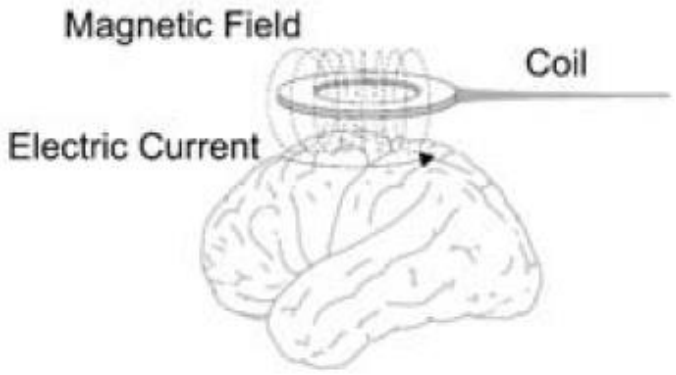

FIGURE 10 – Transcranial magnetic stimulation. Source: Petersen et al. (2003).

One issue of magnetic stimulation is that it is impossible to focus the magnetic field in order to limit the extent of the induced current flow. For example, with a standard round coil, the induced current in the brain flows in an annulus, underneath the coil, which is usually some 8 – 12 cm in diameter. Clearly a large volume of neural tissue may be activated by such a device, although the precise extent is unknown and depends of the stimulation intensity. (Hallett 2000) In order to provide more focal stimulation, coils wound into a figure-eight shape have been used, and in these, the induced electric field under the junction region of the 8 is twice as large as that under the two wings. Nevertheless, the magnetic field falls off rapidly with the distance from the coil: since the cerebral cortex can be 1–2 cm from the surface of the scalp, and the central sulcus itself can be 2 cm deep in man, this means that stimulation is severely attenuated at deep sites such as basal ganglia or thalamus. In addition, since the resistance of white matter is greater than the grey matter, currents induced in the sub-cortical tissues are likely to be small in comparison with those induced in surface layers of cerebral cortex. (Rothwell 1997)

During the last decades, a rapid increase in the application of TMS to study cognition, motor control, pathophysiology of neurologic and psychiatric disorders, among many others has been reported. Thus different paradigms have been created: TMS can be applied one stimulus at a time (i.e. single-pulse TMS), in pairs of stimuli separated by a variable

interval (i.e. paired-pulse TMS), or in trains (i.e. repetitive TMS). Most importantly, considering the large number of subjects and patients who have undergone TMS studies, and the very few cases of reported side effects (seizures are the most serious TMS-related acute side effect), it is very reasonable to affirm that TMS is a safe procedure, if the safety guidelines are followed. (Rossi et al. 2009)

Motor Evoked Potentials (MEP). Stimulation of the motor cortex with single-pulse TMS produces clear short latency responses in the target muscles of contralateral limb. MEPs can be observed with surface electromyography (EMG) and are presumably result from the combination of several volleys coming from different pathways from the cortex. (Nielsen et al. 1993) TMS does not seem to penetrate deep enough into the brain to activate the axons of corticospinal neurons directly; rather it activates them indirectly through synaptic inputs, such as axon collaterals of pyramidal cells, intracortical neurons, among other possible sites (Avela & Gruber, in Komi 2011, 118-119, Hallet 2000). This has been determined by the observation that TMS produces a corticospinal volley with later indirect waves (I-waves) rather than an early direct wave (D-wave), which can be evoked by transcranial electrical stimulation (Di Lazzaro et al. 1998). Although TMS mainly elicits I-waves, high intensity TMS is also able to elicit D-waves (Di Lazzaro et al. 1998; 2003; Nakamura et al. 1997).

According to Petersen et al. (2003), in order to evaluate changes in the excitability of the corticospinal cells, TMS needs to excite the neurons at the soma or trans-synaptically. If TMS primarily activated the axons of the corticospinal cells, the evoked muscular responses would not be affected by the excitability of the cells, except for the rather short-lasting refractory time in the axons following an AP. Whether the exact mode of activation is indirect, via other cortical cells which supply synaptic inputs to the corticospinal tract cells, or direct, at the soma or at the axon hillock of the corticospinal cells, it is not important, since the evoked responses would be influenced by changes in cortical excitability in any case. TMS-induced MEPs constitute a composite effect of excitability stemming from both spinal and cortical regions (Oya et al. 2008), thus to draw conclusions about the site of excitability changes, different methodological approaches can be utilized. The following paradigms have been utilized during natural and sports-specific movements,

only a list for the sake of completeness will be provided, for a complete description refer to Avela & Gruber, in Komi 2011, 123-134.

1 – Comparison of MEPs evoked by TMS with other evoked potentials like those evoked by transcranial electrical stimulation, cervicomedullary stimulation, and Hoffmann reflex stimulation (e.g. Abbruzzese et al. 1994; Duclay et al. 2011; Gandevia et al. 1999; Gruber et al. 2009; Sacco et al. 1997; Taylor & Gandevia 2004).

2 – Conditioning of MEPs evoked by TMS with subthreshold TMS prior to the suprathreshold stimulus, resulting in short intracortical inhibition or intracortical facilitation (e.g. Ilic et al. 2002; Kujirai et al. 1993; Nakamura et al. 1997).

3 – TMS with a subthreshold intensity to suppress the EMG of an active muscle (e.g. Davey et al. 1994; Petersen et al. 2001).

4 – Conditioning of MEPs evoked by TMS with ipsilateral TMS stimulation (e.g. Febert et al. 2002; Irlbacher et al. 2006).

5 – Conditioning of the H-reflex by subthreshold TMS (e.g. Morita et al. 2000; Petersen et al. 2003; Taube et al. 2006).

Rest motor threshold (RMT) and the input-output curve. In order to standardize the input intensity during TMS measurements, the RMT is usually defined as the minimum TMS intensity that elicits reproducible MEPs of at least 50μV in about 50% of 5 – 10 consecutive trials. The RMT reflects the global excitability of the corticospinal pathway, including large pyramidal cells, cortical excitatory and inhibitory interneurons and spinal motoneurons. (Avela & Gruber, in Komi 2011, 120) The relationship between stimulus intensity and motor response (i.e. static input-output relationship) can be determined by recording several MEPs starting at RMT and then progressively increasing the stimulation intensity and recording the responses (Devanne et al. 1997; Sekiguchi et al. 2001). Several experiments have shown that the TMS recruitment curve represents a sigmoidal increase in

MEP amplitude with increasing stimulus intensity until it reaches a plateau (Figure 10A; Devanne et al. 1997). The non-linearity of the input-output curve must reflect at least in part, the well known fact that stimuli of increasing strength recruit motoneurons with increasing MU potentials (Capaday et al. 1997, Henneman 1957). Three parameters have been suggested to completely characterize the input-output curve, providing general measures of corticospinal excitability: threshold, plateau and the slope. Any task-related difference in the involvement of the motor cortex can be demonstrated by statistical differences in one or more of these parameters, at comparable levels of muscle activity. (Devanne et al. 1997; Sekiguchi et al. 2003) Figure 11a shows two input-output curves of tibialis anterior muscle evoked responses measured 1.5 hours apart: there is no statistical difference between them. Furthermore, figure 11b shows two averaged (n=8) superimposed MEPs obtained 1.5 hours apart in response to stimuli of 32% of maximum stimulator output. Thus, figures 11a and 11b show that responses obtained later in the experiment were no more variable than at the beginning. In addition, larger responses are inherently less variable than smaller ones; the coefficient of variation was typically around 0.5 for threshold responses, and 0.1 for MEPs of maximal amplitude (Figure 11c; Devanne et al. 1997). Although reproducible for the same subject, the values for the input-output curve and RMT can vary considerably among different subjects (Bawa et al. 2002).

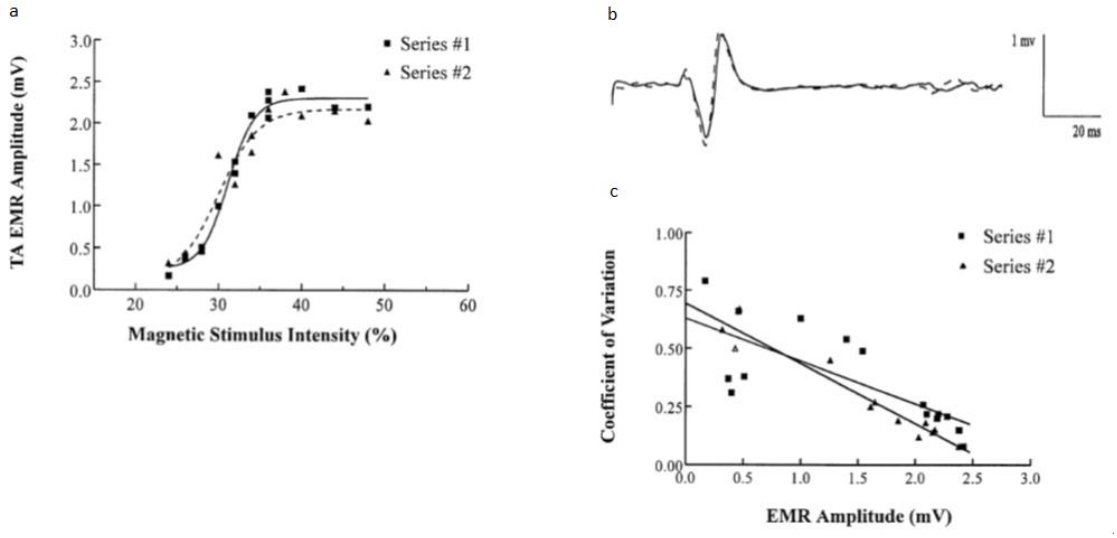

FIGURE 11. (a) Input-output curves for tibialis anterior muscle measured 1.5h apart in the same subject, (b) 2 averaged (n=8) superimposed MEPs obtained around 1.5h apart in response to stimuli

of 32% of the maximum stimulator output, (c) relationship between coefficient of variation and MEP amplitude. Source: modified from Devanne et al. (1997).

Coil positioning. Due to the motor cortex topographic organization, the position of the stimulating coil must be adjusted in order to evoke MEPs in different target muscles. The main goal after the position is attained, is to maintain and later replicate exactly the same position, since the failure to do so may lead to stimulation of different corticospinal cells, and the recording of different motor unit responses. (Avela & Gruber, in Komi 2011, 121) Manual fixation against a reference grid marked on the scalp is the simplest way to maintain the position of the stimulating coil on the head, and this method has been used successfully during voluntary activity while sitting, postural maintenance and walking (Capaday 1997). Recently, combining brain imaging information to TMS (i.e. Navigational TMS) has provided a platform for targeting systems which allow repetition of a constant stimulus to a certain location in the cortex by utilizing magnetic resonance image (Säisänen et al. 2008).

Muscle activity modulation on MEP. Voluntary muscle activation increases the size and number of epidural volleys evoked by a given intensity of TMS. It also decreases the latencies and threshold stimulation intensity at which they are evoked. (Di Lazzaro et al. 1998) The MEPs also show a large increase (Abbruzzese et al. 1994), which is disproportional compared with the increase of volley size, suggesting an important contribution of spinal excitability in the overall EMG response (Matthews 1999). The decrease in MEP latency and increase in amplitude is most likely explained by an easier activation of the preactivated motoneuronal pool compared with the resting condition (Morita et al. 2000). The MEP modulation by muscle activity shows marked differences among different muscle groups: for biceps brachii muscle, MEP amplitude increased sharply with contractions up to 50% of maximal root mean square (RMS) EMG, then reached a stable plateau until MVC (Sacco et al. 1997). In contrast soleus had a continuously increase in MEP amplitude all the way to MVC (Oya et al. 2008), which coincides with its upper MU recruitment limit (Oya et al. 2009). Muscles in which the majority of MNs are recruited early are more likely to show anomalous inverse effects (i.e.

saturation or reduction of the summed population response with increased mean activity) than those whose MN recruitment thresholds are widely staggered and show only small changes in firing rate (Matthews 1999). Possibly, MEP amplitude increment peaks at the upper recruitment limit, because newly recruited MUs are still capable of responding to additional stimulus, and MUs that are already firing at high rates are progressively more incapable of responding (i.e. refractoriness). Motoneurons from different muscles also displays distinct levels of synaptic noise, duration of afterhyperpolarization period and mean firing rate, and all of these variables affects the MU's probability to respond to a given stimulus (Matthews 1996).

Silent Period (SP). TMS of human motor cortex during a voluntary muscle action induces a MEP followed by a pause in EMG activity (Figure 12), known as the silent period (Inghilleri et al. 1993). To determine SP, three latencies are marked by visual inspection: the onset of the MEP, the beginning of the silence (i.e. MEP offset), and the beginning of any level of EMG activity. This way, both the absolute SP (i.e. excluding MEP) and the relative SP (i.e. including MEP) can be defined. (Säisänen et al. 2008) Säisänen et al. (2008) suggested that absolute SP best reflects the grade of intracortical inhibition, although defining its onset can be complicated due to the gradual decay of the preceding MEP. In both measures, additional problem exists in defining the reappearance of the EMG background especially during low muscle forces. Considerable interindividual variation in SP has been reported for first dorsal interosseous and tibialis anterior muscles, but no differences in SP between homonymous muscles of both sides (Roick et al. 1993). Furthermore, Roick et al. (1993) also demonstrated that SP duration was not modulated by any changes of finger or ankle joint position. It was identical irrespective of whether muscle actions were isometric or dynamic, with or without joint fixation. Regarding the coil position over the scalp, it was demonstrated that the SP duration and MEP amplitude were maximal at identical coil positions.

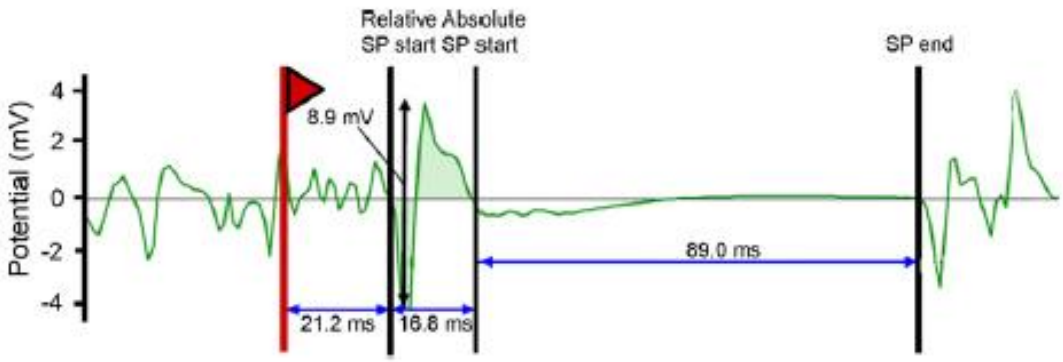

FIGURE 12 – Raw EMG data with a typical MEP and SP response to TMS. The flag indicates the stimulation moment. The MEP is elicited at 21.2 ms latency, amplitude is 8.9 mV. The duration of absolute SP is 89.0 ms and relative SP 105.8 ms. Source: Säisänen et al. (2008).

SP duration progressively increases with increasing TMS intensity. Roick et al. (1993) reported that for first dorsal interosseous muscle, SP increased concomitantly with stimulation intensity from 30 to 100% of maximum stimulator output, unlike the MEPs which plateaued at 60-70%. Interestingly, muscle activation had a negligible role on the duration of SP; Säisänen et al. (2008) reported that varying from 20 to 80% of MVC for the thenar musculature had no effect on SP.

There is a continuous debate about the origin of the SP; several studies have suggested that the initial part of SP is mainly due to reduced spinal excitability, while the late part of the SP is of cortical origin (Chen et al. 1999; Inghilleri et al. 1993; Sacco et al. 1997). There is conflicting evidence that spinal excitability is decreased during the early part of the SP: H-reflex can be reduced (Chen et al. 1999) or show no changes (Roick et al. 1993) during the early part of the SP. Stimulation of the motor tracts at the cervicomedullary junction produces a small SP of around 43 ms, and it appears to depend mostly on spinal inhibitory mechanisms that follow neuronal excitation: afterhyperpolarization and recurrent inhibition. Since this type of stimulation bypasses the cortex, the SP in this form of stimulation is not influenced by the level of cortical excitability. The same spinal mechanisms probably operate in the first 40 – 50 ms of the silent period after cortical stimulation. (Inghilleri et al. 1993) MN refractoriness after nerve stimulation may last only up to 8 ms, and may therefore be neglected in a discussion of the origin of an inhibitory

period lasting up to 250 ms. However, it is not clear if the TMS induced refractoriness lies in the same time range. (Roick et al. 1993)

It has been proposed that SP results from transsynaptic activation of inhibitory interneurons projecting to the pyramidal cells of the motor cortex (Inghilleri et al. 1993, Roick et al. 1993). Davey et al. (1994) demonstrated that inhibition of voluntary action by TMS can occur at lower stimulation strengths than those needed to induce MEPs, consistently at longer latencies, suggesting an oligosynaptic intracortical pathway. Furthermore, the coil position with the lowest threshold for excitatory responses and for suppression of EMG were the same. This finding suggests that underlying cortical structures being stimulated during suppression and excitation not only have shared locations, but also that the neuronal elements involved have similar orientations. This result demands caution, since the resolution in this particular experiment was only 45 degrees, and smaller differences in orientation of structures would not be detected. (Davey et al. 1994)

Further experiments were conducted to investigate whether TMS-evoked suppression of EMG has a cortical component that reduces corticospinal output or if the stimulus excites a descending corticospinal volley that inhibits motoneurons at the spinal cord level. Davey et al. (1994) stimulated the spinal cord electrically at the neck to produce test responses in the thenar muscles. TMS stimulus at an intensity shown to produce suppression of EMG was applied prior to the test shock at the spinal cord, which was adjusted to be very small (10% of maximum), ensuring that any weak inhibitory effect would not be obscured by a strong excitatory drive. The absence of any effects in rest or during muscle activation suggests that TMS was not producing inhibition of motoneurons via corticospinal activation of inhibitory interneurons at the spinal cord level. Additionally, TMS could simultaneously activate other muscles, inducing a corticospinal volley that would activate interneurons at the MNs level. Ia inhibitory interneurons mediating reciprocal inhibition are excited by corticospinal neurons (Jankowska et al. 1976) and the discharge of motoneurons could excite recurrent inhibitory interneurons. However, if the suppression of voluntary activity observed in response to TMS was a result of a corticospinal volley activating spinal inhibitory interneurons, then the observed suppression of EMG in one muscle should have been

accompanied by facilitation or excitation of other muscles. To test this hypothesis, subthreshold TMS during simultaneous voluntary activation of the 2nd and 3rd dorsal interrossei muscles (which are antagonist because they cause abduction of the middle finger in different directions) was performed to check if facilitation of one muscle would occur during EMG suppression of the other. There was a pronounced suppression in both muscles, with no evidence of prior excitatory responses in either muscle. Thus, TMS-evoked excitation to one of these muscles was not a prerequisite for the inhibition of the other. However, not enough is known about the Ia inhibitory pathways regulating MN discharge to different muscles to generalize these findings. (Davey et al. 1994)

2.5.2 Hoffman reflex

Originally described by Paul Hoffmann in 1910, and later given his name, the H-reflex is an electrically induced spinal reflex (Bischoff 2002). Percutaneous electrical stimulation of a mixed peripheral nerve (i.e. containing both motor and sensory axons), will evoke preferentially an afferent sensory volley (i.e. from the Ia fibers to the αMNs) that by depolarizing αMNs will result in an efferent motor response (i.e. from αMNs to the muscle fibers) recorded in EMG, called the H-reflex (Figure 13a). If the stimulation intensity is increased, motor axons will also depolarize, generating a compound muscle action potential denominated M-wave (i.e. muscle response; Figure 13b). The higher intensity required to elicit an M-wave is explained by the motor axon's intrinsic properties and smaller diameter which results in a higher depolarization threshold (Latash 1998). The M-wave has a shorter latency than the H-reflex since it is generated via direct stimulation of the nerve and hence there is no synaptic delay (Bischoff 2002). The H-reflex and M-wave do not activate the same αMNs: Ia afferent input will recruit MNs according to the size principle (Henneman 1957), while the electrical stimulation that elicits the M-wave activates initially larger diameter axons from fast MUs. (Knikou 2008; Pierrot-Deseilligny & Mazevet 2000) The amplitudes of the H-reflex and M-wave will both increase fairly linearly with the stimulation intensity until the maximum H-reflex (Hmax), representing the fullest extent of reflex activation, and, at higher stimulation intensities, the maximum M-wave (Mmax), representing the maximal muscle activation (Crone et al. 1999). The APs generated in the

motor axons not only travels orthodromically (i.e. towards the muscle), but also antidromically (i.e. towards the αMNs). Therefore, after plateauing, the H-reflex will progressively decrease until completely disappear, as a result of the collision between the orthodromic and antidromic volleys (Figure 13c). Since the muscle spindle is not been stimulated, the H-reflex is not modulated by changes in spindle sensitivity via beta or gamma motoneuron modulation. (Palmieri et al. 2004)

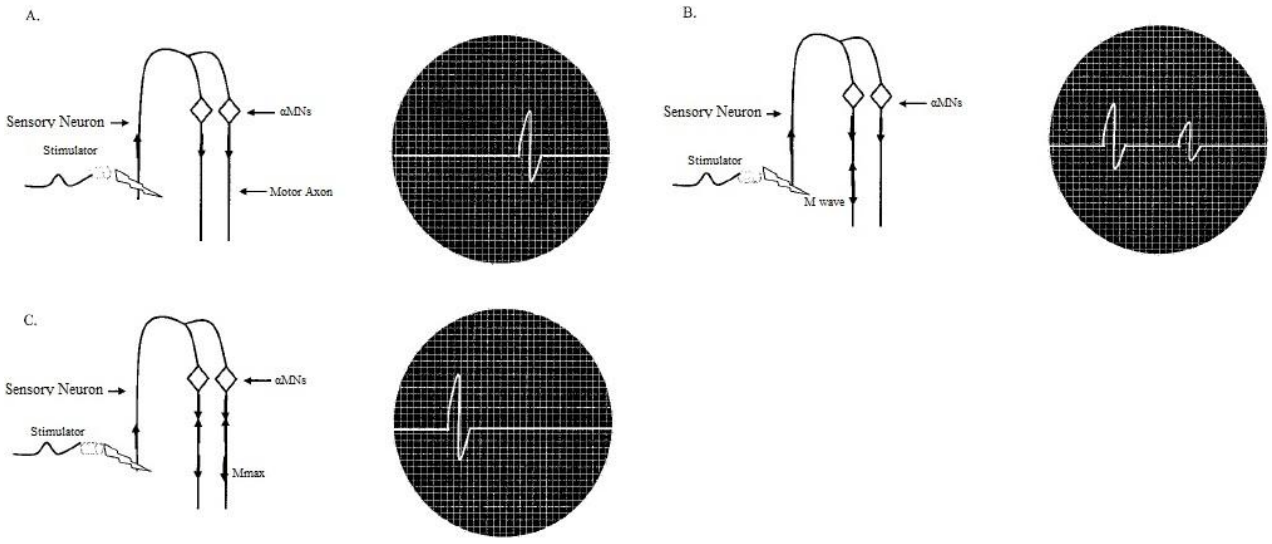

FIGURE 13 – Summary of events leading to the appearance of the H-reflex and M-wave, and the disappearance of the H-reflex. (A) Electric stimulus elicits a response only in Ia afferent fibers, causing an orthodromic volley toward the spinal cord and resulting in the firing of the αMNs and the appearance of the H-reflex on the EMG. (B) Electric stimulus elicits a response in Ia afferents and also directly activates some motor axons, generating the M-wave (orthodromically) and reducing the H-reflex (antidromically). (C) Activation of all motor axons, antidromic collision blocks all APs that were result of the Ia orthodromic activity, therefore only the Mmax appears on the EMG. Source: modified from Palmieri et al. (2004).

Motoneuron excitability is an intrinsic property that depends on the total membrane conductance, the membrane potential relative to threshold, and the presence of neuromodulators (Capaday 1997; Heckman et al. 2008; 2009). Due to the direct synaptic connection of Ia afferents and αMN, it has been tempting for researchers to assume that the H-reflex represents faithfully the excitability of the MN pool under study (Misiaszek 2003; Zehr 2002). However, the synaptic connection between Ia afferents and αMN is itself under

modulation of presynaptic inhibition (PSI), which functions as a variable gain regulator of the Ia/αMN synapse (Meunier & Pierrot-Deseilligny 1998). It is now fairly well established, from both electrophysiological and morphological investigations, that the terminal arborizations of large cutaneous and muscle afferents in the vertebrate spinal cord are targets of specific sets of axo-axonic connections with GABAergic (i.e. gamma aminobutyric acid) interneurons. PSI is mediated by the action of a GABA inhibitory interneurons acting on the Ia afferent terminals, leading to a reduction in neurotransmitter release and a concomitant reduction in motoneuron depolarization induced by Ia activity. Thus, afferent transmission can be altered without a corresponding effect on the postsynaptic (i.e. motoneuron) membrane. (Rudomin & Schmidt 1999) PSI can alter the afferent signal that actually evokes the H-reflex and thus can lead to a separate pattern of modulation of reflex and motoneuron excitability (Misiaszek 2003; Zehr 2002). Therefore, the H-reflex could be described more accurately as a measure of efficacy of the synaptic transmission in the Ia reflex arc (Capaday 1997).

Assuming a consistent level of presynaptic and postsynaptic inhibition or facilitation, the amplitude of the H-reflex will vary directly with the afferent volley arriving at the Ia/αMN synapse. Therefore it is critical that the synaptic input received by the αMNs is constant (i.e. same number of Ia afferent axons must be activated by electrical stimulation). Movement of the stimulating electrodes or of the nerve relative to the electrodes (e.g. during postural change or continuous movement) will alter the relative activation of the Ia afferent axons, thereby leading to changes in the H-reflex, independent of changes in synaptic efficacy or other factors. Monitoring the stimulating current alone does not guarantee that the same number of nerve fibers are stimulated during different trials, even if the task is kept strictly the same. Furthermore, the constant current stimulator will maintain the stimulus current constant over relatively large changes of load impedance, but because of the possible changes in cathode orientation relative to the nerve, different fibers may be stimulated. To safeguard against this methodological error, H-reflexes should be evoked at an intensity of stimulation that also evokes an M-wave. The amplitude of the M-wave measured immediately after the delivery of the stimulus can then be monitored as a mean of estimating and controlling stimulus consistency. (Pierrot-Deseilligny & Mazevet 2000;

Zehr 2002) The stimulation frequency should also be carefully controlled, since delivering stimuli too close together decreases the amplitude of the H-reflex because of previous activation of Ia afferents and depletion of neurotransmitters, a phenomenon known as postactivation depression (Knikou 2008). The stimulus duration is also an important parameter since it allows the separation of the threshold of Ia fibers to that of motor fibers. Stimulus duration of 0.5 ms to 1 ms has been suggested to clearly separate the thresholds and avoid unpleasant sensations to the subject. (Capaday 1997)

In order to compare H-reflexes between subjects and conditions, reflex amplitude is often normalized to the Mmax. However it has been demonstrated that Mmax can vary considerably throughout an experiment. Therefore it is critical that the Mmax is evoked for each condition and in each test position to be used as a reference in that specific condition/position. (Crone et al. 1999)

Muscle activity modulation on H-reflex. H-reflex responses are modulated by the activity of the motoneuron pool: in general, increasing the level of motoneuron depolarization will increase the H reflex amplitude (Morita et al. 2000; Romanó & Schieppati 1987). The following MUs can contribute to an H-reflex superimposed on an activated muscle: (1) MUs that are not tonically firing but are discharged by the H-reflex stimulus and (2) MUs that are tonically active but are not refractory at the arrival of the afferent volley (Rüegg et al. 1990). Therefore, although an increasing trend is often verified, at high depolarization levels it can decrease or plateau, since MNs are firing rapidly and hence refractory for a substantial fraction of time (Stein 1995). Furthermore, for a given level of voluntary activation, the H-reflex amplitude will also be modulated by muscle action type (more details in section 2.8; Duclay & Martin 2005; Nordlund et al. 2002) and velocity (Duclay et al. 2009; Romanó & Schieppati 1987). Phase specific modulation of H-reflex has also been demonstrated for stepping, walking and running at varying speeds (Simonsen & Dyhre-Poulsen 1999; Trimble et al. 2000).

2.6 Muscle-tendon complex (MTC)

The skeletal muscle is composed of thousands of muscle fibers, and each fiber is a single multinucleated cell that is enclosed in a cell membrane (i.e. sarcolemma; Figure 14). The muscle fiber is composed of sarcoplasm, which contains myofibrils that are the contractile structures of a muscle fiber, and nonmyofibrilar structures such as ribosomes, glycogen, and mitochondria, that are required for cell metabolism.

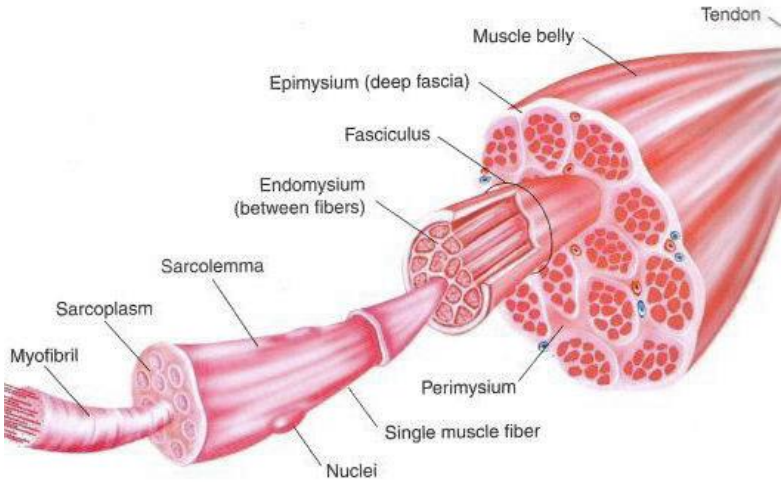

FIGURE 14 – Diagrammatic representation of the structural composition of the skeletal muscle tissue. Source: Baechle & Earle (2008).

The basic contractile unit that composes the myofibril is called sarcomere (Figure 15): a multi-protein complex located between two Z-lines, composed of contractile proteins actin and myosin and non-contractile proteins that provide stability and structure for the sarcomere. The actin filaments have two twisted chains of monomers bound by tropomyosin helical polypeptide chains. Another protein called troponin is also attached to the actin strand. The myosin filament consists of bundles of molecules with polypeptide tails and globular heads, and it is surrounded by a hexagonal array of six actin filaments. (McArdle et al. 2010, 357-364) There are about 15 different non-contractile proteins within the sarcomere structure, and the functional understanding of many is still in early stages. Desmin is a non-contractile protein that connects the sarcomeres in parallel through the Z-lines and to the costameres in the sarcolemma. These connections maintain the structural

and mechanical integrity of the cell and also permits force transmission along the fiber and to adjoining fibers. Another important non-contractile protein is titin, which connects the Z-lines to the middle part of the myosin filament, called the M-line. Titin resists the deformation of the sarcomere during lengthening, thus contributing to the passive stiffness of the muscle, and also serves to maintain the myosin filament centralized in the sarcomere, providing stability during the contraction and thus permitting that the myosin filament has optimal interaction with all the surrounding actin filaments. (Patel & Lieber 1997; Gajdosik 2001)

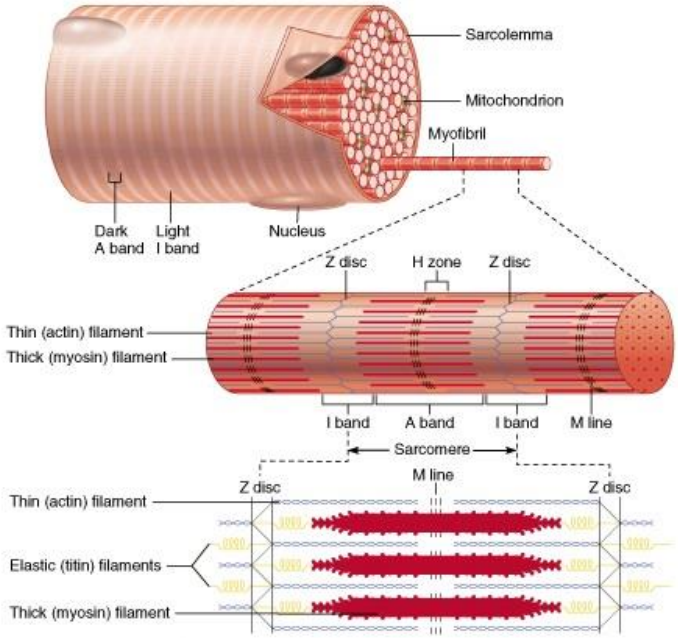

FIGURE 15 – Sarcomere structure. Source: Benjamin Cummings (2001).

The connective tissue called endomysium consists of a dense weave network of collagen fibers (100–120 nm in diameter) that surround the surface of individual muscle fibers and attach into the basement membrane of the sarcolemma. It also attaches perpendicularly to adjacent muscle fibers and interconnects with the perimysium (Patel & Lieber 1997). The primary role of endomysium is to transfer the in series tension between the contractile components and the tendons (Trotter & Purslow 1992). Groups of muscle fibers (i.e. fascicles) are covered by another layer of connective tissue called perimysium. It consists of tightly woven bundles of collagen fibers (600-1800 nm in diameter) that interconnects groups of muscle fibers (Gajdosik 2001). The perimysium has the function of redistributing

the tension in a uniform manner across the various muscular components and to prevent that the muscle lengthens excessively (Magnusson 1998). The endomysium and perimysium are continuous with the outer connective tissue sheath called the epimysium, which envelops the entire muscle (Gajdosik 2001).

Tendons are responsible for transmitting contractile forces developed by muscles to the skeleton, generating joint movement or stabilization (Maganaris et al. 2004). The structure of tendons, like other connective tissues, can be divided into cellular content (for tendons fibrocytes or fibroblasts, depending on the cell synthetic activity) and an extracellular matrix that can be further divided into fibrilar and interfibrilar components. The fibrilar component is mainly constituted of collagen and a small quantity of elastin. The interfibrilar component is composed by a hydrated network of glycoproteins (i.e. compounds containing a carbohydrate covalently linked to a protein; Levangie & Norkin 2005, 78-79).

The basic building block of collagen is the triple helix of polypeptide chains called tropocollagen, and many of these structures are combined together to form fibrils. Intramolecular and intermolecular crosslinks stabilizes and strengthens the enlarging fibrils into successively larger subunits, to form primary bundles known as fibers. Groups of fiber bundles, enclosed by a loose connective tissue called the endotendon, form a secondary bundle called fascicle (Figure 16). The endotendon also encloses nerves, lymphatic and blood vessels supplying the tendon. The sheath that encloses the entire tendon is called epitendon and there is also the paratendon, which is a double-layered sheath that is loosely attached to the outer surface of the epitendon. The paratendon protects the tendon, enhances its movement on adjacent structures, and provides a source of cells if the tendon is injured. (Levangie & Norkin 2005, 76-78)

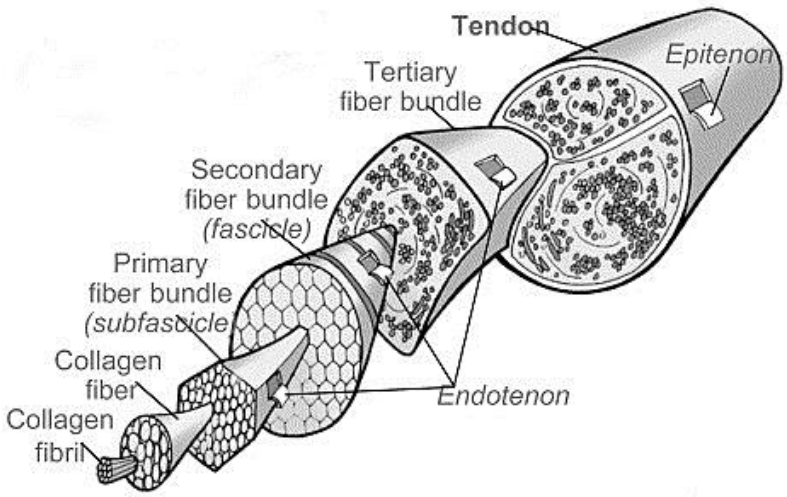

FIGURE 16 – Diagrammatic representation of the structural composition of the tendon.
Source: Ashe et al. (2004).

Because the loads are applied by the in series attached muscle, the collagen fibers in tendons have a largely unidirectional alignment parallel to these tensile forces, although there are also crossed and spiral arrangements. The attachment of tendon to muscle at the myotendinous junction comprises an interdigitation between collagen fibers and muscle fibers. Surface friction and the direct connections between collagen, proteoglycans, basal lamina and integrins in the muscle cell membrane create a strong and stable interaction. The endotendon blends into the endomysium, and the epitendon blends into the epimysium, forming a meshwork of connective tissue around the muscle fibers. The attachment of tendon to bone at the osteotendinous junction is accomplished through the gradual transition between tendon to fibrocartilage and its further transition to mineralized fibrocartilage and finally bone. (Levangie & Norkin 2005, 78-79)

2.6.1 Muscle architecture and force transmission

Muscle architecture. The arrangement of muscle fascicles varies among muscles. Muscles that have a parallel fiber arrangement to its long axis are designated fusiform muscles, and those with a fiber arrangement oblique to its long axis are called pennate muscles. Fusiform muscles tend to have longer fascicles, capable of producing forces over large length ranges and at high shortening speeds, because they have a large number of simultaneously

contracting, serially arranged sarcomeres. Moreover, since the shortening speed of each sarcomere in a fiber or fascicle would be slower for a given speed of whole-fiber shortening when there are more sarcomeres in series, sarcomere force would not decrease as rapidly as fiber-shortening speeds increase, according to the force-velocity relationship. Regarding pennate muscles, the fascicle pennation decreases the amount of force that is directed along the long axis of the muscle (Figure 17). Thus, only a given amount of force effectively produces motion of the bony lever. This decrease in muscle force is a function of the cosine of the pennation angle, meaning that the greater the pennation angle, the greater is the force loss. However, this potential decrease in force production capability is offset by at least three main mechanisms. First, for the same muscle volume, a muscle with larger fascicle pennation will have a greater physiological cross sectional area (increases as a function of the sine of the pennation angle) and thus a greater force-generating capability. Physiological cross sectional area is defined as the magnitude of muscle fiber area perpendicular to the longitudinal axis of the individual muscle fibers multiplied by the cosine of the pennation angle. Second, fascicle angulation probably increases force by allowing fibers to operate closer to their optimum length. Fibers in pennate muscle rotate as they shorten so tendon excursion is greater than shortening distance of the individual fibers. According to the length-tension relationship, there will be an optimum sarcomere length at which fibers produce their greatest active force. Since the optimum sarcomere length seems to occur at lengths where the highest forces are required, fibers that shorten less for a given tendon excursion are likely to stay closer to their optimum for force generation. Third, since the time-dependent shortening distance of a fiber decreases during a contraction, the shortening speed will also decrease and force increases as per the force-velocity relationship for muscle. Thus, in a pennate muscle, a more forceful contraction might be possible from the exploitation of both the force-velocity and force-length relationships in addition to the greater quantity of contractile material that can attach to the tendon or aponeurosis. (Aagaard et al. 2001; Blazevich 2006; McArdle et al. 2010, 360–361)

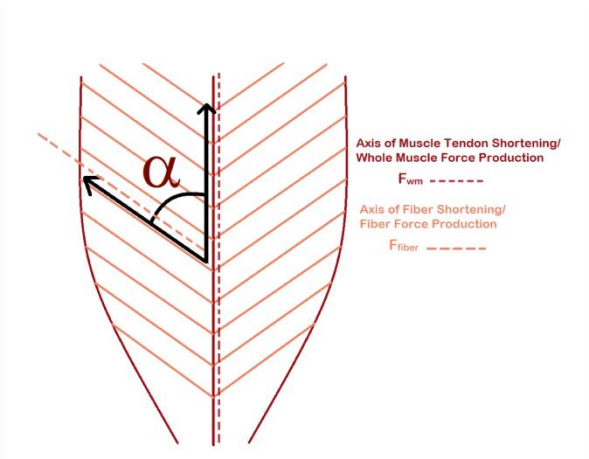

FIGURE 17 – Pennate muscle diagram. Source: Wikimedia Commons.

Force transmission. The actual path of force transmission from actomyosin interaction to tension at the osteotendinous insertion site is not yet consolidated. "Within the muscle cell, endo and exosarcomeric cytoskeletal proteins create a meshwork across which force can be transmitted in practically any direction with respect to the fiber axis. When myosin interacts with actin, force is directly transmitted between molecules at their interface, and a tensile force is transmitted from the myosin filament via titin and actin to the Z- disk (Figure 18a). This force is transmitted longitudinally within the myofibril, between the sarcomere generating force and its neighbor but also to adjacent parallel myofibrils, because adjacent Z-disks and M-lines are mechanically connected via the intermediate filament system (Figure 18b). Thus, the force generated by a single sarcomere can easily be transmitted radially, as well as longitudinally. This arrangement provides a great deal of mechanical redundancy within the muscle cell, and even in a situation where a serial sarcomere is inactive or damaged, longitudinal force can still be transmitted via the intermediate filament network, either longitudinally around the damaged sarcomere within the same myofibril or laterally to adjacent myofibrils. The intermediate filament system connects adjacent myofibrils and then forms connections with the cell surface membrane via specialized focal adhesion sites (i.e. costameres; Samarel 2005) along the fiber length (Figure 18c) and at the muscle-tendon junction (Figure 18d). Finally, muscle fibers themselves have a complex mode of force transmission to the extracellular matrix via lateral connections to the endomysial connective tissue (Figure 18e) or even via muscle fiber to muscle fiber connections in series. (Pater & Lieber 1997) The longitudinal tensile

stiffness of the endomysium near resting length is low, and therefore it would be too compliant to transmit contractile force along the muscle fiber direction, especially when a fiber produces high forces with little or no length change (Purslow & Trotter 1994). This apparently contradictory notion that, whilst force transmission between intrafascicularly terminating muscle fibers of series fibered muscles must be via the intervening endomysium, the tensile properties of this structure are inadequate to fulfill this role (Purslow & Trotter 1994). Trotter and Purslow (1992) postulated that force transmission between laterally adjacent muscle fibers that overlap for typically two-thirds to three-quarters of their length is most likely to be by shear rather than tension.

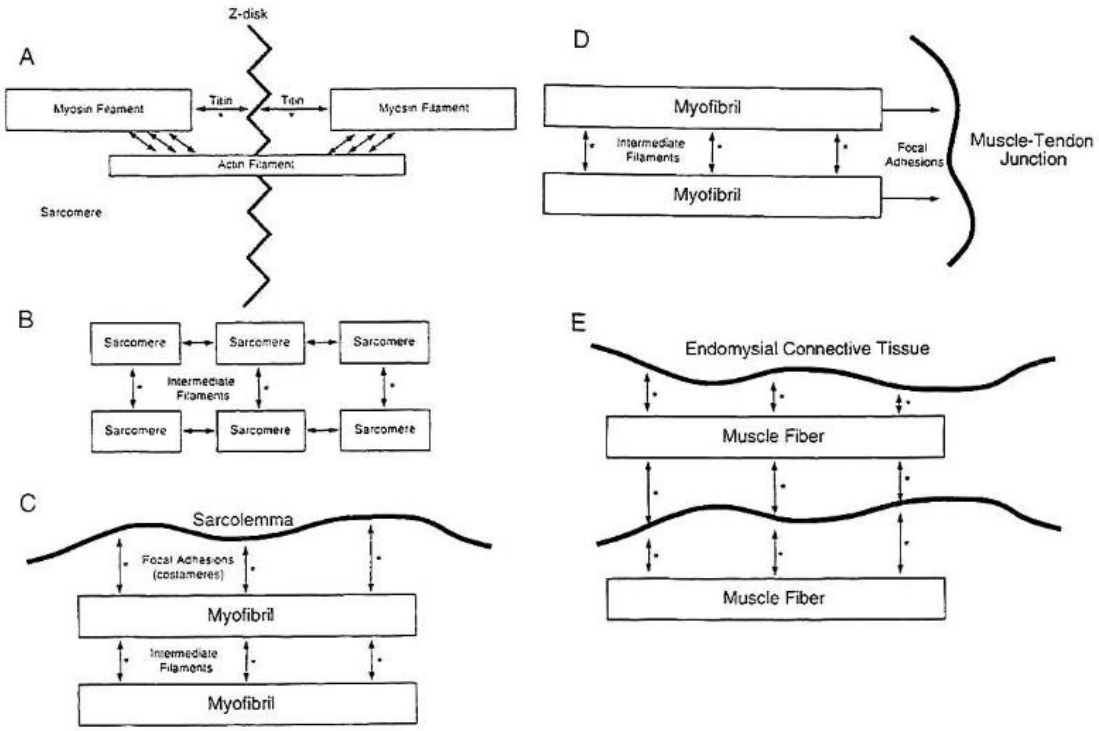

FIGURE 18 – Schematic representation of potential paths of force generation and transmission in skeletal muscle. Arrows with asterisks represent current research areas, whereas solid arrows represent accepted paths of muscle force transmission. Source: Patel & Lieber (1997).

2.7 Assessment of muscle tendon complex mechanical behaviour

Ultrasonography is a non-invasive tool frequently used in medicine, and in the past decades has been increasingly utilized in the research field. The ultrasound waves created by the probe are propagated into the desired area and the echoes received are interpreted by a transducer to create a digital image. The sound waves are created by electrical pulses that vibrate piezoelectric transducers (usually a type of ceramic), creating a single arc-shaped sound wave from the sum of all individual pulses emitted by the transducer. (Hedrick et al. 2004, 9-15) To make sure that the sound is transmitted efficiently into the body (i.e. impedance matching), the transducer face has a rubber coating and a water-based gel is placed between the probe and the subject's skin (Szabo 2004, 23). The sound wave is partially reflected from the interface between different tissues and returns to the transducer. The strength of the reflected sound depends on the difference in the acoustic impedance between the adjacent structures. The acoustic impedance of a tissue is related to its density; the greater the difference in the acoustic impedance between tissues, the more reflective the boundary will be. The spatial resolution of ultrasonography depends on the material and the frequency of the ultrasound: the wavelength (l) equals the speed of sound transmission (n; 1540m/s) divided by the frequency of the ultrasound (f; 1-10MHz) (l = n/f). Therefore, the spatial resolution of an ultrasound image in the human body can be theoretically from 0.15 to 1.54 mm. (Hedrick et al. 2004, 5-15) The choice of frequency is a trade-off between spatial resolution of the image and imaging depth: lower frequencies produce less resolution but image deeper into the body. Higher frequency sound waves have a smaller wavelength and thus are capable of reflecting or scattering from smaller structures. Higher frequency sound waves also have a larger attenuation coefficient and thus are more readily absorbed into the tissue, limiting the depth of penetration of the sound wave. (Szabo 2004, 22) Superficial structures such as muscles and tendons are typically imaged with a 7.5 MHz B-mode linear array probe (Aagaard et al. 2001; Finni et al. 2001), although there can be some variations depending on the methodology and aimed studied structures.

Briefly, in order to generate the image, the ultrasound scanner needs to determine which transducer element received the echo, how strong the echo was and how long it took to

receive the echo after it was emitted. Once these three parameters are analyzed, the scanner can locate each pixel in the image and give it the according brightness (for a complete description refer to Szabo 2004, 225-227).

Fascicle length, velocity and pennation angle. The ultrasound probe is placed on the surface of a muscle and the examiner moves the scanning probe on the dermal surface proximally and distally in order to visualize fascicles along their lengths from the superficial to the deep aponeurosis (Fukunaga et al. 1997). Care is taken to align the plane of the ultrasonogram in parallel to the fascicles (Kawakami et al. 2002); otherwise the fascicle length may be overestimated (Scott et al. 1993). The fascicle length is defined as the distance between the insertions of the fascicle into superficial and deep aponeurosis (Kubo et al. 2000; figure 19), and it is sometimes regarded as equivalent to muscle fiber length, based on observations of tendon-to-tendon fiber arrangement within a fascicle (Kawakami et al. 2002). Although the orientation of fascicles is the same as muscle fibers, the fascicle length may not correspond to the muscle fiber length, because muscle fibers may terminate mid-fascicularly (Ounjian et al. 1991). In spite of the fact that fascicle length measurements do not give accurate representation of fiber or sarcomere function due to inhomogeneities in sarcomere lengths in series and compliance of connective tissue, the ultrasonographic method can give more detailed information about muscular function in vivo as compared to the muscle-tendon unit length estimations alone (Finni et al. 2001).

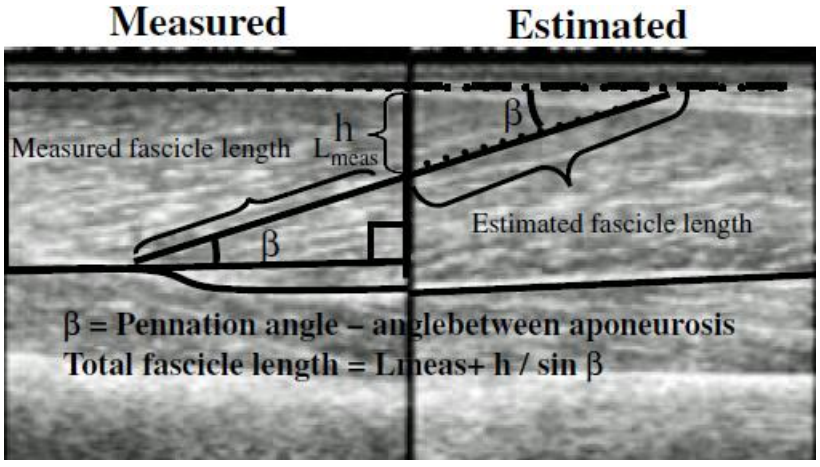

FIGURE 19 – Fascicle length and pennation angle determination. Source: Ishikawa et al. (2003).

Utilizing the same positioning, the muscle fiber pennation angle (β) can also be determined. It is defined as the angle between the fascicle and the deep aponeurosis (Fukunaga et al. 1997; Figure 19). Additionally, fascicle velocity can be obtained by differentiating the corresponding length change value (i.e. fascicle length tracked through a determined number of frames) with time (Kurokawa et al. 2001; 2003). The measurements can be performed with a digital curvimeter (Fukunaga et al. 1997) and with specialized image programs (Cronin et al. 2011; Kubo et al. 2000). They are usually performed three to five times (Aagaard et al. 2001; Morse et al. 2008), and the coefficient of variation has been reported to range from 0% to 9.8% between measures (Fukunaga et al. 1997; Klimstra et al. 2007; Narici et al. 1996) and from 0.2% to 6.2% between trials in different days (Kubo et al. 2000).

Main error sources. When performing ultrasound measurements, the first potential source of error arise from the need to match skin landmarks precisely. Additionally, other sources of error include the difficulty in recognizing muscle borders and varying the angle and/or pressure of the transducer. Because planimetry involves averaging large numbers of operator estimates of the muscle boundary, the constant error due to border estimation is reduced. Variations in the angle at which the transducer is held relative to the surface of the skin result in error that increases with the size and depth of the imaged muscle. This error becomes much greater if the muscle size or shape changes along the axis perpendicular to the plane of the image, particularly if the muscle is deep under the skin. (Dupont et al. 2000; Klimstra et al. 2007) In some muscles, like vastus lateralis, the entire length of the fascicle may not be visualized in one imaged area. A possible alternative is to estimate the fascicle length utilizing trigonometry, assuming a linear continuation (Finni et al. 2001; Ishikawa et al. 2003; Figure 19). Finni et al. (2003) compared the estimation method with the actual fascicle length and reported an estimation error of 0–7% due to aponeurosis non linearity.

Muramatsu et al. (2002) quantitatively demonstrated muscle fascicle curvature in human skeletal muscle in vivo. Fascicle curvature increased as the force level (i.e. %MVC)

increased, and also increased when muscle length decreased (i.e. changing joint angle). This information is important since many researchers use a two-point fascicle model whereby the points are placed at each end of the muscle fascicle, thereby failing to account for fascicle curvature (Cronin et al. 2011). Fascicle length has also been estimated utilizing the pennation angle and muscle thickness, this method will also produce error if the fascicle exhibits curvature. This second method generated an average underestimation of 6% for medial gastrocnemius, which might not seem to be critically large. However, the error would be substantially larger in muscles with long fascicles such as vastus lateralis and triceps brachii. (Muramatsu et al. 2002)

Ultrasound is a planar (i.e. two-dimensional) imaging technique, therefore obvious limitations exists when trying to understand the three-dimensional muscle architecture. Considering the three-dimensional internal arrangement of the muscle fascicles, and their possible curvatures, it is reasonable to suppose that the contraction-induced spatial movements occur off a simple planer image in human skeletal muscles. To overcome this limitation, Kurihara et al. (2005) recorded transverse serial images as composite video signal, while an electromagnetic sensor provided positional information at the same frequency. A specialized software was then utilized to build the three-dimensional image. They reported an error range of 0.3% – 1.6% for both vertical and horizontal directions, in superficial and deep regions. The coefficient of variance for repeated measures was reported to range from 1.7 – 3.3%. Therefore, this methodology was demonstrated to be accurate and reproducible. (Kurihara et al. 2005)

2.8 Muscle actions

The interaction of the force developed by muscle groups with the external forces (e.g. mass of the body parts, gravity) will result in muscle actions that produce static exercise (i.e. no movement about the related joints) or dynamic exercise (i.e. involving either a decrease or an increase in joint angles). Static exercise of activated muscle is traditionally described as an isometric action. In this condition, the torque due to the load is matched by muscle-exerted torque that is equal in magnitude but opposite in direction. Although in an isometric

action there is no angular change at the joint level (i.e. no change in muscle-tendon complex length), the muscle shortens due to the elasticity of the in-series tendon. (Komi 2003, 5)

When a muscle is activated and the torque it exerts is different from the load torque, there is a change in the muscle-tendon complex length and the muscle performs a dynamic action. The terms concentric and eccentric are used to identify muscle actions in which the muscle-tendon complex shortens and lengthens respectively. (Komi 2003, 5)

Human locomotion and performance seldom involves pure forms of isolated concentric, eccentric or isometric actions. This is because the body segments are periodically subjected to impact forces, as in running or jumping, or because some external force such as gravity causes the muscle to lengthen. Often the muscles first act eccentrically with a concentric action following immediately. The combination of eccentric and concentric action forms a natural type of muscle function called stretch–shortening cycle. (Komi 2003, 6)

2.8.1 Force–velocity Relationship

Force/Torque. The actual force a muscle can produce is not solely a function of the activation level produced by the voluntary command, but also depends on the speed at which the muscle changes its length: designated the "force-velocity relationship" (Edman 1988; Hill 1938; Figure 20). This relationship was obtained with an isolated muscle preparation that received constant supramaximal electrical stimulation against different mechanical loads. Briefly, the muscle was stimulated until maximal isometric force was reached, then it was suddenly released and depending on the magnitude of the extra load, the resulting shortening/lengthening speed could be determined (Komi 2011, 10-13). In this relationship, the maximal force decreases in the concentric mode in a curvilinear fashion, as a function of the shortening speed. During eccentric actions, maximal force has been reported to increase as a function of stretching velocity until reaching a plateau and becoming unaffected by changes in velocity. (Flitney & Hirst 1978; Harry et al. 1990; Lombardi & Piazzesi 1990)

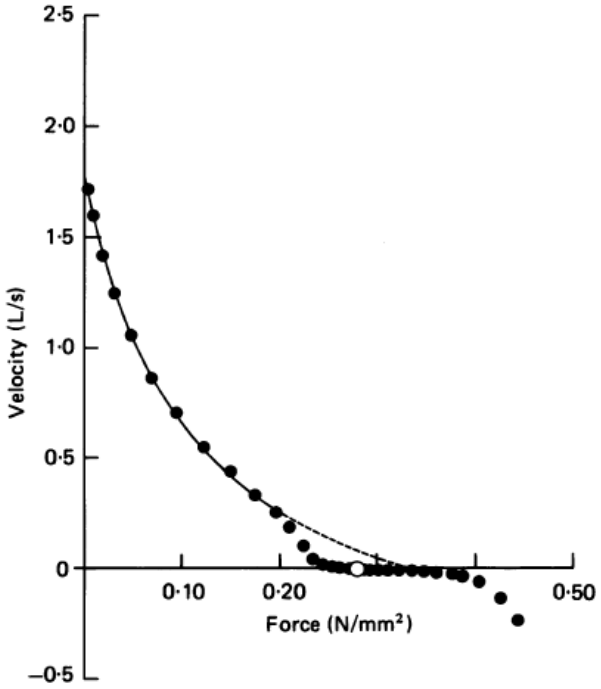

FIGURE 20 – Force-velocity relation in a single muscle fiber including data at loads greater than the maximal isometric force, Po (O). Hyperbola fitted to values truncated at 0.78 Po. Negative velocity means fiber lengthening. Resting sarcomere length: 2.10µm. Fiber length: 8.35 mm. Temperature: 1.0°C. Source: Edman (1988).

According to the *in vitro* force-velocity relationship depicted for a maximally activated muscle, force generated during muscle lengthening substantially exceeds maximal isometric force, which in turn exceeds force produced during muscle shortening (Edman et al. 1978; Katz 1939; Levin & Wyman 1927). Researchers have postulated that differences in actomyosin cross-bridge cycling and engagement processes might allow for greater moment production during eccentric actions compared with concentric or isometric actions (Lombardi & Piazzesi 1990). The presence of a series elastic component related to cross-bridge engagement may be the most significant factor responsible for the enhanced moment generation observed during eccentric actions (Blange et al. 1972; Flitney & Hirst 1978).When, after an isometric action, the muscle is forcibly lengthened, the first part of the lengthening movement is opposed by a steep rise in tension. In the later part of the movement, however, the resisting force increases slowly and may actually decrease. A converse effect is observed during shortening, the tension falls steeply at first but much more gradually during the later part of the movement. The large force changes at the

beginning of the movement are attributed to distortion but not breakage of the crossbridges. (Flitney & Hirst 1978; Rack & Westbury 1974)

Moment-angular velocity relationships derived from *in vivo* studies demonstrate that the isometric MVC exceeds peak and angle-specific concentric moments that decrease with increasing velocity of shortening (Linnamo et al. 2002; Pinniger et al. 2000; Westing et al. 1988). However, the ratio of eccentric to isometric moments is not consistent with that observed for *in vitro* force-velocity relationship. Peak forces generated during muscle lengthening *in vitro* have been shown to reach 150 – 190% of maximum isometric force (Edman et al. 1978, Harry et al. 1990), whereas human studies have generally found that maximal voluntary eccentric moments were not statistically greater than the isometric MVC (Gruber et al. 2009; Pinniger et al. 2000). Studies have employed electrical stimulation and quick muscle stretches to achieve eccentric magnitudes similar to *in vitro* data. The eccentric-to-isometric ratio achieved in these studies has at most reached the lower range of that observed *in vitro*. (Amiridis et al. 1996; Pinniger et al. 2000; Webber & Kriellaars 1997)

EMG/Voluntary activation. During maximal isokinetic actions, muscle activation (e.g. EMG integral or RMS) is often greater during concentric than eccentric actions, even though eccentric force levels are consistently higher (Enoka 1996; Linnamo et al. 2002; Westing et al. 1991; Figure 21). Westing et al. (1991) has suggested that the incomplete muscle activation reflects a "tension-limiting" mechanism that maintains muscle tension within safe limits (i.e. preservation of musculoskeletal integrity). Furthermore, there is evidence that muscle is activated differently during eccentric actions as compared to isometric and concentric actions (Duchateau & Enoka 2008; Enoka 1996; Nardone & Schieppati 1988; Nardone et al. 1989). Additionally, Bishop et al. (2000) reported that although mean integrated EMG was lower in eccentric actions, EMG peaks and peak-to-mean ratio were significantly higher in eccentric as compared with concentric actions. This finding is consistent with higher levels of spindle afferent feedback and a more synchronized MU activation during eccentric actions (Burke et al. 1978).

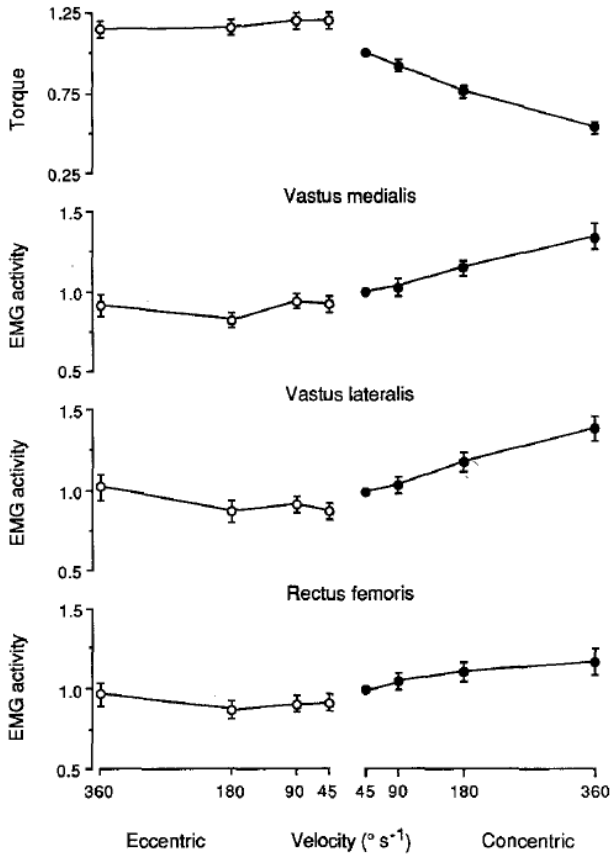

FIGURE 21 – Mean and standard error of measurement for torque- and EMG-velocity relationships for knee extensors during various eccentric (open symbols) and concentric (filled symbols) tests. Points represent amplitude-averaged signals between 30° and 70° of knee angle. Torque and full-wave rectified EMG signals were normalized by dividing all scores by the respective concentric 45°/s values (n = 14). Source: Westing et al. (1991).

Voluntary activation level (AL) is usually assessed by tetanical stimulation of a motor nerve superimposed on a maximal voluntary action. This technique assumes that the MUs that are not recruited or not discharging at their maximal frequency (i.e. incomplete activation) should yield a detectable force increment as a consequence of the stimulation of their axons. The voluntary AL is usually expressed as a percentage and can be calculated as:

AL = [1 – (superimposed twitch/ rest twitch)] x 100 (Babault et al. 2001).

A similar voluntary AL has been reported between eccentric (88.3%) and concentric (89.7%) actions, and both were significantly lower than isometric actions (95.2%). According to the proposed "tension-limiting" mechanism, maximal concentric actions should have induced higher voluntary AL compared with both isometric and eccentric actions. Nevertheless, this apparent contradiction may be explained by the fact that the "tension-limiting" mechanism would be mostly dependent on the type of muscular actions (i.e. isometric or dynamic) rather than on the maximal torque output. During submaximal eccentric, isometric, and concentric actions, the AL was reported to increase linearly with increasing tension level. Eccentric actions had a significantly lower slope in the AL-torque relationship. Thus, it seems that voluntary activation reduction is not limited to maximal eccentric efforts but may be an inherent characteristic of the command to activate lengthening muscles voluntarily. (Babault et al. 2001; Pinniger et al. 2000)

Spinal and Supraspinal Mechanisms. The reduced neural drive during eccentric actions may be accomplished by feedback from peripheral receptors such as joint receptors, free nerve endings in the muscle, cutaneous and pain receptors, and Golgi tendon organs (Westing et al. 1991). Regarding sensory feedback, a few important considerations are needed: (1) the excitatory influences of the Ia inputs from muscle spindles on MNs became less relevant with increasing concentric velocity, whereas it should greatly increase power during eccentric actions. Consequently gating the presynaptic Ia afferent input could attenuate elicitation of unwanted stretch reflex at the spinal cord level, thus preventing further increase in force and tissue damage (Burke et al. 1978; Fang et al. 2004). (2) The inhibitory influences of Ib afferents (i.e. Golgi tendon organs) should theoretically increase as a function of EMG level, but instead the greatest inhibition of the reflex was reported when the EMG level was very low at the termination of the eccentric action, and vice versa. (3) Cutaneous inputs should remain relatively constant during all action types; this is because the pressure exerted by the foot plate on the foot (i.e. recorded torque) is fairly constant, specially comparing isometric and eccentric actions. (Romano & Schieppati 1987) Unique central commands for lengthening muscle actions have also been suggested as a possible origin of differences between eccentric and concentric torque/velocity relationships in man (Duchateau & Enoka 2008; Enoka 1996). Further evidence suggests

that modulation of motoneuron excitability via PSI of Ia afferent inputs contributes to the differences in torque output observed during eccentric and concentric muscle actions (Abbruzzese et al. 1994; Romano & Schieppati 1987). Although neurophysiological evidence is still lacking, an orchestration of several afferent and efferent signals seems likely to explain the differences between action types (Westing et al. 1991).

The reduced neural drive (i.e. reduced EMG and AL) may be due to changes at supraspinal and/or spinal levels. Duclay and Martin (2005) demonstrated a reduced H-reflex during eccentric MVC as compared with isometric and concentric MVC, despite similar EMG activity in the three action types. In contrast, the V-wave response, a variant of the H-reflex, which depends on the level of efferent descending neural drive (Aaggard et al. 2000), remained unchanged (Duclay & Martin 2005). The contrasting results between H-reflex and V-wave led the authors to suggest that spinal excitability may be specifically modulated by the supraspinal centers and/or neural mechanisms located at the spinal level. Other research groups have also reported decreased H-reflex and MEPs produced both by TMS and transcranial electrical stimulation, and also a lower plateau and slope in the input/output curve utilizing TMS during eccentric actions. These results corroborate with the idea that task-related changes in excitability of cortical neurons play a minor role. Thus, cortical control of two different action types might be exerted by an appropriate "setting" action on the excitability of the spinal circuitry, or by a change in the excitability profile of the MN pool. (Abbruzzese et al. 1994; Duclay et al. 2011; Romano & Schieppati 1987; Sekiguchi et al. 2001; 2003) Interestingly, the decrease in spinal excitability observed during passive stretching was attenuated when subjects performed voluntary eccentric actions, suggesting that the neural control of the spinal excitability by the supraspinal centers can be specifically modulated (Nordlund et al. 2002). Taken together, the results suggest that the unique modulation of the activation signal during eccentric actions seems to involve both supraspinal and spinal mechanisms (Duchateau & Enoka 2008).

To differentiate specific modulation in excitability of supraspinal and spinal centers during lengthening and isometric MVCs, Gruber et al. (2009) compared MEPs elicited in biceps brachii and brachioradialis by TMS and cervicomedullary motor–evoked potentials

(CMEP) obtained by electrical stimulation of the corticospinal tract. Variations in MEP size reflect changes that can occur at both cortical and spinal levels, whereas the CMEPs were used to probe MN excitability, based on the assumption that the corticospinal pathway is free from PSI (Nielsen & Petersen 1994). The results showed that lengthening MVCs were associated with reduced amplitudes of MEPs and CMEPs. Furthermore, the decrease was significantly greater for CMEP, in both biceps brachii and brachioradialis, reflecting greater inhibition at the spinal level. The MEP/CMEP ratio (i.e. index of cortical excitability) was significantly increased during lengthening MVCs. It was therefore suggested by Gruber et al. (2009) that the increase in cortical excitability could partially counteract spinal inhibition. This is consistent with electroencephalogram data showing greater amplitude of movement-related cortical potential, and a larger area involved in the control process during lengthening compared with shortening MVCs for the elbow flexors (Fang et al. 2001; 2004).

Muscle-tendon mechanical behavior. At rest, pennation angle and fascicle length are closely dependent on joint position. When a muscle shortens, fiber pennation angle increases and fascicle length decreases, the opposite is true for muscle lengthening. When pennation angle is plotted against fascicle length, it can be observed that the increase in pennation angle is tightly accommodated by the concomitant decrease in fascicle length. Increasing isometric action intensity (i.e. % of MVC) causes a linear increase in pennation angle accompanied by a linear decrease in fascicle length. (Fukunaga et al. 1997; Narici et al. 1996)

In tibialis anterior muscle, at a constant ankle joint angle, concentric MVC with lower velocity (50°/s) had similar values for pennation angle and fascicle length as the isometric MVC. Increasing the concentric velocity increased fascicle length and decreased pennation angle at the same joint angle. This behavior can be explained by the compliance of the series elastic components (SEC): during the isometric action, the greater force causes fascicle shortening, stretching the SEC and increasing the pennation angle. As angular velocity increases during concentric actions, less force is produced, resulting in less fascicle shortening, and a smaller proportion of the central aponeurosis being pulled proximally,

causing a decreased pennation angle. During eccentric MVC, the tibialis anterior muscle fascicles acted quasi-isometrically. Pennation angles during eccentric actions were reduced compared with those during isometric actions. Both fascicle length and pennation angle were independent of angular velocity during eccentric actions. The quasi-isometric behavior of fascicles during eccentric muscle actions reveals an important role for the SEC in acting as a mechanical buffer during this type of action. (Reeves & Narici 2003) This may be an important factor for preventing injury to muscle fibers during rapid, high-force eccentric actions (Roberts & Azizi 2010).

Chino et al. (2006) reported higher fascicle shortening and lengthening velocities for medial gastrocnemius compared with soleus, for movement velocities from 120°/s to 240°/s. This could be due to differences in fascicle architecture and/or the existence of mechanical linkages between these muscles. However, the effective fascicle shortening and lengthening velocities (before differentiation with time, length is multiplied by the cosine of the pennation angle, giving velocity in the longitudinal direction of the muscle) showed no consistent variation between the MG and SOL at any of the tested velocities. The length change of tendinous tissues in both concentric and eccentric trials contributed as much as 50% to total changes in MTC length, but there was considerable intra-subject variability. These results suggest that the assumption that angular velocity of limb movement can be used to calculate muscle velocity should be carefully reconsidered. Likewise, assuming the same mechanical configuration (i.e. fascicle length and pennation angle) in a specific angle for two different muscle actions may be misleading.

3 MANUSCRIPT FOR PUBLICATION

3.1 Introduction

According to the in vitro force-velocity relationship described for maximally activated muscle, peak forces generated during muscle lengthening have reached 150 – 190 % of maximum isometric force (Edman et al. 1978; Harry et al. 1990; Levin & Wyman 1927; Lombardi & Piazzesi 1990; Katz 1939). However, human in vivo studies generally found that maximal eccentric moments were not statistically greater than isometric (Chino et al. 2006; Duclay et al. 2011; Gruber et al. 2009; Pinniger et al. 2000; Westing et al. 1988). Furthermore, twitch interpolation technique and electromyography (EMG) have shown voluntary inability to fully activate the involved muscles, demonstrated by a higher electrically evoked torque and lower EMG during eccentric actions, respectively (Amiridis et al. 1996; Babault et al. 2001; Linnamo et al. 2002; Pinninger et al. 2000; Westing et al. 1990).

The reduction of neural drive during eccentric muscle actions may be accomplished by spinal and/or supraspinal modulation. Several research groups have reported decreased Hoffman reflex (H-reflex), and decreased motor evoked potentials (MEPs), the latter produced by both transcranial magnetic and transcranial electrical stimulation (TMS/TES). Additionally, a lower plateau and slope in the input-output curve utilizing TMS during eccentric actions has also been reported. (Abbruzzese et al. 1994; Duclay & Martin 2005; Duclay et al. 2011; Nordlund et al. 2002; Romano & Schieppati 1987; Sekiguchi et al. 2001, 2003) Furthermore, Gruber et al. (2009) compared MEPs elicited in biceps brachii and brachioradialis by TMS and cervicomedullary motor-evoked potentials (CMEPs) obtained by electrical stimulation of the corticospinal tract. Variations in MEP size reflect changes that can occur at both cortical and spinal levels, whereas the CMEPs were used to test motoneuron excitability, based on the assumption that the corticospinal pathway is free from presynaptic inhibition (Nielsen & Petersen 1994). Maximal eccentric actions had reduced MEPs and CMEPs compared with isometric actions, however the decrease was

significantly greater for CMEP, for both muscles, reflecting greater inhibition at the spinal level. Furthermore, the MEP/CMEP ratio, which is an index of cortical excitability, was significantly increased during eccentric actions.

Unlike the direct comparison between MEP and CMEP, another approach is to compare the H-reflex with MEP. The rationale is that H-reflex responses are modulated by motoneuron excitability and Ia synaptic transmission (Romano & Schieppati 1987), while MEPs reflect corticospinal excitability. A clear separation of spinal from cortical components is not attainable with this methodology because supraspinal centers continuously modulate spinal excitability through presynaptic inhibition and neuromodulatory inputs (Heckman et al. 2008, 2009; Rudomin & Schimidt 1999; Stein 1995). However, since the H-reflex is a measure of efficacy of the synaptic transmission in the Ia reflex arc (Capaday 1997), it carries relevant information to be discussed with the corticospinal modulations revealed by the MEP. Duclay et al. (2011) reported decreased MEPs and H-reflexes for soleus (SOL) muscle during maximal eccentric actions as compared with concentric and isometric actions, but no differences were found for medial gastrocnemius (MG). Furthermore, Duclay et al. (2011) reported a decreased silent period (SP) during the eccentric action for SOL, which being an index of cortical inhibition (Chen et al. 1999; Davey et al. 1994; Inghillerj et al. 1993; Roick et al. 1993), corroborates with the increased cortical excitability proposed by Gruber et al. (2009).

It is generally assumed that when different muscle action types are tested in a specific joint angle, the muscle-tendon complex (MTC) mechanical configuration, characterized by the fascicle length, pennation angle, and tendon/aponeurosis length will be similar (Nordlund et al. 2002; Pinniger et al. 2001). Muscle-tendon mechanical configuration depends on the dynamics between the produced force and compliance of the series elastic component (SEC; Narici et al. 1996; Reeves & Narici 2003). SEC stiffness is dependent on the applied force magnitude and velocity (Fukunaga et al. 1997; Pearson et al. 2007; Theis et al. 2012). Since the muscle's force production capability varies with velocity (Edman 1988; Hill 1938), and SEC behavior can also vary, the same joint angle may represent different mechanical configurations for different action types in different intensities and velocities.

Furthermore, the assumption that angular velocity of limb movement can be used to calculate muscle velocity needs to be carefully reconsidered, as the movement velocity and the intensity of the muscle action will affect the fascicle velocity (Chino et al. 2006; Finni et al. 2001, Fukunaga et al. 1997; Narici 1996; Reeves & Narici 2003). Movement velocity during both passive joint movement and voluntary muscle actions have been reported to modulate H-reflex amplitude. Generally, higher velocity eccentric actions and passive muscle lengthening have significantly lower H-reflexes for soleus muscle, while diverging results have been shown for medial gastrocnemius (Duclay et al. 2009; Nordlund et al. 2002; Romano & Schieppati 1987). It remains unclear if the different velocities were capable of modifying muscle-tendon dynamics, and if the different afferent inputs would explain the larger inhibition at higher velocities, even though an increase in muscle spindle activity is expected (Burke et al. 1978). Thus, in order to further elucidate the corticospinal modulation during different muscle action types, it is crucial to have a clear picture of the muscle-tendon mechanical configuration at the moment of corticospinal excitability testing.

The aim of the current study was to investigate SOL corticospinal excitability during maximal isometric and eccentric plantarflexion actions with two different velocities (100°/s and 25°/s), while monitoring the muscle-tendon mechanical behavior. We hypothesized that a different mechanical configuration between the two eccentric velocities would exist although corticospinal excitability would be equally lower in both as compared with the isometric action. Furthermore, we also hypothesized that the mechanical configuration between slow eccentric and isometric would be the same, adding further evidence of a unique activation strategy for the eccentric actions (Enoka 1996; Duchateau & Enoka 2008). To this end, MEP and H-reflex modulation for SOL were compared between the isometric and two eccentric protocols, while ultrasonography was utilized to access SOL and MG fascicle length, pennation angle and fascicle velocity.

3.2 Methods

Subjects. Ten healthy male subjects (mean ± S.D. for age, height and weight were 23.8 ± 2.4 yr, 1.81 ± 0.05 m, 81.1 ± 5 Kg, respectively) with no history of neurological injuries or diseases participated in the present study. All subjects gave their written informed consent after explanation of the experiment and the risks involved. The procedures were approved by the local university ethics committee and performed according to the *Declaration of Helsinki.*

Study design. The study consisted of five testing sessions, all separated by 48 – 72 hours. The first day was a familiarization session, in which the subjects performed several trials of the different maximal muscle action types (isometric, fast and slow eccentric), experiencing TMS, H-reflex, ultrasonography and EMG procedures. In the second day, maximal isometric actions were performed at four different ankle joint angles, from 110° (plantarflexion) to 80° (dorsiflexion) in 10° steps. Force and ultrasound data were recorded for each test joint angle in order to obtain a force-length and force-pennation angle relationship for both SOL and MG. The remaining three days were randomized and consisted of one isometric and two eccentric test protocols. At the beginning of each session a standard warm up, consisting of 10 muscle actions (matching the action type of the particular experimental protocol) with progressively higher intensities (60-100% of maximal voluntary contraction; MVC) were performed. This procedure was important not only to prepare the subjects for the upcoming series of MVCs, but to take into account tendon conditioning with consecutive muscle actions, in an attempt to reduce tendon elongation variability in the initial MVC trials (Maganaris 2003).

Mechanical data. Subjects were seated with the knee joint fully extended, hip joint at 120° of extension and the ankle joint at an initial position of 90° (the sole of the foot at right angles to the tibial axis) in an ankle dynamometer (Neuromuscular Research Center, University of Jyväskylä, Finland). The right foot was firmly attached to a footplate mounted on the rotation platform so that the rotation axes of the ankle joint and the motor driven platform coincided. The left leg rested quietly on a wooden support. Subjects were

securely stabilized by an assembly of straps which fastened both shoulders and connected to a waist belt. An additional strap with a foam support prevented the right knee joint from flexing. The torque around the rotational axis of the motor was measured by a piezoelectric crystal transducer (Kistler Holding AG, Winterthur, Switzerland) and the angular movement of the ankle joint with respect to the ergometer plane was monitored by a linear potentiometer. Furthermore, a small stiff metal wire attached to a spring system, located at the calcaneus level, continuously monitored heel displacement from the footplate throughout the experiments. Torque, joint angle and heel displacement signals were sampled at 1 KHz utilizing a 16-bit AD converter (CED power 1401, Cambridge Electronics Design Limited, Cambridge, UK) and stored for later analysis.

Experimental Protocols. For the isometric protocol, while the subject was completely relaxed, the foot plate moved from the starting position (90°) to 80° at 10 °/s. Upon arrival, the subject performed a maximal isometric action lasting three seconds, after it the foot plate returned to the initial position (10 °/s) and the subject was instructed to relax. For the eccentric protocols, while the subject was completely relaxed, the foot plate moved from the starting position to 110° at 20° °/s. At 110°, the subject performed a maximal isometric action lasting two seconds and then the foot plate moved to 75° at 25 °/s (slow eccentric) or 100 °/s (fast eccentric) while the subject maintained maximal effort throughout the movement. Upon arrival at 75°, the foot plate returned to the initial position (20 °/s) and the subject was instructed to relax. The total number of trials for all 3 experimental protocols varied from twenty to forty (mean = 32 trials). Ten trials were performed utilizing TMS, and a variable number of trials (never exceeding thirty) were used to gather H-reflex and Mmax data. Three minutes of rest were allowed between trials, and the test session had a mean duration of 130 minutes (min/max: 110/160). After a maximum of fifteen trials, the subject would rest for 15 minutes to eliminate any possible fatiguing effects. Electrical nerve stimulation and TMS were delivered at 80° ankle joint angle for the 3 experimental protocols, in an attempt to minimize length changes in the muscle that could affect the neural measures (Gerilovsky et al. 1989). Stimulation timing for the isometric condition was 2 sec after the initiation of the MVC, during a stable torque plateau. For the eccentric actions, stimulation happened at 2.3 and 3.2 seconds after the initiation of the MVC for fast

and slow eccentric actions, respectively. Maximal effort duration did not differ much between protocols: 3s for isometric, 2.35s for fast eccentric and 3.4s for slow eccentric. This was an important factor to control since effort duration significantly affects tendon mechanical properties (Pearson et al. 2007), and large variations in effort duration could introduce another variable (i.e. magnitude of creep) into the muscle-tendon mechanical behavior.

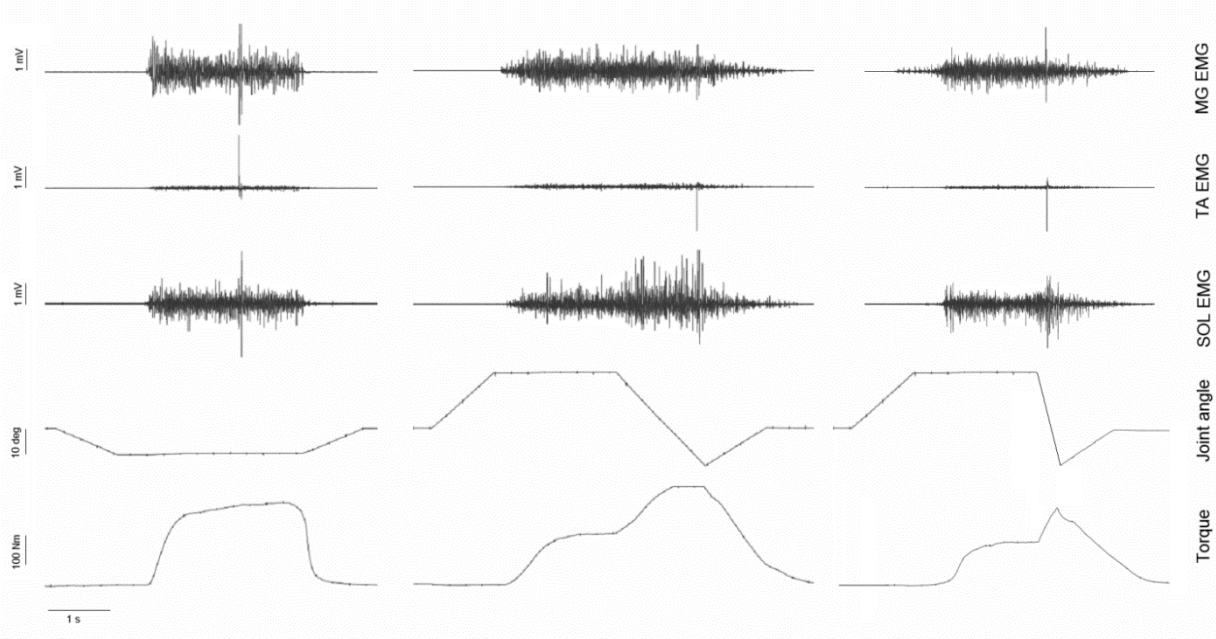

FIGURE 20 – Representative recordings of torque and EMG for isometric (left), slow eccentric (middle) and fast eccentric (right) protocols. From top to bottom: EMG activity for medial gastrocnemius, tibialis anterior and soleus muscles, joint angle displacement and torque during maximal plantarflexions. EMG spikes were caused by the stimulation pulse.

Electromyography. EMG activity was recorded from SOL, MG and tibialis anterior (TA) of the right leg using self-adhesive electrodes (Blue Sensor N, Ag/AgCl, 0.28 cm², Ambu, Ballerup, Denmark) in a bipolar setting, and a ground electrode was placed on the head of the tibia. Additionally, a pseudomonopolar setting for SOL with a reference on the bony surface of tibia was employed. The pseudomonopolar electrode configuration was chosen to acquire SOL MEPs as it provides consistent waveforms, facilitating peak-to-peak and area analyses. Furthermore, although having the disadvantage of not having a common-

mode rejection, the signal to noise ratio was still high, allowing easy MEP recognition and analysis.

Electrode placement and skin preparation were performed according to SENIAM. For SOL, electrodes were placed at 2/3 of the line connecting the medial condyle of femur and the medial malleolus, 2 cm bellow the myotendinous junction, which was previously identified with ultrasonography. MG electrodes were placed at 1/3 of the distance between the medial femoral condyle and the medial malleolus, at the most prominent bulge of the muscle. TA electrodes were placed at 1/3 proximally on the line between the head of the fibula and the medial malleolus. Reference lines with a scale were drawn and a picture was taken to provide accurate replacement of the electrodes on the following sessions. The electrodes were adjusted on the muscle belly in accordance with the underlying fiber direction (interelectrode distance = 2 cm; interelectrode resistance < 2 kΩ). Alignment of the electrodes was checked according to the shape of the M-wave. It was ensured that each subject had a smooth bipolar M-wave during the maximal voluntary actions. EMG signals were amplified and high pass filtered (x1000, 10Hz) by a preamplifier (NL824, Digitimer Ltd., Hertfordshire, UK) then bandpass filtered (10 Hz to 1 KHz) by a differential amplifier (NL900D/NL820A Digitimer Ltd., Hertfordshire, UK). The signals were acquired on a personal computer at a rate of 5 KHz via a 16-bit AD converter (CED power 1401, Cambridge Electronics Design Limited, Cambridge, UK).

Transcranial magnetic stimulation. TMS was delivered using a single pulse, monophasic Magstim 200² stimulator with a 9-cm double batwing coil (Magstim, Whitland, UK), oriented to deliver posterior–anterior directed current to the motor cortex. The coil was optimally positioned to elicit at rest SOL MEPs with the greatest amplitudes while eliciting minimal TA MEPs (less than 50% of SOL MEP amplitude). A custom-made coil holder with a neck support and two elastic bands passing around the subject's chin were utilized to keep the coil's position constant. Additionally, marks were drawn on the subject's scalp to facilitate monitoring coil position throughout the testing session, and to enable accurate coil repositioning in the following sessions. Resting motor threshold (RMT) was defined as the lowest stimulus intensity to elicit a visible MEP with a peak-to-peak amplitude of 70μV

(noise level = 42.6 ± 6.9 µV) in three out of five consecutive trials. All experiments were performed with an intensity of 120% RMT, corresponding to 67.1 ± 9.7 % of maximal stimulator output (min/max 51.5%/ 85.2%).

Electrical stimulation. H-reflexes and M-waves were evoked in SOL by percutaneous electrical stimulation of the tibial nerve. A single rectangular pulse (1ms) was delivered from a constant current stimulator (DS7AH, Digitimer Ltd., Hertfordshire, UK). A circular cathode with a pickup area of 0.77 cm² (Unilect 4535M, Ag/AgCl, Unomedical Ltd., Redditch, UK) was placed over the tibial nerve on the popliteal fossa, and an oval shaped, 5.08 x 10.16 cm anode (V-trodes, Mettler Electronics corp., Anaheim, USA) was placed above the patella. The stimulation site providing the greatest amplitude of the evoked responses in SOL while evoking minimal evoked responses in TA was first located by a hand-held cathode electrode. Three to five trials utilizing supramaximal stimulation (80–120 mA) were performed in order to obtain Mmax in each test protocol. After achieving a plateau, stimulation intensity was further increased by 50%, and the mean value of three stable trials were calculated as the Mmax. Stimulation intensity was then decreased (10 – 40 mA) to elicit H-reflexes preceded by a M-wave with acceptable size of 20 ± 2% of Mmax. At this range of the recruitment curve, the M-wave is sensitive to changes in stimulus conditions and may reliably measure the efficiency of the stimulus intensity (Pierrot-Deseilligny & Mazevet 2000; Zehr 2002). Furthermore similar normalized M-wave amplitudes (M-wave/Mmax) were maintained between experimental conditions in order to allow comparisons.

Ultrasound. A real-time ultrasonic apparatus (SSD-α10, Aloka, Tokyo, Japan) was used to record continuously longitudinal ultrasonic images of SOL and MG during each test session. A B-mode linear array probe (scanning frequency = 7.5 MHz; scanning length = 8 cm) was firmly fixed on the right leg using a foam pad and elastic tapes. The probe was coated with a water-soluble transmission gel to provide acoustic contact without depressing the dermal surface. For SOL, the probe was positioned at 50% of the distance between the popliteal crease and the lateral malleolus. Exceptionally, for one subject, the probe was positioned proximally at 30% of the same reference line, as only at this position the fascicle

visualization was sufficiently clear for the measurements. For MG, the probe was positioned proximally at 30% of the distance between the popliteal crease and the medial malleolus. Probe position was carefully selected so that the plane of the ultrasonogram was parallel to the muscle fascicles (Kawakami et al. 2002), avoiding fascicle length overestimation (Scott et al. 1993). The images were obtained at 100 Hz in a 8 s window. Electronic pulses were used to synchronize analog and video data.

3.2.1 Data analysis

Data analysis was performed off-line utilizing the Spike2 v4. software (CED, Cambridge, UK). The same software was programed to synchronize and control automatically the stimulation, dynamometer movement, ultrasound and EMG.

Torque, heel displacement and EMG. For all experimental protocols, peak torque and mean heel displacement were calculated in a 50 ms window prior to stimulation (80°). Ten trials were selected randomly and the variables torque, heel displacement and EMG were averaged. SOL, MG, and TA EMG activity were quantified using root mean square (RMS) values of the EMG signal over a 50 ms window prior to the stimulation. TA coactivation was normalized by TA RMS obtained in a 50 ms window during maximal dorsiflexions at the same ankle joint angle with the same velocity. Three maximal isometric, slow concentric (25°/s) and fast concentric (100°/s) dorsiflexions were performed at the end of the isometric, slow and fast eccentric protocols, respectively.

H-reflex. Peak-to-peak amplitudes for Mmax, M-wave and H-reflex were calculated between the initial deflection of the EMG from the baseline (i.e. response latency) to the second crossing of the horizontal axis (i.e. response duration). H-reflex amplitude values were normalized by the preceding M-wave of the same trial, and was expressed as H/M. Both Mmax and M-wave were expressed as absolute values. Three trials were averaged.

MEP and SP. Peak-to-peak amplitudes and areas of MEPs were calculated between the initial deflection of the EMG from the baseline (i.e. MEP onset) to the second crossing of

the horizontal axis (i.e. MEP duration). Peak-to-peak amplitude and area were normalized by the mean Mmax value measured in the same action type. Results for peak-to-peak amplitudes and areas were similar, and thus only peak-to-peak amplitudes are reported. The duration of the absolute silent period (SPa) was measured from the MEP second crossing of the horizontal axis (i.e. MEP offset) to the return of EMG activity. The relative silent period (SPr) was measured from the MEP onset to the return of the EMG activity inspected visually always by the same examiner. Additionally, MEP onset and duration were calculated for each muscle action type. TMS trials (n = 10) were averaged for all variables.

Fascicle Behavior. Fascicle length (FL) was defined as the distance between the insertions of the fascicle into the superficial and deep aponeurosis. Pennation angle (PA) was defined as the angle between the fascicle and deep aponeurosis. Measurements of FL and PA were performed manually utilizing a public domain National Institutes of Health image program (Image J, Bethesda, Maryland, USA). Moreover, FL and fascicle velocity (FV) were measured utilizing an automatic fascicle tracking method based on the Lucas-Kanade optical flow algorithm with an affine optic flow extension (Cronin et al. 2011; Gillett et al. 2013). FV was obtained by differentiating the corresponding length change value (FL tracked through 10 frames) with time. Automated processing of the entire ultrasound video was performed in a custom written Matlab graphical user interface (Matlab, The MathWorks Inc. Massachusetts, USA). FL values acquired every 100 ms were used to construct a visual representation of FL dynamics throughout the trials in all three experimental protocols. FV was measured only by the automatic method due to its time consuming nature, and because this method has already been shown reliable and repeatable (Cronin et al. 2011; Gillett et al. 2013).

In each trial, three measurements were performed at the frame corresponding to 80°, immediately before electrical or magnetic stimulation. For each experimental condition, 3 trials were analyzed and averaged for each muscle. A trial was accepted if the muscle fascicles, deep and superficial aponeurosis could be clearly visualized at 80° ankle joint angle, which was immediately checked by one of the researchers. After 3 acceptable trials

for a given muscle were acquired, the ultrasound probe was repositioned on the other muscle.

Repeatability and reliability of measurements. Since data were collected during 3 experimental sessions, it was important to verify repeatability for all the involved variables. A pilot study (n = 4) consisting of 2 pairs of testing sessions with identical protocols for isometric and slow eccentric was performed. Intraclass correlation coefficient (ICC) was calculated using a 2-way fixed-effect model addressing random error (ICC 3.1, Weir 2005). Additionally, the coefficient of variation (CV) and standard error of mean (SEM) was calculated for each variable. CV and ICC are reported in table 1. Another important concern was to compare unfatigued maximal voluntary actions. Trials were only accepted when torque values were within a specific range from the mean of the first 6 trials. The acceptance range was determined utilizing 2 criteria: (1) 2 standard deviations; (2) minimal difference (MI) constructed for a 99% confidence interval (CI).

MI 99% CI = standard error of the mean X 2.575 X $\sqrt{2}$,

The total number of trials for the 3 experimental sessions was 964, in which 42 (4.36%) were excluded due to low torque levels. A non-significant ($P > 0.05$) Pearson correlation coefficient ($r = -0.313$) between trial number and number of exclusions makes the point that excluded trials had a random distribution throughout the experiments and were not associated with fatigue.

TABLE 1. ICC between two testing sessions and CV for the experimental variables.

	CV (%)			ICC 3.1	
Variables	Iso	Slow Ecc	Fast Ecc	Iso	Slow Ecc
Torque	3.4	4.9	4.4	0.99	0.94
SOL RMS	13.6	12.5	15.1	0.89	0.85
MG RMS	13.4	14	16		
TA RMS	12.4	13.9	13.5	0.91	0.92
HD	26.4	34.5	31.7	0.69	0.94
MEP(amp/area)	19.7/23.3	20.8/20.4	20.3/18.8	0.85	0.99
MEP latency	4.2	5.3	5.8	0.74	0.76
MEP duration	14.8	12.2	13.6	0.96	0.96
SPa	6.9	11.2	10.9	0.95	0.99
SPr	6.3	8.3	9.3	0.95	0.99
Mmax	4.3	6.1	4.7	0.85	0.77
M-wave	8.1	6.9	6.4	0.82	0.69
H/M	9.3	23.2	25.4	0.77	0.82
FL (manual/auto)	8.4/6.8	6.5/9.1	6.5/8.0	0.99	0.99
PA	8.9	6.2	8.0	0.84	0.88

Iso = Isometric; Ecc = Eccentric; SOL, MG and TA RMS = soleus, medial gastrocnemius and tibialis anterior root mean square; HD = heel displacement; MEP = motor evoked potential; SP = silent period (absolute and relative); FL = fascicle length; PA = pennation angle.

Statistical analysis. All data are presented as mean ± SD. Data normality was tested using the Shapiro-Wilks test. All variables except heel displacement and SOL EMG had a normal distribution. One-way repeated-measures ANOVA with a Holm-Sidak post hoc test was used to test differences between muscle action types (isometric, fast and slow eccentric) for all variables. The non-parametric analog Friedman repeated-measures ANOVA on Ranks test was used when appropriate. Two-way repeated-measures ANOVA with a Holm-Sidak post hoc was used to test differences between muscle action types and muscles for FL, FV and PA. All statistical analysis were performed using SigmaPlot v.10 (Systat Software Inc., San Jose, USA). Significance level was set at $P < 0.05$.

3.3 Results

Torque, HD and EMG. Peak torque for slow eccentric protocol was statistically higher than fast eccentric (P = 0.037), while no differences between the two eccentric and isometric protocols were found (P > 0.05). HD during the trials were similar among the three experimental protocols (P = 0.458). Regarding EMG, there were no differences in SOL activity between the experimental protocols (P = 0.150), and similar levels of TA coactivation were found (P = 0.160). MG activity during the isometric protocol was statistically higher than fast eccentric (P = 0.040), and not different from slow eccentric (P > 0.05). MG activity was similar between the two eccentric protocols (P > 0.05).

TABLE 2. Effect of muscle action type on mechanical and neural variables.

Variables	Isometric	Slow Eccentric	Fast Eccentric
Torque (Nm)	312.6 ± 50.3	318.8 ± 53.4*	276.1 ± 48.3
HD (mm)	4.4 ± 4.4	3.1 ± 1.9	3.6 ± 2.4
SOL RMS (mV)	0.318 ± 0.154	0.274 ± 0.073	0.296 ± 0.068
MG RMS (mV)	0.305 ± 0.061*	0.247 ± 0.079	0.242 ± 0.077
TA (coactivation %)	10.3 ± 3.42 %	11.9 ± 4.83 %	14.1 ± 4.42 %
MEP (amp/Mmax)	0.351 ± 0.212	0.327 ± 0.147	0.350 ± 0.184
MEP Latency (ms)	33.1 ± 2	32.0 ± 2	32.1 ± 2
MEP Duration (ms)	16.1 ± 4	17.8 ± 5	19.0 ± 4
SP Absolute (ms)	63.7 ± 9.6†	55.0 ± 6.9	54.5 ± 15.1
SP Relative (ms)	79.8 ± 9.2	72.9 ± 10.2	73.5 ± 18.0
Mmax (mV)	12.3 ± 5.85	12.3 ± 3.77	12.8 ± 3.24
M-wave/Mmax	0.198 ± 0.008	0.200 ± 0.006	0.197 ± 0.006
Hreflex (mV)	6.556 ± 3.842 †	3.958 ± 2.085	3.291 ± 1.539
H/M	2.741 ± 0.933 ‡	1.611 ± 0.663	1.323 ± 0.545

Data are mean ± SD. HD = heel displacement; SOL = soleus; MG = medial gastrocnemius; TA = tibialis anterior; RMS = root mean square; MEP = motor evoked potential; SP = silent period; Mmax = maximal M-wave; H/M = h-reflex normalized by the preceding M-wave. *Significant at P

< 0.05: Fast Eccentric vs. Isometric or Slow Eccentric. † Significant at P < 0.05: Isometric vs. Fast and Slow Eccentric. ‡ Significant at P < 0.001: Isometric vs. Fast and Slow Eccentric.

TMS. As depicted in figure 21, analysis of repeated measures for normalized MEP amplitude revealed no differences between muscle actions types (P = 0.750). Furthermore, muscle action type had no effect on MEP latency (P = 0.154) and MEP duration (P = 0.248). The isometric condition had a significantly longer absolute silent period than fast and slow eccentric conditions (P = 0.009). Regarding the relative silent period, no differences were found between the three experimental conditions (P = 0.142).

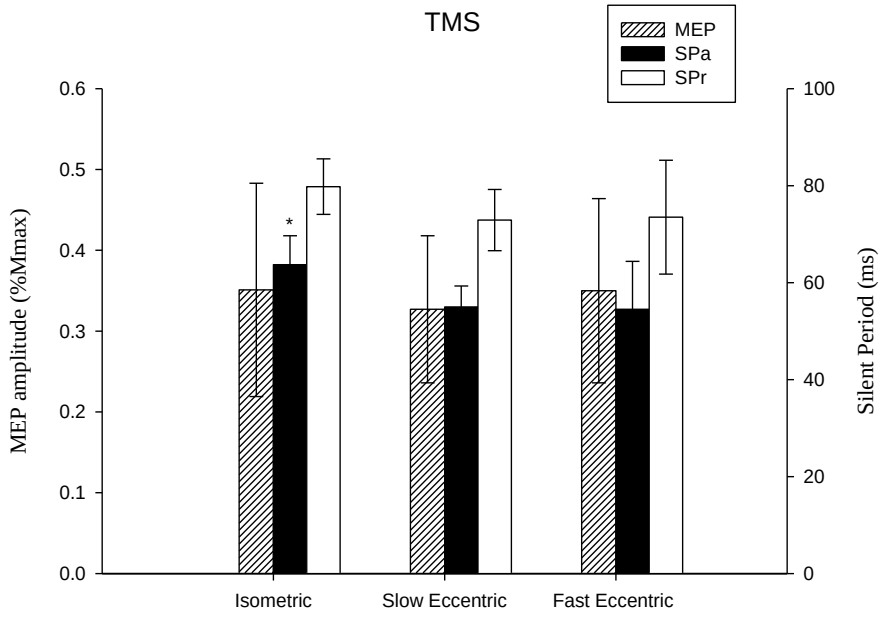

FIGURE 21 – Effect of action type on normalized MEP amplitude and silent period. Data presented as mean ± 95% CI. MEP = motor evoked potential; SPa= absolute silent period; SPr = relative silent period. * Significant at P = 0.009: Isometric vs. Fast and Slow Eccentric.

Electrical Stimulation. Mmax had similar amplitude values among the three experimental protocols (P = 0.950). Likewise, no differences were found between the absolute values of M-wave (P = 0.528) or values normalized to Mmax (P = 0.981). The isometric protocol had a significantly higher (P < 0.001) H/M as compared with both eccentric protocols. No

differences were found between fast and slow eccentric ($P > 0.05$). Figure 22 presents the results for electrical stimulation in the three experimental protocols.

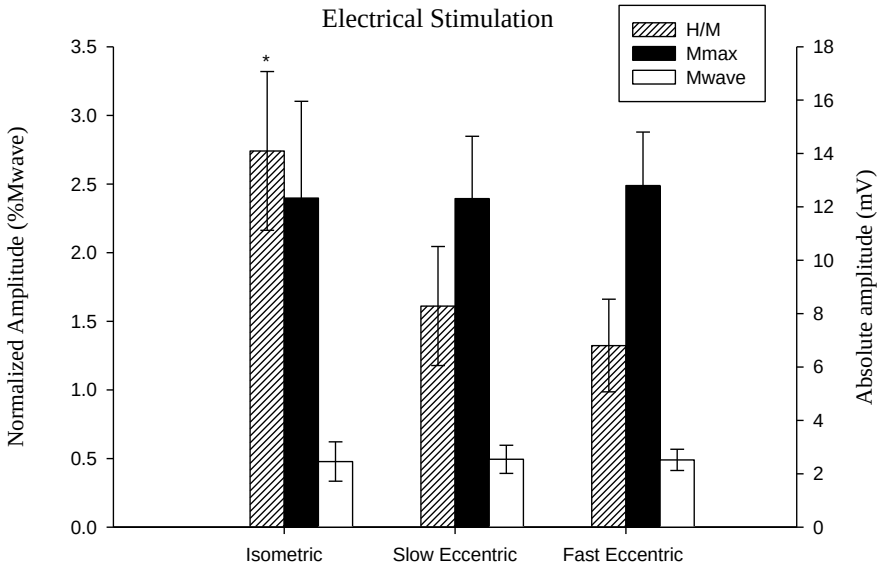

FIGURE 22 – Effect of muscle action type on absolute M-wave and Mmax amplitude, and on normalized H-reflex amplitude. Data presented as mean ± 95% CI. * Significant at $P < 0.001$: Isometric vs. Fast and Slow Eccentric.

Force-Length curves. Group mean values for tendon force, FL and PA were compared between four ankle joint positions (second testing session). Tendon force values at 80° and 90° were significantly higher than 100° and 110°, likewise tendon force values at 100° were also higher than 110° ($P < 0.001$). Fascicle length for SOL was significantly greater at 80° and 90° compared to 110°; moreover, 80° had greater values than 100° ($P < 0.001$). FL for MG was significantly different for every joint angle ($P < 0.001$). Results for tendon force and FL are presented in figure 23a. Pennation angle for SOL was significantly smaller at 80° than at 100° and 110° ($P < 0.001$); moreover, PA was also smaller for 90° as compared to 110° ($P < 0.001$). PA for MG was significantly different for every joint angle ($P < 0.001$). Results for tendon force and PA are presented in figure 23b.

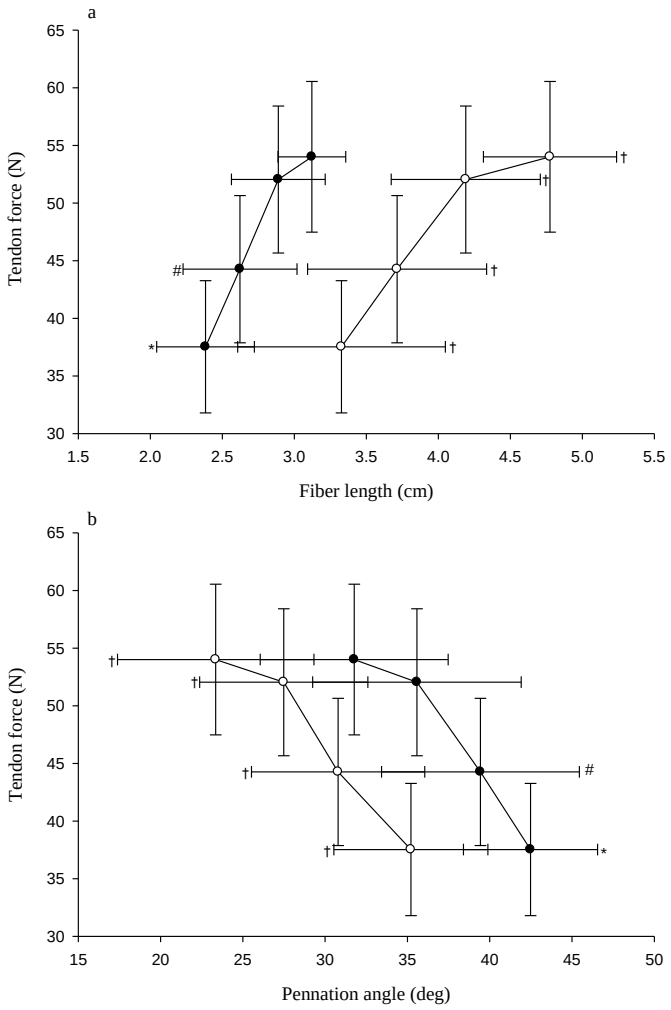

FIGURE 23 – Effect of ankle joint angle on tendon force, FL and PA for SOL (●) and MG (o). Data presented as mean ± 95% CI. (a) Tendon force and FL for joint positions of 110°, 100°, 90° and 80° from left to right respectively. (b) Tendon force and PA for joint positions of 80°, 90°, 100° and 110° from left to right respectively. Tendon force values at 80° and 90° were significantly higher than 100° and 110°, likewise tendon force values at 100° were also higher than 110° ($P < 0.001$; not depicted in graph for clarity purposes).* Significant at $P < 0.001$: 110° vs. 80° and 90°, # Significant at $P < 0.001$: 100° vs. 80°, † Significant at $P < 0.001$: all joint positions were statistically different.

Automatic fascicle tracking. Figure 24 shows the mean values for FL calculated in 100 ms windows during the last 900 ms before stimulation for the three experimental protocols.

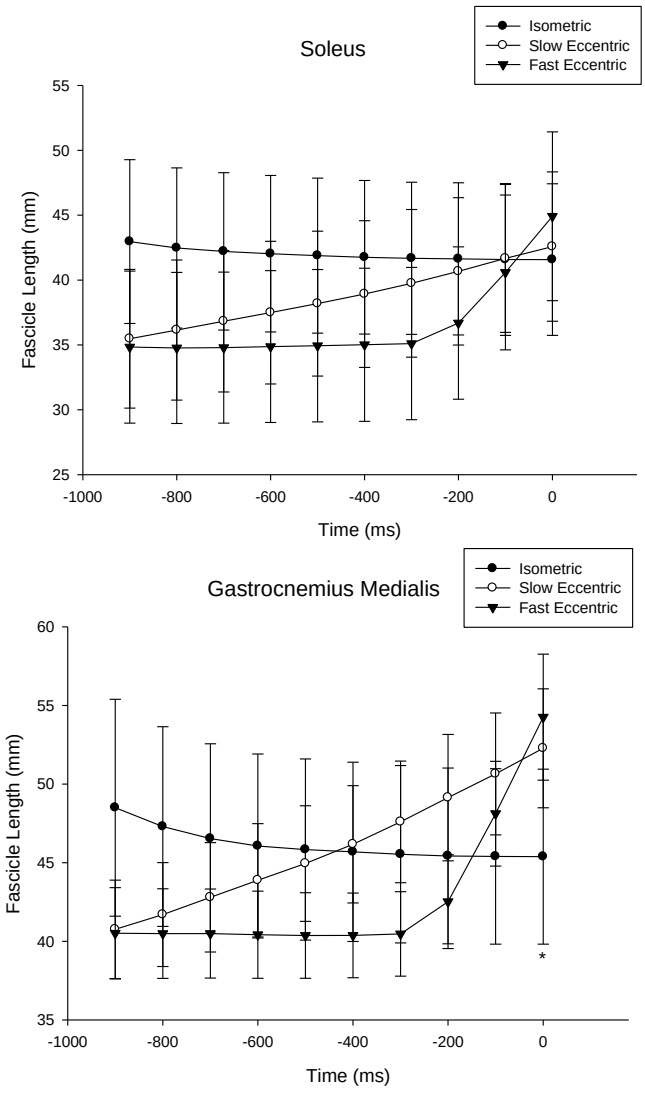

FIGURE 24 – Fascicle length values for SOL (●) and MG (o) aquired at the last 900 ms before electrical of magnetic stimualtion. Data points are presented as mean ± 95% CI of 100 ms window. * Significant at P = 0.03: Isometric vs. Fast Eccentric.

Mean values for FL and FV at the test position (80°), measured with the automatic fascicle tracking software, were compared between the experimental protocols and muscles. There were no differences for FL among the experimental protocols for SOL, but FL in MG was significantly longer in fast eccentric as compared to isometric (P = 0.043). Furthermore, MG had significantly longer FL than SOL for slow eccentric (P = 0.032), in the other two treatments SOL and MG had similar FL values. There was no statistically significant interaction between factors treatment and muscle (P = 0.426) Regarding FV in SOL, fast

eccentric had significantly higher values as compared with the other two experimental protocols ($P < 0.05$), while no differences between slow eccentric and isometric were found. In MG, all experimental protocols had significantly different FV values, with fast eccentric having the highest values and isometric the smallest ($P < 0.05$). FV values were similar in both muscles for the isometric protocol, while MG had significantly higher FV in both eccentric protocols as compared to SOL ($P < 0.05$). There was a statistically significant interaction between factors treatment and muscle ($P = 0.015$).

The upper part of Figure 25 shows the mean FL values calculated in 100 ms windows during the entire trial for the three experimental protocols. The lower part of the figure despicts the ankle joint angle throughtout the trials.

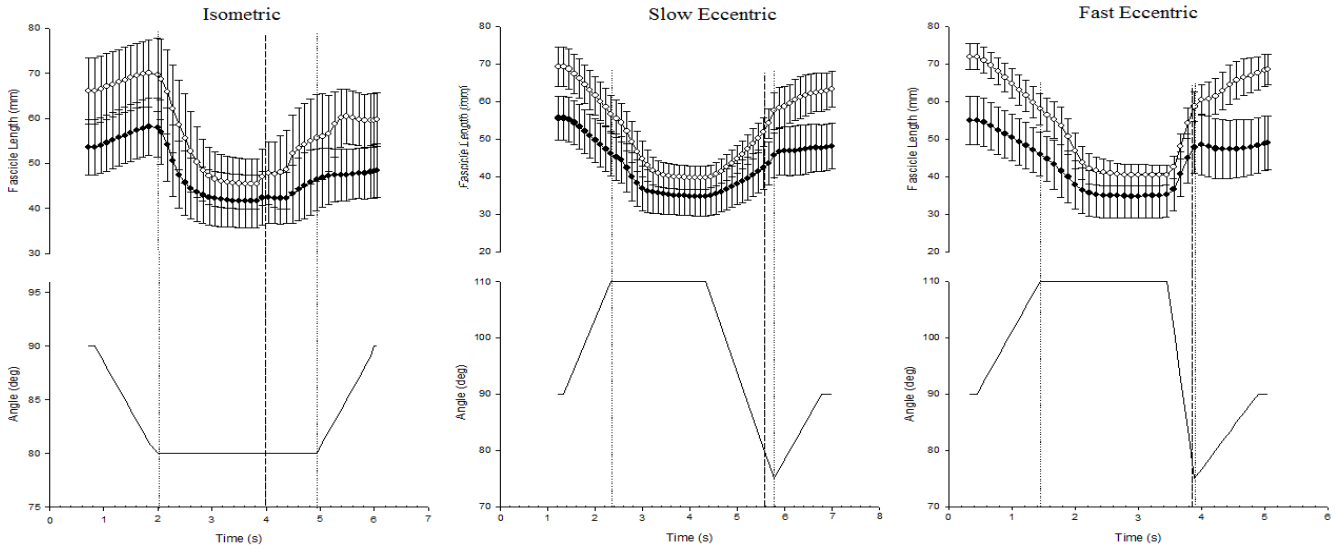

FIGURE 25 – Fascicle length values for SOl (●) and MG (o) aquired throughout the entire trial for all three protocols. Data points are presented as mean ± 95% CI of 100 ms window. Dashed line depicts electrical or magnetic stimulation timing. Dotted lines show maximal voluntary contraction beginning and end.

Manual fascicle measurements. Manual measurements yielded very similar results comparing to automatic tracking. There were no differences for FL among the experimental protocols for SOL and MG ($P = 0.569$). In all experimental protocols MG had significantly longer FL than SOL ($P < 0.001$). There was no statistically significant interaction between

factors treatment and muscle (P = 0.992). Regarding FV in SOL, fast eccentric had significantly higher values as compared with the other two experimental protocols (P < 0.001), while no differences between slow eccentric and isometric were found. In MG, all experimental protocols had significantly different FV values, with fast eccentric having the highest values and isometric the smallest (P < 0.001). FV values were similar in both muscles for all three protocols (P = 0.768). There was no statistically significant interaction between factors treatment and muscle (P = 0.505). There were no differences for PA among the experimental protocols for SOL and MG (P = 0.293). In all experimental protocols MG had significantly smaller PA than SOL (P < 0.001). There was no statistically significant interaction between factors treatment and muscle (P = 0.768).

TABLE 3. Effect of action type on FL, FV and PA for SOL and MG utilizing manual and automatic measurement techniques.

	Isometric SOL/MG	Slow Eccentric SOL/MG	Fast Eccentric SOL/MG
Manual			
FL (cm)	3.3 ± 0.7/4.6 ± 1.2#	3.3 ± 0.67/4.6 ± 0.87#	3.1 ± 0.5/4.4 ± 0.9#
PA (°)	31.2 ± 5.3/23.9 ± 6.9#	32.0 ± 4.9/24.4 ± 4.6#	32.7 ± 4.2/26.6 ± 6.2#
Automatic			
FL (cm)	4.2 ± 0.8/4.5 ± 0.8*	4.2 ± 0.9/5.0 ± 0.6#	4.4 ± 0.9/5.2 ± 0.6
FV (cm/s)	-0.01 ± 0.10/0.01 ± 0.03†	0.85 ± 0.31/1.56 ± 0.49†#	3.7 ± 1.56‡/5.3 ± 1.71†#

Data are mean ± SD. FL = fascicle length; FV = fascicle velocity; PA = pennation angle; SOL = soleus muscle; MG = medial gastrocnemius muscle. Negative values for FV = fascicle shortening. * Significant at P < 0.05: Isometric vs. Fast Eccentric. ‡ Significant at P < 0.05: Fast Eccentric vs. Isometric and Slow Eccentric. † Significant at P < 0.05: All protocols were statistically different. # Significant at P < 0.05: MG vs. SOL.

3.4 Discussion

Corticospinal excitability and fascicle behavior. The main finding was that SOL H/M was depressed during both fast and slow eccentric protocols as compared to isometric, while no differences in fascicle length and pennation angle were found among protocols. Furthermore, although the fast eccentric protocol had greater fascicle velocity than slow eccentric, there were no differences in H/M. Reduced H-reflex during eccentric muscle actions compared with isometric and concentric muscle actions have been consistently reported (Abbruzzese et al. 1994; Duclay & Martin 2005, Duclay et al. 2009, 2011; Romano & Schieppati 1987). Duclay et al. (2009, 2011) reported the only exception for MG muscle, in which no differences in H-reflex were found between concentric, eccentric and isometric muscle actions. However, these authors utilized the SOL passive recruitment curve to define the electrical stimulation intensity yielding the maximal H-reflex (Hmax). The effective stimulation intensity (preceding M-wave) was considerably different between the two muscles (12-21% and 22-42% of Mmax for SOL and MG, respectively). Since the test reflex depends on the stimulation intensity (Crone et al. 1990; Matthews 1999), comparisons between the two muscles are hindered, and the lack of difference among the muscle action types in MG may be due to a higher effective stimulation intensity that may have rendered the test reflex insensitive to excitability changes. Furthermore, utilizing the stimulation intensity to elicit Hmax with a quiescent MN pool as a parameter for stimulation during the MVCs seems troubling, as the stimulus-response curve may change drastically between these two conditions (Matthews 1999, Stein 2007). Regarding muscle action velocity, higher eccentric velocities (studied velocity range: 12°/s - 60°/s) have been reported to have a significantly lower H-reflex than slower velocities (Duclay et al. 2009; Romano & Schieppati 1987). Duclay et al. (2009) compared two eccentric velocities (20°/s vs. 60°/s) during MVCs for the triceps surae muscles. An important difference in the testing protocols was that after the MVC, the subjects rested at the final position (60° ankle angle) for 30 seconds, while in the present study the entire rest was done at 90°, and the subjects were allowed to relax their legs and move them slightly during the testing session. Since a considerable number of trials were performed (16 to 20) in Duclay's study, it is reasonable to assume that stretch-induced effects may have taken place. Passive stretching may have

caused neuromechanical alterations in the triceps surae, especially in the monoarticular soleus, affecting the muscle-tendon dynamics and sensory information processing (Avela et al. 1999; Fowles et al. 2000; Guissard et al. 2001; Weir et al. 2005). In the present study, a larger range of eccentric velocities was utilized (25°/s vs. 100°/s), and although there was a tendency for lower values of H/M in fast eccentric, it was not significantly different. Although FV was higher in the fast eccentric protocol, FL and PA remained constant at the test position, further consolidating the role of the in-series tendinous tissues as mechanical buffers (Finni et al. 2003; Reeves & Narici 2003; Roberts & Azizi, 2010).

In the present study, the effect of muscle action type and velocity was more pronounced in MG as compared to SOL, demonstrated by differences in FL and FV. Duclay et al. (2011) reported no differences in MEP or Hreflex for MG during maximal eccentric muscle actions, contrasting with a consistent reduction of both parameters for SOL. Since the testing position was the same as in our study, it is plausible to suggest that the mechanical changes in MG did not affect corticospinal excitability, and that most likely it was centrally mediated.

TMS. Lower MEPs during maximal and submaximal eccentric muscle actions have often been reported for SOL, biceps brachii and brachioradialis (Abbruzzese et al. 1994; Duclay et al. 2011; Gruber et al. 2009). Contrasting results have been reported only by Duclay et al. (2011) for MG muscle, in which no differences among isometric, concentric and eccentric muscle actions were found. In the present study, no differences were found between the muscle actions, corroborating with Duclay's results, in that they only found significant differences at the highest stimulation intensities, which induced MEPs on or close to the plateau of the input-output curve. Direct comparisons between the effective stimulation intensity in these two experiments are not possible since different coil shapes were utilized (i.e. circular vs. batwing), generating electric fields with different configurations. The circular coil induces a maximal electric field under its border with a minimal at the center, thus stimulating a considerable amount of neural tissue. The batwing coil induces a maximal electric field under its center, with two lower peaks under the outer edges, having

its minimum under the middle of each wing. Therefore, the batwing coil is more adequate for inducing a focal maximal electric field under a well-defined center (Cohen et al. 1990). Although obtaining the whole input-output curve for each muscle action type surely provides much more information about the task-dependent modulation of the corticospinal excitability (Devanne et al. 1997, Duclay et al. 2011; Sekiguchi et al. 2001, 2003, 2007), the number of trials would have been too great for MVCs, probably leading the subjects to fatigue and hindering the study conclusions.

SP. The silent period has been used as an index of intracortical inhibition, as it is generally thought that it results from an early spinal inhibitory mechanism (< 50 ms) followed by later intracortical inhibition (Chen et al. 1999; Inghilleri et al. 1993; Roick et al. 2003). SP has been calculated with different methodologies (Damron et al. 2008; Daskalakis et al. 2003; Säisänen et al. 2008), and although repeatability and reliability issues have been addressed, no consensus has yet been reached about which methodology is the most adequate. In the present study it was demonstrated that non-significant variations in MEP latency and duration were enough to cause significant differences between absolute and relative SP results. Since it is not yet consolidated how much different muscle action types can modulate MEP latency and duration (Abbruzzese et al. 1994), utilizing absolute SP seems more adequate because it is not affected by these variables. Conceptually, Säisänen et al. (2008) arguments that the absolute SP best reflects the grade of intracortical inhibition, since the MEP mechanisms (i.e. excitatory) differ from SP (i.e. inhibitory), being two opposite phenomena. Since in the present study no differences were found in MEP amplitude, which combined with stimulation intensity can modulate SP (Duclay et al. 2011; Inghilleri et al. 1993; Säisänen et al. 2008), the decrease in absolute SP during the eccentric muscle actions suggests decreased cortical inhibition.

Corroborating with these results, Gruber et al (2009) reported an increased MEP/CMEP ratio during eccentric muscle actions, an index of cortical excitability. Additionally, Fang et al. (2001, 2004) reported greater movement-related cortical potential derived from the electroencephalogram during eccentric as compared with concentric MVCs for the elbow flexors. Although these potentials provide only an estimate for brain output, the

demonstrated changes suggest that the brain is more involved in the preparation, planning and execution of the movement and with the processing of sensory input during lengthening actions compared with shortening actions. Interestingly, the eccentric muscle action velocity did not affect SP, suggesting that it does not modulate the intracortical inhibitory mechanisms. Duclay et al. (2011) reported a shorter duration of the relative SP during eccentric muscle actions for SOL, while in the present study no differences were found in this variable. The lack of consistency in this result is most likely explained by the small non-significant variations in MEP duration and latency.

Torque, Heel Displacement and EMG. According to previous in vivo studies with triceps surae muscle, a similar level of maximal torque production between isometric and eccentric muscle actions was expected regardless of movement velocity (Chino et al. 2006; Duclay et al. 2011; Pinninger et al. 2000). In the present study, the fast eccentric protocol had a lower peak torque as compared with slow eccentric, although similar levels of torque and EMG preceding the eccentric phase (i.e. isometric preactivation) were found in both eccentric protocols. Furthermore, at the test angle, similar levels of plantarflexors EMG and TA coactivation were also found between the two eccentric protocols. Since MEP, SP and H/M values were similar between the two eccentric protocols, lower torque production cannot be explained by increased corticospinal inhibition, and thus the most likely explanation for the differences is the available time to produce force. In the slow eccentric protocol, the subject had 1200 ms to achieve peak torque after the preactivation phase, while in the fast eccentric protocol, there was only 300 ms available to increase torque.

There were no differences in heel displacement among the experimental protocols, thus ankle rotation influenced tissue displacement in a similar manner for all experimental conditions. The great variability for the isometric condition is explained by the intentional lack of instruction about how to stabilize the ankle joint during the plantarflexion. During the pilot study, it became very clear that instructing the subject to keep the heel stable increased consistently and significantly the level of TA coactivation. Finally, it has been demonstrated that the stabilization procedures were very efficient, since the highest value for heel displacement was 1.3 centimeters.

According to the force-length curve produced in the second testing session, the test position was near the plateau of the force-length relationship. In order to further elucidate the role of peripheral inputs in modulating corticospinal excitability, future studies should verify systematically different muscle-tendon dynamics (i.e. different test position, muscle action velocity and intensity) while concomitantly probing corticospinal excitability.

Proposed control mechanisms during eccentric muscle actions. Concerning sensory information, several considerations are needed: (1) the inhibitory inputs coming from cutaneous receptors, Golgi Tendon Organs (i.e. autogenic inhibition) and joint capsule mechanical receptors should remain relatively constant due to the similar levels of torque and EMG during the different muscle actions; (2) since the amount of TA coactivation was similar in the different muscle actions, reciprocal inhibition cannot be responsible for the lower corticospinal excitability of SOL during the eccentric muscle actions; (3) a greater afferent input coming from the muscle spindles during the fast eccentric protocol (Burke et al. 1979) was not enough to increase significantly corticospinal excitability, possibly because central pathways controlled the inflow of sensory information from the periphery (Bawa & Sinjær 1999; Nagazawa et al. 1997, 1998). Additionally, fascicle length afferent information coming from the muscle spindles can also be ruled out as possible excitability modulator, since FL was the same among the experimental protocols. (4) small diameter afferents (i.e chemosensors and nociceptors; Gandevia 1998; Garland et al. 1991) should play if any a small role as the maximal effort duration was small (Min:2.35s/Max:3.4s) and the recovery time between trials was long (3 minutes). The torque analysis for each trial throughout the testing session showed no significant effect of fatigue (see methods), therefore it seems that the effort to rest ratio probably was not enough to cause metabolite accumulation and induce peripheral fatigue; (5) corticospinal descending drive produced by TMS reduced PSI of Ia terminals of a relaxed muscle (Meunier & Pierrot-Deseilligny 1998) suggesting that the same cortical sites both activate MNs of a given pool and depress primary afferent depolarization interneurons. Additionally, the decrease of PSI of Ia afferents to MNs of the activated muscle appears 50 ms prior to the contraction, suggesting that it is centrally programmed (Nielsen & Kagamihara 1993). A different central neural

control strategy of PSI may be a possible candidate for lowering spinal excitability during eccentric muscle actions, by reducing the stretch reflex gain (Bawa & Sinjær 1999; Nagazawa et al.1997, 1998).

Corroborating with the present study, other authors have shown evidences that supraspinal excitability seems to be higher during eccentric muscle actions (Duclay et al. 2011, Fang et al. 2001, 2004; Gruber et al. 2009). Despite increased cortical excitability, the responsiveness of the SOL motoneuron pool is lower during eccentric muscle actions, indicating that changes in neural control of muscle activity occurs both at spinal and cortical sites. Furthermore, submaximal eccentric muscle actions have also been reported to be inhibited (Abbruzzese et al. 1994; Gruber et al. 2009; Sekiguchi et al. 2001), suggesting that a potential tension-limiting mechanism during eccentric muscle actions do not depend on the level of activation (i.e. EMG) or torque produced, but rather it is inherent feature of the muscle action type.

3.5 Conclusion

The present study showed a decreased responsiveness of the motoneuron pool to Ia excitatory inputs (i.e reduced H/M) during eccentric muscle actions, while muscle mechanics (i.e. fascicle length and pennation angle) remained the same as compared with isometric muscle action. Additionally, the decrease in absolute silent period during eccentric muscle actions while MEP values remained similar among the different test protocols, suggests that an increased supraspinal excitability compensated the decreased spinal excitability. Although fascicle velocity was greater in the fast eccentric muscle action, no differences were found against the slow eccentric protocol, suggesting that the afferent inputs were not important in modulating the excitability changes.

Taken together, the present results corroborates with the idea that the central nervous system has an unique activation strategy during eccentric muscle actions (Duchateau & Enoka 2008, Enoka 1996), and further refutes the hypothesis that sensory information plays an important role in modulating these actions. The common assumption that MTU's mechanical configuration would be the same during different muscle actions at the same testing position was verified true for SOL, although as already discussed, many factors

could possibly cause significant alterations in its configuration. The combination of detailed mechanical and neural data should be further consolidated in future studies, in order to advance our current knowledge about motor control in different muscle action types.

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5 APPENDICES

Appendix 1 – Consent form

University of Jyväskylä, Department of Biology of Physical Activity.

Study information and consent form

Research Theme: "Effects of muscle action type on corticospinal excitability and soleus muscle architecture".

1. Researcher's contact information

Pedro Frederico Valadão – pedrovaladao@gmail.com

2. Background information about the study

Voluntary muscle actions are usually characterized as isometric or anisometric: in the first case the muscle-tendon unit is kept at the same length throughout the effort, whereas in the second case there can be shortening (concentric) or lengthening (eccentric) of the muscle-tendon unit. The understanding of motor control during human movement requires identification of the involved structures and their behaviour in different muscle actions or tasks. Some primary areas of interest are the motor cortex and the motoneurons – the motor cortex is located at the dorsal part of the precentral gyrus and is responsible for creating the neural signalling which induces movement. The motorneurons are located at the spinal cord, and carry the command from the superior centers to the muscles, and also receive a large amount of information coming from within the body (proprioception and interoception) and from outside of the body (exteroception). All this information may modulate the commands from higher centers and generate a final response that will go to the muscle fibers, resulting in their activation and thus force production.

The transcranial magnetic stimulation (TMS) generates a magnetic field which induces a current in the brain, creating action potentials that travel to the muscle and can be recorded using electromyography. TMS provides a way to measure corticospinal excitability, based on a input-output paradigm, in which the input intensity is kept constant by the researcher and different outputs provides quantitative measures of excitatory/inhibitory effects. The electrical stimulation at a peripheral nerve gives rise to an event called H reflex, which can be used to provide quantitative measure of the efficacy of the transmission in the Ia reflex arc. The combination on both methods is often use in an attempt to discern between cortical and spinal changes in excitability.

Lately many researches have been conducted testing corticospinal excitability at an arbitrary joint angle position, assuming that the muscle architecture (i.e fascicle length and pennation angle) would be the same between different muscle actions, and so far this assumption has not been tested. Accessing muscle architecture concomitantly with the corticospinal excitability testing will give further information about whether the afferent

information from the muscle is important in the specific modulation observed during eccentric muscle actions.

3. Research data saving methods

The research data will be saved by the researcher in charge, who is also responsible for their safe retention. The personal information of the subjects will be kept confidential.

4. Purpose of the study, aim and significance

The purpose of the study is to verify the effects of isometric and eccentric maximal voluntary muscle actions on corticospinal excitability, while concomitantly measuring soleus muscle fascicle length and pennation angle. Better understanding about the muscle's mechanical setup during neural testing is of vital importance, since it is already clear that many afferent input (i.e information coming from receptors in the muscle to the central nervous system) can potentially modulate the corticospinal excitability and thus potentially be the reason for the reported differences between this two muscle actions.

5. Procedures used during the experiment

During maximal voluntary plantarflexion, transcranial magnetic stimulation on the motor cortex, as well as electrical stimulation of the tibial nerve will be used to access corticospinal excitability. An ultrasound probe will be positioned on the right shank of the subject in order to obtain soleus muscle fascicle length and pennation angle. Electromyography of soleus, gastrocnemius medialis and tibialis anterior will be measured, therefore a small area of each muscle will be shaved and rubbed with sandpaper. Other two small areas located on the tibia will also be submitted to the same process to serve as ground electrodes.

6. Benefits and risks for the study's participants

Participants will have the opportunity to get acquainted with the current methods of research and during the measurements they can take advantage of scientific expertise of the researchers. The subjects will receive a summary of the study results. In case they wish to obtain more information about the study itself, or about its outcomes, they may contact the researcher at any time.

The skin preparation for the EMG electrodes placement may cause skin irritation and potentially has a minor risk of infection. The laboratory staff is well acquainted with first-aid procedures, and has access to medical instruments, should an accident of any kind occur. Magnetic stimulation might cause a slight headache if the neck muscles are activated concomitantly and electric stimulation may feel uncomfortable, but both methods are noninvasive and do not offer any risk to the subject.

7. How and where the research results will be used

Results will be used in the master thesis of the student Pedro Valadão. The results will be submitted for publication in a scientific journal, and might also be presented at congresses.

8. Rights of the participants

Participation in the experiment is entirely voluntary. The participants are entitled to suspend the tests at any time, without incurring any penalties. The data obtained are used exclusively by the researcher, and the analysis and report of the results is strictly confidential and anonymous. The participant has the right to request additional information about the study at any moment.

9. Insurance

The participants are insured against accidents due to any external causes. There is no insurance coverage in case of accident due to maximal voluntary muscle effort, as it does not lie in the category of external causes. The laboratory disposes of first-aid equipment, which the staff is able to use.

10. Participant's consent

I understand the study's purpose and content, the potential risks, as well as my rights and insurance protection. I agree to participate in the measurements at my own will, according to the information given to me. I do not have any kind of medical condition which could be aggravated with the study's procedures. I have the right, at any stage of the research, to interrupt my participation. The research results may be used for publication in a scientific journal, in such a form that the identification of the participants is not possible. Following my signature, I receive a copy of the present agreement, which also includes the researcher's signature. The original version remains with the researcher.

_____

Date Participant's signature

_____

Date Researcher's signature

Appendix 2. The declaration of Helsinki

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI
Ethical Principles for Medical Research Involving Human Subjects
Adopted by the 18th WMA General Assembly
Helsinki, Finland, June 1964 and amended by the
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53rd WMA General Assembly, Washington 2002 (Note of Clarification on paragraph 29 added)
55th WMA General Assembly, Tokyo 2004 (Note of Clarification on Paragraph 30 added)
59th WMA General Assembly, Seoul, October 2008

A. INTRODUCTION

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects**,** including research on identifiable human material and data. The Declaration is intended to be read as a whole and each of its constituent paragraphs should not be applied without consideration of all other relevant paragraphs.

2. Although the Declaration is addressed primarily to physicians, the WMA encourages other participants in medical research involving human subjects to adopt these principles.

3. It is the duty of the physician to promote and safeguard the health of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.

4. The Declaration of Geneva of the WMA binds the physician with the words, ―The health of my patient will be my first consideration,‖ and the International Code of Medical Ethics declares that, ―A physician shall act in the patient's best interest when providing medical care.‖

5. Medical progress is based on research that ultimately must include studies involving human subjects. Populations that are underrepresented in medical research should be provided appropriate access to participation in research.

6. In medical research involving human subjects, the well-being of the individual research subject must take precedence over all other interests.

7. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best current

interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

8. In medical practice and in medical research, most interventions involve risks and burdens.

9. Medical research is subject to ethical standards that promote respect for all human subjects and protect their health and rights. Some research populations are particularly vulnerable and need special protection. These include those who cannot give or refuse consent for themselves and those who may be vulnerable to coercion or undue influence.

10. Physicians should consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

B. PRINCIPLES FOR ALL MEDICAL RESEARCH

11. It is the duty of physicians who participate in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects.

12. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

13. Appropriate caution must be exercised in the conduct of medical research that may harm the environment.

14. The design and performance of each research study involving human subjects must be clearly described in a research protocol. The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest, incentives for subjects and provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study. The protocol should describe arrangements for post-study access by study subjects to interventions identified as beneficial in the study or access to other appropriate care or benefits.

15. The research protocol must be submitted for consideration, comment, guidance and approval to a research ethics committee before the study begins. This committee must be independent of the researcher, the sponsor and any other undue influence. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in

this Declaration. The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No change to the protocol may be made without consideration and approval by the committee.

16. Medical research involving human subjects must be conducted only by individuals with the appropriate scientific training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional. The responsibility for the protection of research subjects must always rest with the physician or other health care professional and never the research subjects, even though they have given consent.

17. Medical research involving a disadvantaged or vulnerable population or community is only justified if the research is responsive to the health needs and priorities of this population or community and if there is a reasonable likelihood that this population or community stands to benefit from the results of the research.

18. Every medical research study involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and communities involved in the research in comparison with foreseeable benefits to them and to other individuals or communities affected by the condition under investigation.

19. Every clinical trial must be registered in a publicly accessible database before recruitment of the first subject.

20. Physicians may not participate in a research study involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians must immediately stop a study when the risks are found to outweigh the potential benefits or when there is conclusive proof of positive and beneficial results.

21. Medical research involving human subjects may only be conducted if the importance of the objective outweighs the inherent risks and burdens to the research subjects.

22. Participation by competent individuals as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no competent individual may be enrolled in a research study unless he or she freely agrees.

23. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information and to minimize the impact of the study on their physical, mental and social integrity.

24. In medical research involving competent human subjects, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the

study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information. After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

25. For medical research using identifiable human material or data, physicians must normally seek consent for the collection, analysis, storage and/or reuse. There may be situations where consent would be impossible or impractical to obtain for such research or would pose a threat to the validity of the research. In such situations the research may be done only after consideration and approval of a research ethics committee.

26. When seeking informed consent for participation in a research study the physician should be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent should be sought by an appropriately qualified individual who is completely independent of this relationship.

27. For a potential research subject who is incompetent, the physician must seek informed consent from the legally authorized representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the population represented by the potential subject, the research cannot instead be performed with competent persons, and the research entails only minimal risk and minimal burden.

28. When a potential research subject who is deemed incompetent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorized representative. The potential subject's dissent should be respected.

29. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research population. In such circumstances the physician should seek informed consent from the legally authorized representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research should be obtained as soon as possible from the subject or a legally authorized representative.

30. Authors, editors and publishers all have ethical obligations with regard to the publication of the results of research. Authors have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. They should adhere to accepted guidelines for ethical reporting.

Negative and inconclusive as well as positive results should be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest should be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

31. The physician may combine medical research with medical care only to the extent that the research is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

32. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best current proven intervention, except in the following circumstances:
• The use of placebo, or no treatment, is acceptable in studies where no current proven intervention exists; or
• Where for compelling and scientifically sound methodological reasons the use of placebo is necessary to determine the efficacy or safety of an intervention and the patients who receive placebo or no treatment will not be subject to any risk of serious or irreversible harm. Extreme care must be taken to avoid abuse of this option.

33. At the conclusion of the study, patients entered into the study are entitled to be informed about the outcome of the study and to share any benefits that result from it, for example, access to interventions identified as beneficial in the study or to other appropriate care or benefits.

34. The physician must fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never interfere with the patient-physician relationship.

35. In the treatment of a patient, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available.