MEASUREMENTS OF LOWER EXTREMITY MUSCLE ACTIVATION ON MENOPAUSAL WOMEN

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Abstract

Aging is associated with a variety of changes in the neuromuscular system, which could theoretically reduce maximal voluntary force production. In women, menopause further enhances the loss of muscle mass and strength. These are mainly due to declining estrogen hormone concentrations. Estrogen is capable of affecting muscle function both through the central nervous system or directly through the muscle tissue. The interpolated twitch technique is a common method in the investigation of the ability to activate muscle. Transcranial magnetic stimulation (TMS) on the other hand can be used to study the inhibition-excitation phenomenon in the human brain through a non-invasive procedure. From a research perspective the quantitative assessment of muscle activation is essential in the evaluation of human muscle function.

The aim of this thesis was to study the effects of the stage of menopause on the ability to activate the muscles of the lower extremity during isometric plantar- and dorsiflexion tasks. Electrical nerve stimulation was performed for a maximal voluntary isometric platarflexion task, while the TMS task consisted of a dorsiflexion task at three different force production levels. The subjects in the study consisted of women in the early and late perimenopause phase as well as subjects in the postmenopause phase.

Our results seem to indicate that none of the group-wise differences in the measurement results between subjects in early and late perimenopause can be attributed to serum E2, as the group-wise difference in serum estradiol (E2) levels was not significant ($p = 0{,}597$). In addition no significant differences in the measure of voluntary activation (VA) was found between the early and late perimenopause groups ($p = 0{,}307$). The results also indicate that only about half of participants, five subjects in the early perimenopause group and four subjects in the late perimenopause group, demonstrate a clear postactivation potentiation (PAP) phenomenon.

The late perimenopausal group had generally longer peripheral silent period (PSP) values than the early perimenopausal group (116 ± 22 ms for late vs 92 ± 27 ms for early), which could indicate changes in central drive with advancing menopause. Supporting this is the finding of decreased peak and average voluntary contraction

rate of force development (RFD) between the early and late perimenopausal groups (517 ± 207 N/s avg early vs 315 ± 186 N/s avg late and 3418 ± 1174 N/s peak early vs 2954 ± 909 N/s peak late), which also could be indicative of differences in central drive between the groups. This could also signal a decrease in the subjects capacity to control posture. Our results indicate correlation between the voluntary contraction S-gradient and pre and post twitch maximum force (r = 0,74 for early group pre twitch, r = 0,44 for late group pre twitch, r = 0,70 for early group post twitch and r = 0,42 for late group post twitch), which could reflect submaximal firing frequencies and/or incomplete Ca^{+2} saturation during the early phase of voluntary contraction. The results also indicate significant negative correlation between the serum E2-level and the motor evoked potential (MEP) latency (r = -0,23 early and r = -0,37 late perimenopause), which on the other hand could indicate that serum E2 may act as a mediator in synaptic transmission. The peri- and postmenopause measurement results are somewhat inconclusive and it is therefore our suggestion that in order to identify possible trends in the results it is vital to perform additional studies with a larger cohort.

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Abbreviations

ACSM	American College of Sports Medicine
AD	Analog-to-digital
AP	Action potential
CAR	Central activation ration
CNS	Central nervous system
CSP	Cortical silent period
E2	Estradiol
EMG	Electromyography
ERMA	Estrogen Regulation of Muscle Apoptosis
ES	Electrical stimulation
FMP	Final menstrual period
FSH	Follicle-stimulating hormone
GABA	Gamma-aminobutyric acid
GEREC	Gerontology Research Center
GM	Medial gastrocnemius
ITT	Interpolated twitch technique
LICI	Long-term intracortical inhibition
MEP	Motor evoked potential
MU	Motor unit
MVC	Maximum voluntary contraction
MVF	Maximum voluntary force
PAP	Postactivation potentiation
PSP	Peripheral silent period
RFD	Rate of force development
RFR	Rate of force relaxation
RMT	Resting motor threshold
RLC	Resistor-inductor-capacitor
SD	Standard deviation
SENIAM	Surface EMG for non-invasive assessment of muscles
SICI	Short-term intracortical inhibition
SP	Silent period
$T_{control}$	Size of the control twitch
$T_{interpolated}$	Size of the interpolated twitch

TA	Tibialis anterior
TMF	True maximum force
TMS	Transcranial magnetic stimulation
VA	Voluntary activation

1 Introduction

We are well aware of the fact that aging causes a variety of changes in the central and peripheral nervous system as well as the neuromuscular system. These changes include for example reduced cortical and spinal excitability, altered motor unit discharge properties, reduced motor unit size and numbers, slowing of muscle contractile properties and cross-bridge cycling, impaired excitation-contraction coupling, and decreased tendon stiffness. Theoretically all of the above mentioned changes could reduce maximal muscle strength, power and RFD. It can also be noted that the capacity to produce force rapidly declines even more drastically with aging than maximal muscle strength. The inability to produce explosive force is related to a decreased neuromuscular response capacity in postural control and a decline in motor performance. The strong relationship between lower extremity strength and measures of balance and gait are especially clear for the elderly. The major causes for poor balance seem to be related to ankle and dorsiflexion weakness. As both plantar- and dorsiflexors do play a significant role in balance and gait, the comparison of the gastrocneius and the vastus medialis function could possibly reveal differences in muscle specificity.

The aging associated changes in the the central and peripheral nervous system, as well as in the neuromuscular system, are accompanied by reduced mechanical muscle function due to the loss of muscle mass. The loss of muscle mass is directly related to functional impairment and disability. In women, menopause further enhances the loss of muscle mass and strength through the decline of estrogen hormone concentrations. The peri-menopause phase has been linked to declining levels of estrogen, while the post-menopause phase is associated with low concentration levels of estrogen. The hormonal properties of estrogen enable it to affect muscle function both through the central nervous system (CNS) or directly through the muscle tissue.

Several different biological factors affect the muscle strength measured during a maximum voluntary contraction. One factor of interest is the voluntary activation level of the agonist muscle. In order to assess the level of voluntary activation in a muscle an artificial stimulation needs to be applied to the specific muscle. From a research point of view the quantitative assessment of muscle activation is essential in the evaluation of human muscle function.

The interpolated twitch technique (ITT) is a common method in the investigation of the effects of aging on the ability to fully activate muscle. The method also enables analysis of other muscle performance variables, such as maximum strength, power and RFD. TMS on the other hand can be used to study the inhibition-excitation phenomenon in the human brain through a non-invasive procedure.

This thesis is part of a larger study, "ERMA - Estrogen Regulation of Muscle Apoptosis". The purpose of that study is to understand the effects of timing and molecular mechanisms of estrogen deprivation in the development of muscle weakness in women. The aim of this thesis was to study the effects of the stage of menopause on the ability to activate the muscles of the lower extremity during isometric plantar- and dorsiflexion tasks.

2 Muscle Activation

2.1 Voluntary Activation

VA describes the level of neural drive to a muscle during a contraction (Gandevia et al., 1996) and (Gandevia, 2001). In other words it is the completeness to which a skeletal muscle is activated during a voluntary contraction. Regardless of the assessment method, VA can be said to be less than maximum if during a maximum voluntary contraction (MVC) a superimposed stimulus delivered to the contracting muscle evokes an increase in the produced force (Todd et al., 2003). This may, according to Todd et al. (2004), be due to either incomplete recruitment of the stimulated axons or their discharge at subtetanic rates. On the other hand if the supramaximal stimulus does not increase the produced force, one can conclude that the activation is maximal.

The most common methods for estimation of voluntary activation are based on the twitch interpolation technique introduced by Merton (1954). The method is based on the assumption of a linear relationship between the twitch force and the voluntary force. This relationship has been argued to not hold true by for example Behm et al. (1996). According to de Haan et al. (2009) and Taylor (2009a), the ITT contains inaccuracies regarding the twitch force and voluntary force relationship, the co-activation of antagonists and synergists with stimulation and the intermuscular differences effecting stimulus responses. Nevertheless, the method, despite its methodological inaccuracies, is considered a valid measure in the assessment of the central drive to the muscle and helps detect altered drive to the muscle. However these authors are in debate whether the interpolated twitch provides a valid measure of the percentage of voluntary activation.

The interpolated twitch method was originally described for a supramaximal electrical stimulus applied transcutaneously to the trunk of the motor nerve during an MVC (Merton, 1954). The method is also most commonly used with electrical stimulation of either the nerve trunk or the muscle point (Shield and Zhou, 2004). In general the quantification of the voluntary activation compares the extra force produced by the superimposed twitch to the force produced by the resting muscle with the same stimulus (Lee et al., 2008). The measurement procedure is illustrated

in figure 1. Response 1 represents the stimulation caused direct action potentials in the axons of the α-motoneurons (M-wave). Response 1^* represents the action potentials propagating antidromically along the α-motoneuron axons toward the spinal cord and response 2 depicts the action potentials elicited in the axons of the sensory Ia afferents. Response 3 depicts the action potentials of the evoked reflex response. A more thorough analysis of voluntary activation for different stimulation methods will be given in sections 2.2 and 2.3.

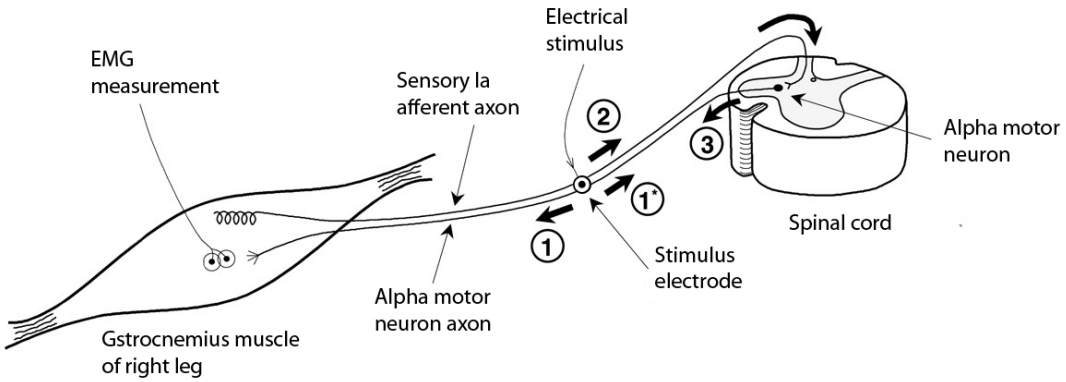

FIGURE 1. Illustration of the ES measurement.

2.2 Voluntary Activation and Electrical Stimulation

According to Merton (1951) the amplitude of the interpolated twitch is determined by mechanisms that are complex and incompletely understood. A supramaximal electrical stimulus applied to the nerve trunk or intramuscular nerve branches of an active muscle during a voluntary contraction causes a twitch-like increment in force from those motor units which have not yet been recruited i.e. motor units firing at sub-maximal rates or whose motoneurons are not in a refractory state (Belanger and McComas, 1981). The near-synchronous twitch of the muscle units in the non-refractory state, is according to Herbert and Gandevia (1999) caused by the orthodromic potentials in the motor axons. Transcranial electrical stimulation is not preferred as only a small fraction of the current applied to the scalp goes through the brain and large currents flowing on the scalp surface cause contractions in nearby muscles which makes the stimulation uncomfortable (Rothwell et al., 1991).

Following the negative relationship between twitch induced force and voluntary force,

described by Merton (1954) and later supported by others (Gandevia and McKenzie, 1988; Lyons et al., 1996), the voluntary activation level of muscle can be calculated by a linear equation. Equation 1 (Allen et al., 1995) describes one linear equation used to quantify voluntary activation:

$$VA(\%) = 100 \times (1 - T_{interpolated}/T_{control}), \tag{1}$$

where $T_{interpolated}$ is the size of the interpolated twitch and $T_{control}$ is the size of a control twitch produced by identical nerve stimulation in the relaxed muscle.

Equation 1 is based on the assumption of linear relationship between twitch force and voluntary force. However, several studies have argued the relationship to be non-linear (Allen et al., 1998; Behm et al., 1996; DeSerres and Enoka, 1998; Rutherford et al., 1986). This would render the estimation of voluntary activation and the force corresponding to maximal central activation invalid as the voluntary activation level could not be determined from a single datum point. Alternative extrapolation methods for quantifying voluntary activation has thus been suggested. For example Behm et al. (1996) suggested that the interpolated twitch - force relationship was best fitted by a hyperbolic curve that would result in insignificant prediction errors for MVC estimation. Even though de Haan et al. (2009) and Taylor (2009a) are in debate whether the interpolated twitch provides a valid measure of %VA, the authors are in agreement that the non-linearities in the relationship between the interpolated twitch and voluntary force are quite common and that extrapolation to predict maximal force is not recommended (de Haan et al., 2009; Taylor, 2009b).

2.3 Peripheral Silent Period

The PSP is an electromyogrphic (EMG) silent period (SP), which follows the interpolated twitch, as can be seen in figure 2. In figure 2 a clear period of silence is evident in both the EMG signal, which has been measured during a voluntary contraction, as well as in its rectified derivative. The high signal peaks right before the SP are due to the voluntary contraction and the superimposed twitches. The PSP may, according to Herbert and Gandevia (1999), be influenced by several different mechanisms. Possible reasons for the period of prolonged inhibition are discussed below.

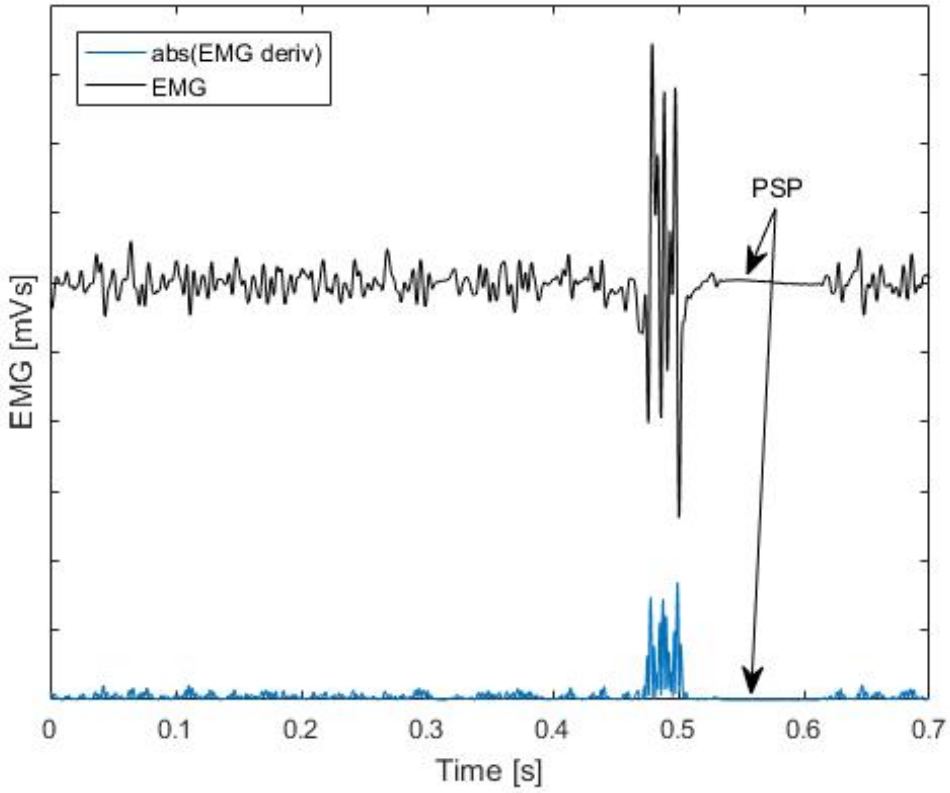

FIGURE 2. Illustration of the PSP in the EMG and EMG derivative signal.

The action potentials, evoked through interpolated stimulation, propagate both ortho- and antidromically (Herbert and Gandevia, 1999). As was previously stated in section 2.2 the orthodromic action potentials produce a near-synchronous twitch in the non-refractory muscle units.

The action potentials (AP) traveling in the antidromic direction may also influence the size of the superimposed twitch due to their collision with voluntarily produced potentials, thus reducing the rate of motoneuron discharge directly following the stimulus (Herbert and Gandevia, 1999). Some antidromic potentials may according to Brook et al. (1952) reach the soma of a motoneuron and cause hyperpolarization. Herbert and Gandevia (1999) also indicate that these antidromic signals may propagate along recurrent branches, which terminate on Renshaw cells. Signals propagating on these recurrent branches could possibly evoke inhibitory postsynaptic potentials in motoneurons. According to Herbert and Gandevia (1999) the interpolated twitch may also effect the motoneuron discharge through short-latency reflex actions of the stimulated sensory axons. Floeter (2003) on the other

hand argues that cutaneous afferents contribute to the terminal portion of the mixed-nerve SP.

2.4 Cortical Silent Period

TMS, when applied to the primary motor cortex, can induce contra-lateral muscle activity. According to Burke et al. (1993) and Day et al. (1989) the stimulus can evoke complex descending volleys in the corticospinal neurons. The onset of the response to these volleys is in many human muscles consistent with monosynaptic corticomotoneuronal excitation (Taylor and Gandevia, 2001). These short-latency excitatory responses, which can be recorded by EMG are termed MEPs (Rotenberg et al., 2014, p 8). The measurement procedure is illustrated in figure 3.

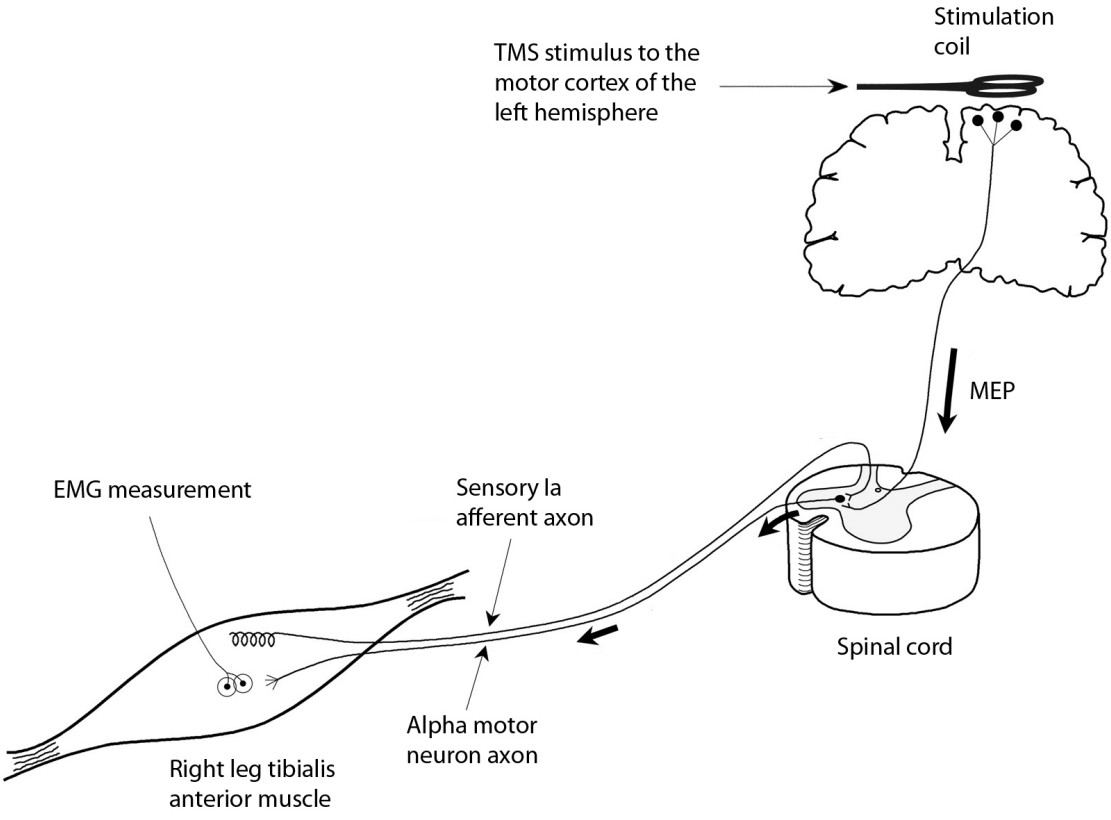

FIGURE 3. Illustration of the TMS measurement.

In some muscles like the soleus the initial response in many motor units is disynaptic inhibition. This disynaptic inhibition may be masked in muscles in which the initial

response is excitation. (Brouwer and Ashby, 1990, 1992) According to Taylor and Gandevia (2001) the descending volleys evoked by TMS from the human cortex depend on both the stimulus intensity and the excitability of cortical cells. The response in the muscle on the other hand depends on the excitatory and inhibitory interneurons and the transmission through them as well as on the excitability of the motoneuron pool. The MEP can be considered a representation of what might be happening in the cortex, if the changes in the distal motor pathway are taken into account. (Taylor and Gandevia, 2001.)

The inhibition-excitation phenomenon in the human brain can also be studied using the non-invasive transcranial magnetic stimulation procedure. Following the TMS depolarization of the motor neuronal population there is a transient suppression of the voluntary muscle action, termed the CSP (Säisänen et al., 2008). The CSP is a period of near silent signal in the electromyogram (Fuhr et al., 1991; Holmgren et al., 1990), as illustrated in figure 4. In figure 4 a clear period of silence is evident in both the EMG signal, which has been measured during a voluntary contraction, as well as in its rectified derivative. The high signal peaks right before the SP are due to the MEP from the TMS. The SP generally lasts for more than 200 ms for a high-intensity stimulus (Inghilleri et al., 1993; Triggs et al., 1993).

According to Säisänen et al. (2008) the mechanisms behind the CSP are poorly understood. It is widely accepted that the initial part of the CSP is of spinal and later part of cortical origin. In their study Taylor and Gandevia (2001) hypothesize that both inhibition of descending drive and reduced excitability of the motoneurons may play a role in the initial part of the SP and that the latter part of the SP may reflect intracortical inhibition. According to Pierrot-Deseilligny and Burke (2012, p 49) the first 50 ms of the CSP is mediated by spinal mechanisms. These mechanisms may include post-spike after-hyperpolarization of the motoneurones and recurrent inhibition (Pierrot-Deseilligny and Burke, 2012, p 49). The later part of the CSP is believed to result from activation of $GABA_B$ inhibitory interneurones at the cortical level (Pierrot-Deseilligny and Burke, 2012, p 49). This is also supported by pharmacological studies by Werhahn et al. (1999) and McDonnell et al. (2006), which suggest that $GABA_B$ receptors mediate long-term intracortical inhibition (LICI), while Ziemann (2003) suggest that short-term intracortical inhibition (SICI) is primarily mediated by $GABA_A$ receptors.

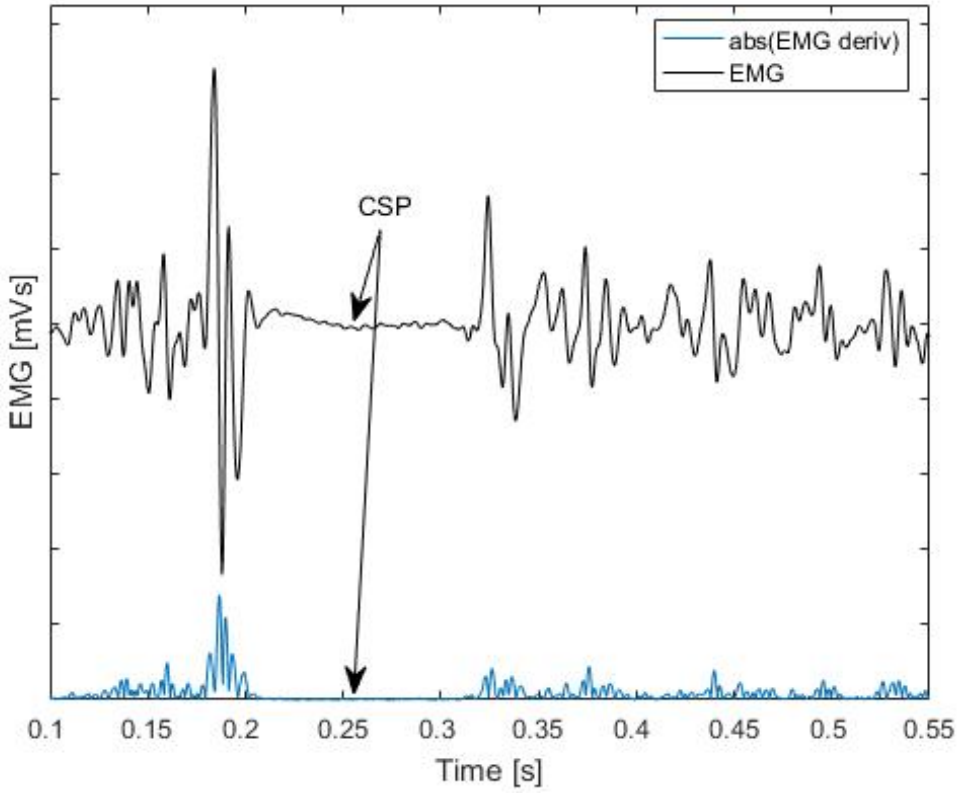

FIGURE 4. Illustration of the CSP in the EMG and EMG derivative signal.

Even though there exists a long history in the recording of the SPs, the effect of muscle contraction level on the duration of the SPs remains unclear (Säisänen et al., 2008). Several studies have concluded that the level of tonic muscle pre-activation does not correlate with SP duration (Haug et al., 1992; Inghilleri et al., 1993; Roick et al., 1993; Taylor et al., 1997; Triggs et al., 1993; Uncini et al., 1993), whereas some studies have concluded a shortened duration in the SP accompanying increasing muscle activity (Stetkarova et al., 1994; Wilson et al., 1993).

According to Stetkarova et al. (1994) to understand the differing results one has to consider the methodology behind the measurements. If complete electromyographic silence is required throughout the whole SP, an increase in the contraction force of the lower extremity results in the shortening of the SP duration. If on the other hand the return of uninterrupted background EMG marks the end of the SP, the trend seems to be a lengthening of the SP with increased force. Thus, for clinically relevant SP measurements, it is essential to agree on the measurement methodology. (Stetkarova et al., 1994.)

Both Kischka et al. (1993) and Hess et al. (1987) state that during a voluntary contraction, both the corticospinal neurons and motoneurons increase their excitability, which enables the same cortical stimulus to evoke a much larger MEPs in a contracting muscle when compared to the MEPs of the same muscle during relaxation. It has also been suggested that during brief isometric contractions the size of the MEP increases with the strength of the contraction, but that this increase depends on the activated muscle (Taylor and Gandevia, 2001). According to DiLazzaro et al. (1998); Kaneko et al. (1996); Mazzocchio et al. (1994); Ugawa et al. (1995) part of the increase in the size of the MEP with contraction may be attributed to an increased output from the motor cortex in response to stimulation. While the change in cortical excitability does not solely depend on the contraction level, it seems that some tasks require a stronger cortical contribution than others, which manifests as an increase in cortical excitability (Flament et al., 1993; Nielsen et al., 1993; Schieppati et al., 1996).

According to Orth and Rothwell (2004) a high amplitude MEP usually leads to a longer SP and it has been shown that the SP can be predicted from both MEP amplitude and area. It can thus be speculated that the MEP and the SP share some common mechanisms. What the exact mechanisms of these inter-relationships are remains however unclear (Säisänen et al., 2008).

2.5 Postactivation Potentiation

PAP, as defined by Robbins (2005), is the increase in the exerted muscle force caused by a previous contraction. The PAP theory proposes that the contractile history of a muscle affects the mechanical performance of the following muscle contractions. More specifically, non-fatiguing contractions with heavy loads for brief periods may improve muscle performance (Stone et al., 2008). For example, according to Hodgson et al. (2005) and Sale (2002), in skeletal muscle the peak torque of an isometric twitch is temporarily increased following a maximal voluntary contraction. The phenomenon is illustrated in figure 5, where the peak twitch force following the voluntary contraction is higher compared to the pre-contraction twitch. This effect may, according to Chiu et al. (2003) and Rixon et al. (2007), last from 5 to 30 minutes. PAP is most commonly evident as an increase in isometric

twitch force following a maximal voluntary isometric contraction (Mitchell and Sale, 2011). Along with increasing muscle force, PAP also increases the rate of force development (Baudry and Duchateau, 2007; MacIntosh et al., 2008; Vandenboom et al., 1995). According to Grange et al. (1993), Vandenboom et al. (1995) and Hamada et al. (2000b), the most important muscle characteristic which affects the PAP magnitude is fiber type, with muscles with the greatest proportion of type II fibers having the greatest potential for PAP enhancement. Also muscles with the shortest twitch contraction have been shown to have greater PAP (Vandenboom et al., 1995; Hamada et al., 2000b). In the elderly a reduction in potentiation capacity is partly explained by the overall slowing of the muscle contractile properties with aging. This potentiation attenuation does, however, only moderately affect the decrement of muscular performance. (Baudry et al., 2005.)

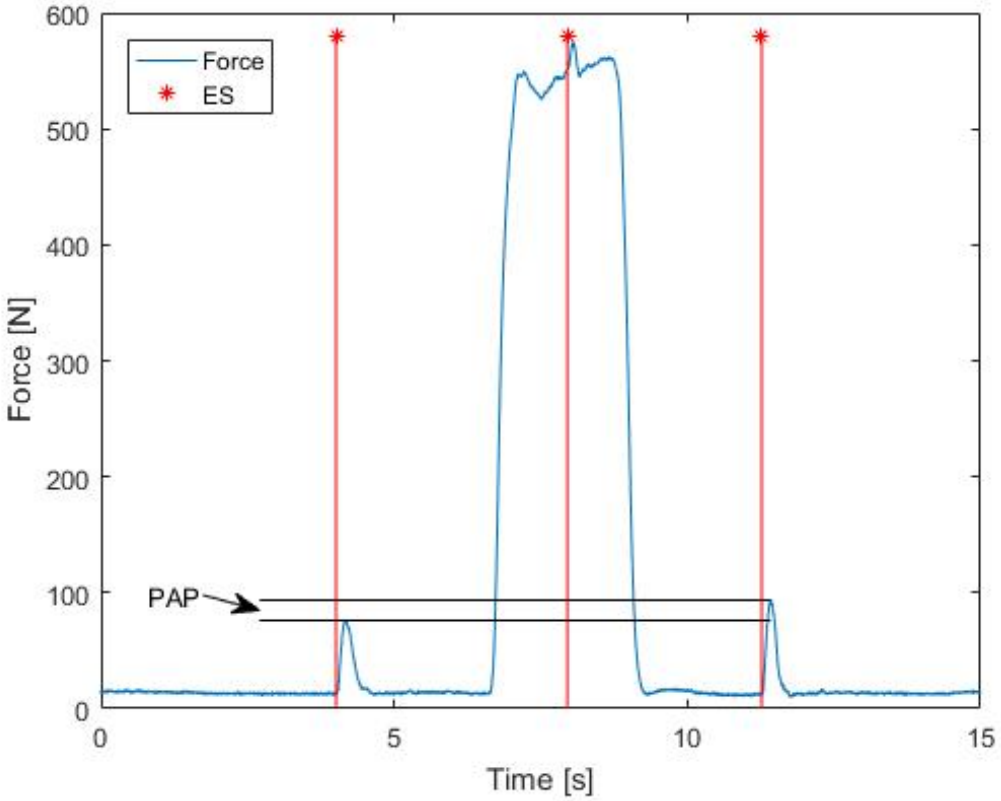

FIGURE 5. PAP phenomenon for an isometric twitch.

Two main theories regarding the mechanisms for PAP have been proposed. The first theory involves the phosphorylation of myosin regulatory light chains during the maximal contraction. This causes the actin-myosin binding to become more

sensitive to the released calcium for the following muscle contractions (Grange et al., 1993; Hamada et al., 2000a; Young et al., 1995). This in turn causes faster rates of force development and increase in force (Chiu et al., 2003). The second theory involves an increase in the synaptic excitation at the motoneuron, due to the maximal contraction, as reflected by changes in the H-reflex. This increases the post-synaptic potentials, thus enabling greater force production. (Hodgson et al., 2005.) A third possible potentiation mechanism, which could contribute to an increase in power and strength after a muscle contraction, is the reduction in the pennation angle of the muscle following the muscle contraction (Mahlfeld et al., 2004).

2.6 Rate of Force Development

For elderly people, according to Foldvari et al. (2000) and Skelton et al. (2002), muscle power is a major indicator of functional motor performance and incidence of falling. The muscle power of the lower limbs has also been shown to decline more rapidly than muscle strength with aging (Skelton et al., 1994; Izquierdo et al., 1999b). Muscle power is the product of muscle force and velocity. One measure of muscle power, the RFD, is defined as the rate of rise in the produced force at the early phase of force production (Häkkinen and Komi, 1986; Sleivert and Wenger, 1994; Thorstensson et al., 1976). The RFD can be calculated from the slope of the force-time curve and it has important functional significance in fast and forceful force production. Even though a short contraction time in rapid joint movements may not enable the production of maximal muscle force, the ability to produce force at a high rate has an important role for the elderly population. This is due to the fact that fast force production may reduce age related incidence of falls caused by impaired control of postural balance (Aagaard et al., 2002). Studies have reported significant correlation between agonist EMG and explosive early phase volitional force (de Ruiter et al., 2006) and relative RFD (Klass et al., 2008). Some indirect results from Tillin et al. (2010) support the possibility of neural drive being an important factor for early-phase explosive force production. Tillin et al. (2010) attributed the greater explosive force capability of explosive power athletes in part to differences in neural drive. VanCutsem et al. (1998) and Aagaard et al. (2002) have also stated that neural drive may be an important factor in maximizing explosive force production.

A measure of the early phase of the RFD is the S-gradient, described by Komi (2002, p 441) as the average RFD at the beginning phase of a muscular effort. The S-gradient can be calculated as the ratio between half the maximum voluntary force and the time to reach this force (Komi, 2002, p 442). Folland et al. (2014) found that twitch force was a better determinant of explosive force during the initial 50 ms of contraction than octet force. They also speculated that the similarities between the early phase of a voluntary contraction and the twitch response could be due to submaximal firing frequencies and/or incomplete Ca^{+2} saturation during the early phase of the voluntary contraction. Andersen and Aagaard (2006) also reported a relationship between twitch force and RFD of the early phase of voluntary contraction. According to Grimby et al. (1981) the level of neutral activation may influence the RFD. Other possible influencing factors include muscle size, fiber-type composition (MHC isoform) (Harridge, 1996), crossbridge cycling rate and tendon stiffness (de Ruiter et al., 2007).

2.7 Motor Unit Activation Order During Stimulation

According to Henneman's size principle the motoneurons activated during voluntary muscle contractions are recruited in an orderly fashion from small motoneurons to large motoneurons (Henneman, 1957; Henneman et al., 1965). This is due to the fact that the Ia excitatory post-synaptic potential in individual motoneurons, evoked by specific afferent signaling, is larger in the small motoneurons than in the large motoneurons (Pierrot-Deseilligny and Burke, 2012, p 4). Since the small motoneurons typically supply slow motor units and the large motoneurons typically supply fast motor units the size principle also states that motor units are recruited in an orderly fashion from slow to fast. The orderly recruitment therefore lays the foundation for the force production of the entire musculoskeletal system. It is also important to realize that as such the MVC of a muscle is the representation of the capacity of the entire neuromuscular system to produce force. It is generally accepted that two main factors affect the motor unit activation order during transcutaneous stimulation, the excitability threshold of motoneuron axons and the relation of the axons to the current field (Knaflitz et al., 1990; Grill and Mortimer, 1995; Feiereisen et al., 1997; Nilsson et al., 1997). However, according to Rodriguez-Falces and Place (2013) studies into the effects of transcutaneous stimulation on the

activation order of motor units have revealed conflicting results. For stimulation of the nerve trunk, studies have obtained results for both reverse order recruitment and recruitment following the size principle. For motor point stimulation, study results include recruitment according to the size principle, reversed order of recruitment and activation with no specific order (Rodriguez-Falces and Place, 2013). Thus the use of artificial stimulation to achieve muscle contraction has created the need to better understand the mechanisms underlying the method. Based on physiological properties of the nerve trunk and stimulation intensity the general consensus is that H-reflex stimulation follows the size principle while M-wave stimulation reverses the order of recruitment (Pierrot-Deseilligny and Burke, 2012, p 5) and (Merletti and Farina, 2016, p 304). The results of Rodriguez-Falces and Place (2013) however support the size principle order of recruitment for nerve trunk stimulation, while the recruitment of motor units through direct muscle activation is not as straight forward. The order of recruitment for direct muscle stimulation seems to be more muscle specific, with the innervating motor branch playing a role in the recruitment order. According to Feiereisen et al. (1997) differences in nerve axon impedance and the size and organization of the axonal branches within a muscle play a significant role in the recruitment of motor units with transcutaneous motor point stimulation. Thus according to Grill and Mortimer (1995) and Knaflitz et al. (1990) the actual stimulation geometry becomes a more determining factor in the depolarization of the motor axon.

The recruitment order is not of importance when the stimulus is used to obtain maximal activation of the target muscle. On the other hand if muscle voluntary activation is studied at submaximal force levels it is possible that different parts of the muscle are activated with successive stimuli (Behm et al., 1996). It should also be noted here that the recruitment order discussed here has been studied using transcutaneous electrical stimulation, but that the discussion is valid for transcutaneous magnetic stimulation as well. This is due to the fact that magnetic stimulation can evoke a similar effect in the nerve and muscle as electrical stimulation (Lampropoulou et al., 2012; Pierrot-Deseilligny and Burke, 2012, p 7).

According to Bawa and Lemon (1993) TMS appears not to act in a specific manner at the human motor cortex. The stimulation activates several motoneuron pools simultaneously, both agonists and antagonists. This activation of the corticospinal

cells is, according to Abbruzzese and Trompetto (2002), transsynaptic, as is evident by the multiple waves in the descending volley evoked by a single TMS (Bawa and Lemon, 1993). These waves are likely caused by both direct and indirect corticospinal neuron activation (Day et al., 1989). On the other hand the response of a single motoneuron pool is likely the result of several different excitatory and inhibitory inputs, thus making the descending input at the motoneuron very complex (Bawa and Lemon, 1993). In their study Bawa and Lemon (1993) examined the effects of TMS on rate coding and motor unit recruitment in hand and forearm muscles. Their results show, that despite the complex nature of the descending corticospinal volleys TMS produces orderly recruitment similar to voluntary activation (Bawa and Lemon, 1993).

3 Considerations Regarding Stimulation

3.1 Considerations with Electrical Stimulation

While the interpolated twitch technique introduced by Merton (1954) still remains the the most widely used method for measurement of muscle activation the parameters and procedural methodologies within the method have not been standardized. This has led to a number of outstanding issues regarding the use of the method. In their study for example Folland and Williams (2007) found that out of the variables they studied three variables (type of extrapolation, timing of the control twitch and timing of the superimposed twitch) had significant influence on the measure of maximum voluntary force (MVF).

3.1.1 Timing of the Stimulation Twitches

The post-contraction potentiation phenomenon is a well known factor which influences the magnitude of the control twitch evoked into passive muscle. It has been unclear weather pre-contraction or post-contraction control twitches should be used to evaluate the level of muscle activation. Studies into the effects of pre-contraction and post-contraction control twitches came to the conclusion that in terms of both reliability and validity the potentiated post-contraction control twitch is more recommendable for activation level assessment. (Folland and Williams, 2007.) It should also be noted here that the superimposed twitch is also as such potentiated and thus using the post-contraction twitch for activation level assessment would seem logical.

Folland and Williams (2007) have also observed that the timing of the superimposed twitch during MVC could influence the assessment of the activation level. Their study analyzed the difference between two superimposed twitches delivered approximately 1 s apart during the MVC. The novel finding was that the timing of the superimposed twitch significantly influences the evaluation of voluntary activation, with the first twitch typically producing more reliable values. Folland and Williams (2007) could however not comment on the reasons for the difference.

Shield and Zhou (2004) emphasize that precautionary steps should be taken to maximize the probability that the stimulus is delivered to the muscle during maximal effort. According to them these steps could include for example automatic triggering and rejection criteria when maximal voluntary contractions are not achieved.

The discussion above is valid for maximal efforts per se and assessment of voluntary activation at submaximal efforts will only need to take into account the effects of pre- and post-contraction control twitches in the measurements.

3.1.2 Number of Stimulation Twitches

As has been stated earlier, the original twitch interpolation technique described by Merton (1954) involved a single stimulus interpolated over voluntary contractions. Recently, however the use of two or more stimuli have become more popular, perhaps because the evoked force increments are larger and more readily detected (Shield and Zhou, 2004).

The effect of stimulation twitch type has been studied quite thoroughly. Behm et al. (1996) concluded in their study that there is no significant difference in predicting true maximum force (TMF) using single, doublet or quintuplet stimulation. Allen et al. (1998) also reported similar findings, with no significant difference in the forces evoked by single, paired or trains of stimuli for force levels above 85% MVC. Allen et al. (1998) did however notice that doublet and trains of stimuli evoked higher forces for force levels below 85% MVC. Studies by Strojnik (1995) and Miller et al. (1999) have on the other hand reported increases in the measured torque for stimulus trains. In the study by Oskouei et al. (2003) the authors recommend a paired stimulation to reduce the variations in the superimposed twitch technique. A comparison of single stimuli versus pulse trains by Suter and Herzog (2001) revealed that once the evoked force was normalized to the corresponding resting twitch the variability in the ITT decreased significantly as the number of stimuli increased. This would indicate that multiple twitches provide a more reliable estimate of voluntary activation. This holds particularly true for fatigue studies as indicated by Place et al. (2007). Contrary to these findings, Knight and Kamen (2008) have suggested that trains of 1 s do not produce greater force, but that stimulation at higher, non-physiological rates can produce greater muscular force, which in turn supports the

idea that a single pulse stimulus may be appropriate for determining the quality of neural activation. This would also, according to Knight and Kamen (2008), support the idea that multiple stimuli at high stimulation rates may produce force enhancements due to Ca^{+2}-related force potentiation, transient increases in stiffness, or other muscle-related issues. An important variable supporting the use of single and paired stimuli is the discomfort associated with the use of trains of stimuli. According to Folland and Williams (2007) this may be a significant distraction from production of a genuine maximum voluntary contraction and thus compromise measurement of muscle activation. Herbert et al. (1997); Herbert and Gandevia (1999) have also criticized the use of multiple stimuli on the grounds that spinal reflexes have more time to influence the superimposed response.

For paired stimuli a 10 ms inter-stimulus interval has often been used, even though the reasoning for this value or the mechanism affecting the response of muscle by varying intervals are not clear (Karimpour, 2013). In her study (Karimpour, 2013) investigated the effect of stimulus interval on muscle activation in young and old. She concluded that an interval of 10 ms seems to elicit highest responses in the elderly and an interval of 5 ms seems to elicit highest responses in young individuals. Her conclusion also states that the above mention values for inter-stimulus intervals result in a significantly higher rate of torque development and which may have an advantage in power-related studies. Based on their results Karimpour (2013); Mayfield et al. (2015) recommend the use of short-interval doublets for ITT.

3.1.3 Stimulation Intensity

Folland and Williams (2007) studied the effects of stimulus magnitudes based on the hypothesis that a larger superimposed stimulus might produce a greater signal-to-noise ratio and increase the validity and reliability of the ITT. In addition, Behm et al. (1996) found no significant difference between TMF with superimposed single, doublet and quintuplet stimuli of different magnitudes. Miller et al. (1999) however reported an increase in sensitivity for the assessment of central activation failure for larger magnitude pulse trains than for single impulses. Folland and Williams (2007) came to the same conclusion as Behm et al. (1996) that the type of twitch stimulus used in their study did not effect TMF or activation. According to Gandevia

(2001) the increased number of pulse stimuli cause greater antidromic activation of motoneurons and Renshaw cells, which may counter any improvement in the signal-to-noise ratio.

According to Rutherford et al. (1986) submaximal stimulation with twitch interpolation can be used to provide reliable estimates of voluntary activation in unfatigued muscle. The use of submaximal stimulation can be justified as it reduces the discomfort associated with supramaximal twitch interpolation. Submaximal stimulation may also in some cases reduce cross talk-contaminated twitches by a more selective recruitment of the agonist with less activation in the antagonistic muscles (Awiszus et al., 1997). Since submaximal stimulation does not recruit all possible motor units (MUs), one possible disadvantage with submaximal stimulation according to Behm et al. (1996) is that successive stimuli may activate different portions of the muscle. According to Vagg et al. (1998) the use of submaximal stimulation is also unsuited for fatigue studies as fatiguing contractions cause an increase in the threshold of motor axons. This will result in fewer activated motor units at specific stimulation intensities (Vagg et al., 1998).

3.1.4 Duration and Type of Stimulation Twitch

According to Veale et al. (1973) and Burke et al. (2001) afferent axons are preferentially recruit over efferent axons with relatively long pulse durations due to the afferent axons lower rheobase and longer strength-duration time constant. The results of Lagerquist and Collins (2008) on the other hand indicated that the mean maximum M-wave decreased when using a pulse-width of 0,05 ms compared with 0,2, 0,5 and 1,0 ms. To determine the effect of stimulus duration on H-reflex measures Panizza et al. (1989) used varying stimulus intensities and pulse durations to stimulate the posterior tibial nerve and the median nerve. Their study included stimulus intensities ranging from 0 to 200 V, while the duration of the rectangular pulse was 0,1, 0,3, 0,5, 0,8, 1,0, 2,0, or 3,0 ms. The results of the study indicate that for stimulation of the tibial nerve the maximal amplitude H-reflex response was largest for pulse durations between 0,3 and 1,0 ms and for median nerve stimulation the maximal amplitude H-reflex response was obtained with 0,5 and 0,8 ms pulse durations. Panizza et al. (1989) also reported that subjects complained of pain or

discomfort as pulse duration exceeded 1,0 ms in duration with an intensity sufficient to elicit M-responses. Miller et al. (1999) also noticed that short pulse duration, in their case 0,1 and 0,2 ms, increased the comfort of the superimposed stimulation.

Different stimulus pulse formats have been compared by Kramer et al. (1984); Walmsley et al. (1984). Kramer et al. (1984) compared asymmetrical bi-phasic rectangular waves, asymmetrical bi-phasic spike waves and symmetrical mono-phasic square waves. Their results indicate that for stimulation of a relaxed muscle the torque obtained with the symmetrical mono-phasic square wave was significantly less than that obtained with the other two current formats. Also, the torque associated with the asymmetrical bi-phasic spike wave was significantly less than that associated with the asymmetrical bi-phasic rectangular wave format. However, under voluntary and superimposed contractions Kramer et al. (1984) found no significant differences in mean torque between the three current formats. Kramer et al. (1984) notes that subjects reported the asymmetrical bi-phasic rectangular wave as being the most comfortable. Walmsley et al. (1984) compared alternating sinusoidal current and bi-phasic impulses and reported no significant difference between the two formats.

3.1.5 Intermuscular Differences

In their study of investigating differences in the ability to activate different muscles Behm et al. (2002) found that the quadriceps had significantly greater inactivation when compared to the to the plantar flexor, dorsiflexor and elbow flexor muscles during isometric contractions. The authors speculate that the reason for greater inactivation of the quadriceps can not be due to differences in the number of activated motor units or differences in muscle architecture. According to Behm et al. (2002) one possible factor explaining the greater quadriceps inactivation is related to the muscle fiber composition differences between the compared muscles. For example, Behm et al. (2002) stated that both Johnson et al. (1973) and Edgerton et al. (1975) have reported greater type II fiber percentages in the vastus lateralis as compared to the plantar flexor muscles. According to Belanger and McComas (1981) and Gandevia and McKenzie (1988) the capacity of full activation was better in dorsiflexor muscles as compared to plantar flexors. The difference in activation capacity was explained to result from either differences in muscle fiber composition

or from the greater series elastic component of the long tibialis anterior tendon.

3.1.6 Method of Extrapolation

As has already been discussed in section 2.2, the interpolated twitch technique has traditionally been assumed to follow a linear relationship between twitch force and voluntary force. Section 2.2 also discussed other possible models for the relationship, with most of the evidence pointing towards a concave curvilinear twitch force-voluntary force function (Folland and Williams, 2007). As was also mentioned in section 2.2, even though de Haan et al. (2009) and Taylor (2009a) are in debate whether the interpolated twitch provides a valid measure of %VA, the authors are in agreement that the non-linearities in the relationship between the interpolated twitch and voluntary force are quite common and that extrapolation to predict maximal force is not recommended (de Haan et al., 2009; Taylor, 2009b).

3.1.7 Influence of Series Elastic Structures, Synergistic and Antagonistic Activation

The ITT exhibits a maximal voluntarily produced torque in the absence of an evoked superimposed twitch response. It has been demonstrated that the biceps brachii could under motor-point stimulation always produce close to the absolute maximal torque of its capability. On the other hand the brachioradialis rarely fully activated during voluntary contraction. They are innervated by different nerves, which may affect the activation of the synergist muscles in elbow flexion tasks, thus contributing to the shape of the relationship between twitch amplitude of the agonist and voluntary force. From the study results it could be concluded that the increase in torque beyond what was achieved with near-maximal activation could be attributed to the variable activation of synergists and/or to the slight lengthening of the active muscle at high voluntary forces. They therefore propose that during maximal contractions assessment of several synergists will provide a better estimate of the global voluntary activation. (Allen et al., 1998.)

Allen et al. (1998) also demonstrated that a nonlinear relationship between voluntary and evoked muscle torque at high voluntary torques was unlikely affected by the

variations in the series elastic component of the muscle and compliance of the myograph system.

To test the effects of series compliance, which allows the muscle to shorten during the superimposed twitch, Loring and Hershenson (1992) measured the force production of the adductor pollicis during voluntary isometric contractions with a non-compliant and a compliant loading device. Their results indicated that the superimposed twitch force was systematically less with the compliant loading device than with the non-compliant device. They also found that the superimposed twitch force vs. voluntary force was often concave upward. From their findings Loring and Hershenson (1992) concluded that the results are consistent with the force-velocity relationship of muscle. This in turn suggests that the results from twitch-interpolation measurements must be interpreted with caution if the muscle is not held isometric during the superimposed twitch. On the other had Allen et al. (1998) reported that their findings support minimal effect from series compliance during strong voluntary efforts.

With the stimulation of muscle by either electrodes placed over the nerve trunk or over the muscle belly it is possible that in addition to activating the studied muscles their antagonists will also be activated (Gandevia and McKenzie, 1988). Activation of antagonists may also be further influenced by placing electrodes too far apart, too close to antagonists and using excessively large electrodes or intensities for the stimulation (Awiszus et al., 1997). According to Carolan and Cafarelli (1992) the co-activation of antagonist muscles can reduce the performance of agonist muscles through opposing mechanical action and according to Crone and Nielsen (1989) through reciprocal inhibition. The torque produced by the antagonist activation may reduce the amplitude of the control twitch or mask small agonistic force increments during maximal voluntary efforts, thus indicating full activation of muscles during submaximal efforts (Awiszus et al., 1997). Kellis (1998) stated that the magnitude of co-activation during MVCs should be assessed by expressing the EMG activity in the antagonist muscle as a percentage of its activity when the muscle is acting as an agonist in a maximal contraction. It should also be mentioned that according to Baratta et al. (1988) a small amount of co-activation is necessary in the stabilization of joints.

3.1.8 Site of Stimulation

Electrical stimulation is typically, according to Hultman et al. (1983), applied via electrodes placed over the nerve trunk that innervates the studied muscle or muscles or over the muscle belly. The percutaneous muscle stimulation activates the muscle via intramuscular nerve fibres. According to Pierrot-Deseilligny and Burke (2012, p 6) the optimal method for evoking an H-reflex through Ia afferent stimulation at lower threshold than motor axons involves placing the cathode over the nerve and the anode on the opposite side of the limb. This setup ensures that the current passes transversely through the stimulated nerve. For nerve stimulation where bipolar stimulation may stimulate additional nerves the cathode should be placed over the nerve and the anode distal or lateral from that to avoid possible anodal block (Pierrot-Deseilligny and Burke, 2012, p 6). However according to Kramer et al. (1993), who tested monopolar anodal and cathodal responses to traditional bipolar cathode stimulation, anodal block does not appear to occur during routine nerve conduction studies.

3.1.9 Different Muscle Actions

ITT is most often applied in isometric conditions (Millet et al., 2011). When ITT measurements have been performed in dynamic conditions, the contraction speeds used have ranged from 15 to $360^o/s$ (Paillard et al., 2005).

According to Paillard et al. (2005) the different muscle actions have a strong influence on the muscle activation level. For concentric muscle actions Strojnik and Komi (1998) have been the only ones to report higher peak torques with percutaneous stimulation compared to voluntary muscle activity, whereas for example Gandevia et al. (1998) reported no influence of the contraction velocity on muscle activation level. For eccentric contractions it has been shown that superimposed electrical stimulation enables the torque to increase over values obtained under voluntary activation conditions for contraction velocities from -30 to -$120^o/s$ (Westing et al., 1990; Amiridis et al., 1996). The authors speculate that voluntary eccentric contractions may be limited by a neural inhibition mechanism protecting the muscle from extreme tension due to co-activation of the antagonist muscles. The eccentric voluntary contractions also stimulate the Golgi tendon organs (Westing et al., 1990)

which could limit voluntary torque production. The eccentric voluntary contraction also inhibits Ia type facilitatory afferents from the muscle spindles to limit tensions (Amiridis et al., 1996). According to Westing et al. (1990) and Amiridis et al. (1996) the superimposition of electrical stimulation could reduce the neural inhibition mechanism by stimulating the cutaneous receptors thus increasing the recruitment of motor units involved in the muscle action. Due to the above mentioned points contraction mode is an important factor that has to be taken into account when explaining effects induced by the superimposed

3.1.10 The Accuracy of the Force Measurements

The detection of a 1% activation deficit requires a force resolution that is 1% of the amplitude of the control twitch (Shield and Zhou, 2004). According to Gandevia and McKenzie (1988), control twitches evoked by single stimuli may be as small as 10% of the MVC force, thus in a muscle that is 99% activated the evoked superimposed force increment may only be 0.1% of the MVC force. The resolution of the force measurements is seldom reported in studies, thus possibly placing the results under some scrutiny (Shield and Zhou, 2004).

To improve the level of resolution, increasing the level of the control response enables the detection of smaller activation deficits and thus some advantage may be obtained by employing more than one stimuli (Shield and Zhou, 2004). Also consequently the sensitivity of twitch interpolation is greater when the stimulated muscle is responsible for the majority of the total force produced at the joint. It is however possible to detect extremely small deficits in activation with single stimuli when appropriate methods are employed to amplify the superimposed response (Hales and Gandevia, 1988).

3.2 Considerations with Magnetic Stimulation

As was stated in section 2.4 the assessment of voluntary activation by magnetic stimulation has been shown to be a reliable and valid method (Todd et al., 2003), (Verges et al., 2009), and (OBrien et al., 2008). Regardless of the reliability and validity of the method, several issues still limit magnetic stimulation from being a

more widespread method in peripheral stimulation or in the assessment of muscle activation (Lampropoulou et al., 2012). According to Peterchev et al. (2012) the current practice is that the electromagnetic stimulation dose is often described relative to individual measures (motor threshold etc.), and/or in terms of summary metrics (total stimulus charge, total stimulus energy, or electrode charge density). To ensures reproducibility of the electromagnetic dose control, documentation of all relevant stimulation device parameters should be implemented (Peterchev et al., 2012).

3.2.1 Magnetic Field Strength

One of the major issues concerning magnetic stimulation relates to the limited field strength of the currently available stimulators (OBrien et al., 2008). Electromagnetic induction follows the inverse cube law, where the power of the magnetic field decreases exponentially with distance from its origin. The current induced in tissue thus also decreases rapidly with distance from the coil (Rotenberg et al., 2014, p 4). The intensity of a transcranial magnetic stimulation pulse is determined by the capacitance voltage of the transcranial magnetic stimulation device. Thus the intensity can be easily adjusted within the limits of the device. The capacitance voltage affects the steepness of the induced magnetic field, thus affecting the amplitude of the induced electrical current in the brain. (Rotenberg et al., 2014, p 79) It has been shown that by increasing the intensity of the transcranial stimulus it is possible to increase the MEP amplitudes, thus modifying the pattern of the descending volley (Rotenberg et al., 2014, p 79). For transcranial stimulation the field strength is not an issue if for any stimulator output a large MEP with a minimum amplitude 50-60 % of M_{max} can be obtained (Todd et al., 2003).

3.2.2 Pulse Parameters

The shape of the magnetic pulse is determined by the circuitry of the magnetic stimulator, and cannot easily be adjusted in most commercially available stimulators without changing the resonant frequency and thus the stimulator hardware (Rothkegel et al., 2010; Rotenberg et al., 2014, p 79). The resistance-inductance-capacitance (RLC) circuit in the simulator determines the resonating frequency, which makes

the pulse shape dependent of the capacitor and inductor of each stimulator device (Rotenberg et al., 2014, p 79). Rothkegel et al. (2010) studied the effect of pulse duration on different physiological parameters of the primary motor cortex excitability using two different pulse lengths. They came to the conclusion that pulse length does not affect the threshold-adjusted single pulse measures of motor cortex excitability. At the same time they did suggest that increasing the pulse duration would produce stronger pulses, which might be required in subjects with a higher threshold due to age, medication or disease (Rothkegel et al., 2010). The author did not find any mention of paired pulse transcranial voluntary activation studies, most likely due to the TMS paired pulse paradigms (i.e. SICI, short interval intracortical facilitation, LICI etc.) described by DiLazzaro et al. (2004). Due to the paradigms a second stimulus of the same exact strength, or repetitive for that mater, can not excite the same number of intracortical axons as the first stimulus did, since any discharges, natural or caused by a conditioning stimulus, will change the excitability of the axons (Pierrot-Deseilligny and Burke, 2012, p 38).

3.2.3 Stimulation Coil

According to Rotenberg et al. (2014, p 80) several physical properties of the coil available for magnetic stimulators can influence neuronal activation. The geometry of the coil determines the focality and pattern of induced current in the tissue. Several different types of coils exist making for the possibility to select an optimal coil for the specific stimulation task. There is however the possibility that similar coil shapes of different manufacturers may produce slightly different results (Rotenberg et al., 2014, p 80).

The orientation of the coil during transcranial stimulation plays a significant role in the tissue activation mechanisms. The orientation of the coil is described by either the current in the coil or by the induced current in the tissue (Rotenberg et al., 2014, p 80). According to Wagner et al. (2004), Pell et al. (2010), Thielscher et al. (2011) and Fox et al. (2004) the direction of the induced current can significantly influence neuronal activation mechanisms, for example the type of neurons recruited or the site of the neuronal depolarization. The effect of coil orientation on the outcomes of transcranial magnetic stimulation has primarily been studied for the motor cortex

Rotenberg et al. (2014, p 81). Along with the orientation, the coil-to-cortex distance also significantly influences the induced current in the tissue as the induced magnetic field decreases exponentially with distance from its origin Rotenberg et al. (2014, p 83).

3.2.4 Antagonist Activation

As with electrical stimulation the possibility of inadvertent antagonist co-activation may influence the validity of voluntary activation measurements (Lee et al., 2008). According to Pierrot-Deseilligny and Burke (2012, p 38) for cortical stimulation the site of stimulation is not focal, and corticospinal cells thus often project to multiple muscles. Due to this the transcranial magnetic stimulation may activate motoneurons that innervate antagonist muscles. The activation of antagonistic muscles could reduce the superimposed twitch force therefore over-estimating the voluntary activation (Lee et al., 2008). In their study Lee et al. (2008) showed that the inadvertent activation of the antagonists by transcranial magnetic stimulation has little effect on the assessment of voluntary activation.

3.3 Common Issues

3.3.1 Familiarisation of Subjects

According to Shield and Zhou (2004) unfamiliarised subjects are typically unable to perform consistent isometric MVCs that display a marked plateau in force. This in turn produces artificially low and highly variable levels of activation. The view of Shield and Zhou (2004) according to unpublished observations is that some subjects unfamiliar with the protocol perform weaker contractions when expecting a stimulus. The reason could presumably be because they are intimidated by the prospect of receiving the stimuli. Shield and Zhou (2004) propose that subjects should be allowed to perform practice MVCs without stimulation during the familiarization and these results should be used during testing to ensure adequate force production under stimulation conditions.

3.3.2 Verbal Encouragement and Feedback

Andreacci et al. (2002) and McNair et al. (1996) have demonstrated that loud verbal encouragement has a significant positive impact on exercise performance. During measurements of maximal voluntary activation the positive impact of verbal encouragement on MVCs has been shown to have particular relevance (McNair et al., 1996). According to Shield and Zhou (2004) it is also important that the subjects are provided with some objective feedback relating to their performance. Shield and Zhou (2004) suggest the possibility to for example use monitors.

4 Effects of Menopause and Aging on Voluntary Activation

The human motor cortex goes through several morphological changes with aging. The MRI studies of Salat et al. (2004) revealed that areas near the primary motor cortex demonstrate prominent atrophy and that cortical thinning seems to occur by middle age. In addition to this the cross-sectional results Marner et al. (2003) demonstrated an approximately 45% loss in the total length of the myelinated fibers in the brain white matter due to aging. The cross-sectional study of Madden et al. (2004) indicates that aging could disrupt white matter integrity. These findings could therefore support the idea that aging effects the functional cortico-cortical and corticospinal connectivity (Clark and Taylor, 2011). Along with the morphological changes the brain also undergoes age related neurochemical changes. Of most interest have been the neurochemical changes within the basal ganglia due to the idea that the changes in the neurotransmitters and their receptors within this area may be connected to decreased cognitive and motor functions (Clark and Taylor, 2011). For example the findings of Mora and DelArco (2008) report age-related changes in the interactions between glutamate, dopamine, and GABA in the nucleus accumbens. This part plays a role in the motor behavior in addition to emotion and motivation and it has been hypothesized to play a role in the reduction of voluntary physical activity with aging (Mora and DelArco, 2008). According to Miwa et al. (1995) and Shaffer and Harrison (2007), in addition to the aging related modifications in the motor areas of the cortex, which may lead to decreased descending drive, the sensory feedback from muscle is reduced due to a reduction in muscle spindle sensitivity. This most likely reduces their excitatory drive to the motor neurons during contraction (Miwa et al., 1995; Shaffer and Harrison, 2007). Studies by Cruz-Sánchez et al. (1998) and Tomlinson and Irving (1977) have indicated a reduction in the motor neuron population, especially the larger neurons in the ventral horn. This loss should reduce the excitatory drive needed in motor neuron recruitment (Clark and Taylor, 2011).

In conjunction with the neurological modifications, aging also induces changes in the neuromuscular system. For example muscle fiber properties tend to shift toward a greater portion of slow twitch fibers (Roos et al., 1997; Brunner et al., 2007). In

addition, for example Frontera et al. (1991) and Lexell (1993) have shown a link between aging and sarcopenia, while Narici and Maganaris (2006) and Narici et al. (2008) have illustrated decreased tendon stiffness with aging.

Theoretically all the above mention modifications could reduce maximal voluntary force production, maximal muscle power and muscle RFD. As a whole, they suggests a general age-related reduction in motor performance. For example the reduced mechanical muscle function due to the loss of muscle mass was shown by Janssen et al. (2002) to be directly related to functional impairment and disability. The aging caused reduction in the capacity to produce force rapidly was on the other hand shown to be related to a decreased neuromuscular response capacity in postural control (Izquierdo et al., 1999a). Muscle strength in the lower extremity has also been associated with measures of balance and gait for the elderly, with lower strength correlating with a higher probability of falls. The study by Wolfson et al. (1995) indicated a strong relationship between lower extremity strength and measures of balance and gait for the elderly. The subjects in the study with a history of falls had significantly less muscle strength at the knee and ankle compared to those without a history of falls. The difference in strength was most prominent for the dorsiflexors. Whipple et al. (1987) showed that the peak torque and power in isokinetic tasks for both the knee and ankle extensors and flexors were significantly less for nursing home residents with a history of falls as compared to their age-matched controls. Here again the dorsiflexors indicated the most pronounced differences. Whipple et al. (1987) also suggest that at functional limb velocities ankle weakness and especially dorsiflexor weakness seems to be a major cause in poor balance. The study by Vandervoort and McComas (1986) concluded that the aging caused loss of strength was, on a relative scale, very similar between plantarflexors and dorsiflexors, while the absolute loss of strength was greater for the plantarflexor muscles.

The aging related atrophy of muscle fibers, especially fast (type II) muscle fibers, has been linked to decreased twitch potentiation. Both Petrella et al. (1989); Vandervoort and McComas (1986) have reported reduced twitch potentiation in the elderly compared to the young. According to Hamada et al. (2000b) the human muscles which exhibit greater PAP, also display shorter twitch contraction times as well as a higher percentage of type II muscle fibers. The reduced PAP capacity in the elderly can therefore, be seen as an indicator of the overall slowing

of the muscle contractile properties. The potentiation attenuation with aging does however, according to Baudry et al. (2005), only moderately influence the decrement of muscular performance.

Extensive studies into the effects of strength training in older people have concluded that systematic strength training can lead to considerable improvements of strength (Komi, 2002, p 414). The interpolated twitch technique, regardless of disagreeing results, is a common method in the investigation of the effect of aging on the ability to fully activate muscle (DeSerres and Enoka, 1998; Roos et al., 1999; Stackhouse et al., 2000, 2001; Stevens et al., 2003). According to Jakobi and Rice (2002) the conflicting results may for example be due to differences in the studied muscle group or differences in study methodologies.

According to some research there is significantly lower voluntary activation in the elderly when compared to young adults during a maximal voluntary contraction (Kent-Braun and LeBlanc, 1996; Stackhouse et al., 2000, 2001; Stevens et al., 2003). Contradicting results have been obtained by Klein et al. (2001b) and Roos et al. (1999). These authors found no significant difference in the ability to activate muscle. They also attributed the weaker muscle contraction of the elderly to greater co-activation of antagonists and reduced specific tension.

Some studies have also suggested that, because of the curvilinear relationship between the produced force and the activation level, small variations in the activation can exhibit large differences in the generated force at near maximal efforts. This could also explain the small voluntary activation deficits reported for the elderly. (Stackhouse et al., 2001; Stevens et al., 2003.)

In addition to the age related loss of muscle mass and strength, **menopause** further enhances these processes. McEwen and Alves (1999) suggested, that due to the pleiotropic nature of the estrogen hormone, it may affect muscle function both through the central nervous system or directly through the muscle tissue. Human skeletal muscle can be said to be a target tissue for estrogen signaling, as estrogen receptors are expresses in muscle cells of both males and females (Lemoine et al., 2003; Wiik et al., 2009). A study performed on ovariectomized rats suggests that estrogen has a preserving function for type II muscle fibers (Kadi et al., 2002). According to the review by Enns and Tiidus (2010), studies involving humans have

not been able to demonstrate estrogen to affect twitch characteristics, tetanic force development or strength. Some animal studies have shown estrogen to affect the twitch characteristics, i.e. peak value and half-relaxation time, and muscle fatigue (Hatae, 2001; McCormick et al., 2004; Schneider et al., 2004).

It has also been suggested by Moran et al. (2007) that estrogen may influence the contractile properties of muscle by directly binding to myosin. According to Kadi et al. (2002) estrogen may modulate force development by affecting specific contractile proteins. In their study on rodents they found that in both fast- and slow-twitch muscles estrogen administration altered the expression patterns of myosin heavy chain proteins. The study by Suzuki and Yamamuro (1985) on the other hand reported that the isometric twitch tension of the extensor digitorum longus muscle was lower in estrogen supplemented and ovary-intact rats when compared to ovariectomized rats. While the extensor digitorum longus muscle primarily consist fast-twitch fibres Suzuki and Yamamuro (1985) also found that estrogen had no effect on isometric twitch tension in the soleus, primarily consisting of slow-twitch fibres. A study by McCormick et al. (2004) however did not find changes in the myosin heavy chain composition with estrogen replacement.

The study by Hatae (2001) indicated an immediate increase in twitch force from estradiol administration in frog skeletal muscle fibers. The study attributed twitch potentiation to unbalanced Ca^{+2} turnover in the cytoplasm. This was reasoned to result from the rate of Ca^{+2} release from the sarcoplasmic reticulum being slightly faster than Ca^{+2} reuptake. On the other hand, according to the study by Wattanapermpool and Reiser (1999) on estrogen-deficient ovariectomized rats, the Ca^{+2} sensitivity may remain the same, while the isometric force is reduced in the soleus fibers.

Cohen et al. (2006) reported that the stage of peri-menopause is linked to declining levels of estrogen, while the stage of post-menopause is associated with low concentration levels of estrogen. By altering either the responsiveness of postsynaptic receptors (Yankova et al., 2001; Maejima et al., 2013) or the presynaptic release of neurotransmitters (Yokomaku et al., 2003), these ovarian hormones have a modulatory effect on synaptic transmission. Estrogen thus alters the neural excitability, by affecting the neurochemical GABA signaling system.

5 Purpose of the Study

At the time of writing, numerous studies demonstrating the role of aging on the changes in the central and peripheral nervous system as well as the neuromuscular system exist (Kamen et al., 1995; Lexell, 1995, 1997; Roos et al., 1997). These changes are accompanied by a decline in motor performance. For example Janssen et al. (2002) has shown that the reduced mechanical muscle function due to the loss of muscle mass can be directly related to functional impairment and disability, especially in older women. The decline in the ability to produce explosive force has also been related to a decreased neuromuscular response capacity in postural control (Izquierdo et al., 1999a). For the elderly Wolfson et al. (1995) showed a clear relationship between lower extremity strength and measures of balance and gait. The same study also indicated, that the most pronounced difference in muscle strength, between subjects who had experienced falls and those who had not, was found in the dorsiflexor muscles (Wolfson et al., 1995).

In addition to the age related declines in muscle mass and function there is, according to Maltais et al. (2009) a great deal of evidence supporting the hypothesis that the increased loss of muscle mass in postmenopausal women is related to the declining levels of estrogen. According to Cohen et al. (2006), perimenopause has also been linked to declining levels of estrogen. For post and peri-menopausal women this would seem to indicate an increased exposure to the degenerative changes in the neuromuscular system and to decreases in motor performance.

As far as the author is aware, no previous studies, examining the role of estrogen in the development of muscle weakness in women nearing postmenopause, exist. The purpose of this study was to shed light into understanding of the effects of the timing mechanisms of estrogen deprivation in the development of muscle weakness in perimenopausal women. More specifically the aim of this thesis was to study the effects of the stage of menopause on the ability to activate the muscles of the lower extremity during isometric plantar- and dorsiflexion tasks.

6 Methods

6.1 Overview and Subject Selection Criteria

This study was part of a larger ongoing study, "ERMA - Estrogen Regulation of Muscle Apoptosis", conducted at the Department of Health Sciences at the University of Jyväskylä and the Gerontology Research Center (GEREC). The invitation to the ERMA study and the pre-questionare was sent to a random sample of 65% of the whole age cohort representing 48-54 year old women living in the area of Jyväskylä (N=5950). The expected exclusion due to not responding or unwillingness to participate was around 50% and due to not fulfilling the inclusion criteria about 40%. The participants of the study (N $\approx$ 1160) were invited to the laboratory for serum sampling to determine their systemic hormone status and to assign them into groups (early peri-, late peri- and postmenopausal) following STRAW +10 guidelines, which takes into account the systemic hormone status and self-reported menstrual cycle (Harlow et al., 2012). The hormone assessments were measured from fasting serum samples taken between 8:00 and 10:00 AM, and in women with menstrual cycle, during cycle days 1-5. The inclusion into a group requires the fulfillment of two of the three criteria; follicle-stimulating hormone (FSH), estradiol (E2) and self-reported menstrual cycle, see figure 6. The limits for the criteria for each group is also depicted in figure 6. In addition all participants went through a basic health and physiological assessment. The overall health, including clinical examination, blood sample, and questionnaires was assessed during a medical examination, which was performed by a nurse practitioner and a physician if needed. The presence of chronic conditions and the use of prescription medication was confirmed using a pre-structured questionnaire and information from current prescriptions. Contraindications were evaluated by the nurse practitioner and/or a physician according to American College of Sports Medicine (ACSM) guidelines (Haskell et al., 2007) and acute conditions such as infections were ruled out by measuring blood count in order to ascertain safe participation in the physiological measurements. To assess health and gynecological status and history, depression and mood, physical activity and daily energy intake validated questionnaires were used (Ronkainen et al., 2009).

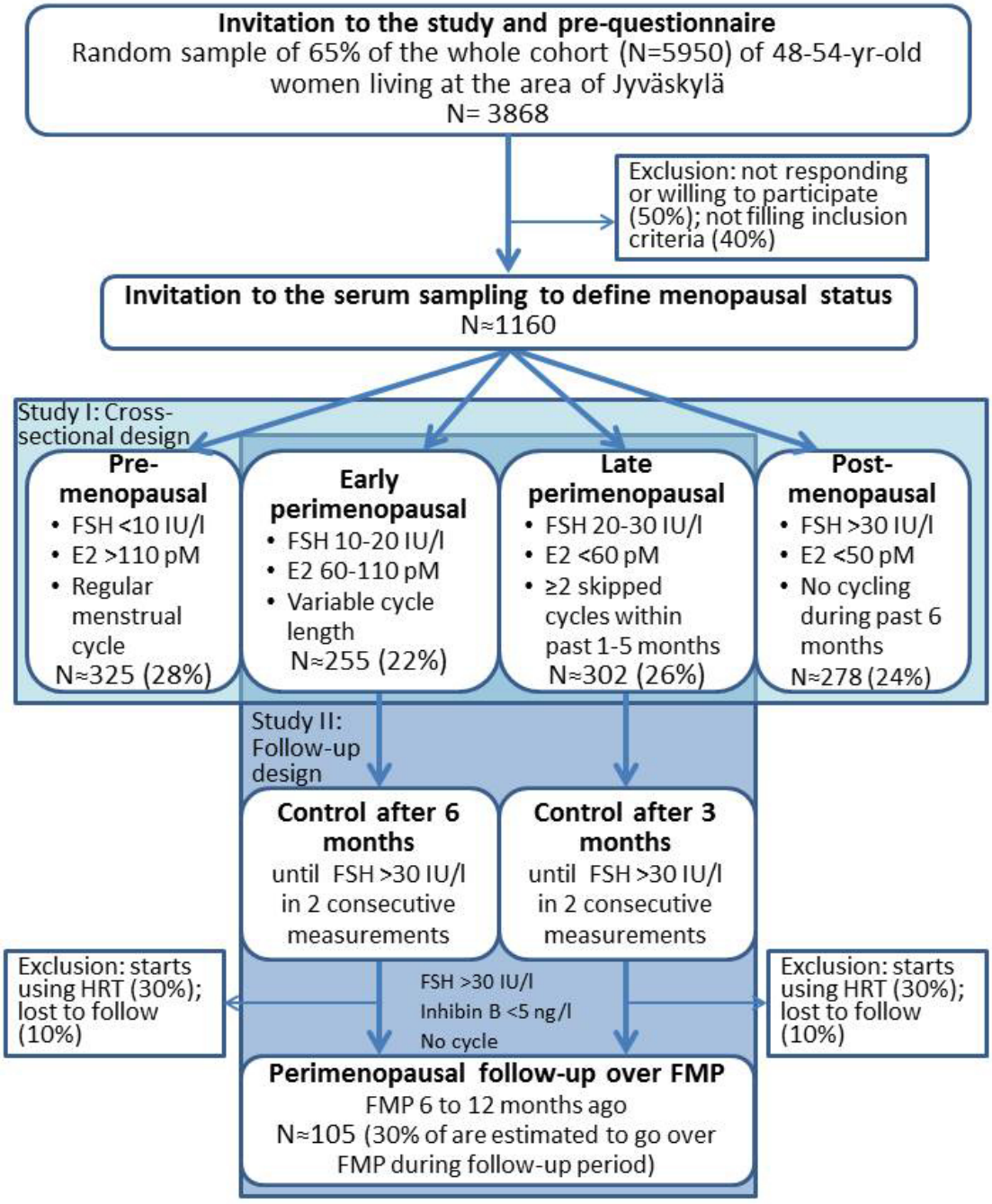

FIGURE 6. ERMA measurement protocol.

The exclusion criteria for the entire ERMA study are: body mass index $> 34{,}9$, currently pregnant or lactating, polycystic ovary syndrome or other conditions affecting ovarian function, current or within past 3 months use of intrauterine contraceptives, external hormone preparations or other medications affecting ovarian function, and chronic diseases or medications affecting muscle function (e.g. diabetes type I and II; type II diabetes with insulin treatment; rheumatic diseases with heavy

medication; asthma with regular medication; cancer during the last 5 years with treatments; severe musculoskeletal disorders affecting everyday physical activity).

The serum sampling and health and physiological assessments enable a cross-sectional comparisons between the groups (study I in figure 6). For study II, of a longitudinal design, early and late perimenopausal women will be followed beyond final menstrual period (FMP), see figure 6.

The entire ERMA study follows the guidelines of good clinical and scientific practice. The approval for the study was given by the Ethics Committee of the Central Finland Health Care District. Informed consent, explaining the possible risks and personal benefits associated with the examinations and a permission to use the data for research purposes and in publications was signed by the subjects at the laboratory before the measurements.

The subjects for our study were the participants in the early peri- (Group 2) and late perimenopausal (Group 3) groups (see figure 6). For the analysis of measurement data 10 subjects from the early peri- (51,9 $\pm$ 2,2 years) and 10 subjects from the late perimenopausal (53,0 $\pm$ 2,0 years) group were chosen on a semi pseudorandom basis using a selection algorithm implemented in Matlab. The semi randomness is due to the fact that two subjects (early perimenopause group) were purposefully chosen for analysis as we were also able to measure these subjects after they had transitioned into the postmenopausal phase.

Our study design was a group-wise comparison to examine the neuromuscular function of the calf and shin muscles (tibialis anterior and medial gastrocnemius) during maximal and submaximal voluntary contractions. By analyzing the two subjects who transitioned into the postmenopausal phase we are also able to do a preliminary pre-test post-test reference for future studies within this project.

6.2 Procedure

The preparation of the subject began by identifying and marking the center of the top of the skull at the intersection of half the distance from nasion to inion with half the scalp distance. The optimal stimulus site for both the plantar- and dorsiflexors can usually be located just lateral to this mark. Next the stimulation anode, a

5×9 cm, rectangular, self-adhering reusable electrode coated with conductive gel, (Axelgaard, Fallbrook, California) was positioned proximal to the superior border of the patella.

All measurements were performed on the right legs of the subjects. Two single use skin surface electrodes (Ambu® BlueSensor N) were placed next to each other on the medial gastrocnemius (MG) muscle, with a 20 mm interelectrode distance to provide a bipolar electromyographic (EMG) setup. The same bipolar setup was also placed on the tibialis anterior (TA) muscle. The electrodes were placed on the belly of the muscle according to surface EMG for non-invasive assessment of muscles (SENIAM) recommendations (Hermens et al., 1999). A single electrode (Ambu® BlueSensor N) was placed on the right vastus lateralis to serve as a ground for the other electrodes. Before the placement of the electrodes the skin under the electrodes was shaved, abraded, and cleaned with alcohol. This was done to reduce the skin resistance.

In order to electrically stimulate the plantarflexor muscles, the optimal stimulation point, on the skin overlying the tibial nerve in the popliteal fossa, was located by moving a stimulating probe around the popliteal fossa while the subject was lying in a prone position. The optimal stimulation point was defined as the location from where the peak-to-peak amplitude of the M-wave and the shape of the M-wave for the given intensity were most repeatable. Nerve stimulation is preferred over percutaneous skin surface muscle stimulation as this may not activate all the muscle fibers (Hultman et al., 1983). A stimulation cathode (Ambu® WhiteSensor 4500M, 79 mm^2) for electrical tibial nerve stimulation was attached to the optimal stimulation location.

6.2.1 Electrical Stimulation

A constant-current stimulator (Model DS7AH; Digimeter, Hertfordshire, UK) was used to deliver double pulses of 1 ms duration, with an inter-pulse-interval of 10 ms, to the tibial nerve to evoke both a superimposed twitch during MVC and a twitch to a relaxed muscle. Supramaximal intensity (150% maximum M-wave) was used for both superimposed and twitches to a relaxed muscle.

6.2.2 Transcranial Magnetic Stimulation

TMS was performed using a Double 70 mm Alpha coil (figure-of-eight coil) attached to a Magstim Rapid^2 stimulator (Magstim, Whitland, UK). Single pulse stimulation was applied over the motor cortex of the left hemisphere to preferentially activate the tibialis anterior muscle. The stimulation intensity was gradually increased until a stimulation intensity eliciting 3 out of 3 MEPs with amplitude > 50 μV was achieved in the relaxed muscle. This stimulation intensity was used for all stimulations. The coil position was adjusted along with the increased stimulation intensity to determine optimal stimulation position. During the measurement stimulations the coil position and orientation were held manually at the optimal stimulation position. In order to obtain four clearly analyzable MEP responses generally around 6 to 10 stimulations during one force production cycle were administered.

6.3 Plantarflexion Torque Measurement Protocol

Maximal voluntary isometric plantarflexion torque and supramaximal electrically induced twitch torque was measured using a custom made ankle dynamometer chair. The dynamometer has the capability to do both isometric and dynamic torque measurements (Avela et al., 2001). Using the two built in straps in the dynamometer the right foot of the subject was secured to the footplate of the measurement device at an ankle joint angle of 90^o. The subjects sat in the chair with the knee extended with their back and head leaning against the backrest of the chair. The backrest was adjusted to provide a firm support for both dorsi- and plantarflexion tasks. The firm support along with the fastened straps also ensured that the subjects calcaneus was not able to rise from the footplate during the tasks. The subjects' hands were instructed to be folded relaxed on their lap. Subjects were allowed to practiced the isometric plantarflexion task with feedback from a force signal provided on a television screen.

After the practice contractions, three trials of maximal voluntary contractions were performed with about 30 s rest intervals. Each trial was composed of a supramaximal stimulus to the relaxed muscle, followed by a maximal plantarflexion contraction, during which, at the time of leveled peak torque, a supramaximal superimposed

twitch was elicited. This was followed by the relaxation of the muscle and a post-contraction supramaximal stimulus to the relaxed muscle. During the contraction strong verbal encouragement as well as visual feedback of the torque level were given to the subject. The post-contraction stimulus was given within 2-5 s after the relaxation of the muscle in order to identify the postactivation potentiation effect as the postactivation potentiation phenomena is maximal immediately following the conditioning contraction where after it declines progressively (OLeary et al., 1997; Klein et al., 2001a). Figure 7 illustrates the the time course of one trial. The blue curve in figure 7 illustrates the produced plantarflexion force and the red $*$-symbols illustrates the stimulation incident.

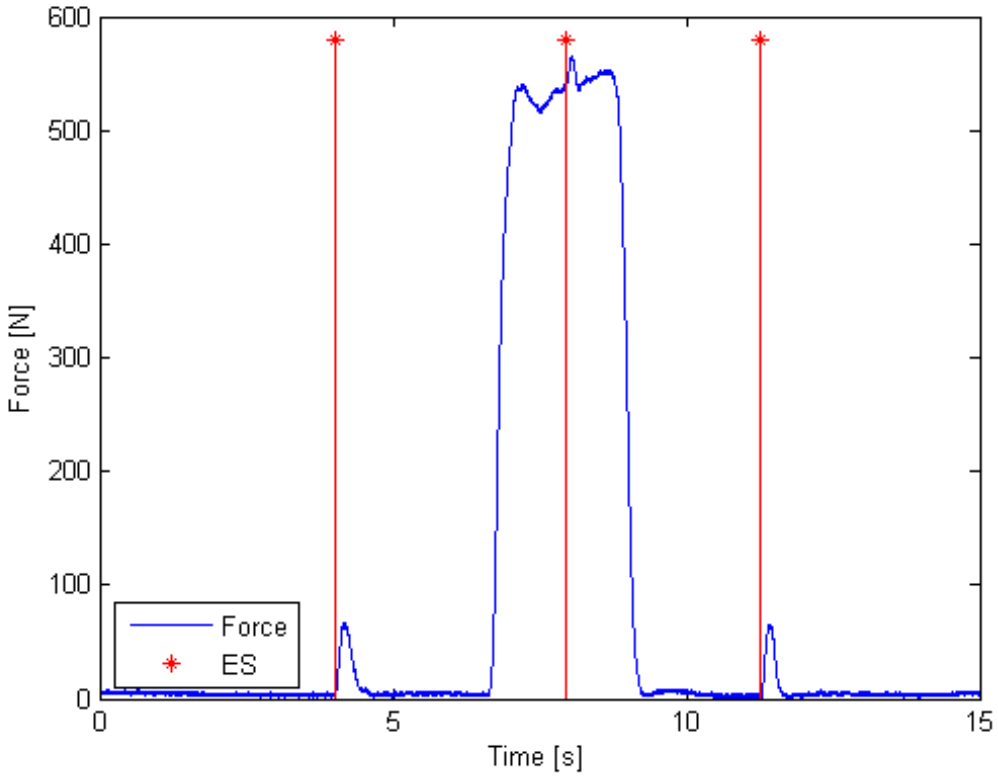

FIGURE 7. ES measurement protocol.

6.4 Dorsiflexion Torque Measurement Protocol

The maximal and submaximal voluntary isometric dorsiflexion torque was also measured using the previously described custom made ankle dynamometer chair. With the subject sitting in the chair as described in 6.3 the subjects were allowed to

practiced the isometric dorsiflexion task with feedback from a force signal provided on a television screen.

After the practice contractions, two trials of maximal voluntary dorsiflexion contractions were performed with about a 20 s rest interval in between. The maximal force production value of the dorsiflexion was used to calculate the force production levels for the actual task. The target force production levels for the actual task were set at 20%, 40%, and 60% of the maximum value. For each target force value the subject was asked to continuously produce a constant force matching one of the target values. To aid in the force production subjects received feedback from a force signal provided on a television screen. During each force production session subjects were administered a minimum of six TMS pulses with a interpulse interval of around 8-10 s as for example Vaseghi et al. (2015) has shown that longer interpulse interval yields larger MEPs. The minimum of six pulses were administered in order to obtain a minimum of four clear responses for analysis. Figure 8 illustrates the procedure for one force level measurement. The blue curve in figure 8 illustrates the produced dorsiflexion force and the red $*$-symbols illustrates the stimulation incidents.

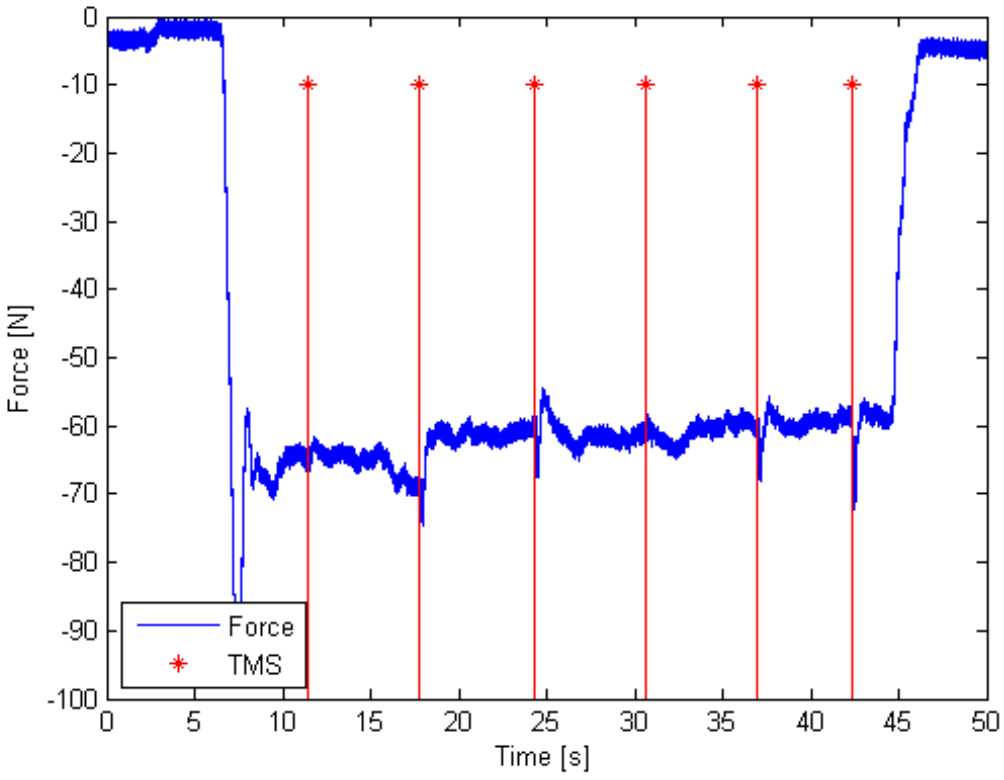

FIGURE 8. TMS measurement protocol.

6.5 Data Collection and Analysis

The data from the dynamometer was measured with a torque-transducer (Kistler, Switzerland). The transducer is mounted between the ankle ergometer servomotor and the platform for the foot (Avela et al., 2001). Surface EMG data was collected using a preamplifier isolator setup (NL824 preamplifier with NL820A isolator, Digitimer) providing a cutoff frequency of 10 Hz and a -3dB point greater than 10kHz with a gain of 1000.

EMG, torque, trigger signals for the electrical and magnetic stimulators and heel trigger signals were fed into a computer via an analog-to-digital (AD) converter (CED 1401; Cambridge Electronics Design, Cambridge, UK). The AD converter sampled the analog signal at a sampling frequency of 2 kHz. The digital signal was stored for offline analysis using Spike2 (version 6.17) software. During the AD conversion the measured torque data was also converted to an equivalent force value by dividing the measured torque value with the length of an approximation of the ankle moment arm. All offline analysis was performed with Matlab (The MathWorks Inc., Natick, MA). Before offline analysis of the EMG signal the raw EMG signal was digitally processed by removing the DC-offset and by zero-phase digitally filtering the raw EMG signal in both the forward and reverse directions with a 5^{th} order Butterworth filter with the low cutoff frequency at 20 Hz and the high cutoff frequency at 500 Hz.

6.5.1 ES Data Analysis

For the electrical stimulation tasks peak forces for the voluntary maximal plantarflexion contractions (maximum value just prior to the superimposed twitch) and the pre- and post-contraction and superiposed twitches were analyzed. To analyze the possible changes in muscle activation the level of voluntary activation for all the three performed maximal contractions was calculated using the equation by Allen et al. (1995):

$$VA(\%) = 100 \times (1 - T_{interpolated}/T_{control}), \tag{2}$$

where $T_{interpolated}$ is the size of the interpolated twitch and $T_{control}$ is the size of a control twitch produced by identical nerve stimulation in the relaxed muscle.

The PAP effect of the voluntary contraction was analyzed by comparing the peak force values of the pre- and post-contraction twitches of the first trial with each other. In addition, the areas of the rectified pre- and post-contraction twitch M-waves were compared with each other. The PAP was calculated as the percentage difference between the post and pre twitches. A PAP measure was also obtained from the areas of the rectified twitch M-waves, comparing the percentage difference between the rectified areas of the post- and pre-twitch M-waves. The pre- and post-contraction twitches were also analyzed for rise times to peak force and half-relaxation times. The twitch rise time was defined as the time from the onset of the twitch to the peak force, while the twitch half relaxation time was measured as the time interval between peak force and the point where the force had decreased to half of the peak value. The average and peak (10 ms sliding window) RFD and rate of force relaxation (RFR) were also analyzed, along with the S-gradient, maximum force and the rectified area of the M-waves for the pre- and post-contraction twitches. The maximum twitch force was defined as the measure of peak force achieved by the twitch. The twitch rise time was measured from the onset of twitch to the point of maximum twitch force and the twitch S-gradient was measured from the onset of the twitch to half the maximum twitch force. The voluntary contractions were also analyzed for rise times to peak force, average and peak (10 ms sliding window) rate of force production, S-gradient, maximum force and the M-wave area during stimulation. The maximum voluntary force was defined as the measure of peak force before the onset of the superimposed twitch for each voluntary contraction. The voluntary rise time was measured from the onset of force production to the point of maximum voluntary force. The voluntary S-gradient was measured from the onset of force production to half the maximum voluntary force.

The EMG signal following the superimposed twitch was also analyzed for PSP duration. The beginning and end of the PSP were analyzed by an semi-automated detection routine as well as manually by visual inspection. The semi-automatic routine for analyzing the PSP was based on logical indexing of the rectified first derivative of each epoch to indicate when the processed signal was below a threshold value, calculated from the pre-stimulation raw rectified EMG signal. The PSP was considered as the longest logical index of the processed signal indicating a value below threshold. Comparing the raw graphical visualizations of the results with those from the semi-automated method, it was noticed that allowing for a 7 ms disruption in the

continuous logical indexing, where the processed signal exceeds the threshold value, resulted in similar analyzed result values for both methods. The threshold was first set at a fixed value (0,5), which was calculated using a 100 ms window starting at 150 ms before the electrical stimulation (ES), but for some measurements the threshold value had to be manually adjusted. This semi-automated routine was implemented in Matlab. Figure 9 illustrates the key points of the analysis. The red plot depicts the measured EMG signal and the blue plot illustrates the rectified derivative of the EMG signal. The magenta $*$-symbol illustrates the ES time point, the black $*$-symbol depicts the beginning of the PSP and the green $*$-symbol illustrates the end of the PSP.

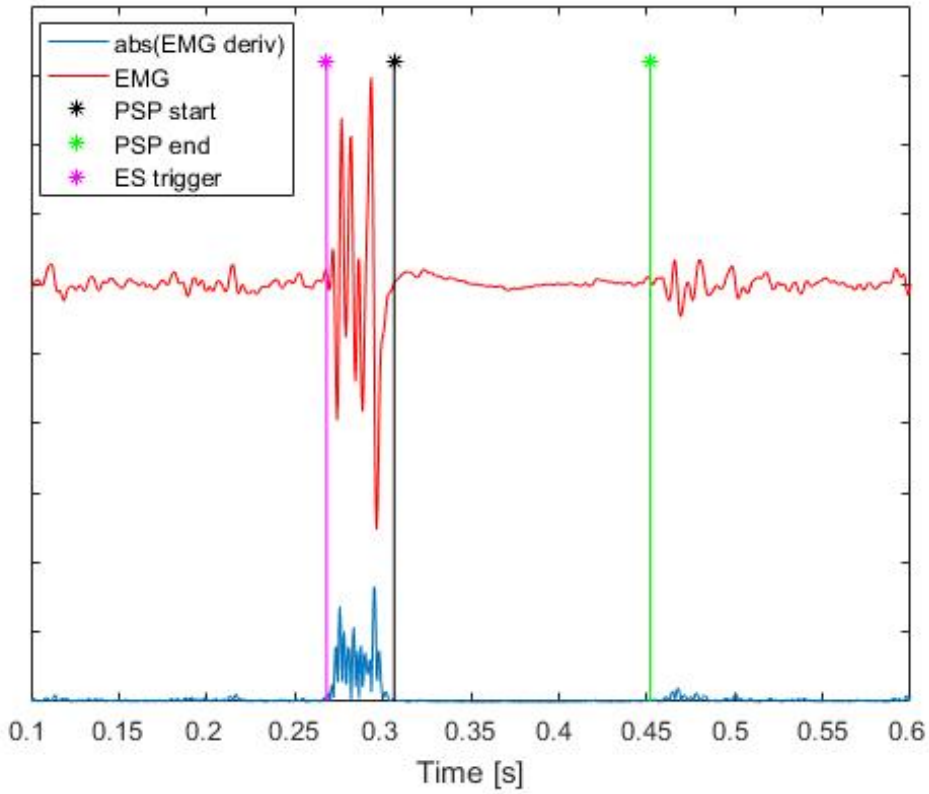

FIGURE 9. ES analysis protocol.

6.5.2 TMS Data Analysis

For each subject, four measurements with clearly distinguishable MEP response and CSP from each force production level were chosen for analysis on a visual basis. From the TMS measurements we analyzed the CSP and the areas of the MEPs.

The continuous EMG was sampled to 700 ms epochs, starting at 150 ms before and continuing to 550 ms beyond the incident of the TMS. The beginning and end of the CSP were analyzed by an semi-automated detection routine as well as manually by visual inspection. The semi-automatic routine for analyzing CSP was based on logical indexing of the rectified first derivative of each epoch to indicate when the processed signal was below a threshold value calculated from the pre-TMS processed signal. The CSP was considered as the longest logical index of the processed signal indicating a below threshold value. According to Groppa et al. (2012), EMG activity exceeding the pre-TMS baseline level for at least 50 ms, marks the end of the CSP. We however noticed that a 30 ms disruption in the continuous logical indexing, where the processed signal exceeds the threshold value, gave similar analyzed result values as visual analysis of the raw graphical data. The semi-automated routine was implemented in Matlab. The routine is somewhat similar to Garvey et al. (2001) and Julkunen et al. (2013). The threshold was set at a fixed value (0,5) of the pre-TMS processed signal, which was calculated using a 100 ms window starting at 150 ms before the TMS.

The beginning of each MEP was also calculated from the rectified first derivative of each epoch using a threshold based edge detector algorithm implemented in Matlab. To verify the results a visual inspection of the results was performed. The MEP response latency was analyzed as the time period between the stimulus trigger and the beginning of the MEP response. The end of the MEP was defined as the starting point of the CSP. Figure 10 illustrates the key points of the analysis. The red plot depicts the measured EMG signal and the blue plot illustrates the rectified derivative of the EMG signal. The cayan $*$-symbol illustrates the TMS time point, the green $*$-symbol illustrates the beginning of the MEP response, the black $*$-symbol depicts the end of the MEP response and the beginning of the CSP and the magenta $*$-symbol illustrates the end of the CSP. The area of the MEP was calculated as the integral of the rectified raw EMG signal from the start of the MEP to the end of the MEP.

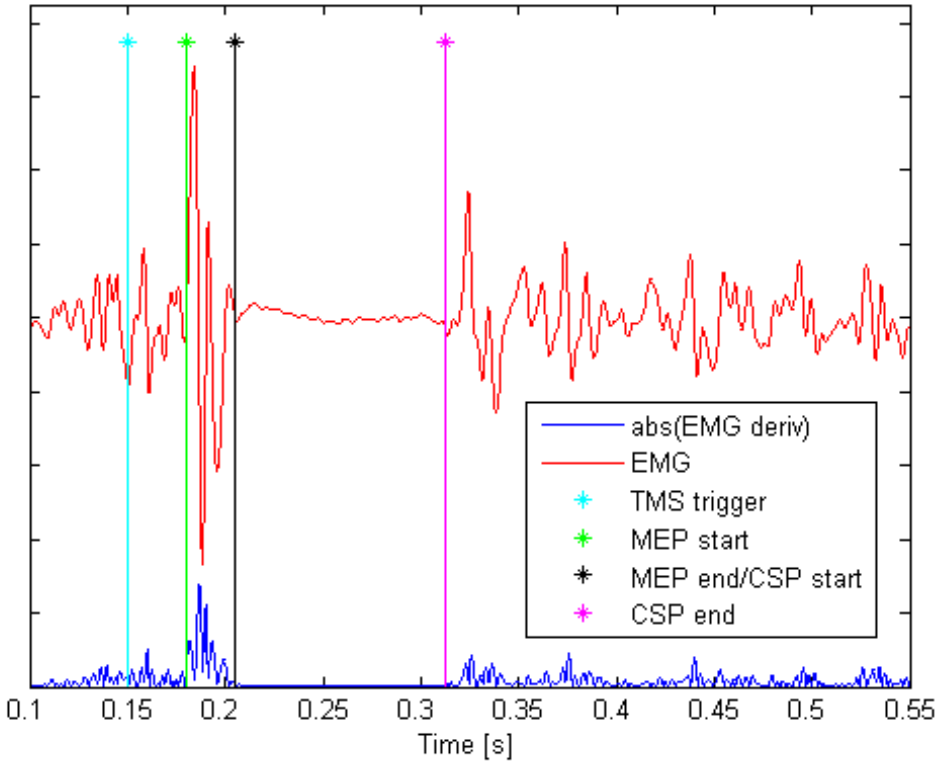

FIGURE 10. TMS analysis protocol.

According to Orth and Rothwell (2004) both MEP amplitude and area have been shown to be satisfactory predictors of CSP. In this thesis we decided to analyze the MEP area. The peak force (maximal value of the two maximal voluntary dorsiflexion contractions) and the force level before each TMS (mean of a 850 ms window prior to TMS) was also analyzed.

6.5.3 Statistical Analysis

A statistical analysis of the measurement data was performed. Normal distribution of the data was checked using the Shapiro-Wilk test. This is, according to Razali and Wah (2011), the most powerful test for checking normality. When the Shapiro-Wilk test could not be performed the normality check was conducted using the Kolmogorov-Smirnov test. The Shapiro-Wilk test was performed with the MuPAD extension to Matlab, while the Kolmogorov-Smirnov test was performed in Matlab. For normally distributed data the group-wise differences were analyzed using an unpaired t-test, while all non-normal data was analyzed using the Wilcoxon

signed-rank test. For subject wise intrapair testing a paired-sample t-test was used for normally distributed data. The Wilcoxon signed-rank test was used to calculate intrapair differences for non-normally distributed data. In cases where the comparable data sets indicated different distributions a Box-Cox transformation (Matlab) of the data was performed in order for the data to have an approximately normal distribution. The Pearson correlation coefficient (Matlab) was used to investigate associations between variables. All analysis were carried out with the significance level set at $p < 0.05$, and all measurement data are reported as mean $\pm$ standard deviation (SD), unless stated otherwise.

7 Results

7.1 Subjects Initial Status

The early and late perimenopause E2 and FSH measurement results used to group the subjects are illustrated in figure 11. The E2 level of the early perimenopause group (275 ± 210 pmol/l) did not significantly (p = 0,597) differ from the E2 level of the late perimenopause group (304 ± 180 pmol/l). The age distribution between the early perimenopause (51,9 ± 2,2 years) and late perimenopausal (53,0 ± 2,0 years) did not significantly (p = 0,255) differ either. On the other hand the FSH serum values for the two groups (early perimenopause 16,2 ± 4,2 UI/l and late perimenopause 45,4 ± 21,6 UI/l) did differ significantly (p < 0,001).

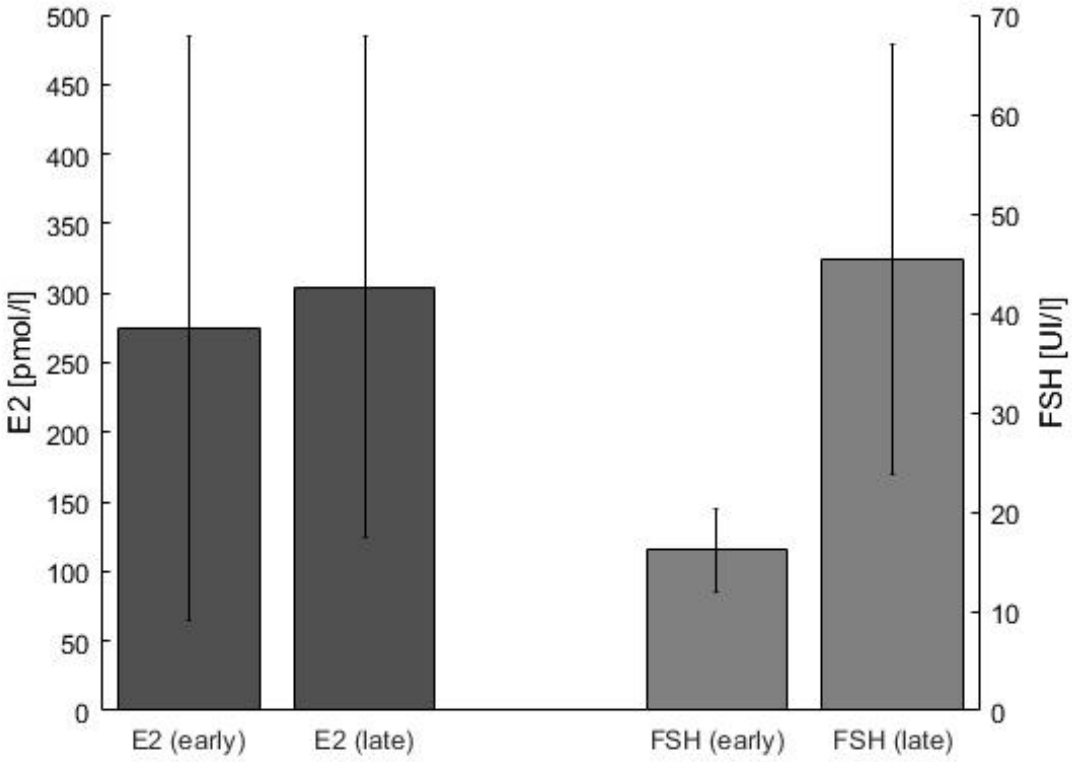

FIGURE 11. Early and late perimenopause group serum E2 and FSH levels.

No statistical analysis could be performed for the single subject serum E2 and FSH values. From the raw measurement values it is clear that the values do shift markedly between peri- and postmenopaus measurements. For subject 7 the serum

E2 decreased from a perimenopausal value of 189 pmol/l to a postmenopausal value of 20,3 pmol/l, while for subject 8 the decrease was from a perimenopausal value of 852 pmol/l to a postmenopausal value of 9,4 pmol/l. The FSH values increased from peri- to postmenopaus. For subject 7 the serum FSH increased from 23,6 UI/l to 162,5 UI/l, while for subject 8 it increased from 63,5 UI/l to 146 UI/l. The time period between the peri- and postmenopause measurements was less than a year. The early peri- and postmenopause E2 and FSH measurement results for subjects 7 and 8 are illustrated in figure 12.

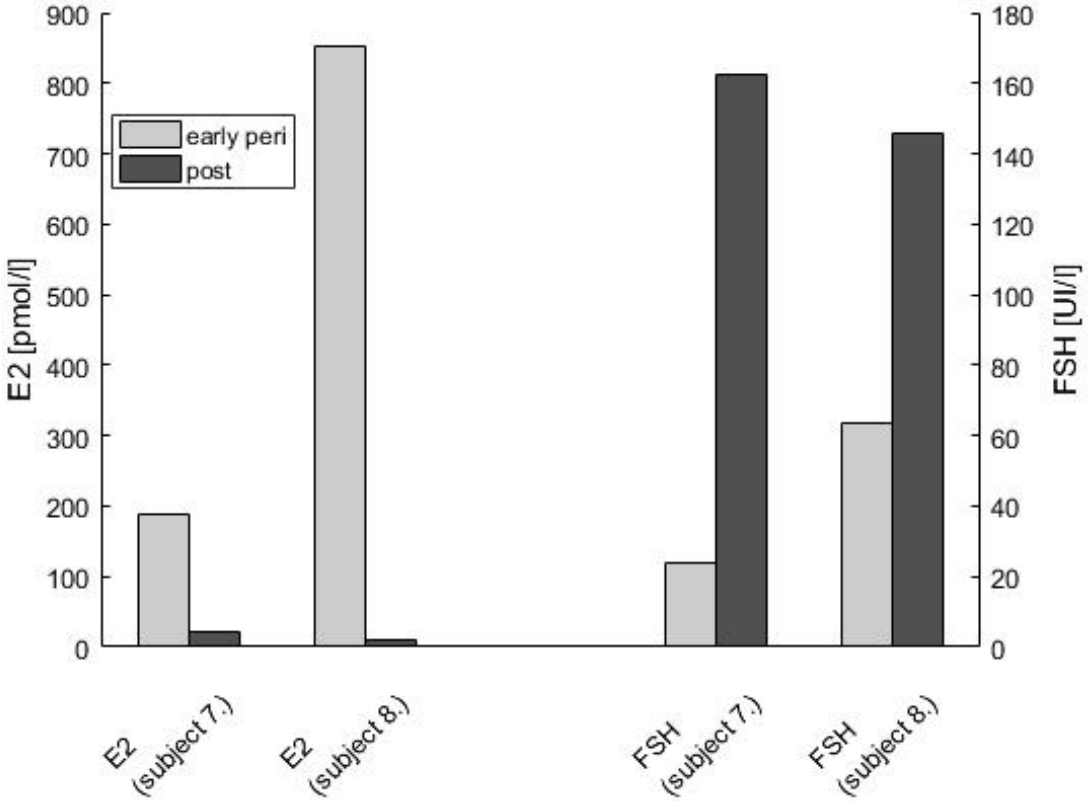

FIGURE 12. Early peri- and postmenopause serum E2 and FSH levels for subjects 7 and 8.

7.2 Measurement Parameter Correlations

The measurement variables, from the electrical stimulation measurement procedure, which indicate significant correlation between each other or with the initial status measurement results, for both the early and late perimenopause groups are listed in table 1. A closer look at the correlation coefficients in the table reveals that all other

correlations, except the correlation between the serum E2-level and the voluntary contraction average RFD (r = -0,38 for early and r = 0,57 for late), display quite similar correlation coefficients, both in sign and magnitude. As is also evident from the table, only the correlations between the serum E2 level and the peripheral silent period (r = -0,37 for early and r = -0,50 for late) and the peripheral silent period and the maximum voluntary force (r = -0,66 for early and r = -0,54 for late) indicate a negative correlation between the variables. All the rest of the correlations in table 1 indicate a positive correlation.

Both groups indicate similar significant correlation between measurement variables. In addition to these, there are also several measurement variables, which indicate significant correlation for only one of the two groups as can bee seen in table 2.

In addition to the correlations in tables 1 and 2, there were also several measurement variables, which were tested for, but did not illustrate correlation as can be seen from table 3. The group-wise variable measurement results can be found in tables 11 - 29 in appendix A.

The correlation results, from the TMS measurements, between measurement variables, as well as the measurement variable correlations with the initial status results for both the early and late perimenopause groups are listed in table 4. As is evident from the table, both the early and late perimenopausal groups indicate, quite similar significant correlation (both sign and magnitude) between the E2 serum level and the MEP latency (r = -0,23, p = 0,012 for early and r = -0,37, $p < 0,001$ for late), between the produced force and the MEP area (r = 0,26, p = 0,005 for early and r = 0,34, $p < 0,001$ for late), and between the MEP area and the CSP (r = 0,38, $p < 0,001$ for early and r = 0,34, $p < 0,001$ for late).

TABLE 1. Early and late perimenopause group measurement variables with significant correlation (* = significant correlation at $p < 0{,}05$).

	Early Perimenopause		Late Perimenopause	
Variables	**Correlation Coefficient**	**Significance Level**	**Correlation Coefficient**	**Significance Level**
E2 - Peripheral silent period	-0,37	0,047*	-0,50	0,005*
E2 - pre twitch rise time	0,43	0,017*	0,39	0,033*
E2 - Voluntary contraction average RFD	-0,38	0,037*	0,57	0,001*
Peripheral silent period - Maximum voluntary force	-0,66	< 0,001*	-0,54	0,002*
Maximum voluntary force - pre twitch max force	0,70	< 0,001*	0,67	< 0,001*
Maximum voluntary force - post twitch max force	0,72	< 0,001*	0,67	< 0,001*
Voluntary contraction S-gradient - pre twitch maximum force	0,74	< 0,001*	0,44	0,016*
Voluntary contraction S-gradient - post twitch maximum force	0,70	< 0,001*	0,42	0,020*
Voluntary contraction peak RFD - pre twitch maximum force	0,77	< 0,001*	0,49	0,006*
Voluntary contraction peak RFD - post twitch maximum force	0,77	< 0,001*	0,49	0,006*
Voluntary contraction avg RFD - pre twitch maximum force	0,74	< 0,001*	0,48	0,006*
Voluntary contraction avg RFD - post twitch maximum force	0,73	< 0,001*	0,46	0,010*
pre twitch average RFD - Voluntary contraction average RFD	0,50	0,005*	0,37	0,046*
post twitch average RFD - Voluntary contraction average RFD	0,41	0,025*	0,47	0,009*
pre twitch peak RFD - Voluntary contraction peak RFD	0,83	< 0,001*	0,49	0,006*
post twitch peak RFD - Voluntary contraction peak RFD	0,75	< 0,001*	0,54	0,002*

TABLE 2. Early and late perimenopause group measurement variable correlations (* = significant correlation at p < 0,05).

	Early Perimenopause		Late Perimenopause	
Variables	**Correlation Coefficient**	**Significance Level**	**Correlation Coefficient**	**Significance Level**
E2 - Maximum voluntary force	-0,27	0,154	0,43	0,017*
E2 - pre twitch half relaxation time	0,60	< 0,001*	-0,12	0,516
E2 - pre twitch average RFD	-0,47	0,008*	-0,07	0,704
E2 - pre twitch peak RFD	-0,38	0,038*	0,03	0,885
E2 - post twitch peak RFD	-0,46	0,011*	0,09	0,648
E2 - pre twitch average rate of force relaxation	0,49	0,006*	-0,25	0,180
E2 - post twitch average rate of force relaxation	0,42	0,021*	-0,31	0,094
E2 - pre twitch peak rate of force relaxation	0,54	0,002*	-0,18	0,353
E2 - post twitch peak rate of force relaxation	0,57	< 0,001*	-0,21	0,254
E2 - post twitch S-gradient	-0,38	0,041*	-0,09	0,639
E2 - Superimposed twitch M-wave area	0,62	< 0,001*	-0,01	0,976
E2 - pre twitch M-wave area	0,60	< 0,001*	0,02	0,934
E2 - post twitch M-wave area	0,66	< 0,001*	-0,04	0,825
E2 - Voluntary contraction S-gradient	-0,17	0,380	0,66	< 0,001*
E2 - Voluntary contraction peak RFD	-0,33	0,071	0,60	< 0,001*
Peripheral silent period - Voluntary contraction superimposed twitch M-wave area	-0,38	0,041*	-0,01	0,978
Peripheral silent period - Voluntary activation	-0,65	< 0,001*	-0,36	0,051

TABLE 3. Early and late perimenopause group measurement variable correlations (* = significant correlation at p < 0,05).

	Early Perimenopause		Late Perimenopause	
Variables	**Correlation Coefficient**	**Significance Level**	**Correlation Coefficient**	**Significance Level**
E2 - VA	0,08	0,678	0,34	0,064
E2 - post twitch rise time	0,23	0,227	0,18	0,339
E2 - post twitch half relaxation time	0,30	0,102	-0,22	0,244
E2 - post twitch average RFD	-0,34	0,066	0,09	0,645
E2 - pre twitch S-gradient	-0,24	0,197	-0,02	0,908
E2 - PAP	-0,22	0,545	0,18	0,625
E2 - PAP (M-wave area)	-0,22	0,550	-0,14	0,701
E2 - pre twitch maximum force	-0,22	0,249	0,05	0,779
E2 - post twitch maximum force	-0,28	0,141	0,09	0,633
E2 - Voluntary contraction rise time	0,32	0,087	-0,23	0,213
PAP - PAP (M-wave area)	-0,17	0,641	0,29	0,121
pre twitch maximum force - pre twitch M-wave area	-0,20	0,283	0,02	0,934
post twitch maximum force - post twitch M-wave area	-0,19	0,323	-0,04	0,825
Maximum voluntary force - Voluntary contraction superimposed twitch M-wave area	0,18	0,354	-0,12	0,524
Voluntary contraction S-gradient EMG integral - pre twitch maximum force	0,28	0,135	-0,22	0,232
Voluntary contraction S-gradient EMG integral - post twitch maximum force	0,33	0,075	-0,23	0,229
Voluntary contraction S-gradient EMG integral - Voluntary contraction S-gradient	-0,07	0,727	-0,29	0,117

TABLE 4. Early and late perimenopause group variable correlations (* = significant correlation at p < 0,05).

	Early Perimenopause		Late Perimenopause	
Variables	**Correlation Coefficient**	**Significance Level**	**Correlation Coefficient**	**Significance Level**
E2 - Maximum dorsiflexion force	-0,55	0,098	0,23	0,515
E2 - Cortical SP duration	-0,12	0,184	-0,38	< 0,001*
E2 - MEP area	-0,38	< 0,001*	-0,05	0,622
E2 - MEP latency	-0,23	0,012*	-0,37	< 0,001*
Produced force - Cortical SP duration	0,15	0,105	0,28	0,002*
Produced force - MEP area	0,26	0,005*	0,34	< 0,001*
Produced force - MEP latency	-0,14	0,130	0,04	0,645
MEP area - Cortical SP duration	0,38	< 0,001*	0,34	< 0,001*

7.3 Group-wise Differences

Comparing the measurement results from the electrical stimulations between the early and late perimenopause groups we find significant group-wise differences in the peripheral silent period ($p < 0{,}001$), maximum voluntary force ($p = 0{,}002$), voluntary rise time ($p = 0{,}009$) and voluntary average RFD ($p < 0{,}001$). As is evident from table 5 the early and late perimenopause groups also indicate significant differences in the post twitch average RFD ($p = 0{,}018$) and pre twitch maximum force ($p = 0{,}032$).

TABLE 5. Group-wise differences between early and late perimenopause groups for ES measurement variables (* = statistically significant difference at $p < 0{,}05$).

Variables	**p-value**
Peripheral silent period	$< 0{,}001^*$
Maximum voluntary force	$0{,}002^*$
Voluntary contraction rise time	$0{,}009^*$
Voluntary contraction average RFD	$< 0{,}001^*$
post twitch average RFD	$0{,}018^*$
pre twitch maximum force	$0{,}032^*$

For the rest of the electrical stimulation results the early and late perimenopause groups do not show significant group-wise differences (see table table 9 in appendix A).

Only the MEP latency ($p = 0{,}015$) displays significant group-wise difference. The produced force levels for the performed tasks (% of maximum dorsiflexion force) ($p = 0{,}053$) is quite close to a the threshold indicating significant difference. The rest of the TMS measurements, CSP duration ($p = 0{,}475$), MEP area ($p = 0{,}521$) and maximum dorsiflexion force ($p = 0{,}337$) indicate similarities between the early and late perimenopausal groups. Table 10 in appendix A lists the measures of the group-wise differences between the measurement results for TMS between the early and late perimenopause groups.

7.4 Voluntary Activation and Peripheral Silent Period

The group averages for the VA level and PSP duration are shown in figure 13. The early perimenopause group achieved an average VA level of 86,4 ± 15,2 %, while the late perimenopause group attained a somewhat lower average VA level of 73,4 ± 29,1 %. For the two subjects measured in postmenopause, the average VA level at the postmenopause stage was 85,8 ± 16,1 %. The average PSP duration for the early perimenopausal group (92 ± 27 ms) is shorter than for the late perimenopausal group (116 ± 22 ms). For the two subjects in postmenopause the average PSP duration (81 ± 19 ms) is even shorter than for the early perimenopausal group.

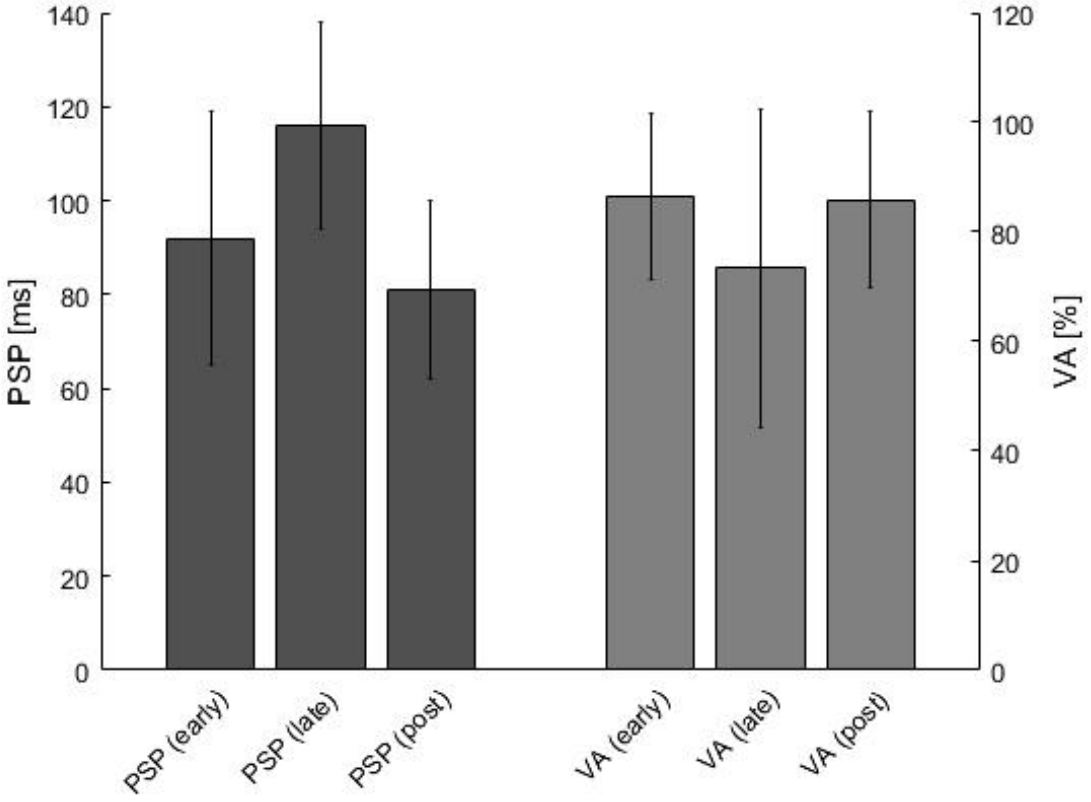

FIGURE 13. Early and late peri-, and postmenopause group PSP duration and VA levels.

7.5 Voluntary RFD

The peak and average voluntary RFD for all groups are displayed in figure 14. The peak RFD is highest for the early perimenopause group (3418 ± 1174 N/s), decreasing to 2954 ± 909 N/s for the late perimenopause group and further

decreasing to 2620 ± 236 N/s for the postmenopause group. The average RFD is also highest for the early perimenopause group (517 ± 207 N/s), but lowest for the late perimenopause group (315 ± 186 N/s), with the postmenopause group having an averge RFD of 418 ± 54 N/s.

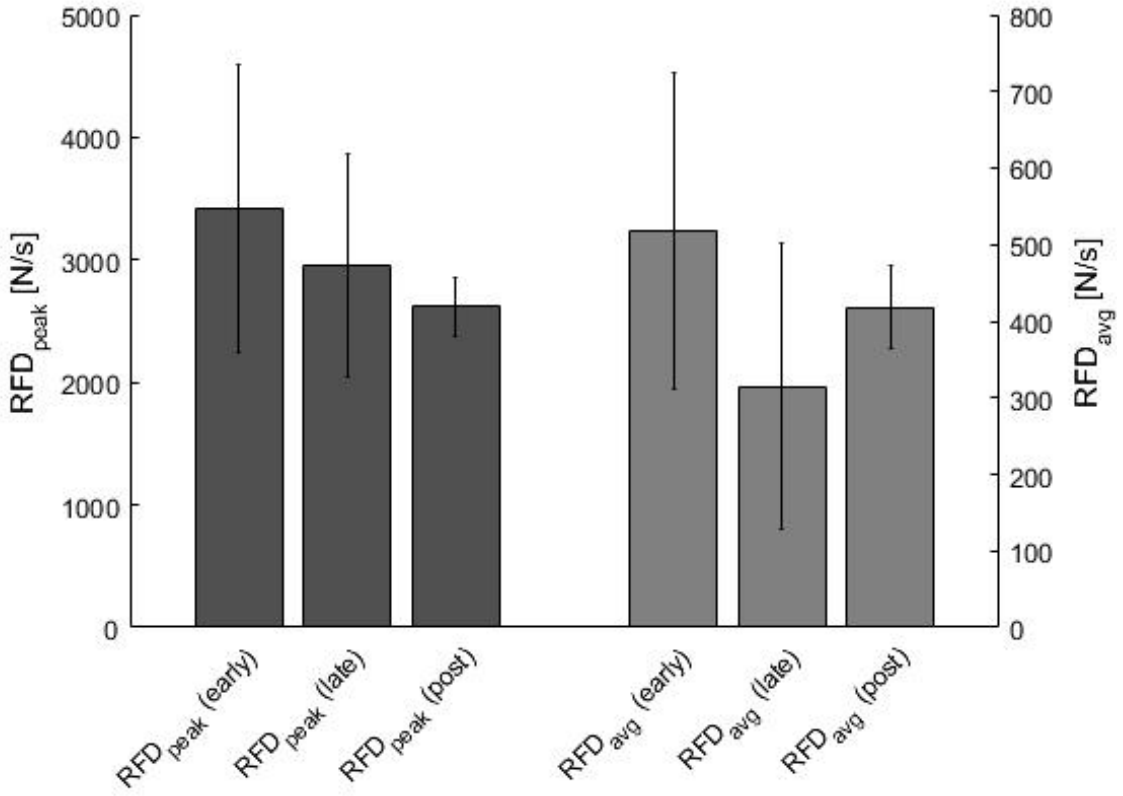

FIGURE 14. Early and late peri-, and postmenopause group peak and average RFD results.

7.6 Postactivation Potentiation

Figures 15 and 16 show the subject-wise results for the PAP measurements. From the results it can be said that from the early perimenopause group a clear PAP phenomenon is evident for five subjects, subjects 3, 4, 6, 9 and 10 (PAP 4,8; 8,0; 6,1; 14,1 and 21,3 % respectively). In the late perimenopause group a clear PAP phenomenon is evident for subjects 4, 5, 7 and 8 (PAP 4,8; 14,1; 9,8 and 5,4 % respectively). A small percentile increase in twitch potentiation is evident for subjects 5 and 8 (PAP 1,6 and 2,2 % respectively) in the early perimenopaus group while subject 1 from the early perimenopause group and subjects 3 and 6 from the late perimenopause group indicate almost no change in twitch force between pre- and

post-contraction twitches (PAP -0,2; 0,3 and -0,6 %). The rest of the results actually indicate a decreasing effect in the postactivation. In the early perimenopause group the lessening effect was -3,3 % for subject 2 and -7,6 % for subject 7, while for the late perimenopause group a decreasing effect was evident for subjects 1, 2, 9 and 10 (PAP -5,7; -15,1; -2,2 and -2,3 % respectively). The subjects measured in the postmenopause phase both indicate a negative postactivation effect, -1,7 % for subject 7 and -4,8 % for subject 8.

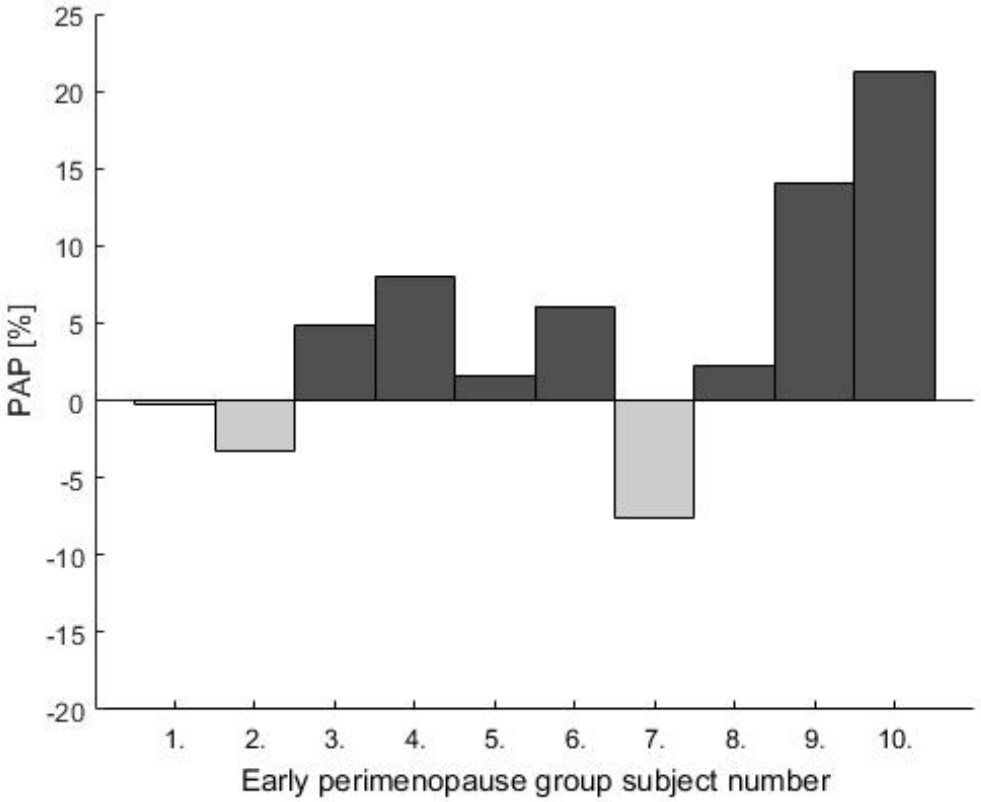

FIGURE 15. Early perimenopause group subject-wise PAP measurement results.

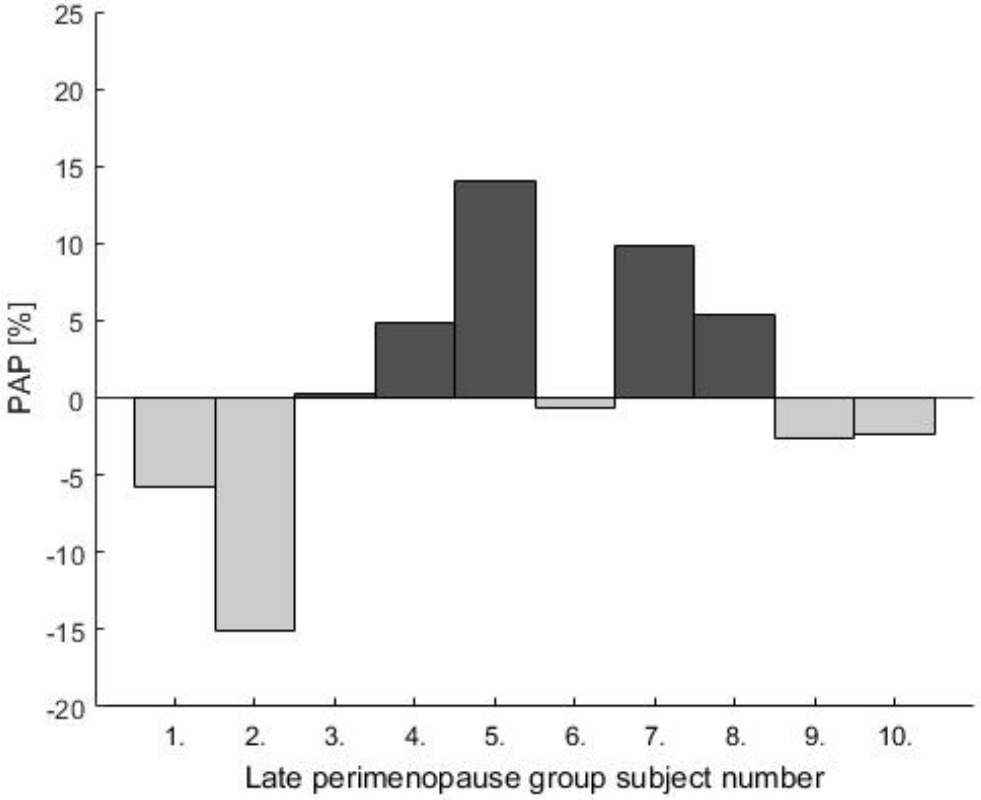

FIGURE 16. Late perimenopause group subject-wise PAP measurement results.

7.7 Peri- and Postmenopause Results

The peri- and postmenopause ES measurement results for subject 7 are listed in table 6. Noticeable changes seem to have occurred between early peri- and postmenopause measurements for several measurement variables. Some notable changes include a decrease in the VA level from an early perimenopause value of 98,7 ± 1,6 % to a postmenopausal value of 72,4 ± 10,6 % and a decrease in maximum voluntary force (806 ± 74 N vs 579 ± 45 N) between the early perimenopausal and postmenopausal measurements respectively.

The peri- and postmenopause ES measurement results for subject 8 are listed in table 7. As is evident from the table clear changes seem to have occurred between early peri- and postmenopause measurements for some measurement variables. Most notably, both the pre and post twitch average and peak RFD have decreased from early perimenopause to postmenopause (pre average from 1144 ± 91 N/s to 693 ± 42 N/s, post average from 1324 ± 137 N/s to 760 ± 53 N/s, pre peak from 2811 ±

202 N/s to 2102 ± 87 N/s and post peak from 2939 ± 214 N/s to 2473 ± 274 N/s respectively). The peri- and postmenopause TMS measurements for subjects 7 and 8 are listed in table 8.

TABLE 6. Peri- and postmenopause ES measurement results for subject 7.

Variables	**Peri**	**Post**	**Units**
Peripheral silent period	94 ± 4	97 ± 13	ms
Voluntary activation	$98,7 \pm 1,6$	$72,4 \pm 10,6$	%
pre twitch rise time	159 ± 12	118 ± 11	ms
post twitch rise time	150 ± 13	118 ± 14	ms
pre twitch half relaxation time	140 ± 10	135 ± 25	ms
post twitch half relaxation time	115 ± 8	101 ± 26	ms
pre twitch average RFD	1123 ± 52	505 ± 36	N/s
post twitch average RFD	1162 ± 29	458 ± 74	N/s
pre twitch peak RFD	2730 ± 36	1118 ± 107	N/s
post twitch peak RFD	2758 ± 143	946 ± 46	N/s
pre twitch average RFR	-639 ± 54	-225 ± 41	N/s
post twitch average RFR	-765 ± 113	-273 ± 47	N/s
pre twitch peak RFR	-1503 ± 189	-618 ± 101	N/s
post twitch peak RFR	-1983 ± 221	-927 ± 289	N/s
pre twitch S-gradient	2207 ± 435	1458 ± 143	N/s
post twitch S-gradient	2836 ± 251	1677 ± 670	N/s
pre twitch maximum force	197 ± 6	79 ± 3	N
post twitch maximum force	199 ± 18	76 ± 5	N
pre twitch M-wave area	$0,0500 \pm 0,0009$	$0,0576 \pm 0,0006$	mVs
post twitch M-wave area	$0,0489 \pm 0,0008$	$0,0572 \pm 0,0004$	mVs
Voluntary contraction M-wave area	$0,0233 \pm 0,0002$	$0,0174 \pm 0,0011$	mVs
Voluntary contraction rise time	$2,25 \pm 0,18$	$1,53 \pm 0,13$	s
Voluntary contraction S-gradient	1245 ± 221	1722 ± 196	N/s
Voluntary contraction peak RFD	2112 ± 488	2701 ± 291	N/s
Voluntary contraction average RFD	340 ± 52	369 ± 12	N/s
Voluntary contraction S-gradient			
EMG integral	$0,021 \pm 0,002$	$0,013 \pm 0,001$	mVs
Maximum voluntary force	806 ± 74	579 ± 45	N

TABLE 7. Peri- and postmenopause ES measurement results for subject 8.

Variables	**Peri**	**Post**	**Units**
Peripheral silent period	56 ± 10	65 ± 1	ms
Voluntary activation	$91,5 \pm 12,8$	$99,2 \pm 1,5$	%
pre twitch rise time	185 ± 17	205 ± 9	ms
post twitch rise time	160 ± 12	187 ± 7	ms
pre twitch half relaxation time	722 ± 823	210 ± 55	ms
post twitch half relaxation time	536 ± 405	207 ± 89	ms
pre twitch average RFD	1144 ± 91	693 ± 42	N/s
post twitch average RFD	1324 ± 137	760 ± 53	N/s
pre twitch peak RFD	2811 ± 202	2102 ± 87	N/s
post twitch peak RFD	2939 ± 214	2473 ± 274	N/s
pre twitch average RFR	-304 ± 214	-353 ± 97	N/s
post twitch average RFR	-272 ± 152	-382 ± 145	N/s
pre twitch peak RFR	-913 ± 192	-980 ± 54	N/s
post twitch peak RFR	-937 ± 278	-1080 ± 167	N/s
pre twitch S-gradient	3008 ± 516	2771 ± 105	N/s
post twitch S-gradient	3129 ± 235	3241 ± 92	N/s
pre twitch maximum force	250 ± 14	186 ± 3	N
post twitch maximum force	245 ± 5	185 ± 5	N
pre twitch M-wave area	$0,0722 \pm 0,0013$	$0,0706 \pm 0,0002$	mVs
post twitch M-wave area	$0,0206 \pm 0,0001$	$0,0208 \pm 0,0007$	mVs
Voluntary contraction M-wave area	$0,0438 \pm 0,0040$	$0,0368 \pm 0,0020$	mVs
Voluntary contraction rise time	$2,39 \pm 0,62$	$1,56 \pm 0,13$	s
Voluntary contraction S-gradient	1323 ± 86	1643 ± 674	N/s
Voluntary contraction peak RFD	1993 ± 491	2540 ± 185	N/s
Voluntary contraction average RFD	263 ± 55	467 ± 5	N/s
Voluntary contraction S-gradient			
EMG integral	$0,023 \pm 0,003$	$0,025 \pm 0,001$	mVs
Maximum voluntary force	721 ± 99	779 ± 42	N

TABLE 8. Peri- and postmenopause TMS measurement results for subject 7. and 8.

	Subject 7.		Subject 8.		
Variables	**Peri**	**Post**	**Peri**	**Post**	**Units**
CSP (60% Max Force)	$108,4 \pm 7,5$	$45,8 \pm 10,3$	$30,6 \pm 8,8$	$49,6 \pm 9,1$	ms
CSP (40% Max Force)	$92,1 \pm 23,4$	$38,3 \pm 15,1$	$43,8 \pm 24,9$	$53,6 \pm 4,8$	ms
CSP (20% Max Force)	$110,1 \pm 6,8$	$51,0 \pm 9,7$	$46,4 \pm 14,5$	$42,8 \pm 6,9$	ms
Actual produced force (60% Max Force)	$60,1 \pm 2,1$	$58,1 \pm 1,8$	$68,2 \pm 0,8$	$52,4 \pm 4,2$	N
Actual produced force (40% Max Force)	$41,9 \pm 1,9$	$41,8 \pm 0,6$	$55,1 \pm 1,8$	$33,8 \pm 0,3$	N
Actual produced force (20% Max Force)	$23,3 \pm 1,6$	$24,0 \pm 0,6$	$38,1 \pm 1,0$	$18,1 \pm 0,9$	N
MEP area (60% Max Force)	$11,8 \pm 1,8$	$8,8 \pm 0,4$	$2,0 \pm 1,8$	$1,9 \pm 0,7$	μVs
MEP area (40% Max Force)	$9,3 \pm 2,2$	$3,8 \pm 1,0$	$1,2 \pm 0,1$	$2,5 \pm 0,9$	μVs
MEP area (20% Max Force)	$3,6 \pm 0,7$	$1,8 \pm 0,2$	$1,2 \pm 0,3$	$1,7 \pm 1,0$	μVs
MEP latency (60% Max Force)	$33,0 \pm 5,5$	$32,4 \pm 0,8$	$30,6 \pm 5,8$	$31,4 \pm 4,3$	ms
MEP latency (40% Max Force)	$34,3 \pm 0,3$	$32,6 \pm 0,9$	$27,0 \pm 5,6$	$29,8 \pm 2,8$	ms
MEP latency (20% Max Force)	$34,6 \pm 2,3$	$31,9 \pm 2,7$	$30,6 \pm 1,3$	$29,1 \pm 2,6$	ms
Maximum dorsiflexion force	265	244	141	152	N

8 Discussion

The aim of this thesis was to study the effects of the stage of menopause on the ability to activate the muscles of the lower extremity muscles during isometric plantar- and dorsiflexion tasks. As is evident from figure 11, the serum E2-levels of the two groups do not significantly differ from one and other ($p = 0{,}597$).

Significant correlation between measurement variables for both the early and late perimenopause groups were found for several measurement variable pairs (see table 1 and 4). The early and late perimenopause groups indicate significant group-wise differences in the peripheral silent period ($p < 0{,}001$), maximum voluntary force ($p = 0{,}002$), voluntary rise time ($p = 0{,}009$) and MEP latency ($p = 0{,}015$).

While the group averages for the measure of VA do not differ significantly between the early and late perimenopause groups ($p = 0{,}307$), the early perimenopause group did however achieve a slightly higher group average $86{,}4 \pm 15{,}2$ % than the late perimenopause group $73{,}4 \pm 29{,}1$ %. In addition neither group showed significant correlation between the serum E2 level and the level of VA ($r = 0{,}08$, $p = 0{,}678$ for early and $r = 0{,}34$, $p = 0{,}064$ for late).

Even though the voluntary contraction peak RFD for the early perimenopause group is clearly higher than for the late perimenopause group (3418 ± 1174 N/s vs 2954 ± 909 N/s respectively) no significant group-wise difference exists ($p = 0{,}092$). There is however a significant group-wise difference for the average voluntary contraction RFD between the two groups ($p < 0{,}001$), with the voluntary contraction RFD value for the early perimenopause group at 517 ± 207 N/s and for the late perimenopause group at 315 ± 186 N/s.

A clear PAP was evident for less than half the subjects, five subjects from the early perimenopause group and four from the late perimenopause group. In addition two subjects from the early perimenopause group show a small percentile increase in twitch potentiation. Three subjects, one from the early and two from the late perimenopause group indicate no change in twitch force between pre- and post-contraction twitches, while the rest (two subjects from the early and four from the late perimenopause group) actually indicate a negative postactivation effect.

Both groups display significant correlation between the serum E2-level and the MEP latency (r = -0,23, p = 0,012 for early and r = -0,37, p < 0,001 for late), between the produced force and the MEP area (r = 0,26, p = 0,005 for early and r = 0,34, p < 0,001 for late), and between the MEP area and the CSP (r = 0,38, p < 0,001 for early and r = 0,34, p < 0,001 for late). These correlations also display similar correlation coefficients in both sign and magnitude. The results from table 10 indicate that the two groups only differ significantly in MEP latency times (p = 0,015).

8.1 Initial status of the subjects

Even though the serum E2-level was used as one inclusion criteria for group assignment at the perimenopausal phase, the major contributing factors in the inclusion criteria appear to be the FSH serum level values (see figure 11) and the self-reported menstrual cycle. Since the serum E2-levels between early and late perimenopause groups do not significantly differ, we must conclude that none of the group-wise differences in the measurement results can be attributed to serum E2.

On the other hand, the results comparing peri- and postmenopausal subjects 12 show clear differences in serum E2-levels. For subject 7 the decreases was from a perimenopausal value of 189 pmol/l to a postmenopausal value of 20,3 pmol/l and for subject 8 the decrease was from a perimenopausal value of 852 pmol/l to a postmenopausal value of 9,4 pmol/l. It should be noted here that the values are single values and no statistical analysis has been performed. In any case, the results E2 serum level wise, support proceeding to the next phase of the ERMA-study, where the intrapair differences of the early and late perimenopausal subjects will be compared to results obtained at the postmenopausal phase.

The group-wise comparison did not reveal a significant statistical difference in age between the groups. On the other hand the average age of the peri- and postmenopausal differ by approximately 1 year (51,9 ± 2,2 years early vs 53,0 ± 2,0 years late perimenopausal), which in some cases can be sufficient time for neural and muscular changes to take effect (Häkkinen et al., 2000; Goodpaster et al., 2006; Clark and Manini, 2008).

8.2 Peripheral Silent Period

Our results indicate s significant correlation between the serum E2 level and the peripheral silent period (r = -0,37 for early and r = -0,50 for late). The PSP values differ significantly ($p < 0{,}001$) with the late perimenopausal group having generally higher PSP values than the early perimenopausal group (116 $\pm$ 22 ms for late vs 92 $\pm$ 27 ms for early). And as was stated earlier the serum E2-level values are similar group-wise. Therefore, no direct causality exists between the parameters. Even without causality, the correlation indicates that the level of serum E2 influences the duration of the PSP. As has been suggested by Cox and Cafarelli (1999) the PSP duration is dependent of the amount of central drive to the α-motor neuron pool. The serum E2 level may influence the amount of central drive by altering the responsiveness of postsynaptic receptors (Yankova et al., 2001; Maejima et al., 2013) or the presynaptic release of neurotransmitters (Yokomaku et al., 2003).

The notion of no direct causality between the PSP and the serum E2-level is further enhanced by the fact that, according to the correlation between the PSP and the maximum voluntary force (r = -0,66 for early and r = -0,54 for late), the duration of the PSP is inversely related to the maximum force. This indicates a clear causality between the maximum voluntary force and the PSP duration.

As was discussed in 2.3 several possibilities may affect the duration of the PSP. All the methods described in section 2.3 function through neurological inhibitory/impediment mechanisms. Neurologically, force production can be increased either by increasing the amplitude (recruiting more MUs) or the firing rate of the action potentials or by increasing both. The higher voluntary force values for the early primenopause group could therefore indicate stronger voluntary nervous system activation, which could better annul the inhibitory effects caused by the superimposed twitch leading to a decrease in the duration of the PSP. This is further supported by the previously mentioned correlation between the PSP and the maximum voluntary force, and therefore these results are in line with those from Cox and Cafarelli (1999).

8.3 Voluntary Activation

There is no statistically significant difference in VA between the early and late perimenopause groups (p = 0,307), but the group average for the early perimenopause group is higher than for the late perimenopause group (86,4 ± 15,2 % for early and 73,4 ± 29,1 % for late), which could be due to reduced central drive. Another possible explanation could be that due to the curvilinear relationship between the produced force and the activation level, small variations in the activation can exhibit large differences in the generated force at near maximal efforts, which could also explain some of the deficit in voluntary activation (Stackhouse et al., 2001; Stevens et al., 2003). Additionally, neither group showed significant correlation between the serum E2 level and the VA (r = 0,08, p = 0,678 for early and r = 0,34, p = 0,064 for late). This would seem to suggest that estrogen plays no modulatory effect on synaptic transmission in maximal VA tasks. The voluntary activation, as measured by the superimposed twitch method does correlate with the PSP for the early perimenopause group (r = -0,65, p < 0,001), but not for the late group (r = -0,36, p = 0,051), where the p-value is just above the limit of the chosen significance level. The reason for this remains unknown.

8.4 Plantar- and Dorsiflexion Force Production

The difference in maximum voluntary plantarflexion force between the groups (869 ± 229 N for the early perimenopause group and 632 ± 244 N for the late perimenopause group) could partially be due to age related changes in the neuromuscular system, even though no significant statistical difference in age between the groups exists. There is however the previously mentioned approximately 1 year difference in the average age between the two groups. The loss of strength could be due to loss of muscle mass associated with aging, but according to Goodpaster et al. (2006) the loss of strength is more likely caused by a decline in muscle quality, i.e. force per unit muscle mass. Aging has been shown to cause a shift in muscle fiber composition toward a greater portion of slow twitch fibers (Roos et al., 1997; Brunner et al., 2007), which also could reduce the maximum voluntary force. Unfortunately, because muscle biopsies were not taken we have no knowledge of the contribution of fiber type to maximum voluntary force. The age related decrease in tendon stiffness

(Narici and Maganaris, 2006; Narici et al., 2008) could also theoretically reduce maximal voluntary force production. Tendon stiffness was also not measured, so we have no knowledge of its contribution the maximum voluntary force. In their study Klass et al. (2005) suggested that the loss in force generating capacity with age is an indication that muscle contractile mechanisms, not increased tendon compliance, are the main cause for the slowing of the muscle contractile properties with age. Their results also indicate that the decrease in force due to aging is caused by muscular alterations and not impaired neural drive.

Vandervoort and McComas (1986) concluded that the aging caused loss of strength was, on a relative scale, very similar between plantarflexors and dorsiflexors, while the absolute loss of strength was greater for the plantarflexor muscles. As was previously mentioned, our results indicate a decrease in plantarflexion maximum voluntary force between the early perimenopause group and the late perimenopause group. The maximum voluntary dorsiflexion force on the other hand has increased slightly between early and late perimenopause (212 $\pm$ 45 N for early vs 237 $\pm$ 65 N for late). Whipple et al. (1987) speculated, that at functional limb velocities ankle weakness and especially dorsiflexor weakness are a major cause in poor balance. If this speculation holds true, and if isometric dorsiflexion force can be used as an indicator of dorsiflexor weakness for functional limb velocities, our results would seem to indicate unchanged or slightly improved balance from peri- to postmenopause.

8.5 Rate of Force Development

Both the average and peak RFD are higher for the early perimenopause group compared to the late perimenopause group (517 $\pm$ 207 N/s avg early vs 315 $\pm$ 186 N/s avg late and 3418 $\pm$ 1174 N/s peak early vs 2954 $\pm$ 909 N/s peak late). Our results also indicate that, in addition to having higher maximum voluntary force values, the early perimenopause group also has, on average, shorter duration voluntary force production rise times (see table 15 in appendix A). This indicates faster maximal voluntary muscle activation, which according to Izquierdo et al. (1999a) is directly related to a persons postural control capacity. Similar changes are not present for the twitch evoked rise times (pre twitch early group 159 $\pm$ 24 ms vs pre twitch late group 153 $\pm$ 20 ms and post twitch early group 148 $\pm$ 26 ms

vs post twitch late group 153 ± 18 ms see table 14 in appendix A), which could be indicative of differences in central drive between the two groups.

According to Klass et al. (2008) the decrease in the voluntary maximal RFD of fast contractions with aging is both due to slowing of the contractile properties of the muscle and reduction in the maximal discharge frequency of MUs. The result of Connelly et al. (1999) indicate that the reduced MU discharge frequency likely limits the maximal RFD but does not seem to effect the force produced during MVC.

According to VanCutsem et al. (1998) and Aagaard et al. (2002), neural drive may play an important role in maximizing explosive force production. One possible cause for differing central drive could be due to differing neural control between the subjects. Both Schmitz et al. (2009) and Chu et al. (2009) have shown in their studies that there exists differences in neural control between subjects. This is however not supported by the correlation results between the maximum voluntary force and the pre and post twitch maximum forces. Our results for both the early and late perimenopause groups indicate strong correlation between MVF and pre and post twitch maximum forces. Since a significant correlation is present it is safe to say that the twitch forces reflect the full voluntary capacity of the muscle, thus indicating similarity in maximum force production for centrally driven and peripheral stimulated contractions. This fact is further supported by the correlations between the voluntary average RFD and pre and post twitch average RFD and the voluntary peak RFD and pre and post twitch peak RFD, which indicate similar force development patterns for centrally driven and peripheral stimulated contractions.

The fact that serum E2-levels correlate with pre twitch rise times ($r = 0{,}43$, $p = 0{,}017$ for early group and $r = 0{,}39$, $p = 0{,}033$ for late group) could be related to the type II muscle fiber preserving function of estrogen as shown by Kadi et al. (2002) on ovariectomized rats. Here again, muscle biopsies would be needed to confirm this hypothesis. It is also possible that the Ca^{+2} kinetics at the sarcoplasmic reticulum play some role, since according to Heilmann and Pette (1979); Fitts et al. (1980); Miyamoto et al. (2012) the rate of release of Ca^{+2} from the sarcoplasmic reticulum is associated with the twitch contraction time. The fact that the rise times of post VA twitches do not correlate with serum E2-levels could reflect changes in Ca^{+2} kinetics at the sarcoplasmic reticulum, possibly due to the PAP phenomenon.

The present correlation between the voluntary contraction S-gradient and pre and post twitch maximum force is in line with the findings of Andersen and Aagaard (2006), who reported a relationship between twitch force and RFD of the early phase of voluntary contraction. This correlation may, according to Folland et al. (2014), reflect submaximal firing frequencies and/or incomplete Ca^{+2} saturation during the early phase of voluntary contraction. High/maximal saturation levels of intracellular calcium are obtained at maximal rates of force development, which in the stimulation case requires high stimulation rates (VanCutsem et al., 1998). For example de Ruiter et al. (2004) indicated that an evoked octet of eight pulses at 300 Hz drives the muscle tendon unit at its maximum RFD. There is however no knowledge how this contractile characteristic is tied to voluntary explosive force production (Folland et al., 2014). According to de Ruiter et al. (2007) it is also quite possible that factors which affect the maximum RFD, including Ca^{+2} kinetics, tendon stiffness or cross-bridge cycling rates differ between different stimulation methods.

Based on the EMG measurements of Folland et al. (2014) neural activation was generally high/maximal at the early phase of voluntary force development (first 50 ms), after which the rise in force was mostly dependent on the contractile properties of the muscle tendon unit. Here again, since muscle biopsies were not taken and tendon stiffness was not measured, we have no knowledge of the contribution of these factors on our results.

We did not find correlation between agonist EMG and relative RFD (r = - 0,07, p = 0,727 for early and r = -0,29, p = 0,117 for late) as Klass et al. (2008) found for young adults. However our results are in line with the results of Klass et al. (2008) for older adults. The decline in the performance of fast voluntary contractions were attributed to both an age-related decline in maximal motor unit discharge frequency, as well as to the slowing of muscle contractile properties. This was due to the fact that the decline in the maximal rate of torque development for fast voluntary contractions was greater than for an electrically evoked twitch. (Klass et al., 2008.) Our results seem to indicate a similar tendency between the two groups.

8.6 Postactivation Potentiation

The results for the PAP measurements in figurs 15-16 indicate that about half of participants demonstrate a clear PAP phenomenon, five subjects in the early perimenopause group and four subjects in the late perimenopause group. Combining these results with those in tables 20 - 25 in appendix A we can make the following conclusions. All of the subjects displaying PAP also display a decrease in twitch rise time between pre and post twitches. Results indicating the same tendency have been obtained by Hamada et al. (2000b); Vandervoort et al. (1983), while for example Miyamoto et al. (2012) and Pääsuke et al. (2007) have reported no such phenomenon. In addition to decreased twitch rise time subjects displaying PAP also indicate an increase in average and peak RFD between pre and post twitches. The increase in RFD is in line with the results obtained by Baudry and Duchateau (2007). Our results are also well in line with Hamada et al. (2000b) who reported that the human muscles which exhibit greater PAP also display shorter twitch contraction times as well as a higher percentage of type II muscle fibers. Several different possibilities may explain the differences in the occurrence and lack of PAP. Aging has been shown to shift muscle fiber fiber properties toward a greater portion of slow twitch fibers (Roos et al., 1997; Brunner et al., 2007). The most important muscle characteristic which affects the PAP magnitude is according to Grange et al. (1993), Vandenboom et al. (1995) and Hamada et al. (2000b) fiber type, with muscles with the greatest proportion of type II fibers having the greatest potential for PAP enhancement. It may therefore be possible that the muscle type composition of the subjects lacking the PAP phenomenon have undergone a shift towards a greater portion of slow twitch fibers. Since muscle biopsies were not taken this can not be fully confirmed. One other possible explanation could be that task familiarization has already potentiated the muscle. The PAP effect may according to Chiu et al. (2003) and Rixon et al. (2007) last from 5 to 30 minutes. The third possibility is that some of the subjects may have been fatigued. The measurements were performed as the last tests of the testing day lasting several hours. According to Rassier and Herzog (2001) and Sale (2002) in addition to reflecting the PAP mechanism the twitch response also reflects fatigue. In addition to this, even if twitch potentiation is at its or close to its highest level shortly after muscle activity, the performance of the maximal contraction may be impaired due to fatigue (Gossen and Sale, 2000; Hamada et al., 2003).

The twitch potentiation observed by Miyamoto et al. (2012) was mainly attributed to the changes in cross-bridge dynamics, which result from phosphorylation of myosin regulatory light chains. Their findings, unlike ours did not show changes in M-wave amplitude, contraction time, or half-relaxation time of twitches. Our result indicating a decrease in rise time between pre and post twitches for subjects illustrating the PAP phenomenon, could therefore be an indication of changes in neuromuscular transmission. The PAP phenomenon could therefore be caused by both phosphorylation of myosin regulatory light chains and changes in synaptic transmission.

8.7 Findings from Cortical Stimulation

The negative correlation between the serum E2-level and the MEP latency (r = -0,23 early and r = -0,37 late perimenopause) indicate that serum E2 may act as a mediator in synaptic transmission. One possible explanation lies in the GABA signaling system. The studies by Yankova et al. (2001) and Maejima et al. (2013) have shown estrogen to alter the responsiveness of postsynaptic receptors while the study by Yokomaku et al. (2003) has shown estrogen to alter the the presynaptic release of neurotransmitters. Since our results indicate that lower levels of estrogen correlate with longer MEP latencies it can be speculated that serum E2-levels influence the speed of synaptic transmission by mediating either the responsiveness of postsynaptic receptors or the presynaptic release of neurotransmitters or both.

The fact that the two groups differ significantly in MEP latency times (p = 0,015), with the early perimenopause group having longer latencies at all three force production levels (see table 28 in appendix A), seems to indicate increased synaptic transmission speed with advanced menopause. This is in direct contrast to the general observation that with advancing menopause the serum E2-level decreases and if the correlation holds this would lead to lengthening MEP latencies. In our case however the group-wise serum E2-levels do not differ significantly (p = 0,597), but the average serum E2-levels of the late perimenopause group are a bit higher than for the early perimenopause group (304 ± 180 pmol/l late vs 275 ± 210 pmol/l early), which could explain at least some of the difference in MEP latency.

Our results indicating a positive correlation between the level of force production and

the MEP area ($r = 0{,}26$, $p = 0{,}005$ early and $r = 0{,}34$, $p < 0{,}001$ late perimenopause) are in line with the findings of for example DiLazzaro et al. (1998); Kischka et al. (1993); Taylor et al. (1997). The increase in MEP size with increased voluntary contraction can partly be attributed to the increased output from the motor cortex in response to stimulation (DiLazzaro et al., 1998; Kaneko et al., 1996; Mazzocchio et al., 1994; Ugawa et al., 1995). According to Taylor and Gandevia (2001) the level of contraction is not the sole cause. There is a notion that some tasks require a stronger cortical contribution than others, which manifests as an increase in cortical excitability (Flament et al., 1993; Nielsen et al., 1993; Schieppati et al., 1996).

Säisänen et al. (2008) state that regardless of a long history in the recording of the silent periods, the effect of muscle contraction level on the duration of the silent periods remains unclear. Taylor and Gandevia (2001) has suggested that during brief isometric contractions the size of the MEP increases with the strength of the contraction, but that this growth is muscle dependent. The MEP has been shown to increase only little as contraction strength increases above 10% of MVC in a hand muscle, while for the biceps brachii the MEP has been shown to increase up 75% MVC (Kischka et al., 1993; Taylor et al., 1997). Our results indicate that in general the MEP in the tibialis anterior increases at least up to 60% MVC (see table 29 in appendix A). This is in line with results reported by Säisänen et al. (2008). One possible explanation for the increase in MEP in relation to contraction strength may lie in the recruitment of additional motor units to increase contraction strength. Kukulka and Clamann (1981) have demonstrated that in the biceps brachii, additional motor units are being recruited in order to increase voluntary contraction strength even at 80% MVC, while for the adductor pollicis it seems most motor units have been recruited by 30% MVC. Our results therefore indicate that for early and late perimenopausal women the increase in voluntary strength in the tibialis anterior, at least to 60% MVC, is achieved by recruiting additional motor units.

Our results indicating a positive correlation between MEP area and CSP duration ($r = 0{,}38$, $p < 0{,}001$ for early and $r = 0{,}34$, $p < 0{,}001$ for late) are in line with the findings of Orth and Rothwell (2004), who suggest that higher amplitude MEPs usually lead to longer silent periods. In addition they state that the silent period could be predicted from both MEP amplitude and area. As was stated by Säisänen

et al. (2008) the exact mechanisms of the inter-relationship between MEP and CSP remains unclear, but it can be speculated that they share some common mechanisms.

Pharmacological studies by Werhahn et al. (1999) and McDonnell et al. (2006) linked $GABA_B$ receptors as mediators in LICI, while Ziemann (2003) found that SICI to be primarily mediated by $GABA_A$ receptors. On the other hand estrogen has been linked to synaptic transmission through estrogens modulatory effect on the neurochemical GABA signaling system. Oddly enough, with both groups showing correlation between MEP area and CSP duration, only the early perimenopause group illustrates a correlation between serum E2-level and MEP area, while only the late perimenogroup illustrates a correlation between serum E2-level and CSP duration.

It has been widely accepted that the initial part of the CSP is of a spinal and later part of a cortical origin (Cantello et al., 1992; Inghilleri et al., 1993; Roick et al., 1993; Taylor et al., 1997; Triggs et al., 1993; Uncini et al., 1993). The initial part of the CSP has been hypothesized to reflect inhibition of descending drive and reduced excitability of the motoneurons, with the later part reflecting intracortical inhibition (Taylor and Gandevia, 2001). According to Cantello et al. (1992); Inghilleri et al. (1993); Taylor et al. (1997) a measurement protocol with continued muscle activity post stimulation may shorten the duration of the CSP due to an additional voluntary effort at the perceived contraction pause. Theoretically this greater voluntary effort may increase excitation to the cortical motoneurons and thus overriding the inhibitory effects more rapidly (Säisänen et al., 2008). Our measurement protocol, aimed at keeping the contraction levels constant during stimulation, may explain some of the shorter duration CSPs. From a measurement perspective it is important to maintain a stable contraction post stimulation in order to allow a clear measurement of the CSP duration.

Several studies, for example Inghilleri et al. (1993); Roick et al. (1993); Taylor et al. (1997); Triggs et al. (1993); Uncini et al. (1993) have indicated no correlation between the level of voluntary contraction and CSP duration. Our results for the early perimenopause group (r = 0,15, p = 0,105) are in line with this, while the results for the late perimenopause group indicate a correlation between the level of voluntary contraction and CSP duration (r = 0,28, p = 0,002). The results of the late perimenopause group are in line with results reported by Stetkarova et al.

(1994) and Wilson et al. (1993). The discrepancy between study results has been attributed to differences in measurement methodologies (Stetkarova et al., 1994). This is however not the case in our measurements, as the same methodology was used throughout the entire study. Why the two groups differ remains unclear.

8.8 Peri- and Postmenopause

The fact that the PSP duration only slightly increases between peri- and postmenopause measurements for subject 7 (from 94 ± 4 ms to 97 ± 13 ms)and clearly increses for subject 8 (from 56 ± 10 ms to 65 ± 1 ms), while the serum E2-level clearly decreases for both subjects (from 189 pmol/l to 20,3 pmol/l for subject 7 and from 852 pmol/l to 63,5 pmol/l for subject 8) seems to suggest that the decreased serum E2-level has no clear function in the mechanisms effecting PSP duration. This is somewhat in agreement with the earlier findings in this study which indicate correlation between serum E2-levels and PSP for both the early and late perimenopause groups, but no causality between the two. It is still completely possible that the decreased serum E2-levels show a correlation with the PSP and this should be verified in further studies.

Both subjects illustrate a decrease in both pre and post twitch peak force from peri- to postmenopause (pre twitch from 197 ± 6 N to 79 ± 3 N and post twitch from 199 ± 18 N to 76 ± 5 N for the early group and pre twitch from 250 ± 14 to 186 ± 3 N and post twitch from 245 ± 5 to 185 ± 5 N for the late group) but only subject 7 displays a clear decrease in maximum voluntary force (from 806 ± 74 N vs 579 ± 45 N). Subject 8 actually displays an increase in voluntary force from the perimenopause value, but it is only a slight increase (from 721 ± 99 N to 779 ± 42 N).

In the case of subject 7 the fact that both voluntary and stimulated maximum force production have decreased could be indicative of either a shift in muscle fiber composition toward a greater portion of slow twitch fibers as suggested by Roos et al. (1997) and Brunner et al. (2007) or due to declined nervous system performance Clark and Taylor (2011). According to Klass et al. (2005) the decrease in force due to aging is more likely caused by muscular alterations and not impaired neural drive. Our results however indicate a decreased M-wave area for the voluntary contraction

superimposed twitch (from 0,0233 ± 0,0002 mVs to 0,0174 ± 0,0011 mVs) and an increase in the pre and post twitch M-wave areas (from 0,0500 ± 0,0009 mVs to 0,0576 ± 0,0006 mVs pre twitch and from 0,0489 ± 0,0008 mVs to 0,0572 ± 0,0004 mVs post twitch), which would indicate alterations in the synaptic transmission. The fact that subject 7 also illustrates a clear decrease in voluntary activation (from 98,7 ± 1,6 % to 72,4 ± 10,6 %) could very well indicate impaired neural drive. In addition however, the significant decrease in maximum voluntary force and pre and post twitch maximum force could indicate a shift in muscle fiber composition toward a greater portion of slow twitch fibers. This could also explain the decreased voluntary rise time (from 2,25 ± 0,18 s to 1,53 ± 0,13 s), as it most likely would take less time to reach a lower force production level. The decrease in pre and post twitch average and peak RFD (pre average from 1123 ± 52 N/s to 505 ± 36 N/s, post average from 1162 ± 29 N/s to 458 ± 74 N/s, pre peak from 2730 ± 36 N/s to 1118 ± 107 N/s and post peak from 2758 ± 143 N/s to 946 ± 46 N/s) is a direct cause of the greater decreased in maximum twitch forces compared to the decreases in twitch rise times. Since muscle biopsies were not taken we have no knowledge of the contribution of fiber type to force production.

For subject 8 the results indicate a slight increase in maximal voluntary force from peri- to postmenopause (from 721 ± 99 N to 779 ± 42 N). The pre and post twitch maximum forces do however indicate a clear decreases (pre from 250 ± 14 to 186 ± 3 N and post from 245 ± 5 to 185 ± 5 N). Our results also indicate a decreased M-wave area for the voluntary contraction superimposed twitch (from 0,0438 ± 0,0040 mVs to 0,0368 ± 0,0020 mVs) but no change in the pre and post twitch M-wave areas (pre twitch 0,0722 ± 0,0013 mVs vs 0,0706 ± 0,0002 mVs and post twitch 0,0206 ± 0,0001 mVs vs 0,0208 ± 0,0007 mVS), which could be indicative of alterations in supraspinal or spinal synaptic transmission. There is however a clear increase in voluntary activation between peri- and postmenopause (from 91,5 ± 12,8 % to 99,2 ± 1,5 %), which would seem to indicate preserved neural function. A decreased neural drive would also not explain the difference between increased voluntary force and decreased twitch force. The decrease in pre and post twitch average and peak RFD (pre average from 1144 ± 91 N/s to 693 ± 42 N/s, post average from 1324 ± 137 N/s to 760 ± 53 N/s, pre peak from 2811 ± 202 N/s to 2102 ± 87 N/s and post peak from 2939 ± 214 N/s to 2473 ± 274 N/s) is a direct cause of the decreased maximum twitch forces and the increased pre and post twitch rise times (pre from

185 ± 17 ms to 205 ± 9 ms and post from 160 ± 12 ms to 187 ± 7 ms). Because muscle biopsies were not taken we have no knowledge of the contribution of fiber type to force production.

The TMS measurement results for subject 7 indicate that pre and post measurement results for the CSP duration have clearly decreased from peri- to postmenopause (from 108,4 ± 7,5 ms to 45,8 ± 10,3 ms for 60% force production, from 92,1 ± 23,4 ms to 38,3 ± 15,1 ms for 40% force production and from 110,1 ± 6,8 ms to 51,0 ± 9,7 ms for 20 %force production). In addition the MEP area measurement values have decreased from peri- to postmenopause for subject 7 (from 11,8 ± 1,8 mVs to 8,8 ± 0,4 mVs for 60%, from 9,3 ± 2,2 mVs to 3,8 ± 1,0 mVs for 40% and from 3,6 ± 0,7 mVs to 1,8 ± 0,2 mVs for 20%). The MEP delay measurement results also indicate a small decrease from peri- to postmenopause for subject 7 (from 33,0 ± 5,5 ms to 32,4 ± 0,8 ms for 60%, from 34,3 ± 0,3 ms to 32,6 ± 0,9 ms for 40% and from 34,6 ± 2,3 ms to 31,9 ± 2,7 ms for 20%). As was evident in table 4 both the early and late perimenopause groups displayed significant correlation between the serum E2-level and the MEP latency ($r = -0,23$, $p = 0,012$ for early and $r = -0,37$, $p < 0,001$ for late), the level of produced voluntary force and the MEP area ($r = 0,26$, $p = 0,005$ for early and $r = 0,34$, $p < 0,001$ for late) and the MEP area and the CSP ($r = 0,38$, $p < 0,001$ for early and $r = 0,34$, $p < 0,001$ for late). The group-wise results indicate that lower levels of estrogen correlate with longer MEP latencies it can be assumed that the decreased serum E2-levels have not caused the decrease in MEP latency between peri- and postmenopause measurements. The decrease in MEP area between peri- and postmenopause measurements for subject 7, with no clear difference in measurement incident force production levels between peri- and postmenopause measurements (60,1 ± 2,1 % vs 58,1 ± 1,8 % for the target of 60% MVF, 41,9 ± 1,9 % vs 41,8 ± 0,6 % for 40% and 23,3 ± 1,6 % vs 24,0 ± 0,6 % for 20%), could indicate a decrease in motor cortex output in response to stimulation (DiLazzaro et al., 1998; Kaneko et al., 1996; Mazzocchio et al., 1994; Ugawa et al., 1995). This may be due to several different reasons such as changes in stimulus intensity and the excitability of cortical cells (Taylor and Gandevia, 2001). Since the stimulus intensity of the stimulation device was the same for pre and post measurements the difference could be due to differing orientations and stimulation sites between peri- and postmenopause measurements. This is due to the fact that the stimulation coil was hand held and without navigation. Since the

site of stimulation is not focal as stated by Pierrot-Deseilligny and Burke (2012, p 38) it is possible that the cortical cells were stimulated with different intensities. The excitability of the cortical cells was not monitored in any way in these measurements so we have no knowledge of the contribution of the excitability on MEP size. With no significant difference in force production levels between peri- and postmenopause measurements the decrease in CSP between peri- and postmenopause measurements is most likely related to the decrease in MEP area.

The TMS measurement results for subject 8 indicate that peri- and postmenopause measurement results clearly differ for the produced force (68,2 ± 0,8 % vs 52,4 ± 4,2 % for the target of 60% MVF, 55,1 ± 1,8 % vs 33,8 ± 0,3 % for 40% and 38,1 ± 1,0 % vs 18,1 ± 0,9 % for 20%). In other words the target force production during measurements differed significantly between peri- and postmenopause measurements, with the perimenopause measurements having been done at higher percentages of the maximum voluntary force. With no clear trends in the measurement results, it is quite difficult to draw any conclusions. As was with subject 7, we can here also conclude that the decreased serum E2-level is not the cause of the decrease in MEP latency between peri- and postmenopause measurements.

Since the results from the peri- and postmenopause measurements for the two subjects are not in agreement with each other, it is vital to perform additional studies with a larger cohort in order to identify possible trends in the results.

8.9 Conclusions

Even though the results did not yield a significant group-wise diffeerence in serum E2-level between the early and late perimenopause groups and thus none of the group-wise differences in the measurement results could be attributed to serum E2, our results did seem to indicate the possibility of alterations in both central drive and muscle contractile mechanisms with advancing menopause.

Our findings indicated that the serum E2-level may influence the duration of the PSP but, there was no direct causality between the two parameters. We were also able to speculate that even without direct causality the group-wise results indicate a possible

reduction in central drive and muscle contractile mechanisms due to the serum E2 level for the late perimenopause group as compared to the early perimenopause group. Reduced central drive could also have been a contributing factor to the lower average level of VA for the late perimenopause group. In addition, the finding of decreased voluntary RFD between the early and late perimenopausal groups could indicate of differences in central drive between the groups, while also being a signal of decreased postural control capacity. Our results also show correlation between the voluntary S-gradient and pre and post twitch maximum forces. This could indicate submaximal firing frequencies and/or incomplete Ca^{+2} saturation during the early phase of voluntary contraction, both of which could negatively effect balance and postural control capacity.

The results of our measurements indicate a decrease in rise time between pre and post twitches for those subjects who displayed the PAP phenomenon. This could indicate of changes in neuromuscular transmission. Thus, the PAP phenomenon in our measurements, could be said to be caused by both phosphorylation of myosin regulatory light chains and changes in synaptic transmission. In addition, the non-correlation between the rise time of the post VA twitch and serum E2-levels could be reflective of changes in Ca^{+2} kinetics at the sarcoplasmic reticulum, possibly due to the PAP phenomenon.

A negative correlation between serum E2-level and the MEP latency may indicate serum E2 mediation in synaptic transmission through the GABA signaling system. Our results indicated that the early perimenopause group generally displayed longer MEP latencies, which could indicate increased synaptic transmission speed with advanced menopause. This was however in direct contrast to the negative correlation between serum E2-level and the MEP latency, and with the general observation of decreased levels of E2 with advancing menopause. Our results show a positive correlation between MEP area and CSP duration, suggesting that higher amplitude MEPs usually lead to longer silent periods. And while the exact mechanisms of the inter-relationship between MEP and CSP remains unclear, the correlation seemed muscle dependent. Related to this, our measurement results also indicated that the increase in MEP size in the tibialis anterior, at least up to 60% MVC, was due to the recruitment of additional motor units.

As estrogen has been linked to synaptic transmission through its modulatory effect

on the neurochemical GABA signaling system, it is odd that while both groups showed a significant correlation between MEP area and CSP duration, only the early group illustrated a correlation between serum E2-level and MEP area, while the late group illustrated a correlation between serum E2-level and CSP duration.

For the peri- and postmenopause measurements the serum E2-levels for both measured subjects had significantly decreased between peri- and postmenopause, while the PSP measurement values had remained relatively stable. These results seemed to therefore suggest that the decreased serum E2-level has no function in the mechanisms effecting PSP duration. The rest of the ES measurement results were somewhat inconclusive. One subject displayed both altered muscular function and impaired neural drive, while the other illustrated results both for and against alterations in the synaptic transmission.

The peri- and postmenopause TMS measurement results for the two subjects were also somewhat inconclusive. While one subject illustrated decreased motor cortex output in response to stimulation, the other displayed results totally contradicting the previously shown correlations between the level of produced voluntary force and the MEP area and the MEP area and the CSP. It is therefore our suggestion that in order to identify possible trends in the results between peri- and postmenopause it is vital to perform additional studies with a larger cohort.

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A APPENDIX

A.1 ES

TABLE 9. Group-wise differences between early and late perimenopause groups for ES measurement variables (* = statistically significant difference at p < 0,05).

Variables	**p-value**
Voluntary activation	0,307
pre twitch rise time	0,276
post twitch rise time	0,378
pre twitch half relaxation time	0,473
post twitch half relaxation time	0,825
pre twitch average RFD	0,169
pre twitch peak RFD	0,322
post twitch peak RFD	0,072
pre twitch average rate of force relaxation	0,503
post twitch average rate of force relaxation	0,646
pre twitch peak rate of force relaxation	0,551
post twitch peak rate of force relaxation	0,325
pre twitch S-gradient	0,279
post twitch S-gradient	0,558
PAP	0,311
PAP (M-wave area)	0,140
Voluntary contraction M-wave area	0,212
pre twitch M-wave area	0,541
post twitch M-wave area	0,880
Voluntary contraction S-gradient	0,148
Voluntary contraction peak RFD	0,092
Voluntary contraction S-gradient EMG integral	0,082
post twitch maximum force	0,062

TABLE 10. Group-wise differences between early and late perimenopause groups for TMS measurement variables (* = statistically significant difference at p < 0,05).

Variables	**p-value**
CSP duration	0,475
MEP latency	0,015*
Produced force levels during task	0,053
MEP area	0,521
Maximum dorsiflexion force	0,337

TABLE 11. Electrical stimulation plantarflexion task pre and post twitch average and peak rate of force production for all groups.

	pre Twitch Average RFD [N/s]	**post Twitch Average RFD [N/s]**	**pre Twitch Peak RFD [N/s]**	**post Twitch Peak RFD [N/s]**
early	1590 ± 333	1702 ± 387	3728 ± 738	4120 ± 897
late	1442 ± 476	1439 ± 444	3472 ± 1192	3652 ± 1211
post	599 ± 108	609 ± 175	1610 ± 546	1710 ± 854

TABLE 12. Electrical stimulation plantarflexion task pre and post twitch average and peak rate of force relaxation for all groups.

	pre Twitch Average Rate of Force Relaxation [N/s]	**post Twitch Average Rate of Force Relaxation [N/s]**	**pre Twitch Peak Rate of Force Relaxation [N/s]**	**post Twitch Peak Rate of Force Relaxation [N/s]**
early	-851 ± 366	-838 ± 449	-1864 ± 549	-2039 ± 656
late	-789 ± 421	-774 ± 465	-1781 ± 592	-1868 ± 643
post	-289 ± 97	-328 ± 113	-799 ± 211	-1003 ± 227

TABLE 13. Electrical stimulation plantarflexion task pre and post twitch maximum forces and s-gradients for all groups.

	pre Twitch Max Force [N]	**post Twitch Max Force [N]**	**pre Twitch S-Gradient [N/s]**	**post Twitch S-Gradient [N/s]**
early	296 ± 60	303 ± 66	3886 ± 1007	4250 ± 908
late	262 ± 83	267 ± 81	3568 ± 1236	4054 ± 1575
post	133 ± 59	130 ± 60	2115 ± 728	2459 ± 958

TABLE 14. Electrical stimulation plantarflexion task pre and post twitch rise times and half relaxation times for all groups.

	pre Twitch Rise Time [ms]	**post Twitch Rise Time [ms]**	**pre Twitch Half Relaxation Time [ms]**	**post Twitch Half Relaxation Time [ms]**
early	159 ± 24	148 ± 26	208 ± 282	257 ± 327
late	153 ± 20	153 ± 18	181 ± 187	197 ± 171
post	161 ± 48	152 ± 39	173 ± 56	154 ± 82

TABLE 15. Maximum voluntary force, voluntary rise time and voluntary s-gradient for all groups during plantarflexion task.

	Maximum Voluntary Plantarflexion Force [N]	**Voluntary Rise Time [s]**	**Voluntary S-Gradient [N/s]**
early	869 ± 229	1,71 ± 0,57	2056 ± 999
late	632 ± 244	2,17 ± 0,86	1710 ± 801
post	679 ± 116	1,54 ± 0,12	1682 ± 446

TABLE 16. Electrical stimulation plantarflexion task pre and post and superimposed twitch M-wave area for all groups.

	pre Twitch M-wave Area [mVs]	**post Twitch M-wave Area [mVs]**	**Superimposed Twitch M-wave Area [mVs]**
early	0,0458 ± 0,0180	0,0448 ± 0,0159	0,0318 ± 0,0079
late	0,0430 ± 0,0180	0,0441 ± 0,0183	0,0306 ± 0,0123
post	0,0391 ± 0,0203	0,0390 ± 0,0199	0,0271 ± 0,0107

A.2 PAP

TABLE 17. PAP measures for early perimenopause group.

Subject number	**PAP (all trials) [%]**	**PAP from M-wave area (first trial) [%]**	**PAP from M-wave area (all trials) [%]**
1.	-4,7 ± 16,6	10,4	-8,0 ± 28,9
2.	-1,5 ± 1,7	-3,3	-1,8 ± 2,0
3.	3,9 ± 0,9	1,1	0,8 ± 2,1
4.	5,7 ± 5,4	1,3	0,7 ± 4,7
5.	-3,6 ± 7,2	1,7	17,3 ± 22,6
6.	5,4 ± 1,1	0,6	1,6 ± 0,9
7.	1,0 ± 11,5	-0,3	-2,2 ± 2,9
8.	-1,5 ± 4,3	-1,2	-2,2 ± 2,0
9.	15,5 ± 3,1	-0,8	14,2 ± 13,4
10.	4,9 ± 16,7	-1,3	-13,3 ± 14,0

Table 18. PAP measures for late perimenopause group.

Subject number	PAP (all trials) [%]	PAP from M-wave area (first trial) [%]	PAP from M-wave area (all trials) [%]
1.	-2,6 ± 3,0	-4,1	-1,1 ± 2,7
2.	-7,7 ± 9,8	3,9	3,6 ± 0,6
3.	3,5 ± 3,0	5,0	2,1 ± 2,8
4.	2,6 ± 2,5	5,8	4,3 ± 6,0
5.	12,9 ± 4,7	7,6	9,1 ± 2,2
6.	5,5 ± 5,4	0,7	1,0 ± 2,1
7.	0,6 ± 8,0	3,4	2,7 ± 1,7
8.	6,7 ± 2,2	0,5	-0,1 ± 0,8
9.	1,5 ± 3,5	4,9	3,5 ± 1,3
10.	1,3 ± 3,9	4,4	4,7 ± 6,8

Table 19. PAP measures for postmenopause group.

Subject number	PAP (all trials) [%]	PAP from M-wave area (first trial) [%]	PAP from M-wave area (all trials) [%]
7.	-4,0 ± 4,4	0,8	1,4 ± 3,1
8.	-0,9 ± 4,7	0,3	-0,7 ± 1,3

TABLE 20. Electrical stimulation plantarflexion task pre and post twitch rise times and half relaxation times for the first trial for each subject in the early perimenopause group.

Subject number	Pre Twitch Rise Time (first trial) [ms]	Post Twitch Rise Time (first trial) [ms]	Pre Twitch Half Relaxation Time (first trial) [ms]	Post Twitch Half Relaxation Time (first trial) [ms]
1.	164	157	133	1627
2.	152	130	148	109
3.	184	166	139	131
4.	170	152	108	93
5.	174	177	141	140
6.	190	184	111	742
7.	170	139	142	124
8.	191	165	213	1003
9.	114	104	159	144
10.	128	124	180	165

TABLE 21. Electrical stimulation plantarflexion task pre and post twitch rise times and half relaxation times for the first trial for each subject in the late perimenopause group.

Subject number	Pre Twitch Rise Time (first trial) [ms]	Post Twitch Rise Time (first trial) [ms]	Pre Twitch Half Relaxation Time (first trial) [ms]	Post Twitch Half Relaxation Time (first trial) [ms]
1.	129	131	138	151
2.	141	157	148	757
3.	140	133	221	158
4.	170	167	102	99
5.	150	132	131	673
6.	174	173	135	127
7.	151	134	97	123
8.	172	156	159	160
9.	154	155	140	584
10.	128	176	158	100

TABLE 22. Electrical stimulation plantarflexion task pre and post twitch rise times and half relaxation times for the first trial for each subject in the postmenopause group.

Subject number	Pre Twitch Rise Time (first trial) [ms]	Post Twitch Rise Time (first trial) [ms]	Pre Twitch Half Relaxation Time (first trial) [ms]	Post Twitch Half Relaxation Time (first trial) [ms]
7.	117	133	164	103
8.	214	193	207	310

TABLE 23. Electrical stimulation plantarflexion task pre and post twitch rise times and half relaxation times for the first trial for each subject in the early perimenopause group.

Subject number	Pre Twitch Average RFD (first trial) [N/s]	Post Twitch Average RFD (first trial) [N/s]	Pre Twitch Peak RFD (first trial) [N/s]	Post Twitch Peak RFD (first trial) [N/s]
1.	1298	1324	2754	2911
2.	1570	1783	3966	4080
3.	1671	1768	4052	4351
4.	1666	1832	3709	4722
5.	1515	1505	3795	4294
6.	1477	1592	3752	4280
7.	1119	1185	2697	2597
8.	1071	1230	2582	2725
9.	1838	2210	3553	4437
10.	1587	1972	3182	4180

TABLE 24. Electrical stimulation plantarflexion task pre and post twitch rise times and half relaxation times for the first trial for each subject in the late perimenopause group.

Subject number	Pre Twitch Average RFD (first trial) [N/s]	Post Twitch Average RFD (first trial) [N/s]	Pre Twitch Peak RFD (first trial) [N/s]	Post Twitch Peak RFD (first trial) [N/s]
1.	1222	1287	2540	2611
2.	2499	1870	5921	5279
3.	1301	1294	3125	3410
4.	2054	2220	5051	5350
5.	1418	1862	3424	4038
6.	1187	1160	2839	3182
7.	958	1113	2782	2953
8.	656	763	1555	1855
9.	1311	1211	3096	2939
10.	2057	1374	3538	3553

TABLE 25. Electrical stimulation plantarflexion task pre and post twitch rise times and half relaxation times for the first trial for each subject in the postmenopause group.

Subject number	Pre Twitch Average RFD (first trial) [N/s]	Post Twitch Average RFD (first trial) [N/s]	Pre Twitch Peak RFD (first trial) [N/s]	Post Twitch Peak RFD (first trial) [N/s]
7.	496	435	1227	913
8.	645	699	2026	2226

A.3 TMS

TABLE 26. Cortical silent period duration for different force production levels for all groups during dorsiflexion task.

	60% Max Force Silent Period [ms]	**40% Max Force Silent Period [ms]**	**20% Max Force Silent Period [ms]**
early	79,5 ± 44,2	75,1 ± 33,1	57,0 ± 28,3
late	80,9 ± 31,6	70,0 ± 34,2	65,1 ± 33,2
post	47,7 ± 9,2	45,9 ± 13,2	46,9 ± 8,9

TABLE 27. Maximum and actual % of maximum dorsiflexion force during measurements for all groups.

	Max Force [N]	**60% Max Force True Value [%]**	**40% Max Force True Value [%]**	**20% Max Force True Value [%]**
early	212 ± 45	48,4 ± 11,9	34,4 ± 10,2	18,0 ± 8,3
late	237 ± 65	56,4 ± 9,2	36,5 ± 6,8	20,7 ± 4,2
post	198 ± 65	55,2 ± 4,3	37,8 ± 4,3	21,0 ± 3,3

TABLE 28. MEP latency for different force production levels for all groups during dorsiflexion task.

	60% Max Force MEP Latency [ms]	**40% Max Force MEP Latency [ms]**	**20% Max Force MEP Latency [ms]**
early	31,9 ± 5,0	31,1 ± 5,2	31,6 ± 4,1
late	29,7 ± 6,1	30,6 ± 3,5	29,5 ± 5,8
post	31,8 ± 2,9	31,2 ± 2,4	30,5 ± 2,9

TABLE 29. MEP area for different force production levels for all groups during dorsiflexion task.

	60% Max Force MEP Area [μVs]	**40% Max Force MEP Area [μVs]**	**20% Max Force MEP Area [μVs]**
early	7,4 ± 4,0	6,2 ± 3,3	4,1 ± 2,7
late	9,0 ± 5,9	6,3 ± 5,9	4,6 ± 5,6
post	5,3 ± 3,7	3,1 ± 1,1	1,8 ± 0,7