ELECTROMECHANICAL DELAY OF THE DORSIFLEXORS IN YOUNG AND OLD WOMEN

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By

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ABSTRACT

PURPOSE: The aim of the current study was to examine the effect on electromechanical delay (EMD) in the dorsiflexors of young and old women during maximal isometric voluntary and electrically evoked contractions, and after a bout of lengthening contractions. METHODS: Nine young (25.1±1.3 years) and nine old (68.3±6.1 years) women performed baseline isometric contractions with evoked twitches followed by a series of dynamic lengthening contractions using a Biodex multi-joint dynamometer. Maximal isometric voluntary and evoked contractions were measured to assess EMD. Time points were recorded at baseline, mid-point of the intervention, post-task termination, and during recovery at 0.5, 2, 10, and 30 minutes. RESULTS: The EMD of the evoked twitches and voluntary contractions were not different in the young and old at baseline. Following the lengthening contractions the EMD of the evoked contractions at the mid-point of fatigue were shorter in the young compared to old, but not different between groups at task termination, or during recovery. There were no differences in the EMD measured from the voluntary maximal isometric contractions in the young and old at any fatigue or recovery time points. CONCLUSION: Shorter evoked EMD in the young during the mid-point of the intervention was possibly a result of potentiation which dissipated by task-termination as fatigue developed. This did not occur in the old women. Results indicate that in the dorsiflexors EMD is not affected by age in women and overall is not affected by fatigue in either group. Also recovery in both measures of EMD was not differently affected by age. Keywords: Electromechanical Delay, Lengthening Contractions, Eccentric, Women, Aging, Dorsiflexors, Fatigue, Strength
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LIST OF ABBREVIATIONS

ACh – acetylcholine
AMP – adenosine monophosphate
ATP – adenosine triphosphate
Ca$^{2+}$ – calcium ion
CC – contractile components
CSA – cross sectional area
EMD – electromechanical delay
EMG – surface electromyography
ES – effect size
FT – fast twitch
ITT – interpolated twitch technique
K$^+$ – potassium ion
MMS – mechanomyography
MU – motor units
MVC – maximal isometric voluntary contraction
$P_t$ – evoked twitch
$P_d$ – evoked doublet twitch
RMS – root mean squared
SD – standard deviation
SE – standard error
SEC – series elastic components
SR – sarcoplasmic reticulum
ST – slow twitch
TA – tibialis anterior
TRT – total reaction time
VA – voluntary activation
VC – voluntary contractions
CHAPTER 1

1.0 General Introduction

The skeletal muscle and the peripheral motor nervous systems have an intimate relationship with one another, each dependent on the other for functional survival. During a voluntary movement, from the motor cortex of the brain, electrical signals propagate along the spinal cord tracts eventually synapsing to ventral alpha motor neurons and are then conducted along the axons toward the peripheral muscles. The interface between the axon and the muscles is at the neuromuscular junction. Transmission across this specialized synapse is facilitated by the release of acetylcholine (ACh) into the synaptic cleft to bind with post-synaptic ACh receptors. When a sufficient amount of ACh is bound to these receptors, the muscle fibre will be depolarized. The wave of sarcolemma depolarization is transmitted to T-tubules where excitation-contraction coupling occurs in which the electrical signal is converted to a chemical response to initiate the process of filament sliding.

1.1 Electromechanical delay

The time between the initiation from the motor cortex and the electrophysiological response at the muscle surface varies depending on the muscle, but for the tibialis anterior (TA) is around 30-33 milliseconds (ms) (Cacchio et al., 2011) (see Fig. 1). During induced electrical stimulation in which a peripheral axon is depolarized, the time from the electrical stimulus to the onset of contraction force varies between 31 – 74 milliseconds and is basically muscle length-dependent and related to the nerve-muscle being studied (Winter & Brookes, 1991; Zhou et al., 1995). For the overall response from brain to force generation the electromechanical delay (EMD) represents a main portion of this time at the peripheral end. This peripheral component is
operationally defined as the time from the post-synaptic muscle depolarization to the onset of myofibril segmental acceleration (Grabiner, 1986) (Fig. 1) and in human skeletal muscles, the cross bridge duty cycle varies between 25 and 30 ms (Gordon et al., 2000).

**Fig. 1.** Various time delays summarized from the motor cortex to the production of force in striated muscle (Boyas & Guevel, 2011; Cacchio et al., 2011; Cooke, 1997; Eisen et al., 1984; Gordon et al., 2000; Katz & Miledi, 1965; Zhou et al., 1995). Bold characters signify factors associated with electromechanical delay portion. Adapted from (Boyas & Guevel, 2011).
It is important to differentiate total reaction time and premotor time from EMD. Total reaction time (TRT) is the overall delay from the onset of a sensory stimulus (auditory, visual, etc) to the motor response of the target muscle or a group of muscles. TRT includes sensory transmission and both the premotor time and the synaptic discharge from the central nervous system to the designated pool of motor units (MU), and the motor time. The peripheral motor time is the EMD and is the time delay of the mechanical properties of skeletal muscle to produce active force following either a change in electrical activity in the target muscle from voluntary excitation (Cavanagh & Komi, 1979; Hill, 1950; Morris, 1977; Weiss, 1965; Winter & Brookes, 1991), or an evoked electrical stimulus. For human limb muscles the EMD ranges from usually 21 to 89 ms and can be influenced by the nerve-muscle under study and importantly by various factors related to muscle activation under acute and chronic situations (Norman & Komi, 1979; Winter & Brookes, 1991; Zhou et al., 1998). A minimal level of contraction of the target muscle, before a larger muscular exertion, is expected to reduce EMD by taking up the ‘slack’ as the background muscle contraction level stretches the serial elastic component (SEC), and therefore, contribution of SEC to EMD can be studied at different levels of isometric muscle contraction (Yavuz et al., 2010). In detail, the EMD consists of electrical, chemical and mechanical processes (Fig. 1). These include (1) conduction of the action potential along the T-tubule system; (2) release of calcium by the sarcoplasmic reticulum (SR); (3) cross-bridge formation between actin and myosin filaments, thus developing tension in the contractile component (CC); and (4) stretching of the series elastic component (SEC) by CC (Cavanagh & Komi, 1979) until external force is recorded.

The current understanding in the literature indicates there may be either shortening or lengthening of EMD depending on type of contraction as the constructs of a muscle may cause a
change in maximal voluntary contraction (MVC) force and rate of force development, which are both partly dependent on muscle fibre type composition (Grabiner, 1986). Furthermore, EMD has been associated with mechanical events such as the active generation of tension between actin and myosin (Grabiner, 1986), and a significant portion of EMD has also been associated with the time necessary for the elastic elements in series with the contractile elements (SEC) to attain their load-dependent length (Hill, 1950). The mechanical delay in this component is generally referred, but not limited to structures such as the tendon, in addition to the intrinsic contractile components responsible for force production (Hill, 1950).

EMD has been most often studied with voluntary contractions (VC) which exhibit a natural pattern of motor recruitment whereas involuntary evoked contractions could be quite different (L. Grimby, 1984; Viitasalo & Komi, 1981). Zhou et al. (1995) tested EMD of the knee extensors and found significantly shorter EMD values of electrically stimulated contractions compared to VC. These differences in EMD could be explained by motor neuron conduction, muscle contractile, and myo-elastic properties or differences the motor unit recruitment patterns (Zhou et al., 1995), or some combination of the above. Motor unit recruitment level may also affect EMD as prolonged EMD may be found at lower excitation levels, primarily explained by the recruitment of slow twitch (ST) motor units; and shorter EMD at higher stimulation levels due to recruitment of fast twitch (FT) motor units (Jakobsson et al., 1988). This may explain the shorter EMD times of the maximal VCs for a specific type of contraction, however, the variation of EMD cannot be assumed to explain the basis of muscle contraction properties and the pattern of motor unit recruitment, thus making it unclear as to the specific factors involved to explain the shorter EMD values found in the involuntary electrical stimulation (Zhou et al., 1995).
1.2 Factors that could affect EMD

Human muscles fibres can be divided into three different types based on their metabolic, physiological, and structural characteristics (Essen et al., 1975) which may relate to the size principle of MU recruitment (Henneman et al., 1965). Type I fibres in humans are highly aerobic and have slow contractile velocities. Type IIb fibres are fast contracting with high actomyosin adenosine triphosphatase activity with low mitochondrial count, and are mostly anaerobic. The third usual category, Type IIa fibres act like a hybrid of the two previous fibre types displaying characteristics of both relatively fast contraction and the ability to aerobically metabolize adenosine triphosphate (ATP) utilizing its mitochondrial capacity (Rogers & Evans, 1993). It has been reported that ST fibres provide greater contribution to muscle stiffness and therefore, as FT fibres fatigue, the stiffness of the muscle will increase (Aura & Komi, 1987). However, increased muscle temperature as a by-product of muscle fatigue may also decrease stiffness and thus elongate EMD (Zhou, 1996). It is the relative combination of all the different types of muscle fibres, the metabolic processes within them, and thus contraction velocities which can influence the EMD of the whole muscle (Grabiner, 1986).

In 1996, a paper by Zhou suggested a change, either shorter or longer, in EMD would be expected when there are substantial alterations in the structural and functional properties of the muscle including the number, type of fibres, and excitation frequency of the motor units recruited from a contraction induced by either acute exercise or a long period of physical training. Although some trends were observed they (Zhou et al., 1996) found no significant differences in voluntary EMD after sprint training for 7 weeks in previously untrained men. Also, although there were no significant voluntary EMD differences between power or endurance trained athletes to untrained individuals (Kamen et al., 1981), other studies suggest
EMD is likely to be influenced by intense training through mechanical and biochemical adaptations to the neural muscle components of the system (Nilsson et al., 1977; Thorstensson et al., 1975). However, Clarkson (1978) suggested a history of physical activity and training was related to shorter EMD during voluntary contractions when comparing active elderly subjects to a non-active elderly group. There is some support for a relationship between training and EMD in that ballistic limb movements demonstrated a shorter voluntary EMD in power-trained athletes and other individuals who have a high percentage of fast twitch muscle fibres (Gabriel & Boucher, 1998). It was suggested that there were changes in mechanical factors that affected the rate of series elastic component shortening such as the initial muscle length and muscle loading (Grabiner, 1986). These components however are not quantifiable non-invasively and therefore have been estimated only using EMD. Most insights about EMD and training rely heavily on studies based on separate populations of chronically trained or non-trained individuals (Tillin et al., 2010; Zhou et al., 1996) in which the chronically trained individuals (sprinters or power lifters) have shorter EMD than the untrained population. However, endurance trained athletes have not been shown to have significant differences in EMD, possibly related to fibre type differences between power and endurance athletes.

1.2.1 Fatigue

Neuromuscular fatigue has been defined as the inability of the muscle or group of muscles to sustain the required or expected force (Bigland-Ritchie & Woods, 1984). It is the difficulty in performing at peak capacity during a physical task and the inhibition of the muscles to continue working at a given level of exercise. The mechanisms of human neuromuscular fatigue are not fully understood and seem to depend highly on the task (Bigland-Ritchie et al., 1995). Fatigue depresses force generating capacity during either a static or dynamic contraction.
and may also be an influential variable in EMD (Yavuz et al., 2010). Possible mechanisms related to fatigue development that may affect EMD include an impairment of action potential propagation along the muscle membrane due to the large potassium ion (K\(^+\)) efflux from contracting muscle cells, a reduction in the tetanic cytosolic calcium concentration due to reduced calcium ion (Ca\(^{2+}\)) release from SR, a reduction in myofibril sensitivity to Ca\(^{2+}\), as well as direct inhibitory effects of phosphate and hydrogen ions on force generation (Cavanagh & Komi, 1979; Zhou et al., 1998). Therefore, any sites that influence muscle fibre conductivity, contractility, or elasticity may alter EMD during acute prolonged activity.

It has been observed that the greater the muscular fatigue, the longer the EMD (Yavuz et al., 2010). With fatigue, t-tubule conduction velocity has been shown to be much slower and would be dramatically slower during fatigue because of an accumulation of K\(^+\) and depletion of Na\(^+\) ions in the t-tubule space due to repeated membrane excitation (McKenna, 1992). This impairment of action potential propagation may explain the prolongation of EMD during fatigue due to the K\(^+\) efflux into the t-tubules during long-standing contraction. In this case, action potentials cannot reach the depth of the muscle and hence Ca\(^{2+}\) release is reduced, which then slows the rate of force development during the contraction (Yavuz et al., 2010). The work of Zhou et al. (1996) suggest that a change in EMD would not be due to central fatigue, because EMG was unchanged while peak force production was substantially reduced.

Despite these ideas, there are discrepancies in changes in EMD with fatigue. According to Zhou et al. (1996), fatigue should prolong EMD which could be attributed to impaired muscle conduction, contractile or elastic properties, as well as increased temperature of the muscle. Increased temperature due to exercise may cause the structural components that affect EMD to become more elastic. Morris (1977) tested the knee extensors using resisted and non-resisted in
isometric and isotonic movements, but found no change in EMD after fatigue despite strength decrements of up to 24%. In another study, after a 38% decline in force following an unresisted task, EMD was longer by ~5% (Wood, 1979). Furthermore, a decrement of relative force in the shoulder flexors after a fatiguing protocol with a maximum torque decline of up to 45% of MVC resulted in EMD elongation of up to 35-45% from pre-exercise values (Sheerer & Berger, 1972). However, studies by Kroll (1974) failed to see any changes associated with EMD in knee extension after force decreased 24%. Cavanagh and Komi (1979) investigated EMD of the elbow flexors and reported no significant differences between fatiguing isometric and concentric contractions, but eccentric contractions resulted in shorter EMD (see below). Therefore, discrepancies in EMD changes seem to be dependent on the type of task and function of muscle groups involved. Overall for isometric and concentric contractions fatigue results in either no change or a lengthening in EMD. Any EMD lengthening with fatigue must result from some combination of changes in excitation-contraction coupling components or the stretching of the SEC (Zhou et al., 1996). Because both muscle force and the active component of SEC depend upon the number of cross-bridges attached, it has been reported that the stiffness of the active component of SEC increases with an increase in muscle tension (Aura & Komi, 1987).

However, the interplay of various factors relating to muscle stiffness and their effect on EMD seem to be not well-understood.

1.2.2 Potentiation

Muscle performance can be enhanced immediately following a strong voluntary or tetanic contraction. This is referred to as post-activation potentiation (PAP) and is usually explained by phosphorylation of myosin regulatory light chains (Grange et al., 1995), leading to a greater cross-bridge recycling rate (due to an increased sensitivity to Ca^{2+} by the contractile
proteins) thus increasing twitch force and its rate of force development (Metzger et al., 1989). Neuromuscular fatigue, discussed above, can be described as a decrease in force generation capacity after a period of repeated muscle activation (Rassier & Macintosh, 2000). Therefore fatigue can compete and has been shown to acutely suppress the effects of PAP induced by isometric contractions (Gossen & Sale, 2000). However PAP recovers usually within 15 seconds post-MVC (Hodgson et al., 2005), and in the quadriceps has been shown to improve (shorten) EMD by enhancing muscle activation (Minshull et al., 2007; Sahlin & Seger, 1995) Thus, fatigue can coexist with PAP which may confound changes in measures of EMD during activity.

1.2.3 Lengthening Contractions

From the onset of exercise, lengthening (eccentric) contractions in addition to instilling fatigue can cause muscle damage, a loss of peak voluntary force production, a reduction in the range of motion, disturbance in the muscular structures and proteins, and muscle soreness (Byrnes et al., 1985; Clarkson & Tremblay, 1988; Hesselink et al., 1996). It is assumed the decrement of force after lengthening contractions is mainly related to the amount of structural damage (Hesselink et al., 1996). Furthermore, fatigue-induced muscle damage due to mechanical stress from inhomogeneities within in the sarcomere may be important factors of influence on EMD especially with eccentric contractions. This results in increased passive tension to non-contractile structures in the myofibres, causing structural deformation (Morgan & Proske, 2006). In addition, the secondary response following muscle damaging (lengthening) contractions is a response of the loss of Ca\(^{2+}\) homeostasis and subsequent exacerbation of damage to the muscle (Yasuda et al., 1997). As seen in voluntary contractions, studies show a reduced ability to generate force with electrically evoked contractions which is considered to be due to the damaged SR. Along with this, swelling of the muscle may induce a shortening of the
damaged muscle but another factor may be an abnormal increase of Ca$^{2+}$ surrounding the cells (Clarkson & Tremblay, 1988). The abnormal accumulation of Ca$^{2+}$ caused by damaged cells or damaged SR may spontaneously activate contractions eliciting a shortened state (Brody, 1969). From the above it is conceivable to expect EMD to be longer after lengthening contractions but it may depend on the time course of immediate versus several hours. For example, increased swelling along with the increased Ca$^{2+}$ may cause shortening of the EMD perhaps due to greater tension on the SEC and greater availability of Ca$^{2+}$ for excitation. Unfortunately there are very few studies that have explored EMD after lengthening contractions (see Table 1).

### 1.3 Aging

There is a natural neurodegenerative process which occurs with age, constantly changing the motor unit (MU) organization and function (Doherty et al., 1993). There are markers of skeletal muscle activity via enzymes which are measured to quantify the effect of aging on the metabolic capacity. The main metabolic pathways involved in muscle activation are; glycolysis, β-oxidation, and ATP synthesis. The literature concerning the effects of aging on glycolysis and glycolytic enzyme concentrations is mixed (Grimby & Saltin, 1983). However, with respect to oxidative enzymatic capacity, there seems to be a decline of about 25% capacity with age (Rogers & Evans, 1993). Aging has been found to have minimal effect on the concentration levels of high-energy phosphate compounds in the muscle such as resting ATP, adenosine monophosphate (AMP), and the energy charge of the cell (Moller et al., 1980). Structurally, with increasing age, the skeletal muscles of the human body undergo changes which generally result in a reduction of muscle volume and consequently strength (Vandervoort & McComas, 1986). Neuromuscular reviews on aging (Grimby & Saltin, 1983; Rogers & Evans, 1993; Vandervoort, 2002) suggest a reduction in all fibre types with age prominent especially after the
7th decade of life, and with a trend towards a greater loss of Type IIb fibres and a preservation of Type I fibres. This reduction in types of muscle fibres may be due to the size of the muscle fibres themselves and not the selective reduction of Type IIb fibres (Grimby & Saltin, 1983). Several other studies have shown preferential atrophy in Type IIb and IIa fibres which may exaggerate the aging process, thus effecting the overall cross sectional area (CSA) of skeletal muscle and reduction of strength with age (Grimby & Saltin, 1983; Larsson, 1978).

Another view on strength loss with aging comes from the literature on the CSA of the muscle (Allman & Rice, 2002; Rogers & Evans, 1993; Vandervoort, 2002). It is well established that significant losses of maximal force development will occur with aging (Allman & Rice, 2002; Cunningham et al., 1987; Larsson, 1978; Power et al., 2012) although there are variabilities in the rate of loss amongst the different muscle groups. Larsson (1978) found age-related changes in maximal isometric and dynamic muscle strength with reductions between 50 and 70 years of age, ranging from 24-36%. They found the participants of age 60 years had a selective muscle fibre atrophy of 36% smaller Type II fibre area than the participants 40 years of age. The capacity to generate force per unit of CSA was lower in older subjects (Allman & Rice, 2002; Rogers & Evans, 1993; Vandervoort, 2002). Moreover, studies on women (Young, Stokes, & Crowe, 1984) suggest there were differences in the intrinsic strength of the older women as there were no significant differences in the CSA between the young and old, however a significant 35% reduction in force in the old compared to the young was observed. Isokinetic strength of the knee and elbow flexors and extensors were measured in 200 men and women to determine the relationship between muscle mass and strength with aging (Frontera et al., 1991). They found both older men and women were significantly weaker than their young counterparts.
and concluded that the loss of muscle is a major factor in the age-related loss of muscle strength instead of a deterioration in the contractile capacity of the muscle.

Important to my thesis is the tibialis anterior as the main contributor (~60%) to dorsiflexion strength and CSA (Fukunaga et al., 1996) which has been observed to undergo neuromuscular remodelling, atrophy, and thus becoming significantly weaker with age (Connelly et al., 1999; Vandervoort & McComas, 1986). Connelly et al. (1999), observed reduced torque production, and slower contractile properties in old men, compared to those of young adults. These findings of age-related muscle characteristics have also been found by Vandervoort and McComas (1986), for the ankle dorsiflexors in both men and women. Reports of decreased numbers of motor units in the TA, and significantly reduced MVC in the very old (men in their 80s) but not old (men in their 60s), suggesting a progressive loss of MU do not reach a reduction of functional significance (i.e., cause a reduction in strength) until a critical threshold (McNeil et al., 2005). This is due to the denervation-reinnervation process of age-related MU remodelling, where cell death will orphan the muscle fibres it was connected to and the neighbouring MU will undergo collateral reinnervation (Gordon et al., 2004) thus creating larger MUs responsible for a greater number of muscle fibres in the total MU pool (Larsson & Ansved, 1995).

In addition, the reduced number of FT fibres and an increased ST fibre composition have been shown to increase muscle tendon stiffness (Aura & Komi, 1987; Vandervoort, 2002). The age-related morphological modification of the musculotendinous system effects the tendinous properties of the parallel elastic component (PEC) which is the main contributor of passive tension, and also SEC which is responsible in the role of the stretch-shortening cycle in both eccentric and concentric contractions (Valour & Pousson, 2003). It has been observed that ST fibres have increased stiffness and with aging, as the overall proportion of FT fibres is less than
for young adults this may contribute to an increase in overall muscle stiffness (Vandervoort, 2002). Furthermore, Cook and McDonagh (1996) report a slower Ca$^{2+}$ recycling rate in the ST fibres, which prolongs tension compared to the FT fibres. Because of age-related alterations in various portions of the neuromuscular system it is not clear how EMD might be affected overall because some changes may tend to prolong EMD whereas changes to other factors may tend to shorten EMD. Thus, despite the many studies that have explored changes to the neuromuscular system with adult aging, few have explored changes in EMD (see Table 1). Longer EMD times have been reported for the knee extensors (Zhou et al., 1995), but other studies in the knee extensors reported no significant differences (Clarkson, 1978; Weiss, 1965). Another recent report showed a trend for older men to have longer EMDs in the quadriceps muscle of men (Conchola et al., 2013).

1.4 Sex

Most studies of EMD have been focused on healthy, relatively young males likely as samples of convenience and, to provide homogeneity of the sample by avoiding the possible differential or protective effects of estrogen (Howatson, 2010) in women on muscle function especially during damaging contractions. In addition, muscle pennation angle, mass, and force output is known to be greater in men (Abe et al., 1998; Maughan et al., 1983). Amongst these, there are other differences between the sexes such as greater tendon elasticity in women which may affect (prolong) EMD. For example, studies on tendon elasticity between men and women found the women have significantly lower stiffness and hysteresis than men (Kubo et al., 2003), and Winter and Brookes (1991) report significant differences in EMD between women (~45 ms) and men (~40 ms) of the plantar flexors supporting perhaps more elasticity in women compared with men. There are however, numerous studies comparing both sexes and much like fatigue,
there are discrepancies indicating either no significant differences (Morris, 1977; Yavuz et al., 2010), or longer EMD values in women compared to men (Bell & Jacobs, 1986; Winter & Brookes, 1991). One possible problem with these studies is that physiological cross sectional areas and rates of torque development (both greater in men) were not taken into account when making comparisons between men and women. (Bell & Jacobs, 1986).

1.5 Voluntary vs. Evoked Measures of EMD

There are two different methods of testing EMD of the human muscle. Voluntary contraction allows maximal and submaximal contractions to be tested with or without joint movements. In addition, voluntary measurements allow normal neuromuscular motor unit activation strategies to operate to grade force output instead of simultaneous axonal activation of all motor units when using electrically evoked stimulation as used in the second method. Evoked contractions allow a more direct focus on biochemical mechanisms such as EC coupling, Ca$^{2+}$ and other ion activity, and voluntary contractions are more representative of the integrated system under normal activities. Neither method can separate the effect of the mechanical factors related to non-contractile aspects of EMD (Behm & Sale, 1994). The choice of method or combination of method is not always explained or rationalized, but likely depends on the question and application. Far fewer studies have used both methods in the same study, a review of the literature (Table 1) has been created below to summarize the literature on EMD in lower limb muscles comparing males and females using various methods. Most studies evaluated EMD using isometric or concentric contractions induced either electrically or voluntarily. It can be noted that only two studies explored eccentric actions in males using induced stimulation.
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<td>Nordez et al., 2009</td>
<td>9 males</td>
<td>Electrical stimulation</td>
<td>Gastrocnemius Medialis</td>
<td>Isometric</td>
<td>N/A</td>
</tr>
<tr>
<td>Grosset et al., 2009</td>
<td>20 males, 10 females</td>
<td>Electrical stimulation</td>
<td>Soleus</td>
<td>Isometric</td>
<td>N/A</td>
</tr>
<tr>
<td>Blackburn et al., 2009</td>
<td>20 males, 20 females</td>
<td>Reaction experiment</td>
<td>Biceps Femoris</td>
<td>Isometric</td>
<td>Hamstrings stiffer in males. No difference in EMD</td>
</tr>
<tr>
<td>Minshull et al., 2007</td>
<td>7 males, 9 females</td>
<td>Supramaximal magnetic stimulation</td>
<td>Biceps Femoris</td>
<td>Isometric</td>
<td>Females &lt; Males</td>
</tr>
<tr>
<td>Minshull et al., 2012</td>
<td>9 males</td>
<td>Supramaximal magnetic stimulation</td>
<td>Biceps Femoris + Vastus Lateralis</td>
<td>Eccentric + isometric</td>
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<tr>
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<td>Isometric</td>
<td>N/A</td>
</tr>
<tr>
<td>Moore et al., 2002</td>
<td>15 males, 15 females</td>
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<td>Vastus Lateralis</td>
<td>Concentric</td>
<td>Females &lt; Males pre fatigue Males &lt; Females post fatigue</td>
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<tr>
<td>Zhou et al., 1998</td>
<td>4 males, 3 females</td>
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<td>Vastus Lateralis + Rectus Femoris</td>
<td>Isometric</td>
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<td>7 males, 4 females</td>
<td>Voluntary contraction + electrical stimulation</td>
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<td>Isometric</td>
<td>N/A</td>
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<tr>
<td>Zhou et al., 1995</td>
<td>16 males, 5 females</td>
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<td>Vastus Lateralis + Rectus Femoris</td>
<td>Isometric</td>
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</tr>
<tr>
<td>Study</td>
<td>Sex</td>
<td>Type of Task</td>
<td>Muscle Groups</td>
<td>Type of Contraction</td>
<td>Outcome</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>--------</td>
<td>------------------------------</td>
<td>--------------------------------</td>
<td>---------------------</td>
<td>------------------------------</td>
</tr>
<tr>
<td>Zhou et al., 1996</td>
<td>6 males</td>
<td>Voluntary contraction</td>
<td>Quadriceps, Femoris</td>
<td>Isometric</td>
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</tr>
<tr>
<td>Winter &amp; Brookes, 1991</td>
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<td>Reaction experiment</td>
<td>Soleus</td>
<td>Isometric</td>
<td>Males &lt; Females</td>
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<tr>
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<td>Quadriceps</td>
<td>Isometric + Concentric</td>
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<td>Knee extensors</td>
<td>Isometric</td>
<td>N/A</td>
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<tr>
<td>Mora et al., 2003</td>
<td>13 males</td>
<td>Electrical stimulation</td>
<td>Peroneus, Longus + Tibialis Anterior + Soleus</td>
<td>Walking</td>
<td>N/A</td>
</tr>
<tr>
<td>Linford et al., 2006</td>
<td>11 males</td>
<td>Reaction experiment</td>
<td>Peroneus</td>
<td>Walking</td>
<td>No difference</td>
</tr>
<tr>
<td>Granata et al., 2000</td>
<td>11 boys</td>
<td>Voluntary contraction + Reflex</td>
<td>Quadriceps + Medial Hamstrings</td>
<td>Isometric + Concentric</td>
<td>N/A</td>
</tr>
<tr>
<td>Chung et al., 2005*</td>
<td>15 males</td>
<td>Voluntary contraction + Electrical stimulation</td>
<td>Tibialis Anterior + Soleus + Gastrocnemius</td>
<td>Isometric</td>
<td>N/A</td>
</tr>
<tr>
<td>Nilsson et al., 1977</td>
<td>12 males</td>
<td>Voluntary contraction</td>
<td>Vastus Lateralis</td>
<td>Concentric</td>
<td>N/A</td>
</tr>
<tr>
<td>Tillin et al., 2010</td>
<td>19 males</td>
<td>Voluntary contraction</td>
<td>Quadriceps + Biceps Femoris</td>
<td>Isometric</td>
<td>N/A</td>
</tr>
<tr>
<td>Kubo et al., 2001</td>
<td>8 males</td>
<td>Voluntary contraction</td>
<td>Quadriceps + Biceps Femoris</td>
<td>Isometric</td>
<td>N/A</td>
</tr>
<tr>
<td>Study</td>
<td>Participants</td>
<td>Contract Type</td>
<td>Muscle(s)</td>
<td>Contraction Type</td>
<td>Outcome</td>
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<td>------------------------------</td>
<td>--------------</td>
<td>---------------</td>
<td>--------------------</td>
<td>------------------</td>
<td>---------</td>
</tr>
<tr>
<td>Morse et al., 2005*</td>
<td>18 males</td>
<td>Voluntary +</td>
<td>Gastrocnemius</td>
<td>Isometric</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Electrical</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yavuz et al., 2010*</td>
<td>15 males</td>
<td>Voluntary +</td>
<td>Soleus</td>
<td>Isometric</td>
<td>No difference</td>
</tr>
<tr>
<td></td>
<td>15 females</td>
<td>Electrical</td>
<td></td>
<td></td>
<td>Increase in EMD with increase in age</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Passive</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>stretching</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Costa et al., 2010</td>
<td>16 males</td>
<td>Electrical +</td>
<td>Soleus + Gastrocnemius</td>
<td>Isometric + Eccentric</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Passive</td>
<td>Medialis</td>
<td></td>
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<td></td>
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<td>stretching</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Clarkson, 1978</td>
<td>60 males</td>
<td>Reaction</td>
<td>Rectus Femoris</td>
<td>Concentric</td>
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<td></td>
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<tr>
<td>Nordez et al., 2009</td>
<td>9 males</td>
<td>Electrical</td>
<td>Soleus + Gastrocnemius</td>
<td>Isometric</td>
<td>N/A</td>
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<tr>
<td></td>
<td></td>
<td>stimulation</td>
<td>Medialis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wood, 1979</td>
<td>18 males</td>
<td>Reaction</td>
<td>Tibialis Anterior</td>
<td>Isometric +</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>experiment</td>
<td></td>
<td>Concentric</td>
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</tr>
<tr>
<td>Sharma et al., 2011</td>
<td>9 males</td>
<td>Electrical</td>
<td>Quadriceps</td>
<td>Isometric</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>1 female</td>
<td>stimulation</td>
<td></td>
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</tbody>
</table>

Reaction experiment describes voluntary contractions in response to a specified sensory signal. Reflex describes involuntary contractions in response to a stimulus. + represents in addition to. For example, concentric in addition to isometric contractions. < represents shorter EMD time. For example, males < females indicates, males had shorter EMD time compared to females. N/A denotes no data available. * studies that compared young and old.
CHAPTER 2

2.0 Introduction

In a given muscle contraction, there is a time delay from the onset of the detectable electrical signal, either electrically induced, or neural activation from a voluntary contraction (EMG), to the onset of torque production. This is referred to as the electromechanical delay (EMD) (Cavanagh & Komi, 1979). Although similar features of EMD may be explored from either voluntary or evoked contractions, the neural excitation of the muscle is not the same. For evoked, all motor units are synchronously activated whereas for voluntary contractions normal recruitment and rate coding mechanisms of force gradation are utilized. This methodological issue is not well-explored and various studies have used one or the other method, and thus comparisons or commonalities from the literature are difficult to make. In addition to the measurement method, EMD can be influenced by many factors which include (1) conduction of the action potential along the T-tubule system; (2) release of calcium by the sarcoplasmic reticulum (SR); (3) cross-bridge formation between actin and myosin filaments, thus developing tension in the contractile component (CC); and (4) stretching of the series elastic component (SEC) by CC (Cavanagh & Komi, 1979) until external force is recorded. Although this delay period is well-described, few studies have explored possible changes in EMD with adult aging or during lengthening contractions, and most studies have focussed on male subjects (see Table 1). EMD may provide insight to post-synaptic events that affect the function of the active and passive properties of the skeletal muscle system following an intervention (Howatson, 2010).

Despite the well-known alterations that occur in an aged neuromuscular system (Vandervoort, 2002), few studies have explored EMD with adult aging. Although some have
reported on changes in EMD at baseline and following fatigue (see Table 1), not one study has explored this factor after lengthening contractions which offer a unique fatiguing task that may also induce muscle damage. Measures also during a period of short term recovery may help explain any changes observed with fatigue. Thus, the purpose of this investigation was to assess the electromechanical delay of the dorsiflexors during maximal isometric voluntary and electrically evoked contractions before, during, and following a brief recovery period of a fatiguing protocol consisting of lengthening contractions in the young and old women. Because of presumed age-related remodelling of the neuromuscular system and changes in musculotendinous elastic properties that occur with adult aging it was hypothesized that baseline EMD will not differ between the young and old women; likely due to the competing changes in the active and passive portions of the system. That is, age-related remodeling features may tend to lengthen EMD, but age-related increases in musculotendinous stiffness may tend to shorten EMD in both voluntary and evoked measures with consequentially no net change. During fatigue EMD will be lengthened more in the old, and recovery of EMD to baseline values will be delayed in the old women compared with the young. These differences during fatigue and recovery will arise because: 1) metabolic recycling rates will be depressed more in old after fatigue due to their presumed lower numbers of FT fibres which are known to quickly replenish Ca\(^{2+}\), K\(^{+}\), and Na\(^{+}\) among other substrates (Grimby & Saltin, 1983) and 2) the blunted oxidation capacity during fatigue associated with aging further limiting available muscle fibres to contract (Rogers & Evans, 1993). The dorsiflexor model was used because there is much neuromuscular information available on this muscle group in adult aging and the accessibility of the fibular nerve is well-suited for evoked measures.
CHAPTER 3

3.0 Methods

3.1 Subjects

Nine young (25.1±1.3 years) and nine old (68.3±6.1 years) women from the university population and local community groups, who were free from musculoskeletal disorders and recreationally active, volunteered for this study. The mean height and mass of the old and young women were 162.0±7.3 cm and 67.7±8.5 kg, and 167.1±7.0 cm and 63.7±10.4 kg, respectively. All participants were asked to refrain from strenuous activity 1 day prior to testing and to avoid caffeine consumption on the testing day. This study was approved by the local University’s Review Board for Health Sciences Research Involving Human Subjects and conformed to the Declaration of Helsinki. Informed oral and written consent was obtained from all participants prior to testing.

3.2 Experimental Arrangement

All testing was conducted on a Biodex multi-joint dynamometer (System 3, Biodex Medical Systems, Shirley, New York). The right foot was strapped tightly to the Biodex ankle attachment footplate, aligning the lateral malleolus with the rotational axis of the dynamometer. Extraneous movements were minimized using non-elastic shoulder, waist, and thigh straps. Participants sat in a slightly reclined position with the hip, knee, and ankle angles set at ~110°, ~140°, and ~30° plantar flexion; thus, both dynamic actions moved through a 30° range of motion.

Surface electromyography (EMG) was collected from the TA and soleus muscles using self-adhering Ag-AgCl electrodes (1.5 × 1 cm; Kendall, Mansfield, MA). The skin was cleaned
forcefully with an alcohol swab prior to the application of the electrodes. A monopolar electrode configuration was used with the active electrode positioned on the proximal portion of the TA over the innervation zone (~7cm distal to the tibial tuberosity and ~2 cm lateral to the tibial anterior border) and a reference electrode placed over the distal tendinous portion of the TA at the malleoli.

Stimulated contractions of the dorsiflexors were electrically evoked using a bar electrode held distal to the fibular head over the deep branch of the common fibular nerve. A computer-triggered stimulator (model DS7AH, Digitimer, Welwyn Garden City, Hertfordshire, UK) set at 400 V provided the electrical stimulation using a pulse width of 50-100 µs.

3.3 Experimental Procedure

Peak twitch torque ($P_t$) was determined by increasing the amplitude of the current until a plateau in M-wave amplitude was reached (30-95 mA), followed by a further 10-15% increase in current to ensure supramaximal stimulation. This stimulation intensity was the same one used for evoked doublet stimulation ($P_d$), 2 pulses with a 10-ms interpulse interval) to assess voluntary activation.

Three MVCs of 3- to 5-s duration were then performed. Three minutes of rest was given between all contractions to avoid fatigue. Participants were provided with visual feedback of the torque via near real-time display and verbally exhorted during all voluntary contractions. Voluntary activation was assessed for all MVCs with the modified interpolated twitch technique (ITT) (Hales & Gandevia, 1988). The amplitude of the interpolated torque evoked during the MVC was compared with a resting twitch doublet torque evoked ~1 s after the MVC. Percent voluntary activation was calculated as:

Voluntary activation (%) = \[1 – (\text{interpolated twitch doublet}/\text{resting twitch doublet})]\ × 100.
Baseline potentiation was calculated using the ratio between the amplitude of the peak twitch torque recorded before and following the isometric MVC. Values from the peak MVC were used for data analysis.

3.4 Fatigue and Recovery Testing Design

Participants performed 5 sets of 30 eccentric dorsiflexion contractions separated by 30 s rest and performed with a load set at 80% MVC. Pilot testing showed 80% to be a compromise between very rapid fatigue and a sufficient contraction intensity to permit several contraction cycles to occur before achieving task failure. Participants were provided with visual feedback of velocity and instructed to resist while lowering the footplate through the 30° range of motion over a 1 s period. The foot was then returned to the neutral ankle position by the investigator over a period of 2 s. The voluntary and electrically evoked responses of the dorsiflexors were recorded at baseline, during the fatigue protocol, immediately after each of the five sets, and throughout the recovery period at 0.5 min, 2 min, 10 min, and 30 min (Fig. 2).
**Fig. 2.** Schematic representation of the experimental protocol. Gray bars are maximum isometric voluntary contractions (MVC). Open profiles are electrically evoked contractions [twitches (small triangles), doublets (large triangles)]. Filled black profile is the dynamic eccentric contraction protocol at 80% MVC. Recovery time points: post task termination, 0.5, 2, 10, and 30 min. Modified from Power et al., (2011).
3.5 Electromechanical Delay Assessment

The EMG of the TA was assessed offline using Spike 2 software (version 7.10, Cambridge Electronic Design Ltd.). To quantify the EMG, the raw EMG channel was converted to an RMS signal using a time constant of 0.02 s. The electromechanical delay was defined as the time difference between the onset of EMG and the onset of torque production (see figure 3). To determine the onset of EMG, vertical cursors were placed 50 ms – 1 s prior to target recording and prior to the main onset of EMG activity to measure the mean resting EMG. A threshold value of 2 standard deviation (SD) above the mean resting activity was used to determine the onsets of EMG activity and torque production (Howatson, 2010). The onset of electrical activity was defined at the first point in time to cross the horizontal cursor placed at the 95% confidence limits (2 SD) of the EMD channel. The onset of torque production was defined at the first point in time to cross the horizontal cursor placed at the 95% confidence limits (2 SD) of the torque channel (Fig. 3). Evoked contractions were analyzed similarly by using the large deflection of the stimulus artifact on the EMG channel as the starting point. (Howatson, 2010).
Fig. 3. Raw schematic representation of measuring EMD from an isometric voluntary maximal contraction. The inset to the left shows the MVC with an interpolated doublet.
3.6 Data Reduction and Analysis

Torque and position data were sampled at a rate of 100 Hz. All data were converted to digital format with a 12-bit analog-to-digital converter (model 1401 Plus, Cambridge Electronic Design, Cambridge, UK). Surface EMG signals were preamplified (×100), amplified (×2) and band-pass filtered (10-1,000 Hz), and sampled online at 2,500 Hz using Spike 2 software (Version 6.10, Cambridge Electronic Design Ltd.). Surface EMG from the MVC was expressed as root mean squared (RMS) and values were obtained from a 1 s time period about the peak torque. All subsequent MVC RMS values were normalized to the level obtained during baseline. Post-activation potentiation was determined by calculating the ratio between the amplitude of the peak twitch torque recorded before and following the isometric MVC. Spike 2 software was used off-line to determine peak twitch torque ($P_t$), peak doublet torque ($P_d$), maximal isometric voluntary contraction torque (MVC), and electromechanical delay (EMD).

3.7 Statistical Analysis

The SPSS software (version 16, SPSS Inc. Chicago, IL) was used for a two-way (age × time) repeated measures analysis of covariance to assess all neuromuscular data. The voluntary activation values were not normally distributed so a Mann–Whitney $U$ test was used, and an unpaired $t$ test was applied for subject characteristics and baselines measures to assess group differences. The level of significance was determined to be $P<0.05$. When there was a significant main effect or interaction, a post hoc analysis using unpaired $t$ tests was employed using a modified Bonferroni correction factor using the Holm’s procedure to determine where the significant differences existed. The tables are presented as a means ± SD and figures as a means ± SE values, normalized to baseline (pretest).
CHAPTER 4

4.0 RESULTS

4.1 Baseline Measures

As shown in Table 2, the old (68.3 ± 6.1 years) women had lower MVC torque (P=0.02) than young (25.1 ± 1.3 years), but voluntary activation (VA) was high at 98-99% in both groups and there were no significant differences in VA (P=0.68) or doublet twitch torque ($P_d$) (P=0.69) between groups. Evoked twitch potentiation was greater for the young (124.6 ± 17.2%) compared to the old (105.8 ± 6.0%). As mentioned in methods, there were no differences in height and weight between the young (167.1 ± 7.0 cm, 63.7 ± 10.4 kg), and old (162.0 ± 7.3 cm, 67.7 ± 8.5 kg) women.

4.2 Fatigue and Recovery Measures

All participants completed all contractions and finished the protocol. For both groups, the fatiguing protocol consisted of 150 lengthening contractions split into 5 sets (30 contractions per set). For dorsiflexion isometric MVC, there was a main effect only for time (P<0.05). Torque decreased similarly by ~19% for both young and old women following the first set of 30 lengthening contractions, and following each set, continued to decrease until it was reduced to ~28% at End (task termination) (see Figure 4A). At 30 min recovery, both the young and old women regained 9% of their post-fatigue MVC but were still significantly lower than baseline (Fig. 4A) indicative perhaps of muscle damage. Voluntary activation did not change throughout the fatigue and recovery protocol for both young and old (P=0.91). $P_d$ torque decreased ~20% in the young and ~30% in the old at task termination and was reduced equally in both groups by
~40% throughout the 30-min recovery period (Fig. 4B). For $P_d$ torque, there was only a significant effect for time ($P<0.05$).

4.3 EMD Measures

There were no significant differences in the EMD of the voluntary MVC between young and old (Fig. 5A) ($P>0.05$) at any time points during the protocol. Thus, for young and old respectively values at baseline were (75 ± 4 ms, 79 ± 6 ms). During the lengthening contractions; mid-point (65 ± 3 ms, 76 ± 5 ms); at task termination (End) (69 ± 3 ms, 77 ± 5 ms), and during recovery time points; 0.5 min (70 ± 4 ms, 75 ± 4 ms), 2 min (75 ± 6 ms, 78 ± 6 ms), 10 min (80 ± 5 ms, 85 ± 4 ms), and 30 min (76 ± 4 ms, 80 ± 5 ms) ($P>0.05$). For figure 5, raw EMD measures are presented in panel A and were normalized as a function of the value at 100% MVC at baseline for panel B.

For EMD of the evoked contractions, there were no significant differences in the young and old at baseline (58 ± 4 ms, 59 ± 3 ms), but at mid-point the young had shorter (51 ± 2 ms) $P_d$ EMD ($P<0.05$) with a shortening of EMD of ~16% from baseline but not in the old (59 ± 3 ms) $P_d$ EMD ($P>0.05$) (Fig. 6A, B). At task termination (End) there were no differences between young and old (55 ± 2 ms, 56 ± 3 ms) and during recovery; 0.5 min (58 ± 2 ms, 55 ± 3 ms), 2 min (59 ± 2 ms, 60 ± 3 ms) 10 min (58 ± 2 ms, 61 ± 3 ms), and 30 min (61 ± 3 ms, 60 ± 3 ms). For figure 6, raw EMD measures are presented in panel A and were normalized as a function of the value at 100% MVC at baseline for panel B.
Table 2. Baseline strength and EMD values

<table>
<thead>
<tr>
<th>Groups</th>
<th>MVC (N m)</th>
<th>MVC EMD (ms)</th>
<th>( P_t ) (N m)</th>
<th>( P_d ) (N m)</th>
<th>( P_d ) EMD (ms)</th>
<th>VA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young (9)</td>
<td>34.0 ± 4.9</td>
<td>75 ± 12</td>
<td>4.0 ± 0.9</td>
<td>9.1 ± 2.7</td>
<td>58 ± 1.2</td>
<td>99.2 ± 0.9</td>
</tr>
<tr>
<td>Old (9)</td>
<td>28.2 ± 4.9*</td>
<td>79 ± 17</td>
<td>3.9 ± 1.6</td>
<td>8.6 ± 2.3</td>
<td>59 ± 1.0</td>
<td>98.9 ± 2.1</td>
</tr>
</tbody>
</table>

* Denotes significant difference between young and old women. MVC is maximum voluntary contraction, \( P_t \) is twitch torque, \( P_d \) is evoked doublet twitch, and VA is voluntary activation.
Fig. 4. Changes in maximal voluntary isometric contraction (MVC) normalized to 100% of baseline (PRE) values (A), and evoked doublet torque ($P_d$) normalized to baseline (PRE) values (B). Young (solid symbols) and old (open symbols). R indicates recovery time points in minutes. Significant effects for time (*$P<0.05$). There were no age effects detected. Values are means ± SE.
Fig. 5. Maximal isometric voluntary contraction EMD in ms during fatigue and recovery (A). Maximal voluntary isometric contraction (MVC) EMD normalized to baseline (PRE) values (B) for young (solid symbols) and old (open symbols) women. No significant time or group effects were detected. Values are means ± SE.
Fig. 6. Changes in evoked doublet twitch ($P_d$) EMD in ms (A), and evoked doublet twitch ($P_d$) EMD normalized to baseline (B) for young (solid symbols) and old (open symbols) women. R indicates recovery time points in minutes. Significant effects for time and age in the young group at one point (†$P<0.05$). Values are means ± SE.
CHAPTER 5

5.0 DISCUSSION

This study was designed to test whether the electromechanical delay would differ when evaluated during either a voluntary or electrically stimulated activation, between young and old women following a lengthening contraction protocol, and throughout a period of short term recovery. It was hypothesized that there will be no change in EMD with age in both voluntary and evoked measures at baseline. During fatigue, EMD will be lengthened more in the old and recovery of EMD to baseline values will be delayed in the old women. In accordance to my expectations, the maximal voluntary contraction EMD and evoked EMD were not different between young and old women at baseline, but unlike my hypothesis, after fatigue and recovery there were no changes or age-related differences in these parameters whether assessed from the MVC or evoked contractions. However, it was observed that the young women had a shortened $P_d$ EMD at mid-point of the lengthening contractions, but which then became longer at task termination and not different from the old women.

5.1 Baseline Measures

With no anthropometric differences between the groups, the dorsiflexors for the old women was ~28% weaker in MVC, and the evoked doublet torque ($P_d$) was 5% lower compared to the young women. These results agree with the literature (Power et al., 2012; Vandervoort, 2002). To complement these findings, the VA of the both groups were consistently high (~99%), and similar which has been observed previously in other studies involving the dorsiflexors (Connelly et al., 1999; Power et al., 2012). The baseline EMD values observed here are comparable to reports from other studies for this muscle group in both evoked and voluntary
contractions in women (Norman & Komi, 1979; Winter & Brookes, 1991; Zhou et al., 1998).

Very few studies have reported changes in EMD with adult aging and the results are not consistent depending on the muscle group, age, sex and testing method. In this study, there were no age-related differences in baseline EMD using either technique with values ranging from 75-79 ms for voluntary EMD and 58-59 ms for evoked EMD measures. As stated in the Introduction, the variability in EMD can be attributed to many factors such as the target muscle of study, temperature, acute level of previous activity, and the inherent mechanochemical properties of the muscle within the individual. We have no measures of muscle stiffness, but it is suggested that with aging the passive elastic properties of the system become stiffer (Rogers & Evans, 1993; Vandervoort, 2002) which should shorten the EMD measures. On the other hand, features related to age-related remodelling described previously would tend to lengthen EMD with adult aging, but the possible influence of these changes may have been counteracted by the stiffness changes, resulting in no overall change in this measure.

Many studies report that twitch potentiation is decreased in older adults, although the ages of the subjects are generally older (>70y) and most have focussed on men. For the dorsiflexors previous reports of twitch potentiation between young and old range from 38% to 37% in men and women respectively, with no difference with age (Hicks et al., 1991; Vandervoort & McComas, 1986). In this study we found only a slight trend ($P=0.07$) for baseline twitch potentiation be lower in these older women.

5.2 Fatigue and Recovery

In the present study the amount of fatigue induced was reflected by a 28% decline in MVC torque after 150 lengthening contractions and was not different between young and old women. Neuromuscular fatigue can be attributed to many sites and mechanisms within the
neuromuscular system (see Fig. 1 in Intro). Despite the decline in MVC torque, the maintained high level of activation as assessed by the ITT technique indicates that most of the fatigue occurred in peripheral sites. This loss of MVC torque with maintained high activation has been reported for this muscle previously in both young and old (Pasquet et al., 2000), using both concentric and eccentric contractions.

The decline in eccentric power was 19% in the old, and 8% in the young. The greater loss of power in the old has been reported previously for isokinetic (Baudry et al., 2007) and isotonic (McNeil et al., 2007) contractions. Although, lengthening contractions are known to be less energetically demanding than isometric and dynamic shortening contractions (Abbott et al, 1952; Ryschon et al., 1997), they can induce fatigue and also cause associated muscle damage (Baudry et al., 2007; Choi & Widrick, 2009). In this study the MVC did not recover by 30 minutes and in agreement with other studies is indicative of muscle damage (Armstrong et al., 1991; Clarkson & Hubal, 2002; Gandevia, 2001) with no influence by age. In one other study with young and older women, similar trends were observed in isometric and eccentric torque as both parameters failed to recover fully to baseline values at 30 minutes recovery after an ~30% reduction in isometric and eccentric torque at task termination (Baudry et al., 2007).

Overall, in the present study EMD, using either the evoked or the voluntary measure, was not affected by the fatigue or during recovery for either group. However, there was a trend ($P=0.06$) of ~10% elongation of MVC EMD, and ~5% elongation of evoked ($P_d$) EMD after fatigue. Although previous studies have not explored EMD changes with eccentric contractions in young or older women there is some support for EMD to be elongated after fatigue. It is understood that action potential propagation along the t-tubules becomes impaired with peripheral fatigue due to $K^+$ efflux during maximum contraction (McKenna et al., 1993;
Sjøgaard et al., 1985) exhibited in the current study by a reduced amplitude of the evoked doublet ($P_d$). In addition, Zhou et al. (1996) indicated a slowing of muscle fibre conduction velocity with fatigue of the quadriceps in young men after sprint training which likely contributed to their 15% prolongation of voluntary EMD. Furthermore, in a study consisting of young men, a prolonged EMD post fatigue of isometric contractions of the quadriceps was observed (Ce et al., 2013).

In addition, excitation-coupling mechanisms are a major factor in lengthening of EMD with fatigue (Zhou et al., 1996). With increasing acidosis induced by intense, high force generating contractions, sarcoplasmic reticulum Ca$^{2+}$ release along with Ca$^{2+}$ sensitivity and force production is reduced and may prolong EMD (Horita & Ishiko, 1987; MacLaren, Gibson, Parry-Billings, & Edwards, 1989). This reduced Ca$^{2+}$ release also causes an impairment of membrane conductivity of the SR and therefore, force generation is blunted with fatigue (Westerblad et al., 1991). Ca$^{2+}$ rates of release from the SR during the initiation of forming cross-bridges is different depending on the muscle fibre type. FT fibres have been shown to recycle Ca$^{2+}$ faster than ST fibre types and this influences the rate of torque production and maintenance of submaximal torque (Harigaya & Schwartz, 1969). Presumably, with age-related remodelling of MUs (Rogers & Evans, 1993; Vandervoort, 2002) the older women might have a greater relative area of ST fibres and therefore would have reduced Ca$^{2+}$ cycling rates than the young. This would prolong the rate of torque development thus taking longer to initiate measurable force production which could be reflected in a longer EMD. However, significant changes in EMD were not observed in either group and likely eccentric contractions do not induce the same degree of chemical alterations in the system that could affect EMD compared with isometric or
concentric fatigue; although other factors related to sex and elastic properties could influence these measures.

According to Zhou et al. (1996) it is unclear how the elastic property affects EMD during fatigue. It has been reported that, the serial elastic components and total compliance is affected by both muscle and tendon compliance. These are separated into two components referred to as the passive component which makes up the majority of the elastic tension, and the active component, producing varying tension dependant on the number of cross-bridges formed by the contractile proteins (Cook & McDonagh, 1996). With regard to eccentric contractions, Zhou et al., (1998) reported elongation of EMD with fatigue after eccentric contractions in both young men and women of the quadriceps muscle but credited the majority of the effect to increase in muscle temperature, which affects the non-contractile components of the SEC (Ce et al., 2013). For the dorsiflexors the relatively small muscle mass may not have induced a substantial increase in temperature with the eccentric contractions used in this study.

Furthermore, it has been suggested that ST muscle fibres play a greater role in maintaining tension level as the cross-bridges of ST fibres are the first to be recruited, have a longer duration, and as the FT fibres fatigue, the increase in muscle stiffness is due more to active ST fibres (Aura & Komi, 1987). It is then speculated with the increase in ST fibres with age (Vandervoort, 2002) the old women would have greater muscle stiffness as well as increased tendinous stiffness compared to young, thus partly compensating for the prolonged age-related EMD (as discussed above) due to the decrease in mechanochemical efficiency with aging and fatigue. Therefore no overall difference in either evoked or voluntary EMD might result because of the balance between inherent age-related increased muscle-tendon stiffness tending to shorten EMD, and fatigue-related prolongation of EMD.
The transient speeding of the evoked EMD in the young woman during fatigue may be due to muscle potentiation that dominated the first half of the fatiguing protocol before fatiguing processes described above began to play a larger role. Although in this group of women we did not observe a difference in baseline potentiation, it has been reported with age, that there is an impairment of potentiation (Behm & Sale, 1994; Power et al., 2012). This could have explained the lack of significant shortening of evoked EMD in the older women during the mid-point of fatigue compared with the younger women. Thus, it could be that although the inherent muscle potentiation was not different, the young women were less affected early by the competing fatigue processes and potentiation dominated for a longer period of time compared with the older women.

Although overall there were no differences found between the young and old woman in MVC EMD measures during the lengthening protocol and throughout recovery, there was a trend at the mid-point of the protocol in which the young were shorter compared with the old. The MVC EMD may be influenced by other central drive factors related to recruitment and rate coding alterations during fatigue that may have masked the effects of muscle potentiation per se which was more directly reflected in the evoked EMD measure. During evoked contractions all units are simultaneously activated (Solomonow, 1984). Minshull et al. (2007) attempted to explain the inherent ‘reserve capacity’ of unused motor units only deployed artificially or under strenuous conditions. This capacity to utilize the preserved emergency motor units may be different depending on the down-regulation of the potential protective mechanisms of the central and peripheral neuromuscular inhibitory mechanisms which limit the access to all of the large high threshold motor units during a voluntary contraction (Tsuji & Nakamura, 1988; Zhou et al., 1995). Perhaps with age, the reduced cycling rates of the mechanochemical efficiency may
predispose the old to an earlier onset of fatigue compared to the young, thus muting the effects of potentiation during the lengthening contractions.

Although the MVC and $P_d$ torques were not recovered, indicating muscle damage, unlike the hypothesis there were no significant changes after the fatiguing task in the EMD measures. Therefore, the recovery period did not provide the expected opportunity to explore age-related differences in EMD after this task. The age related reduction in aerobic capacity of ~25% (Rogers & Evans, 1993) and with the greater presumed proportion of ST fibres in the old women, their recovery was expected to be delayed due to blunting of the biochemical mechanisms responsible for contractions, and thus greatly relying on tension development from the ST fibres to create shorter EMD times. However, apparently short term muscle damage does not affect either evoked or voluntary overall EMD measures. Because EMD is affected by two main factors - transmission and E-C coupling processes, and passive musculotendinous factors - it is conceivable however that similar to other aspects of this study that some factors may have tended to slow EMD while other factors may have tended to elongate EMD creating no net change in overall EMD.

5.3 Conclusion

In summary, electromechanical delay was similar between voluntary and evoked contractions at baseline and not affected between young and old. Despite a transient age-related difference in one measure of EMD during the development of fatigue, overall there were no changes in EMD or group differences after fatigue induced by 150 lengthening contraction, or during a 30 minute period of recovery. Perhaps the relatively younger age of the women in this study did not express the expected age-related changes to the neuromuscular system as might
have been expected or that has been reported in other studies using older subjects. Also few studies have tested EMD in women under these conditions and so unlike isometric or dynamic induced fatigue results reported mainly from men, lengthening contractions may not create the same fatigue factors, but may induce more muscle damage. Because EMD is affected by two main factors of the peripheral force generating system some of the results could be explained by opposing factors so it appears that the overall EMD is unaffected. This thesis highlights some of strengths and limitations of the EMD measure and provides a first description of the effect of lengthening contractions on EMD in young compared with older women.
 CHAPTER 6

6.0 LIMITATIONS AND FUTURE DIRECTIONS

Because EMD is affected by various factors it was not possible to separate these using the current design and thus the mechanisms of EMD at baseline and during fatigue were not directly investigated in this study. There were no direct measures of muscle damage, metabolic alterations or elastic properties that might have independently and at different times affected EMD. Although of great interest it would be challenging in humans to separately evaluate these factors concurrently. Some recent studies have used mechanomyography (MMG) to attempt to separate the non-contractile and tendon properties from the E-C coupling properties (Ce et al., 2013).

Another possible limitation to this study was the lack of information and consensus about the advantages and limitations, and rationale for methods used to assess EMD in humans. Differences in protocol or method of study, i.e., voluntary versus evoked measures of EMD, make comparisons in the literature difficult. Most studies have used involuntary stimulation and have not used both voluntary and evoked contractions in one study. This was compounded by the lack of agreement or limited information about changes in EMD with aging and fatigue, especially muscle damaging fatigue. The advantages or disadvantages of the dorsiflexor model are not understood in comparison to other muscles studied. Perhaps the dorsiflexors with their relatively long tendons have different elastic properties compared to proximal muscles often studied such as the elbow flexors and knee extensors. There are several reports on EMD in other lower limb muscles (see Table 1) but none have studied the effects of lengthening contractions in aging and women.
Activity level (training status) is a factor not usually reported in many studies (Zhou et al., 1996). The women in this study were relatively active for their respective ages but none were highly or systematically trained and so comparison with the literature especially for the dorsiflexor muscles should be made cautiously. Furthermore, activity level prior to testing (Weiss, 1965), and even temperature increase of 2°C in the testing room, or indeed within the muscle due to exercise enhancement of limb blood flow elongate EMD (Zhou et al., 1998).

In the future, an increased sample size might provide a better representation of the population, importantly including subjects over 75y of age that might more completely express age-related changes to the system. Markers of muscle damage via blood draws or imaging techniques could be implemented to quantitatively assess muscle damage. With this protocol, repeating the measures after 24 or 48h might be useful to assess the effects of muscle damage related to delayed-onset of muscle soreness on EMD measures.
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