OPTIMAL INTER-STIMULUS INTERVAL FOR INTERPOLATED TWITCH TECHNIQUE WHEN USING DOUBLE PULSE STIMULATION

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ABSTRACT

Optimal inter-stimulus interval for Interpolated twitch technique when using double pulse stimulation.

Interpolated twitch technique is a method frequently used to assess voluntary activation. This method uses electrically evoked twitch superimposed on the voluntary activity and its comparison with the twitch in rested muscle i.e. control twitch, to evaluate completeness of muscle activation. The purpose of this study was to investigate the effect of interval in paired stimulation on control twitch in young and elderly individuals with bent and flexed knee positions. Supramaximal electrical stimulation consisted of one single stimulus and eight paired stimulation with intervals from 5 to 40ms, with increments of 5ms. In evoked twitches normalized peak torque amplitude (NPT), time to peak torque (TPT), half-relaxation time (HRT), maximal rate of torque development (RTD) were calculated. Although all parameters were slightly different in young adults compared to elder ones, the difference reached significance only in shorter TPT and HRT for the former, a phenomenon attributed to the slowing of muscle contractile mechanics with ageing. In both groups the highest RTD and NPT were observed with shorter inter-stimulus intervals i.e. 5 and 10ms. This may be attributed to a summation effect and/or a less time available for the longer-latency reflex effects of electrical stimulation to affect the torque.

Contents

ABBREVIATIONS

ITT: Inerpolated twitch technique

TPT: Time to peak torque

HRT: Half-relaxation time

RTD: Maximal rate of torque development

NPT: Normalized peak torque

Sol: Soleus

LG: Lateral gastrocnemius

MG: Medial gastrocnemius

1 INTRODUCTION

Interpolated twitch technique (ITT) is a non-invasive method to investigate the level of voluntary activation in muscle. In a voluntary contraction of muscle force is produced by recruitment of the motoneurons through increased descending drive from motor cortex. When a supramaximal electrical stimulus is applied to the nerve trunk or intramuscular nerve branches of an active muscle during a voluntary contraction, those motor units firing at sub-maximal rates and whose motoneurons are not in a refractory state respond with a twitch- like increment in force. The more motor units recruited and the faster they are firing, the fewer motor units that are available for recruitment via stimulation and thus the smaller the superimposed twitch. In other words, with higher intensities of voluntary contraction the superimposed twitch declines. The alternative term "twitch occlusion" comes from this diminishing twitch size as contraction force increases. ITT was first used by Merton(Merton, 1954) to detect possible muscle inactivation. Quantification of voluntary activation of muscle with the use of ITT is based on the inverse relationship between the amplitude of the twitch evoked by an interpolated stimulus superimposed on a voluntary contraction and voluntary force output at the moment of stimulation. To calculate the level of inactivation the superimposed twitch is normalized to a control twitch that is evoked in the resting muscle. In this study the effect of stimulation on resting twitch was investigated. Different inter-stimulus intervals and modes of stimulation, e.g., single, double and triple and trains have been used in the literature. Although a doublet with a 10ms inter-stimulus interval has been typically applied the reasons behind it and the mechanism affecting the response of muscle by varying intervals are not clear. The aim of this study was to investigate the effect of stimulus interval in order to find whether there is an optimal interval and if age has a determining role in it.

2 INTERPOLATED TWITCH TECHNIQUE:

ITT has been used to compare muscle activation under a variety of conditions such as fatigue(Bülow et al., 1995; Burnley et al., 2012; Place et al., 2008; Smith et al., 2007; Strojnik & Komi, 2012),ageing(Sophie J De Serres et al., 2012; Griffin et al., 2012; Roos et al., 1999; Stackhouse et al., 2000, 2001; Stevens et al., 2003), diverse pathological processes(D. Behm et al., 2001; M Bilodeau et al., 2001; Pap et al., 2004; Urbach & Awiszus, 2002), and training modes(e.g. strength and resistance training)(Reeves et al., 2003). Some studies that used ITT and their conclusions are briefly brought in the following paragraphs.

2.1 Quantifying voluntary activation

Methods by which "voluntary activation", defined as the completeness of muscle activation, is usually appraised are described in the following paragraph.

2.1.1 Voluntary activation index

Activation level is conventionally calculated by a linear equation, which is based on the negative linear relationship between twitch size and voluntary force, originally described by Merton(Merton, 1954) and further supported by others(Gandevia & McKenzie, 1988; Lyons et al., 1996). The equation expresses voluntary activation (VA) as a percentage according to the following formula:

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

where $T_{interpolated}$ is the superimposed twitch noted during a maximal contraction at the time of stimulation and $T_{control}$ is that evoked by the supramaximal stimulation delivered to the potentiated relaxed muscle at the same site. In order for the assumption of equal muscle potentiation between control and superimposed twitches to be acceptable, con-

trol stimulations are delivered 1.5 to 5 seconds after the MVC. Figure 1 shows a representative torque trace of a twitch superimposed on a peak voluntary isometric contraction.
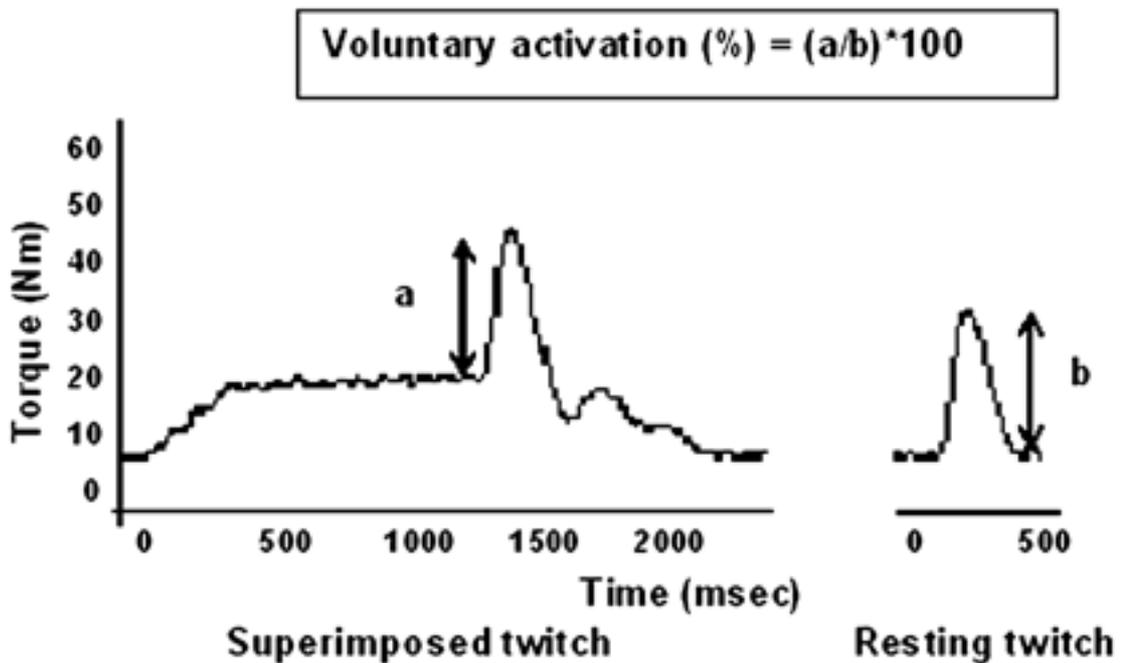

FIGURE 1.Torque trace acquired during maximal voluntary isometric contractions with interpolated twitch to quantify muscle activation deficit. A single supramaximal intensity electrical stimulus was superimposed on a maximal voluntary isometric contraction (a), as well as on a resting, potentiated plantar flexor muscle (b). From: "Lower extremity skeletal muscle function in persons with incomplete spinal cord injury" by Jayaraman et al., 2006, *Journal of Spinal Cord*, 44, p. 684

Based on the inverse linear relationship between evoked and voluntary force maximum potential bite forces of humans was estimated by Lyon et al. in jaw closer muscles(Lyons et al., 1996).

However some studies have shown the evoked-voluntary force relationship to be non-linear(D. G. Behm et al., 1996; Nørregaard et al., 1994; Rutherford et al., 1986) and thus claimed the aforementioned equation for estimation of voluntary activation is invalid and the force corresponding to maximal central activation, "true maximum force", cannot be determined from a single datum point.

2.1.2 Extrapolating the evoked-voluntary force relationship

An alternative method of quantifying voluntary activation involves modeling the relationship between evoked and voluntary force and extrapolating it to the estimated true maximum, where the curve crosses the voluntary force axis, i.e. the point where no extra force is evoked by superimposed stimulation and thus muscle is fully activated(D. G. Behm et al., 1996; Bülow et al., 1993; S J De Serres & Enoka, 1998; Dowling et al., 1994; Rutherford et al., 1986). The evoked- voluntary torque relationship has been modeled in various ways. For instance, Behm et al. suggested that interpolated twitch ratio-force relationship was best fit by a shallow hyperbolic curve as shown in Figure 2, that resulted in insignificant MVC prediction errors(D. G. Behm et al., 1996). Bulow et al. reported a linear relationship between twitch size and force level only for force levels greater than 25% of MVC(Bülow et al., 1993). While In another study by De Serres et al. the relationship was linearized by transforming the evoked force to [normalized evoked force] [k], where k gave the best linear fit to the data for each individual (S J De Serres & Enoka, 1998). This is while Dowling et al. suggested an exponential function that never reached zero as the best fit (Dowling et al., 1994).
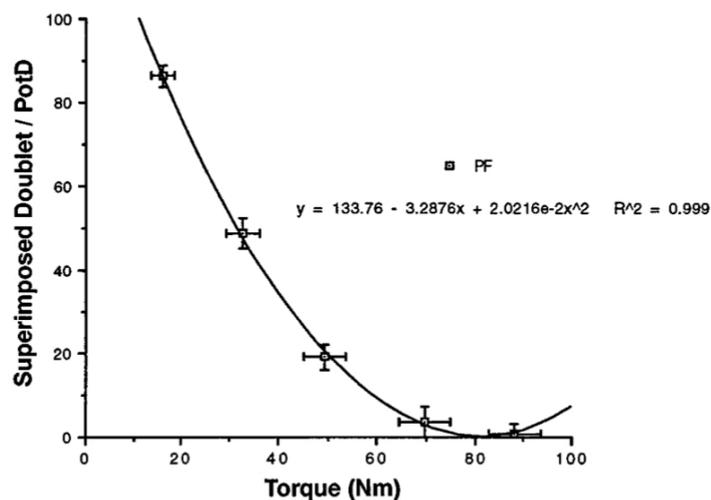

FIGURE 2. Combined plantar flexor IT ratio-voluntary force relationship. Data are means ± SE. Superimposed doublet/potentiated doublet (PotD) = IT ratio. From: "Muscle inactivation: assessment of interpolated twitch technique" by Behm et al., 1996, *Journal of Applied Physiology*, 81(5), p. 2269

The limitations of curve fitting such as the need for numerous experiments and the high

sensitivity of polynomial functions that are usually used to this end also limit the application of this method of quantitation. In some studies estimation of maximal (true) muscle force from the relationship between experimental interpolated twitch amplitude and voluntary force has been even less than the actual MVC (Herbert & Gandevia, 1999) or unrealistically larger (up to 181% MVC)(S J De Serres & Enoka, 1998). Furthermore, to have proper data to fit a curve several submaximal as well as maximal trials are required. Therefore, this method is time consuming and not suitable for fatigue studies where instantaneous results are required, for example when fatigue is studied by applying stimulation and assessing VA during repetitive maximal contractions.

2.1.3 Central activation ratio:

The other commonly used index of muscle activation "central activation ratio" (CAR (Kent-Braun & Le Blanc, 1996; Kent-Braun et al., 2002) is calculated according to the following formula:

$$CAR(\%) = \frac{MVC}{Total\ force(MVC + superimposed\ stimulus\ force)} \times 100$$

Although this index has been used in several studies(Kent-Braun et al., 2002; Reeves et al., 2003; Stackhouse et al., 2000, 2001) and its sensitivity in comparison with AL has been investigated(Bampouras et al., 2006; D. Behm et al., 2001; O'Brien et al., 2008), Sheild et al. have argued that fundamental flaws constitutes its notion(Shield & Zhou, 2004). For instance Stackhouse et al. demonstrated that the relationship between CAR and the percent maximum voluntary effort (% MVE) during isometric quadriceps femoris was best described by a second-order polynomial. Although Knight et al. reported insignificant difference between CAR and AL index in terms of correlation with maximal firing rates(Knight & Kamen, 2008), studies that investigated activation level have reported higher values of CAR (of up to 16% in shorter muscle lengths(Bampouras et al., 2006)) compared to AL index(Bampouras et al., 2006; D. Behm et al., 2001). Bampouras et al. suggested that the mechanism behind this difference is dependent on joint angle and number of stimuli. CAR is more sensitive to the number of stimuli because the evoked force increases due to summation of twitch contractile responses. This is

while AL index that expresses the response of muscle to stimulation in relation to the same stimulation at rest is less likely to be affected by the number of stimuli. On the other hand, AL index was more sensitive to changes in joint angle since joint angle variations change the series elastic component properties of the system thus affecting the force transmitted during rest. Consequently CAR would be less sensitive to changes in joint angle. Comparison of these two indices with the central activation obtained from magnetic stimulation method (MS), only AL index was comparable with the results from MS(O'Brien et al., 2008). They suggested the overestimation of activation level by magnetic stimulation, also observed in CAR, might have been a result of power limitations of magnetic field. The overestimation of activation level by CAR has also been attributed to the fact that MVC force is typically determined by a number of synergists while only the stimulated muscles produce the force increment(Shield & Zhou, 2004).

2.1.4 Limitation

Computer modeling has suggested that the nonlinearity of evoked-voluntary force is caused by collision of antidromic with orthodromic signals and by spinal effects of stimulation (Herbert & Gandevia, 1999). By application of electrical stimulation action potentials propagate antidromically and orthodromically. While near-synchronous orthodromic action potentials contribute to the muscle twitch, some antidromic potentials collide with voluntary potentials and reduce the rate of motoneuron firing and consequently the force. Spinal effects of stimulation including reflexes, for example by stimulating the sensory axons or those due to mechanical effects of twitch, may also reduce the evoked force. The contribution of spinal effects and action potential collision is greatest in forces between 40%MVC and 80%MVC. In near maximal contractions rising phase of interpolated twitches is complete before spinal effects can affect, therefor only collision reduces the twitch force. During weak contractions the probability of collision is lower.

2.2 Applications

*Ageing.*Twitch interpolation technique has frequently been used to investigate the effect of ageing on the ability to fully activate muscle (Sophie J De Serres et al., 2012; Griffin et al., 2012; Roos et al., 1999; Stackhouse et al., 2000, 2001; Stevens et al., 2003). The disagreement in the results of some age-related studies may be due to methodological differences or, as suggested by Jakobi and Rice, the muscle group under study(Jakobi & Rice, 2002). Their study showed a greater age-related reduction of activation level in elbow extensors as compared to elbow flexors. While some researchers have detected a significant lower voluntary activation in elderly compared to young adults(Stackhouse et al., 2001; Stevens et al., 2003), some others found no significant difference in the ability to activate muscle and attributed weaker muscle contraction of the elderly to other factors such as greater co-activation or reduced specific tension(Klein et al., 2001; Roos et al., 1999). Some differences may be related to the mode of stimulation used. Studies that have detected significant difference activation level between young and old typically used a train of stimuli. This is while studies that used paired stimuli did not detect significant difference and attributed the weaker force production of the elderly to other age-related properties of muscle (e.g. atrophy). It is also worth noting that these studies reported submaximal activation for both groups. Some authors suggest that due to curvilinear relationship between force and activation, even a small deficit in activation can mean a large difference in the force generated in near maximal efforts, which would explain the small VA deficit reported for the elderly (Stackhouse et al., 2001; Stevens et al., 2003).

Joint pathologies and rehabilitation measures. Completeness of muscle activation under the influence of joint injuries(Huber et al., 1998; Pap et al., 2004; Urbach & Awiszus, 2002), arthritis(Lewek et al., 2004) or muscle pain(Nørregaard et al., 1994; Rutherford et al., 1986) has also been studied with ITT. For instance, it has been shown that isolated rupture of the anterior cruciate ligament caused a minor voluntary activation (VA) deficit and severe knee injuries more severe VA-deficits of the quadriceps muscles(Urbach & Awiszus, 2002). It has also been shown that patients with unilateral knee injuries exhibit significant quadriceps VA deficit in both legs, depending on the

severity of the injury(Urbach & Awiszus, 2002). Other studies demonstrated that linear extrapolation of the twitch-voluntary force relation to the zero level of twitch, which corresponds to a "true" maximal voluntary contraction, reveal a decreased quadriceps strength and voluntary activation level in patients with fibromyalgia and/or a history of knee or joint injury(Nordlund et al., 2004; Rutherford et al., 1986).

Another application of ITT has been in evaluation of surgical procedures(Machner et al., 2002; Urbach et al., 2001), rehabilitation approaches on voluntary activation of muscle(Vivodtzev et al., 2008). For example, in patients with anterior cruciate ligament (ACL) deficiency VA showed a significant improvement two years after reconstruction of ACL, however it remained less than that of normal control group(Urbach et al., 2001). Immobilization has been reported to cause a significant reduction of up to 9% in voluntary activation(Gondin et al., 2004; Y Kawakami et al., 2001). As a countermeasure to the decline in voluntary activation and strength associated with immobilization, resistance exercise has been shown to be effective (Y Kawakami et al., 2001; Oates et al., 2010).

Fatigue. ITT has also been used during different fatiguing tasks and conditions(Bülow et al., 1995; Burnley et al., 2012; Place et al., 2008; Smith et al., 2007; Strojnik & Komi, 2012) to assess the role of more central mechanisms. Nevertheless in application of ITT in fatigue studies and interpreting its results, limitations of ITT and its sensitivity must be noted(de Haan et al., 2009; Herbert & Gandevia, 1999; Taylor, 2009).

In a study by Stronjik and Komi possible sites of fatigue were examined by applying single, double and a train of stimuli after an intensive stretch-shortening cycle exercise(Strojnik & Komi, 1998). Following observations were made: Twitch decline in single stimuli, no change in twitch by double stimuli and a significant decline by trains of stimuli. They associated the torque decline of single stimuli with a reduced Ca^{++} release from the sarcoplasmic reticulum and/or reduced capability of cross bridges to form strong binding. This is while the Ca^{++} transient was longer with two consecutive stimuli, thus making the twitch torque less sensitive to changes in the Ca^{++} transient. They explained the torque decline of high frequency by impaired high-frequency action potential propagation over the sarcolemma.

In fatigue studies ITT has also been used to investigate the role of central and peripheral fatigue in force loss. Central fatigue is commonly quantified as a reduction in voluntary activation (de Haan et al., 2009; Gandevia, 2001; Taylor & Gandevia, 2008). Whereas peripheral fatigue is represented by decrease in potentiated interpolated twitch at rest(Burnley et al., 2012). Various studies have shown that in the contribution of central and peripheral mechanisms to fatigue several factors are involved such as continuous/intermittent nature of the task (Martin Bilodeau, 2006), age and/or sex of the subjects (M Bilodeau et al., 2001; Yoon et al., 2007), and the contraction intensity(Yoon et al., 2007).

In a similar approach the detection of source of fatigue has been addressed by stimulation of different sites of nervous system including electrical stimulation of motor point. For example the declines in forces evoked by cortical stimulation (transcranial magnetic stimulation) and motor point stimulation have been compared as indicators of supraspinal and central fatigue, respectively (Gandevia et al., 1996; Smith et al., 2007). Smith et al. observed a 90% reduction of voluntary activation measured by cortical stimulation vs. 80% of that measured by electrical stimulation of motor point(Smith et al., 2007). They concluded that about two-thirds of fatigue was due to supraspinal mechanisms.

The controversy around use of ITT to study fatigue has been partly associated to the low sensitivity of this technique in maximal and near maximal contractions due to the nonlinear input-output relationship of motoneuron pool (de Haan et al., 2009; Herbert & Gandevia, 1999). Computer model of Herbert et al. revealed a sigmoidal relationship between motoneuronal excitation and interpolated twitch amplitude which means large increases in motoneuronal excitation produce only small reductions in interpolated twitch amplitude during high-intensity contractions(Herbert & Gandevia, 1999). This s-shaped relationship between excitation and force, as shown in Figure 3, leads to relatively large evoked twitches in isometric force during submaximal contractions (with nonsaturating intracellular calcium concentrations, $[Ca^{++}]_i$) but only very small increases can be expected when maximal effort is approached.
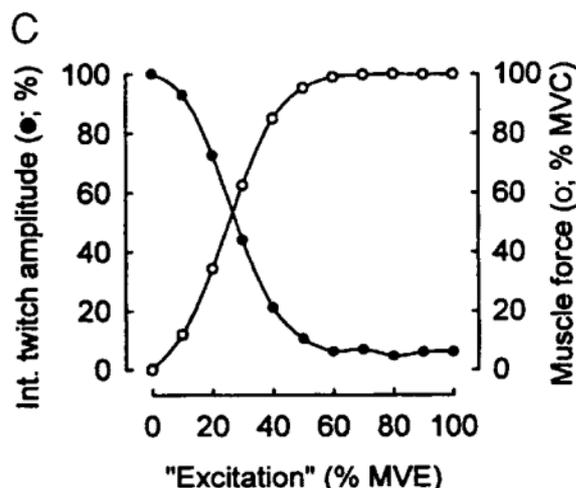
FIGURE 3: Relationship between interpolated twitch amplitude (•, as a percentage of whole muscle resting twitch), muscle force (o, as a percentage of maximal voluntary force) and motoneuron pool excitation. From: "Twitch interpolation in human muscles: mechanisms and implications for measurement of voluntary activation", by Herbert and Gandevia, 1999, *Journal of Applied Physiology*, 82, p. 2279

Furthermore, Place et al. reported increase in interpolated twitch size with fatigue in a single mouse muscle fiber which was at least partly due to moving in the sigmoidal Ca^{++}-force relationship (Figure 4) rather than an increase in the $[Ca^{++}]_i$ induced by ITT (Place et al., 2008). In other words the increase in twitch force was attributed to peripheral mechanisms, such as changes in $[Ca^{++}]_i$ or Ca^{++} sensitivity, as opposed to central mechanisms generally linked with ITT (Place et al., 2008). This questions the validity of comparison between fresh and fatigued muscle. However, the results from mouse studies may not be fully relevant to human muscle studies (Taylor, 2009). For example recently Gandevia et al. who utilized ITT with maximal tetanic stimulation to ulnar nerve reported different behavior in human muscle fibers (Gandevia et al., 2013). They observed a progressive decline of superimposed twitch during two different tetanic stimulations of physiological range frequencies. This decrease was observed regardless of M-wave decline suggesting that decrease in excitability probably takes place in sites distal to the sarcolemma, probably in t-tubules. Therefore action potential failure, which is manifested by decrease in twitch amplitude, is not determined by changes in Ca^{++}-force relationship. Fatigue in central mechanisms is a more relevant cause of this decline.
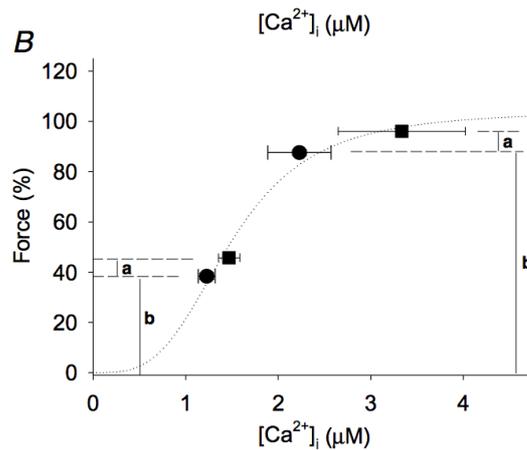
FIGURE 4: Force–$[Ca2^+]i$ relationship obtained during the fatigue protocol with standard (•) and interpolated (■) tetani corresponding to 90 and 40% of the initial force. Relative force was for each fibre calculated as a/b throughout the fatigue run; the same approach was used to calculate relative Δ $[Ca2^+]i$. All the forces were normalized to the force measured at 70 Hz in control conditions. From: Interpolated twitches in fatiguing single mouse muscle fibers: implications for the assessment of central fatigue, by Place et al., 2008, Journal of Physiology, 586(11), p. 2802

2.3 Effect of number of stimuli on ITT

Several studies have investigated the effect of number of stimuli on the sensitivity of ITT and the variability of the results obtained by this method; Different modes of stimulation including single stimulus, multiple and trains of stimuli have been compared (Allen et al., 1998; D. G. Behm et al., 1996; Kent-Braun & Le Blanc, 1996; Suter & Herzog, 2001). Behm et al. reported similar MVC predicted with either single twitch, doublet or quintuplet(D. G. Behm et al., 1996). Similarly Kent-Braun et al. reported no significant difference in CAR values obtained by single or paired stimulation(Kent-Braun & Le Blanc, 1996),however their study showed a significant difference in CAR measured by train of stimuli. Allen et al. reported no significant difference in the forces evoked by single, paired or trains of stimuli for force levels above 85%MVC(Allen et al., 1998). Whereas in force levels below 85% MVC doublet and train of stimuli evoked higher forces. Equivalence of single and double pulse for calculation of level of voluntary activation with nerve trunk stimulation was also reported by Scaglioni et al. (Scaglioni & Martin, 2009).

Paired rather than single stimulation has been recommended because of its higher reliability and validity(Oskouei et al., 2003), particularly in fatigue studies due (Place et al., 2007) to disproportionate decline of force elicited by single stimulus probably caused by low frequency fatigue(Edwards et al., 1977; McKenzie et al., 1992). Therefor fatigue-related studies have typically used doublets(Place et al., 2004; Smith et al., 2007; Strojnik & Komi, 1998). Moreover Edwards et al. suggested that due to properties of mammalian muscle such as positive staircase potentiation (i.e. a progressive increase in force elicited in response to identical stimuli) a remarkable variability is observed in the results of single stimulation(Edwards et al., 1977).

Trains of stimuli have been used in several studies(S J De Serres & Enoka, 1998; Miller et al., 2003; Stackhouse et al., 2001). However Knight et al. have suggested that as train of 1ms did not produce greater force. Rather the force increments produced by trains of stimuli are likely to be a result of Ca^{++}-related force potentiation, transient increases in stiffness, or other muscle-related issues as muscle response to stimulation at higher, non-physiological rates(Knight & Kamen, 2008). Moreover the discomfort associated by use of trains of stimuli is a downside which makes single and paired stimulation the most frequently used modes particularly in studies involving maximal efforts because of hindering the subject from performing their maximum voluntary contraction and thus compromising the method.

Suter et al. reported that as the number of stimuli increase the variability in the results of ITT decrease(Suter & Herzog, 2001). In agreement with Allen et al., their study showed that once the evoked forces are normalized to the corresponding resting twitch single and multiple stimulations were similar. The variability, on the other hand, decreased significantly in 3 vs. 1 and in 4 vs. 1 stimuli (Suter & Herzog, 2001). Based on the findings of aforementioned study, several succeeding researchers opted for triple stimuli (Beltman et al., 2004; Kooistra et al., 2007; Mademli & Arampatzis, 2008).

Stimulus number has been reported to affect CAR significantly more than VA index, whereas VA index is more sensitive to changes in joint angle(Bampouras et al., 2006) (see Quantifying Voluntary Activation).

Mechanism of effect of stimulation mode

 Suter et al. hypothesized that the variability is partly due to the stochastic nature of the timing of twitch application relative to pulses of voluntary excitation trains(Suter & Herzog, 2001). Their hypothesis was based on the fact that occurrence of the superimposed twitch constitutes a stochastic event relative to the stimulation trains that may be activating hundreds of motor units in a muscle during voluntary contraction. From the results of two sets of experiments designed to investigate the effect of timing and number of stimuli they concluded that the variability of ITT results is at least partly due to variations that are an inherent part of this technique.

On the other hand with trains of stimuli when an initial, brief, high-frequency burst of stimuli is applied at the beginning of an imposed subtetanic train of pulses an augmentation of forces is observed. This phenomenon is termed "catch-like" property of muscle; it is called "catch-like" to be distinguished from the true catch property first observed in molluscan muscle which describes the force production without the need for continuous stimulation(Astrand et al., 2003; van Lunteren & Sankey, 2000).

Another explanation on the effect of train stimulation compared to single stimuli is based on basic physiological phenomena occurring during muscle contraction: Maximal muscular force is generated by the contribution of three mechanism: 1-recruitment of a maximal number of slow twitch and fast twitch motor units (MUs) 2-rate coding/ frequency modulation and 3- synchronization of MUs. The contributions of recruitment versus rate coding are different in various muscles. Usually small muscles reach 100% MU recruitment in less than 100% MVC, further force production thereafter depends on rate coding. It is also a well-established principle that motoneuron recruitment is controlled by the size of motoneurons (i.e. Henneman's size principle). Small motoneurons innervating fatigue resistant slow twitch fibers are recruited first and fire at a steady frequency of about up to 15 Hz. In case lagers forces are needed the larger motoneurons innervating fast-twitch fatigable muscle fibers are recruited. Their mean firing frequency may reach 50 Hz during the initiation of a strong voluntary contraction(Basmajian & Luca, 1985).

Given the above physiological principles during a maximal effort when a muscle is at suboptimal MU recruitment or firing rate multiple supramaximal stimuli is more likely to affect the firing frequency of the motoneurons and cause a force increment than a single electrical stimulus is. The force increments produced by the MUs summate and create a greater force increment of total muscle.

3 MUSCLE FIBER CONDUCTION VELOCITY

Muscle fiber conduction velocity (MFCV) is an important diagnostic parameter because it reflects fiber membrane properties and their change with fatigue(Merton, 1954; Smith et al., 2007), pathological condition (Blijham et al., 2011; Hogrel, 2005; M J Zwarts et al., 2000), and exercise(Strojnik & Komi, 1998; Urbach & Awiszus, 2002). For example a decrease in MFCV has been shown to be an indicator of peripheral fatigue. The application of MFCV and its changes as an indicator of the effect of different conditions, e.g. fatigue and ageing, will be explained further below.

 MFCV can be measured in two ways, an invasive and a surface electromyography (sEMG) method. In the invasive method muscle fibers are directly stimulated with a monopolar needle electrode. A recording electrode is placed at some distance in the stimulated bundle. MFCV is calculated from the distance between the stimulating and recording electrodes and the latency of the evoked action potential. Alternatively, non-invasive surface EMG recordings, typically from two or more electrodes, are analyzed – in various ways- in order to detect the latency shift between the two EMG signals for MFCV estimation. Zwarts et al. (Machiel J Zwarts, 1989) suggested that the mean values obtained by the two methods showed a clear correlation. The invasive method has been reported to be more sensitive especially in patients; for example in patients with myopathy it demonstrated some very slow conducting fibers that were not detected by sEMG method (van der Hoeven et al., 1994; Machiel J Zwarts, 1989). However the variability of MFCV was much higher with the invasive technique. Moreover the sEMG is easier to perform. In the following paragraphs we are looking at the sEMG method.

Estimation of MFCV from surface EMG recordings is a complex task. Different sources of noise corrupt the EMG signals that have to be detected and analyzed with advanced systems. This method is based on the detection of action potentials along their line of propagation i.e. from the innervation zone through muscle fibers to the tendon. Ideally the shape of action potentials should remain unchanged along their propagation; however the measurements are affected by several experimental conditions such as electrode placement.

In the following paragraphs some of the studies that have calculated MFCV changes under various conditions are briefly discussed.

3.1 Effect of ageing

Yamada et al. compared EMG variables including MFCV and median frequency (MDF) and averaged rectified value (ARV) in the young and elderly subjects during fatiguing contractions of the tibialis anterior muscle (Yamada et al., 2000). They reported a highly greater MFCV, AVR and MDF in the young compared to the elderly group. They attributed the lower MFCV and MDF of the elderly to the atrophy of the fast twitch muscle fibers previously reported by histochemical studies. Lower AVR in the aged suggested a lower capacity for recruiting motor units. The combined effect of fast twitch muscle fibers and decreased capacity resulted in weaker force production. With the development of submaximal fatigue, MFCV and MDF decreased and AVR increased in both groups with a greater change in the young subjects. It was suggested that lower changes in the aged group was due to a different neuromuscular response between the two groups. In other words higher proportion of fast twitch muscle fibers in the young group and the fact that their 60% MVC is greater cause a greater accumulation of by-products such as lactic acid and hence the greater drop in MFCV and MDF. Based on these results they concluded that aged subjects cannot be fatigued as much as the young.

Similar conclusion suggesting less fatigability of the elder subjects was driven by Bazzuchi et al. during sustained contractions of 20%, 50% and 80 % MVC of elbow flexion and knee extension (Fig. 5) (Bazzucchi et al., 2004). They compared rate of change of MFCV and MDF (%/s) which was defined as the percentage ratio between

the slopes of the regression lines and their initial values at time 0, i.e. "normalized slopes", between the young and elderly group. While 20% and 50% MVC efforts did not show any significant difference, the MVCF and MDF normalized slopes were smaller in aged than young group at 80% MVC. Although in elbow flexion this difference did not reach statistical significance, which was in accordance with the results reported by Merletti et al. (Roberto Merletti et al., 2002). This insignificant decrease of MFCV in the old compared with the young has been ascribed to predominantly different activation strategies in addition to different fiber size or fiber type constituency (Roberto Merletti et al., 2002). The smaller slope of MFCV compared to MDF has been attributed to the motor unit firing synchronization that compensates the reduced contractile capability of motor units(Bazzucchi et al., 2004; Roberto Merletti et al., 2002).
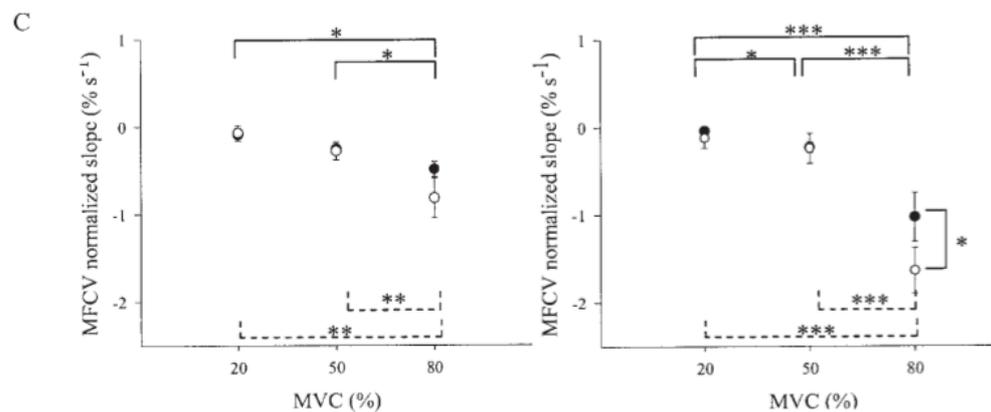

FIGURE 5: MFCV (C) in young women (open circles) and older women (filled circles) at 20%, 50%, and 80% MVC for elbow flexion (left panel) and knee extension (right panel). *P < 0.05; **P < 0.01; *** P < 0.001. From: "Differences between young and older women in maximal force, force fluctuations, and surface emg during isometric knee extension and elbow flexion", by Bazzucchi et al., 2004, Muscle & Nerve, 30 (5), p. 630

The effect of ageing on MFCV has also been investigated between trained and untrained individuals during incremental cycling exercise (Lenti et al., 2010). This study showed an increased MFVC with exercise intensity in young individuals. On the other hand, no significant MFVC increase was observed in the old groups with increasing exercise intensities and the decline started by exhaustion. Moreover the MFCV in untrained elderly group was significantly lower than both untrained and trained young individuals. The authors suggested that the lack of MFCV increase in the old group especially in the un-

trained subjects could reflect the reduced proportion of type II muscle fibers as well as reduced fiber diameter.

3.2 Effect of fatigue

During sustained isometric exercise at relatively high intensity contractions (above 40% MVC) a decrease of MFCV has been reported(Farina et al., 2002; Masuda et al., 1999; R Merletti et al., 1990; M J Zwarts et al., 1988). This decrease has been explained by the accumulation of metabolic byproducts such as lactate, extracellular $[K^+]$ intracellular and subsequent decreased pH which reduce the excitability of sarcolemmal excitability(R Merletti et al., 1984; Machiel J Zwarts & Stegeman, 2003). This has been supported by the studies reporting a higher rate of MFCV decline in muscles with higher proportion of type II muscle fibers (Kupa et al., 1995) that have higher lactate accumulation (TESCH et al., 1983). Also, Linssen et al. (LINSSEN et al., 1990)reported that patients with Mcardle's disease who lack the ability to consume glycogen in the glycolytic pathway and therefor have failure of lactate formation, did not exhibit the reduction in MFCV as quick as normal subjects did (LINSSEN et al., 1990). On the other hand during low intensity isometric contraction MFCV has been reported to remain constant or increase (Krogh-lund & Jergensen, 1992; M J Zwarts & Arendt-Nielsen, 1988). The increase in MFCV has been suggested to be due to the recruitment of fresh, predominantly type II, muscle fibers that were previously inactive(Houtman et al., 2003). This is in accordance with the size principle that indicates the recruitment of larger diameter fibers, typically type II, in later stages of contraction.

More recently with the advent of multichannel adhesive EMG electrode arrays investigation of fatigue during dynamic contractions has become possible. However, the results of these studies are contradicting. For example, during fatiguing cycling exercise MFCV has been reported to decrease (Farina, Pozzo, et al., 2004; Sbriccoli et al., 2009), or remain constant(Macdonald et al., 2008). Small changes in MFCV during exercise have been attributed to the relatively lower acidity caused by dynamic exercise compared to isometric contractions(Schmitz et al., 2012).

Effect of Stimulation: As electrical stimulation has found application such as increasing strength the possible effect of electrical stimulation on MU recruitment has been investigated. To this end, EMG variables, including conduction velocity of MUAPs, have been studied. However the results from studies have been conflicting. For example, Trimble and Enoka studied the elicited H-reflex and M-response in muscle which they explained to typically produce slow and fast twitch responses respectively (Trimble & Enoka, 1991). Their results showed different trends in H-reflex and M-wave in response to stimulation versus voluntary activation. Stimulation resulted in an increase in time-to-peak and peak amplitude of M-wave, but a decrease in H-reflex time-to-peak. Based on these findings, Trimble and Enoka suggested that electrical stimulation, as compared to voluntary contraction, causes the activation of a faster-contacting population of motor units. It is well known that motor units with larger fibers are innervated by larger axons, which have a lower threshold of electrical excitability. Therefore it seems reasonable to expect the larger motor units to be recruited at lower stimulation levels. This seems to be case with direct stimulation of nerve(Kim et al., 1995). However Kim et al. concluded that, although this neurophysiological phenomenon may hold true for direct stimulation of the motor nerve in vitro or in situ, orientation of the peripheral nerves may not favor the activation of these fibers with cutaneous electrical stimulation, thus resulting in a more random pattern of recruitment during surface electrical stimulation(Kim et al., 1995) . In contrast to these findings for instance, Knaflitz et al. reported that only in 28% of the cases increasing the intensity of motor point electrical stimulation caused the conduction velocity to decrease indicating a reverse order of recruitment (Knaflitz et al., 1990). They concluded that motor point stimulation in general does not cause a reverse recruitment order. The authors suggested that the discrepancy might be a result of geometric factors, e.g. position of stimulating electrodes, or the lack of correlation between axonal branch diameter and the diameter of the parent motoneuron axon. The effect of geometric factors was further supported by the study of Henriksson-Larsen et al. (Henriksson-Larsen et al., 1985)which showed that motor units in the deep region of tibialis anterior have an average 13% larger diameter than that of the superficial motor units. Simulation studies and comparison with experimental data have shown that higher intensities of stimulation might induce recruitment of progressively deeper motor units(Farina, Blanchietti, et al., 2004; Mesin et al., 2010).It was suggested that MUs were recruited from low to high CV (small to large fibers MUs) and from superficial to deep muscle layers and that MUs located closer to stimulating electrodes are re-

cruited first. In other words the controversies pertaining MU recruitment during electrical stimulation can be explained by different MU distribution in muscles as well as the inter-individual differences(Mesin et al., 2010).

4 AIM OF STUDY

The purpose of this study was to investigate the effect of inter-stimulus interval used in ITT. The question is whether there is an optimal interval when using ITT, how muscle response is affected by changing the interval and whether that can affect the choice of stimulus mode. Furthermore, young and elderly adults were compared in order to study the effect of factors such as subjects' age on twitch properties in order to standardize the stimulation mode. Also array EMG electrodes were used in order to estimate the muscle fiber conduction velocity and compare its possible role in the changes between the two age groups. As quantification of activation level -regardless of the type of activity performed- involves a comparison with control twitch, i.e. response of resting muscle to the same stimulation, in this study as well the effect of stimulus interval was examined in the calf muscles in a resting position with no voluntary activation.

5 METHODS

5.1 Subjects

Twenty one young adults (Y: 11 female and 10 male, 26.4 ±3.2 years, 173.8 ± 7.6 cm, 70.1 ± 11 kg) and nineteen elderly adults (E: all male, 71.9 ± 5.1 years, 174.2 ± 5.1 cm, 75.9 ± 10.2 kg)) participated in the study. Before testing, all subjects were informed about the procedures and they signed informed consent. The study was approved by the University ethics board in conformity with the Declaration of Helsinki.

5.2 Experimental Design

Subjects were seated on a custom-built ankle dynamometer (University of Jyväskylä) with the right leg resting on a footplate and the left leg in an extended position. The data in this study were acquired from two trials completed with knee in bent (90°) and extended (180°) position in random order while right ankle and hip were fixed at 90° and 120° respectively. As a short warm-up, subjects performed several plantar flexion and dorsiflexion movements before the experiments. Then eighteen supramaximal electrical stimulations were randomly administered to the tibial nerve with a rest period of approximately 20 seconds between two consecutive stimulations. The electrical stimulation trials consisted of one single pulse stimulation (S) and eight doublets with intervals of 5 to 40 milliseconds with increments of 5 milliseconds, (5, 10, 15,20 25, 30, 35 and 40) each of which was delivered two times. The twitch torque of ankle plantarflexors was measured for each trial. EMG was recorded from soleus (SOL) and tibialis anterior (TA) and lateral gastrocnemius (LG) muscles. Also an array electrode was used to record the EMG activity from medial gastrocnemius (MG) muscle.

5.3 Procedures

Electrical stimulations were delivered to tibial nerve using a constant-current stimulator (DS7A, Digitimer, Hertfordshire, UK). Optimal stimulation site where the highest twitch amplitude was attained with relatively low stimulation intensity was determined by moving a stimulating probe near the popliteal fossa in prone position and was then marked. A circular cathode with a pickup area of 77 mm^2 (Unilect short-term ECG Electrodes, Ag/AgCl, Unomedical Ltd., UK) was placed over the stimulation site and an oval-shaped, 5,08 cm x 10,16 cm, anode (V-trodes neurostimulation electrodes, Mattler Electronics corp., USA) was placed above the patella. Responses to single electrical pulses were visualized in real time. The maximal stimulation intensity was determined as the one that consistently produced the largest torque and M-wave in SOL. To ensure supramaximal stimulation, intensity was further increased to 150 %.

5.4 Data collection

Bipolar Ag/AgCl electrodes were used to measure EMG from the TA, SOL and LG muscles. To detect the EMG activity of SOL muscle an electrode pair was placed longitudinally between the muscle mid belly and tendon. Inter-electrode distance was 2cm; electrode shape was circular and diameter 5 mm. A reference electrode (Unilect) was placed over the lateral malleolus. A bipolar electrode was place over the TA muscle belly to the side of tibia bone. Also to record the EMG activity of LG muscle a bipolar electrode was placed on the muscle belly where the bulge was prominent when the subject was asked to do a plantar flexion while pressure was applied against the forefoot. The skin under the electrodes was shaved, abraded and cleaned with alcohol to reduce the inter-electrode resistance. An adhesive array electrode was placed over MG. The best position for the placement of array electrode, where the action potential in different channels were similar in shape indicating the alignment with muscle fiber orientation, was first determined with a silver bar electrode. The position was marked for the placement of an adhesive array EMG electrode. The EMG activity from the SOL, TA, LG, MG and torque from the force platform of the footplate were stored simultaneously on a personal computer. EMG was amplified (gain = 1000) and band-pass filtered (10-1000 Hz) (Cambridge Electronics Design Limited, UK). Data was collected into the computer via 16-bit AD converter (CED Power 1401, Cambridge Electronics Design Limited, UK) with a sampling frequency of 2 kHz for torque, 2 kHz for bipolar electrode and 3 kHz for array.

5.5 Data Analysis

Torque data were stored on a personal computer with Spike 2 software (CED, Cambridge, UK) for offline analysis. The following twitch characteristics were determined: (1) Normalized twitch peak torque (NPT) defined as the baseline-to-peak twitch amplitude divided subject's body mass, (2) time to peak torque defined as the time elapsed from onset (1% of twitch amplitude) to the peak torque (PT), (3) half relaxation time (HRT) defined as the time elapsed from the peak twitch torque to 50% peak twitch torque and (4) maximal twitch rate of torque development (RTD) which was determined as the peak value of first derivative of the curve fitted to torque data. Curve Fitting Tool

of Matlab software was used to fit an eight-term Fourier curve to the torque data in order to reduce the effect of noise and avoid noise amplification in calculating RTD. First derivative of the fitted curve was calculated to measure the maximal rate of torque development. For each stimulation mode the values measured from the two repetitions were averaged.

5.6 Statistical Analysis

Results were compared with repeated measures of ANOVA. T-test was conducted to compare the data from young and elderly adults. The significance level was set at $P < 0,05$. Unfortunately probably due to errors in positioning of the EMG array electrode, its data resulted in CV values outside the physiological ranges (CV ranges: 3-6 ms^{-1}(Andreassen & Arendt-Nielsen, 1987)) and therefore those results are not reported here.

6 RESULTS

6.1 Normalized Peak Twitch Torque

Bent knee. In bent knee position, in both young and elderly adults NPT of single pulse stimulation was significantly lower than that of doublet stimuli trials (Figure 6). In elderly adults inter-stimulus interval of 10ms resulted in a significantly higher NPT than 5ms. Elderly subjects had slightly smaller NPT (3.58 ±0.2 N.m.kg^{-1}s^{-1}) than the young (3.74±0.2 N.m.kg^{-1}s^{-1}), but this difference did not reach significance.
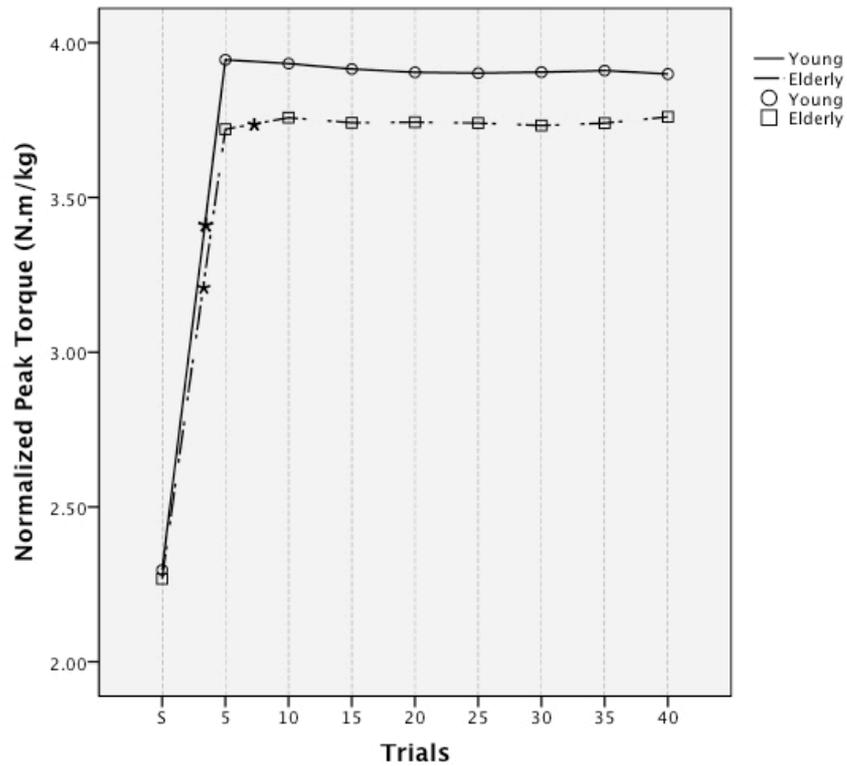
FIGURE 6. Normalized peak torque with knee at 90° for young and elderly subjects. ‡: significant difference between Y and E in a trial, *: significant difference between two consecutive trials shown in the figure.

Extended knee. Similarly, with knee in extended position, single stimulus resulted in a significantly lower NPT than doublets and no significant difference was found between Y (3.85 ±0.3 N.m.kg^{-1}s^{-1}) and E (3.63 ±0.3 N.m.kg^{-1}s^{-1}) (Figure 7). NPT of both groups were slightly higher in this position compared to that of flexed knee position.
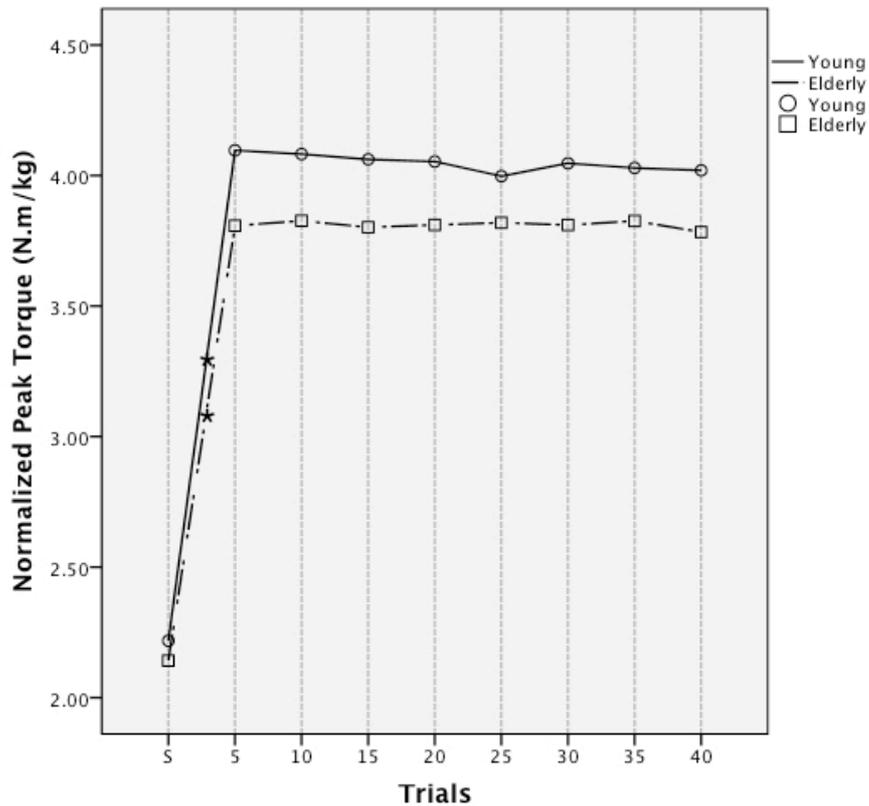
FIGURE 7. Normalized peak torque with knee at 180° for young and elderly subjects. ‡: significant difference between Y and E in a trial, *: significant difference between two consecutive trials shown in the figure.

6.2 Maximal Rate of Torque Development

Bent knee. In 90-90 position doublet stimulus with inter-stimulus interval of 5ms resulted in a significantly higher RTD, (3.99 N.m/s), in young adults compared to other stimulation modes (Figure 8). In elderly subjects there was no significant difference between RTD produced by paired stimuli with intervals of 5ms and 10ms, nevertheless RTD in both of those trials were significantly higher than other stimulation modes. No significant difference was observed between Y and E across the trials.
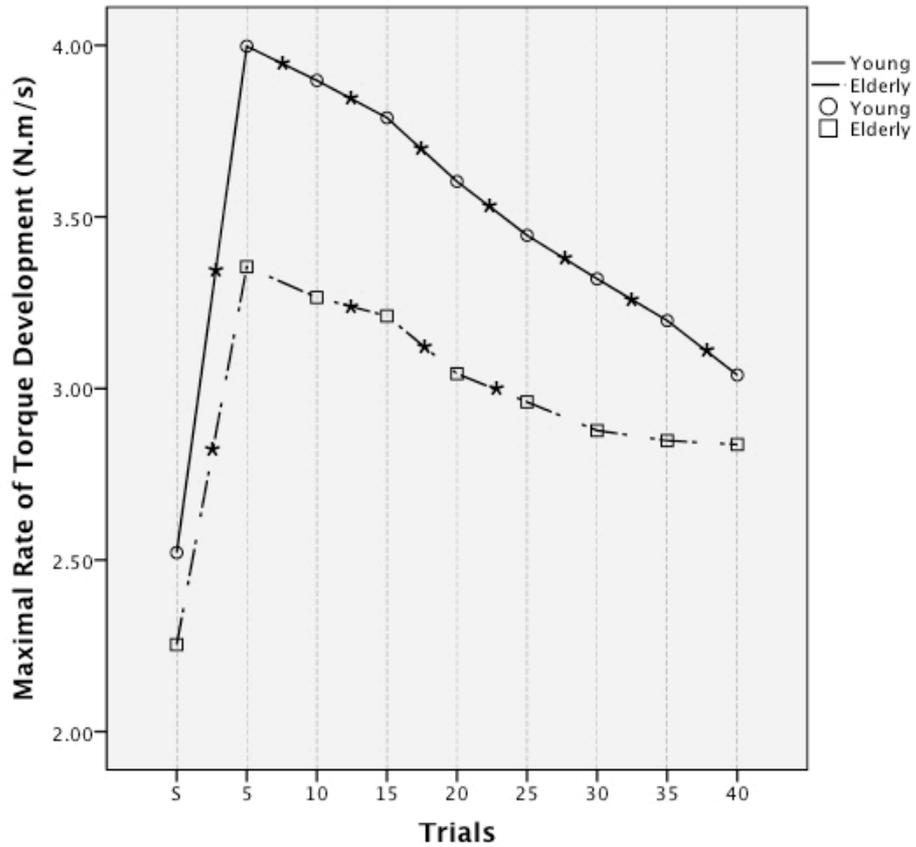
FIGURE 8. Maximal rate of torque development with knee angle at 90° for young and elderly subjects. ‡: significant difference between Y and E in a trial, *: significant difference between two consecutive trials shown in the figure.

Extended knee. In 180 - 90 position in elderly adults RTD produced by doublets with 5 and 10ms interval were not significantly different (3.55 and 3.54 N.m/s respectively), but were significantly higher than other stimulation modes (Figure 9). Similarly in young adults, the RTD produced by doublets with 5 and 10ms intervals (3.9 and 3.88 N.m/s respectively), which did not show any significantly difference were significantly higher than that of other trials. No Significant difference was observed between the RTD of Y and E.
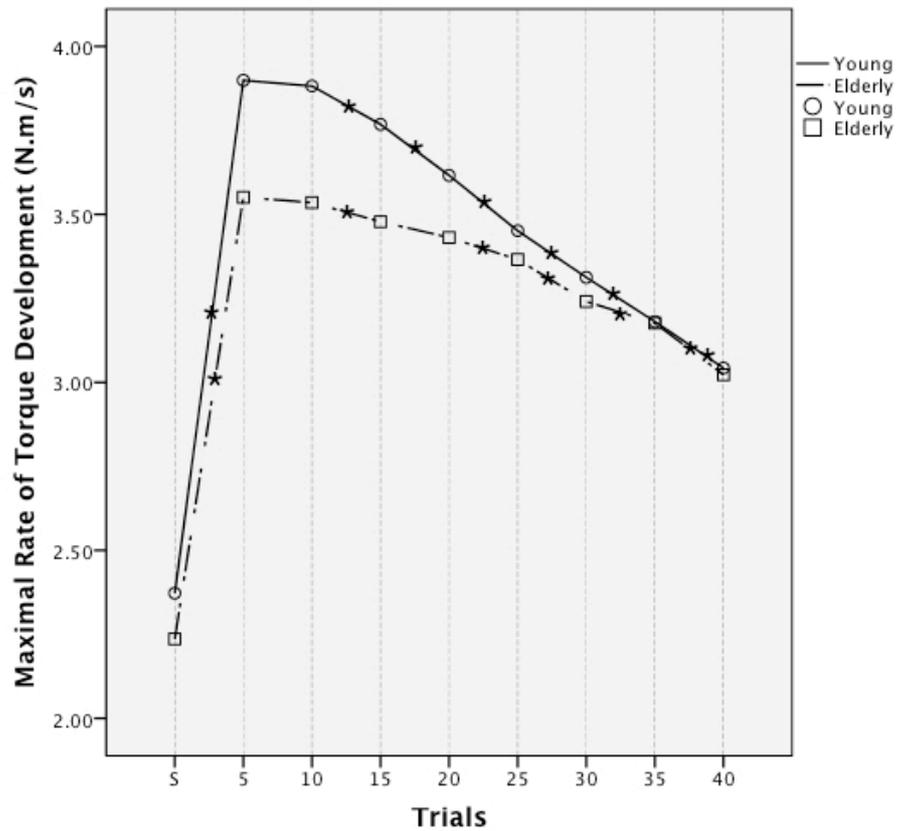
FIGURE 9. Maximal rate of torque development with knee angle at 180° for young and elderly subjects. ‡: significant difference between Y and E in a trial, *: significant difference between two consecutive trials shown in the figure.

6.3 Time to Peak Torque

Bent knee. In flexed knee position, TPT of doublet with 5ms interval (78.7 ms) was significantly longer than single stimulus (65.7 ms) and significantly shorter than other doublets in young adults (Figure 10). In the elderly adults, no significant difference was observed between TPT prduced by 5 and 10 ms intervals. TPT of Y was significantly shorter than that of E in all stimulation modes with an overall average value of 79.9 ± 2.6 and 99.9 ± 2.6 ms respectively. In both groups single stimulus resulted in a significantly lower RTD than other stimulation modes, 65± 2.1ms in Y and 82.4 ± 2.1ms in E.
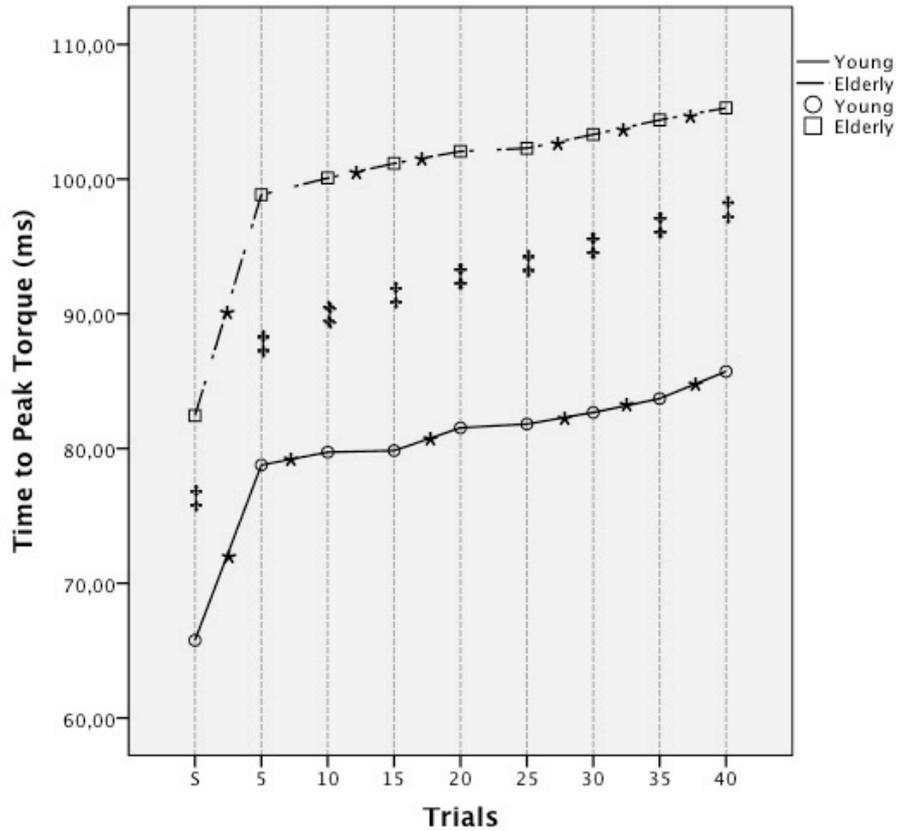
FIGURE 10. Time to peak torque with knee angle at 90° for young and elderly adults.
‡: significant difference between Y and E in a trial, *: significant difference between
two consecutive trials shown in the figure.

Extended Knee. Similarly, in extended knee position for all stimulation modes there was
a significant difference between TPT of young and elderly adults (Figure 11). Their
average values over all trials were 78.7 ± 2.5ms in Y and 91.6 ± 2.6ms in E. In both
groups, single stimulus resulted in a significantly shorter TPT compared to other stimu-
lation modes with a mean value of 64.72 ± 7.5ms in young and 77.2 ± 12 ms in elderly
adults.

FIGURE 11. Time to peak Torque with knee angle at 180° for young and elderly adults. . ‡: significant difference between Y and E in a trial, *: significant difference between two consecutive trials shown in the figure.

6.4 Half Relaxation Time

Bent knee. In the 90-90 position no significant difference was observed in HRT among the trials in the E (Figure 12): The average value over all trials were 54.13 ± 5.7, 73.3± 6 ms for young and elderly individuals respectively. In young adults single stimulus had a significantly lower HRT than other stimulation modes. Also there was a significant difference in HRT between Y and E in all trials.
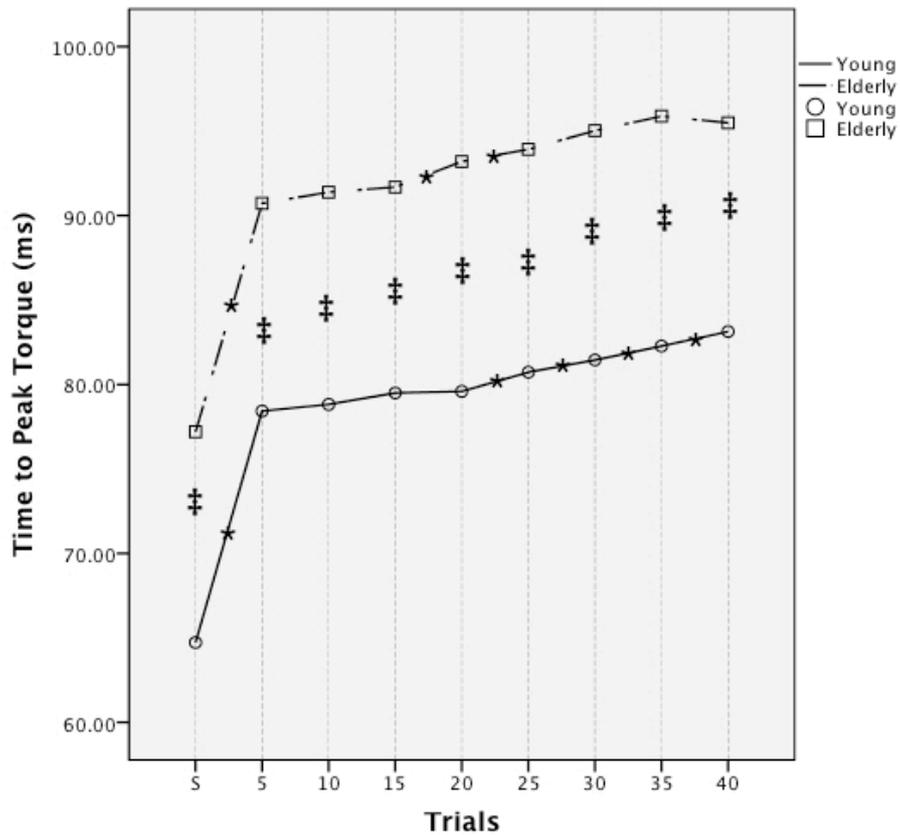
FIGURE 12. Half relaxation time with knee angle at 90° for young and elderly adults. ‡: signif-
icant difference between Y and E in a trial, *: significant difference between two consecutive
trials shown in the figure.

Extended knee. Similarly, a significant difference between HRT in young and elderly
adults was observed in 180-90 position (Figure 13), with an overall average of 56.4 ±
2.5, 67.8 ± 2.6 ms for Y and E respectively. In both groups single pulse stimulus result-
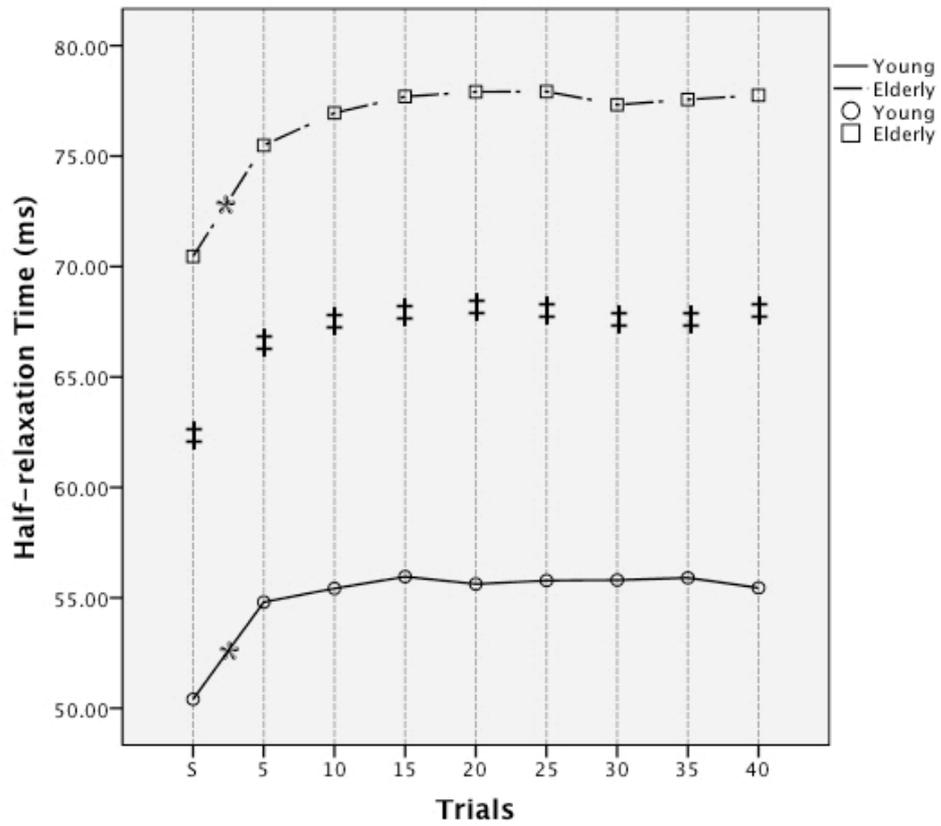ed in a significantly shorter HRT compared to other stimuli.
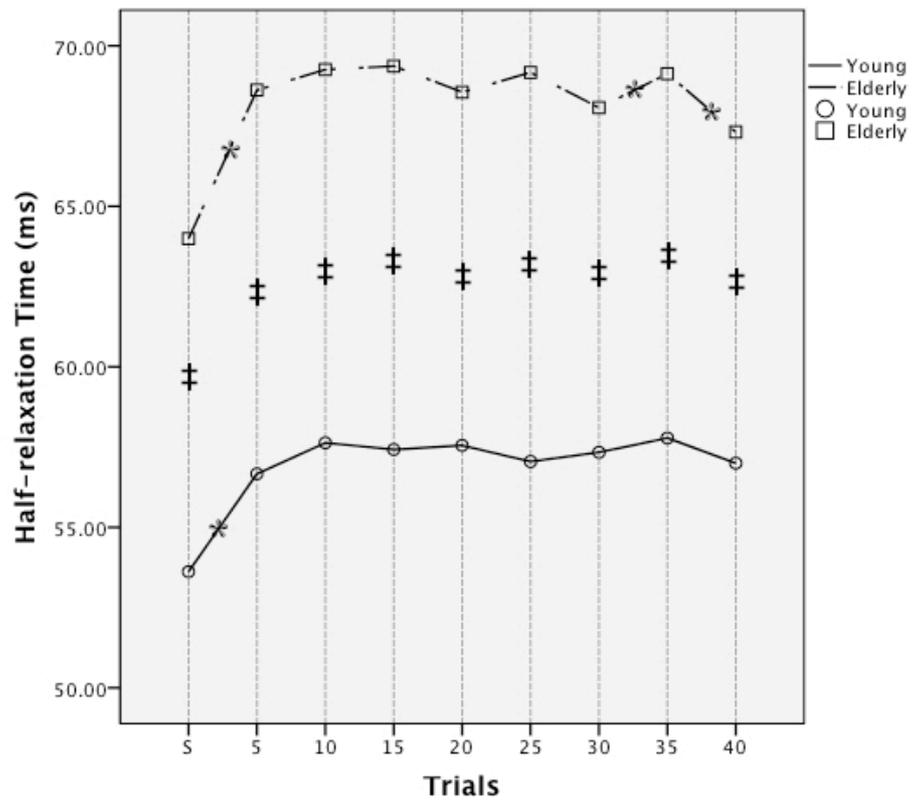
FIGURE 13. Half relaxation time with knee angle at 180° for young and elderly adults. ‡: significant difference between Y and E in a trial, *: significant difference between two consecutive trials shown in the figure.

7 DISCUSSION

The main findings in this study include: 1) significantly shorter TPT and HRT, as well as higher RTD and NPT in the young adults compared to the elderly in all stimulation modes 2) shortening of TPT and HRT and greater NPT for both age groups in extended vs. flexed knee position 3) an apparent achievement of highest response with doublet intervals of 10 and 5 ms in elderly and young adults respectively.

The relatively parallel changes in doublet-evoked torque properties e.g., the gradual drop in RTD and prolongation of TPT, with increasing the interstimulus interval may be due the "silent period" produced by the first stimulus. In addition to collision with or-

thodromic volley, antidromic current of stimulus may activate Renshaw cells and cause recurrent inhibition. Also Ib afferent stimulation and unloading of muscle spindles may contribute to a period of inhibition after the electrical stimulation(Sophie J De Serres et al., 2012; Herbert & Gandevia, 1999). These effects may be mitigated in shorter inter-stimulus intervals with a summation effect and little time for the longer-latency reflexes to take effect and thus the decline in twitch properties are less. Summated torques of the doublets were significantly "more rapid" (had higher RTD) and larger than single stimulus ones.

Discrepancy in the literature regarding the effect of ageing on twitch amplitude may be due to different muscles being studied(Clark & Taylor, 2011) . While several studies have reported an age-related decline in evoked twitch amplitude(Thom et al., 2005; Winegard et al., 1997), lack of difference also observed in this study is in accordance with studies of (Klass et al., 2005; McNeil et al., 2007; Narayanan et al., 1996). Klass et al. suggested that this observation alongside with a well-documented loss of force generating capacity in the elderly indicates a greater contribution of slowing of muscle contractile mechanism, rather than age-related increase in tendon compliance(Narici & Maganaris, 2006), to slowing of muscle contractile kinetics in elderly.

Ankle planter flexion has been reported to decrease with increasing knee flexion angle (Arampatzis et al., 2006; Cresswell et al., 1995; Dalton et al., 2013). This reduction has mainly been attributed to shortened muscle length of Gastrocnemius and a possible consequent impairment in neuromuscular transmission propagation(Cresswell et al., 1995) or alternatively to a critical force–length potential of all three muscles of the triceps surae (Arampatzis et al., 2006). Greater NPT values of Y in extended knee position may be attributed to this greater contribution of gastrocnemius.

Torques in extended knee compared to flexed knee position showed slight shortening of TPT and HRT, and a greater NPT. These observations can be attributed to greater contribution of gastrocnemius muscle- also a faster-twitch muscle as compared to soleus- in extended knee position. Ankle planter flexion has been reported to decrease with increasing knee flexion angle (Arampatzis et al., 2006; Cresswell et al., 1995; Dalton et al., 2013). This reduction has mainly been attributed to shortened muscle length of gastrocnemius and a possible consequent impairment in neuromuscular transmission prop-

agation(Cresswell et al., 1995) or alternatively to a critical force–length potential of all three muscles of the triceps surae (Arampatzis et al., 2006). Moreover histochemical analysis has shown that gastrocnemius is comprised of approximately equal proportions of fast twitch (with shorter contraction time(Close, 1967)) and slow twitch fibers while soleus is mainly comprised of slow twitch fibers (Gollnick et al., 1974). Therefore less contribution of this muscle to the plantar flexor torque in flexed knee position (Cresswell et al., 1995; Yasuo Kawakami et al., 1998), may prolong the twitch. This is in accordance with the significant positive relationship between percentage of slow twitch fibers and TPT (Rice et al., 1988).Furthermore, selective atrophy of fast fibers with age(Porter et al., 1995) which would mean a relatively greater atrophy of gastrocnemius may be a causative factor to greater prolongation of TPT in the elderly in extended knee position.

Prolongation of TPT and HRT with age shown in this study are in accordance with previous studies(Pääsuke et al., 2000; Simoneau et al., 2005; van Schaik et al., 1994; Vandervoort & McComas, 1986). It has been suggested that while TPT is related to the rate of release of Ca^{2+} from the sarcoplasmic reticulum, HRT is associated with the rate at which Ca^{2+} is sequestered(Fitts et al., 1980; Miyamoto et al., 2012). The effect of ageing on sarcoplasmic reticulum and its function in muscle fibers has been investigated however the reports are divergent. For example, Narayanan reported no age-related modification of sarcoplasmic reticulum of Gastrocnemius and a significant reduction in Ca^{2+} uptake properties of sarcoplasmic reticulum in soleus(Narayanan et al., 1996), whereas Larson reported that no age-related differences occur in slow twitch muscle fibers(Larsson & Salviati, 1989)). Although the mechanism is not clear, a significant prolongation of HRT and TPT with age as an indicator of slowing of muscle kinetics has frequently been reported and was also shown in this study.

Although in both positions the maximum RTD was higher for Y compared to E in all stimulation modes, this difference did not reach significance. In literature development rate of electrically evoked torque has been reported to decrease (Klass et al., 2005; Wilder & Cannon, 2009), or remain unchanged with age(Callahan & Kent-Braun, 2011; Lanza et al., 2004; van Schaik et al., 1994). Change in RTD may be attributed to alterations in the MHC composition of the muscle(Harridge et al., 1996) and sarcoplasmic reticulum Ca^{2+} kinetics(Kugelberg & Thornell, 1983). On the other hand, age-

related increase in tendon compliance which has been suggested to contribute to slowing of muscle in older individuals (Narici & Maganaris, 2006) may obscure the effect of age on contractile characteristics of single muscle fiber. Discrepancy in the literature may also be due to different methods of RTD calculation or time epochs from which rate is calculated.

The differences between young and elderly adults may have been masked by the fact that Y included female subjects while E was comprised of solely male adults some of whom had previous background of chronic training. Elimination of female subjects in extended knee position resulted in a ~6% increase in mean peak torque value of the young adults.

In conclusion, doublets evoke larger responses, and can therefore be more readily detected for use in ITT. An interval of 10ms for the elderly seems to elicit highest responses; while in young individuals an interval of 5ms results in a significantly higher rate of torque development and therefore may be better for power-related studies. Based on the results from these studies short-interval doublets can be recommended for using ITT.

8 REFERENCES:

Allen, G. M., McKenzie, D. K., & Gandevia, S. C. (1998). Twitch interpolation of the elbow flexor muscles at high forces. *Muscle & nerve*, *21*(3), 318–28.

Andreassen, S., & Arendt-Nielsen, L. (1987). Muscle fibre conduction velocity in motor units of the human anterior tibial muscle: a new size principle parameter. *The Journal of physiology*, *391*, 561–71.

Arampatzis, A., Karamanidis, K., Stafilidis, S., Morey-Klapsing, G., DeMonte, G., & Brüggemann, G.-P. (2006). Effect of different ankle- and knee-joint positions on gastrocnemius medialis fascicle length and EMG activity during isometric plantar flexion. *Journal of Biomechanics*, *39*(10), 1891–1902.

Astrand, P.-O., Rodahl, K., Dahl, H. A., & Stromme, S. B. (2003). *Textbook of Work Physiology-4th: Physiological Bases of Exercise* (p. 656). Human Kinetics.

Bampouras, T. M., Reeves, N. D., Baltzopoulos, V., & Maganaris, C. N. (2006). Muscle activation assessment: effects of method, stimulus number, and joint angle. *Muscle & nerve*, *34*(6), 740–6. doi:10.1002/mus.20610

Basmajian, J. V., & Luca, C. J. De. (1985). *Muscles Alive: Their Functions Revealed by Electromyography* (p. 561). Williams & Wilkins.

Bazzucchi, I., Felici, F., Macaluso, A., & De Vito, G. (2004). Differences between young and older women in maximal force, force fluctuations, and surface EMG during isometric knee extension and elbow flexion. *Muscle & nerve*, *30*(5), 626–35. doi:10.1002/mus.20151

Behm, D. G., St-Pierre, D. M. M., & Perez, D. (1996). Muscle inactivation: assessment of interpolated twitch technique. *J Appl Physiol*, *81*(5), 2267–2273.

Behm, D., Power, K., & Drinkwater, E. (2001). Comparison of interpolation and central activation ratios as measures of muscle inactivation. *Muscle & nerve*, *24*(7), 925–34.

Beltman, J. G. M., Sargeant, A. J., van Mechelen, W., & de Haan, A. (2004). Voluntary activation level and muscle fiber recruitment of human quadriceps during lengthening contractions. *Journal of applied physiology (Bethesda, Md. : 1985)*, *97*(2), 619–26. doi:10.1152/japplphysiol.01202.2003

Bilodeau, M, Erb, M. D., Nichols, J. M., Joiner, K. L., & Weeks, J. B. (2001). Fatigue of elbow flexor muscles in younger and older adults. *Muscle & nerve*, *24*(1), 98–106.

Bilodeau, Martin. (2006). Central fatigue in continuous and intermittent contractions of triceps brachii. *Muscle & nerve*, *34*(2), 205–13. doi:10.1002/mus.20572

Blijham, P. J., van Engelen, B. G. M., Drost, G., Stegeman, D. F., Schelhaas, H. J., & Zwarts, M. J. (2011). Diagnostic yield of muscle fibre conduction velocity in myopathies. *Journal of the neurological sciences*, *309*(1-2), 40–4. doi:10.1016/j.jns.2011.07.036

Bülow, P. M., Nørregaard, J., Danneskiold-Samsøe, B., & Mehlsen, J. (1993). Twitch interpolation technique in testing of maximal muscle strength: influence of potentiation, force level, stimulus intensity and preload. *European journal of applied physiology and occupational physiology*, *67*(5), 462–6.

Bülow, P. M., Nørregaard, J., Mehlsen, J., & Danneskiold-Samsøe, B. (1995). The twitch interpolation technique for study of fatigue of human quadriceps muscle. *Journal of neuroscience methods*, *62*(1-2), 103–9.

Burnley, M., Vanhatalo, A., & Jones, A. M. (2012). Distinct profiles of neuromuscular fatigue during muscle contractions below and above the critical torque in humans. *Journal of applied physiology (Bethesda, Md. : 1985)*, *113*(2), 215–23. doi:10.1152/japplphysiol.00022.2012

Callahan, D. M., & Kent-Braun, J. A. (2011). Effect of old age on human skeletal muscle force-velocity and fatigue properties. *Journal of applied physiology (Bethesda, Md. : 1985)*, *111*(5), 1345–52. doi:10.1152/japplphysiol.00367.2011

Clark, B. C., & Taylor, J. L. (2011). Age-related changes in motor cortical properties and voluntary activation of skeletal muscle. *Current aging science*, *4*(3), 192–9.

Close, R. (1967). Properties of motor units in fast and slow skeletal muscles of the rat. *J. Physiol.*, *193*(1), 45–55.

Cresswell, A. G., Löscher, W. N., & Thorstensson, A. (1995). Influence of gastrocnemius muscle length on triceps surae torque development and electromyographic activity in man. *Experimental brain research. Experimentelle Hirnforschung. Expérimentation cérébrale*, *105*(2), 283–90.

Dalton, B. H., Power, G. A., Allen, M. D., Vandervoort, A. A., & Rice, C. L. (2013). The genu effect on plantar flexor power. *European journal of applied physiology*, *113*(6), 1431–9. doi:10.1007/s00421-012-2560-0

De Haan, A., Gerrits, K. H. L., & de Ruiter, C. J. (2009). Counterpoint: the interpolated twitch does not provide a valid measure of the voluntary activation of muscle. *Journal of applied physiology (Bethesda, Md. : 1985)*, *107*(1), 355–7; discussion 357–8. doi:10.1152/japplphysiol.91220.2008a

De Serres, S J, & Enoka, R. M. (1998). Older adults can maximally activate the biceps brachii muscle by voluntary command. *Journal of applied physiology (Bethesda, Md. : 1985)*, *84*(1), 284–91.

De Serres, Sophie J, Enoka, R. M., Manini, T. M., Clark, B. C., Biol, J. G. A., & Med, S. (2012). Older adults can maximally activate the biceps brachii muscle by

voluntary command muscle Older adults can maximally activate the biceps brachii muscle by voluntary command, 284–291.

Dowling, J. J., Konert, E., Ljucovic, P., & Andrews, D. M. (1994). Are humans able to voluntarily elicit maximum muscle force? *Neuroscience Letters*, *179*(1), 25–28.

Edwards, R. H., Hill, D. K., Jones, D. A., & Merton, P. A. (1977). Fatigue of long duration in human skeletal muscle after exercise. *The Journal of physiology*, *272*(3), 769–78.

Farina, D., Arendt-Nielsen, L., Merletti, R., & Graven-Nielsen, T. (2002). Assessment of single motor unit conduction velocity during sustained contractions of the tibialis anterior muscle with advanced spike triggered averaging. *Journal of neuroscience methods*, *115*(1), 1–12.

Farina, D., Blanchietti, A., Pozzo, M., & Merletti, R. (2004). M-wave properties during progressive motor unit activation by transcutaneous stimulation. *Journal of applied physiology (Bethesda, Md. : 1985)*, *97*(2), 545–55. doi:10.1152/japplphysiol.00064.2004

Farina, D., Pozzo, M., Merlo, E., Bottin, A., & Merletti, R. (2004). Assessment of average muscle fiber conduction velocity from surface EMG signals during fatiguing dynamic contractions. *IEEE transactions on bio-medical engineering*, *51*(8), 1383–93. doi:10.1109/TBME.2004.827556

Fitts, R. H., Winder, W. W., Brooke, M. H., Kaiser, K. K., & Holloszy, J. O. (1980). Contractile, biochemical, and histochemical properties of thyrotoxic rat soleus muscle. *The American journal of physiology*, *238*(1), C14–20.

Gandevia, S. C. (2001). Spinal and supraspinal factors in human muscle fatigue. *Physiological reviews*, *81*(4), 1725–89.

Gandevia, S. C., Allen, G. M., Butler, J. E., & Taylor, J. L. (1996). Supraspinal factors in human muscle fatigue: evidence for suboptimal output from the motor cortex. *The Journal of physiology*, *490 (Pt 2*, 529–36.

Gandevia, S. C., & McKenzie, D. K. (1988). Activation of human muscles at short muscle lengths during maximal static efforts. *The Journal of physiology*, *407*, 599–613.

Gandevia, S. C., McNeil, C. J., Carroll, T. J., & Taylor, J. L. (2013). Twitch interpolation: superimposed twitches decline progressively during a tetanic contraction of human adductor pollicis. *The Journal of physiology*, *591*(Pt 5), 1373–83. doi:10.1113/jphysiol.2012.248989

Gollnick, P. D., Sjödin, B., Karlsson, J., Jansson, E., & Saltin, B. (1974). Human soleus muscle: A comparison of fiber composition and enzyme activities with other leg muscles. *Pflügers Archiv European Journal of Physiology*, *348*(3), 247–255. doi:10.1007/BF00587415

Gondin, J., Guette, M., Maffiuletti, N. A., & Martin, A. (2004). Neural activation of the triceps surae is impaired following 2 weeks of immobilization. *European journal of applied physiology*, *93*(3), 359–65. doi:10.1007/s00421-004-1225-z

Griffin, L., Garland, S. J., Ivanova, T., Kleine, B. U., Stegeman, D. F., Schelhaas, H. J., … Kamen, G. (2012). Discharge patterns in human motor units during fatiguing arm movements tasks Discharge patterns in human motor units during fatiguing arm movements, 1684–1692.

Harridge, S. D., Bottinelli, R., Canepari, M., Pellegrino, M. A., Reggiani, C., Esbjörnsson, M., & Saltin, B. (1996). Whole-muscle and single-fibre contractile properties and myosin heavy chain isoforms in humans. *Pflügers Archiv : European journal of physiology*, *432*(5), 913–20.

Henriksson-Larsen, K., FRIDEN, J., & WRETLING, M.-L. (1985). Distribution of fibre sizes in human skeletal muscle. An enzyme histochemical study in m tibialis anterior. *Acta Physiologica Scandinavica*, *123*(2), 171–177. doi:10.1111/j.1748-1716.1985.tb07574.x

Herbert, R. D., & Gandevia, S. C. (1999). Twitch interpolation in human muscles: mechanisms and implications for measurement of voluntary activation. *Journal of neurophysiology*, *82*(5), 2271–83.

Hogrel, J.-Y. (2005). Clinical applications of surface electromyography in neuromuscular disorders. *Neurophysiologie clinique = Clinical neurophysiology*, *35*(2-3), 59–71. doi:10.1016/j.neucli.2005.03.001

Houtman, C. J., Stegeman, D. F., Van Dijk, J. P., & Zwarts, M. J. (2003). Changes in muscle fiber conduction velocity indicate recruitment of distinct motor unit populations. *Journal of applied physiology (Bethesda, Md. : 1985)*, *95*(3), 1045–54. doi:10.1152/japplphysiol.00665.2002

Huber, A., Suter, E., & Herzog, W. (1998). Inhibition of the quadriceps muscles in elite male volleyball players. *Journal of sports sciences*, *16*(3), 281–9. doi:10.1080/026404198366812

Jakobi, J. M., & Rice, C. L. (2002). Voluntary muscle activation varies with age and muscle group. *Journal of applied physiology (Bethesda, Md. : 1985)*, *93*(2), 457–62. doi:10.1152/japplphysiol.00012.2002

Kawakami, Y, Akima, H., Kubo, K., Muraoka, Y., Hasegawa, H., Kouzaki, M., … Fukunaga, T. (2001). Changes in muscle size, architecture, and neural activation after 20 days of bed rest with and without resistance exercise. *European journal of applied physiology*, *84*(1-2), 7–12. doi:10.1007/s004210000330

Kawakami, Yasuo, Ichinose, Y., & Fukunaga, T. (1998). Architectural and functional features of human triceps surae muscles during contraction. *J Appl Physiol*, *85*(2), 398–404.

Kent-Braun, J. A., & Le Blanc, R. (1996). Quantitation of central activation failure during maximal voluntary contractions in humans. *Muscle & nerve*, *19*(7), 861–9. doi:10.1002/(SICI)1097-4598(199607)19:7<861::AID-MUS8>3.0.CO;2-7

Kent-Braun, J. A., Ng, a V, Doyle, J. W., & Towse, T. F. (2002). Human skeletal muscle responses vary with age and gender during fatigue due to incremental isometric exercise. *Journal of applied physiology (Bethesda, Md. : 1985)*, *93*(5), 1813–23. doi:10.1152/japplphysiol.00091.2002

Kim, C. K., Bangsbo, J., Strange, S., Karpakka, J., & Saltin, B. (1995). Metabolic response and muscle glycogen depletion pattern during prolonged electrically induced dynamic exercise in man. *Scandinavian journal of rehabilitation medicine*, *27*(1), 51–8.

Klass, M., Baudry, S., & Duchateau, J. (2005). Aging does not affect voluntary activation of the ankle dorsiflexors during isometric, concentric, and eccentric contractions. *Journal of applied physiology (Bethesda, Md. : 1985)*, *99*(1), 31–8. doi:10.1152/japplphysiol.01426.2004

Klein, C. S., Rice, C. L., & Marsh, G. D. (2001). Normalized force, activation, and coactivation in the arm muscles of young and old men. *Journal of applied physiology (Bethesda, Md. : 1985)*, *91*(3), 1341–9.

Knaflitz, M., Merletti, R., & De Luca, C. J. (1990). Inference of motor unit recruitment order in voluntary and electrically elicited contractions. *Journal of applied physiology (Bethesda, Md. : 1985)*, *68*(4), 1657–67.

Knight, C. A., & Kamen, G. (2008). Relationships between voluntary activation and motor unit firing rate during maximal voluntary contractions in young and older adults. *European journal of applied physiology*, *103*(6), 625–30. doi:10.1007/s00421-008-0757-z

Kooistra, R. D., de Ruiter, C. J., & de Haan, A. (2007). Conventionally assessed voluntary activation does not represent relative voluntary torque production. *European journal of applied physiology*, *100*(3), 309–20. doi:10.1007/s00421-007-0425-8

Krogh-lund, C., & Jergensen, K. (1992). , u , o ,.. A p p l i e d Physiology Modification of myo-electric power spectrum in fatigue from 15 % maximal voluntary contraction of human elbow flexor muscles , to limit of endurance : reflection of conduction velocity variation and / or centrally medi, 359–370.

Kugelberg, E., & Thornell, L. E. (1983). Contraction time, histochemical type, and terminal cisternae volume of rat motor units. *Muscle & nerve*, *6*(2), 149–53. doi:10.1002/mus.880060211

Kupa, E. J., Roy, S. H., Kandarian, S. C., & De Luca, C. J. (1995). Effects of muscle fiber type and size on EMG median frequency and conduction velocity. *Journal of applied physiology (Bethesda, Md. : 1985)*, *79*(1), 23–32.

Lanza, I. R., Russ, D. W., & Kent-Braun, J. A. (2004). Age-related enhancement of fatigue resistance is evident in men during both isometric and dynamic tasks. *Journal of applied physiology (Bethesda, Md.: 1985), 97*(3), 967–75. doi:10.1152/japplphysiol.01351.2003

Larsson, L., & Salviati, G. (1989). Effects of age on calcium transport activity of sarcoplasmic reticulum in fast- and slow-twitch rat muscle fibres. *The Journal of physiology, 419*, 253–64.

Lenti, M., De Vito, G., Sbriccoli, P., Scotto di Palumbo, a, & Sacchetti, M. (2010). Muscle fibre conduction velocity and cardiorespiratory response during incremental cycling exercise in young and older individuals with different training status. *Journal of electromyography and kinesiology: official journal of the International Society of Electrophysiological Kinesiology, 20*(4), 566–71. doi:10.1016/j.jelekin.2010.02.004

Lewek, M. D., Rudolph, K. S., & Snyder-Mackler, L. (2004). Quadriceps femoris muscle weakness and activation failure in patients with symptomatic knee osteoarthritis. *Journal of orthopaedic research: official publication of the Orthopaedic Research Society, 22*(1), 110–5. doi:10.1016/S0736-0266(03)00154-2

LINSSEN, W. H. J. P., JACOBS, M., STEGEMAN, D. F., JOOSTEN, E. M. G., & MOLEMAN, J. (1990). MUSCLE FATIGUE IN McARDLE'S DISEASE. *Brain, 113*(6), 1779–1793. doi:10.1093/brain/113.6.1779

Lyons, M. F., Cadden, S. W., Baxendale, R. H., & Yemm, R. (1996). Twitch interpolation in the assessment of the maximum force-generating capacity of the jaw-closing muscles in man. *Archives of oral biology, 41*(12), 1161–8.

Macdonald, J. H., Farina, D., & Marcora, S. M. (2008). Response of electromyographic variables during incremental and fatiguing cycling. *Medicine and science in sports and exercise, 40*(2), 335–44. doi:10.1249/mss.0b013e31815b491e

Machner, A., Pap, G., & Awiszus, F. (2002). Evaluation of quadriceps strength and voluntary activation after unicompartmental arthroplasty for medial osteoarthritis of the knee. *Journal of orthopaedic research: official publication of the Orthopaedic Research Society, 20*(1), 108–11. doi:10.1016/S0736-0266(01)00068-7

Mademli, L., & Arampatzis, A. (2008). Effect of voluntary activation on age-related muscle fatigue resistance. *Journal of biomechanics, 41*(6), 1229–35. doi:10.1016/j.jbiomech.2008.01.019

Masuda, K., Masuda, T., Sadoyama, T., Inaki, M., & Katsuta, S. (1999). Changes in surface EMG parameters during static and dynamic fatiguing contractions. *Journal of electromyography and kinesiology: official journal of the International Society of Electrophysiological Kinesiology, 9*(1), 39–46.

McKenzie, D. K., Bigland-Ritchie, B., Gorman, R. B., & Gandevia, S. C. (1992). Central and peripheral fatigue of human diaphragm and limb muscles assessed by twitch interpolation. *The Journal of physiology*, *454*, 643–56.

McNeil, C. J., Vandervoort, A. A., & Rice, C. L. (2007). Peripheral impairments cause a progressive age-related loss of strength and velocity-dependent power in the dorsiflexors. *Journal of applied physiology (Bethesda, Md. : 1985)*, *102*(5), 1962–8. doi:10.1152/japplphysiol.01166.2006

Merletti, R, Knaflitz, M., & De Luca, C. J. (1990). Myoelectric manifestations of fatigue in voluntary and electrically elicited contractions. *Journal of applied physiology (Bethesda, Md. : 1985)*, *69*(5), 1810–20.

Merletti, R, Sabbahi, M. a, & De Luca, C. J. (1984). Median frequency of the myoelectric signal. Effects of muscle ischemia and cooling. *European journal of applied physiology and occupational physiology*, *52*(3), 258–65.

Merletti, Roberto, Farina, D., & Gazzoni, M. (2002). EFFECT OF AGE ON MUSCLE FUNCTIONS INVESTIGATED WITH, (January), 65–76. doi:10.1002/mus.10014

Merton, P. A. (1954). Voluntary strength and fatigue. *The Journal of physiology*, *123*(3), 553–64.

Mesin, L., Merlo, E., Merletti, R., & Orizio, C. (2010). Investigation of motor unit recruitment during stimulated contractions of tibialis anterior muscle. *Journal of electromyography and kinesiology : official journal of the International Society of Electrophysiological Kinesiology*, *20*(4), 580–9. doi:10.1016/j.jelekin.2009.11.008

Miller, M., Downham, D., & Lexell, J. (2003). Effects of superimposed electrical stimulation on perceived discomfort and torque increment size and variability. *Muscle & nerve*, *27*(1), 90–8. doi:10.1002/mus.10289

Miyamoto, N., Fukutani, A., Yanai, T., & Kawakami, Y. (2012). Twitch potentiation after voluntary contraction and neuromuscular electrical stimulation at various frequencies in human quadriceps femoris. *Muscle & nerve*, *45*(1), 110–5. doi:10.1002/mus.22259

Narayanan, N., Jones, D. L., Xu, A., & Yu, J. C. (1996). Effects of aging on sarcoplasmic reticulum function and contraction duration in skeletal muscles of the rat. *The American journal of physiology*, *271*(4 Pt 1), C1032–40.

Narici, M. V, & Maganaris, C. N. (2006). Adaptability of elderly human muscles and tendons to increased loading. *Journal of anatomy*, *208*(4), 433–43. doi:10.1111/j.1469-7580.2006.00548.x

Nordlund, M. M., Thorstensson, A., & Cresswell, A. G. (2004). Central and peripheral contributions to fatigue in relation to level of activation during repeated maximal voluntary isometric plantar flexions. *Journal of applied physiology (Bethesda, Md. : 1985)*, *96*(1), 218–25. doi:10.1152/japplphysiol.00650.2003

Nørregaard, J., Bülow, P. M., & Danneskiold-Samsøe, B. (1994). Muscle strength, voluntary activation, twitch properties, and endurance in patients with fibromyalgia. *Journal of neurology, neurosurgery, and psychiatry, 57*(9), 1106–11.

O'Brien, T. D., Reeves, N. D., Baltzopoulos, V., Jones, D. A., & Maganaris, C. N. (2008). Assessment of voluntary muscle activation using magnetic stimulation. *European journal of applied physiology, 104*(1), 49–55. doi:10.1007/s00421-008-0782-y

Oates, B. R., Glover, E. I., West, D. W., Fry, J. L., Tarnopolsky, M. A., & Phillips, S. M. (2010). Low-volume resistance exercise attenuates the decline in strength and muscle mass associated with immobilization. *Muscle & nerve, 42*(4), 539–46. doi:10.1002/mus.21721

Oskouei, M. A. E., Van Mazijk, B. C. F., Schuiling, M. H. C., & Herzog, W. (2003). Variability in the interpolated twitch torque for maximal and submaximal voluntary contractions. *Journal of applied physiology (Bethesda, Md. : 1985), 95*(4), 1648–55. doi:10.1152/japplphysiol.01189.2002

Pääsuke, M., Ereline, J., Gapeyeva, H., Sirkel, S., & Sander, P. (2000). Age-related differences in twitch contractile properties of plantarflexor muscles in women. *Acta physiologica Scandinavica, 170*(1), 51–7. doi:10.1046/j.1365-201x.2000.00753.x

Pap, G., Machner, A., & Awiszus, F. (2004). Strength and voluntary activation of the quadriceps femoris muscle at different severities of osteoarthritic knee joint damage. *Journal of orthopaedic research : official publication of the Orthopaedic Research Society, 22*(1), 96–103. doi:10.1016/S0736-0266(03)00128-1

Place, N., Lepers, R., Deley, G., & Millet, G. Y. (2004). Time course of neuromuscular alterations during a prolonged running exercise. *Medicine and science in sports and exercise, 36*(8), 1347–56.

Place, N., Maffiuletti, N. A., Martin, A., & Lepers, R. (2007). Assessment of the reliability of central and peripheral fatigue after sustained maximal voluntary contraction of the quadriceps muscle. *Muscle & nerve, 35*(4), 486–95. doi:10.1002/mus.20714

Place, N., Yamada, T., Bruton, J. D., & Westerblad, H. (2008). Interpolated twitches in fatiguing single mouse muscle fibres: implications for the assessment of central fatigue. *The Journal of physiology, 586*(Pt 11), 2799–805. doi:10.1113/jphysiol.2008.151910

Porter, M. M., Vandervoort, A. A., & Lexell, J. (1995). Aging of human muscle: structure, function and adaptability. *Scandinavian journal of medicine & science in sports, 5*(3), 129–42.

Reeves, N. D., Maganaris, C. N., & Narici, M. V. (2003). Effect of strength training on human patella tendon mechanical properties of older individuals. *The Journal of physiology, 548*(Pt 3), 971–81. doi:10.1113/jphysiol.2002.035576

Rice, C. L., Cunningham, D. A., Taylor, A. W., & Paterson, D. H. (1988). Comparison of the histochemical and contractile properties of human triceps surae. *European Journal of Applied Physiology and Occupational Physiology*, *58*(1-2), 165–170. doi:10.1007/BF00636621

Roos, M. R., Rice, C. L., Connelly, D. M., & Vandervoort, a. a. (1999). Quadriceps muscle strength, contractile properties, and motor unit firing rates in young and old men. *Muscle & nerve*, *22*(8), 1094–103.

Rutherford, O. M., Jones, D. A., & Newham, D. J. (1986). Clinical and experimental application of the percutaneous twitch superimposition technique for the study of human muscle activation. *Journal of neurology, neurosurgery, and psychiatry*, *49*(11), 1288–91.

Sbriccoli, P., Sacchetti, M., Felici, F., Gizzi, L., Lenti, M., Scotto, A., & De Vito, G. (2009). Non-invasive assessment of muscle fiber conduction velocity during an incremental maximal cycling test. *Journal of electromyography and kinesiology : official journal of the International Society of Electrophysiological Kinesiology*, *19*(6), e380–6. doi:10.1016/j.jelekin.2009.03.008

Scaglioni, G., & Martin, A. (2009). Assessment of plantar flexors activation capacity: nerve versus muscle stimulation by single versus double pulse. *European journal of applied physiology*, *106*(4), 563–72. doi:10.1007/s00421-009-1049-y

Schmitz, J. P. J., van Dijk, J. P., Hilbers, P. a J., Nicolay, K., Jeneson, J. a L., & Stegeman, D. F. (2012). Unchanged muscle fiber conduction velocity relates to mild acidosis during exhaustive bicycling. *European journal of applied physiology*, *112*(5), 1593–602. doi:10.1007/s00421-011-2119-5

Shield, A., & Zhou, S. (2004). Assessing voluntary muscle activation with the twitch interpolation technique. *Sports medicine (Auckland, N.Z.)*, *34*(4), 253–67.

Simoneau, E., Martin, A., & Van Hoecke, J. (2005). Muscular Performances at the Ankle Joint in Young and Elderly Men. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, *60*(4), 439–447. doi:10.1093/gerona/60.4.439

Smith, J. L., Martin, P. G., Gandevia, S. C., & Taylor, J. L. (2007). Sustained contraction at very low forces produces prominent supraspinal fatigue in human elbow flexor muscles. *Journal of applied physiology (Bethesda, Md. : 1985)*, *103*(2), 560–8. doi:10.1152/japplphysiol.00220.2007

Stackhouse, S. K., Dean, J. C., Lee, S. C., & Binder-MacLeod, S. a. (2000). Measurement of central activation failure of the quadriceps femoris in healthy adults. *Muscle & nerve*, *23*(11), 1706–12.

Stackhouse, S. K., Stevens, J. E., Lee, S. C., Pearce, K. M., Snyder-Mackler, L., & Binder-Macleod, S. A. (2001). Maximum voluntary activation in nonfatigued and fatigued muscle of young and elderly individuals. *Physical therapy*, *81*(5), 1102–9.

Stevens, J. E., Stackhouse, S. K., Binder-Macleod, S. a, & Snyder-Mackler, L. (2003). Are voluntary muscle activation deficits in older adults meaningful? *Muscle & nerve*, *27*(1), 99–101. doi:10.1002/mus.10279

Strojnik, V., & Komi, P. V. (1998). Neuromuscular fatigue after maximal stretch-shortening cycle exercise. *Journal of applied physiology (Bethesda, Md. : 1985)*, *84*(1), 344–50.

Strojnik, V., & Komi, P. V. (2012). Neuromuscular fatigue after maximal stretch-shortening cycle exercise Neuromuscular fatigue after maximal stretch-shortening cycle exercise, 344–350.

Suter, E., & Herzog, W. (2001). Effect of number of stimuli and timing of twitch application on variability in interpolated twitch torque. *Journal of applied physiology (Bethesda, Md. : 1985)*, *90*(3), 1036–40.

Taylor, J. L. (2009). Point: the interpolated twitch does/does not provide a valid measure of the voluntary activation of muscle. *Journal of applied physiology (Bethesda, Md. : 1985)*, *107*(1), 354–5. doi:10.1152/japplphysiol.91220.2008

Taylor, J. L., & Gandevia, S. C. (2008). A comparison of central aspects of fatigue in submaximal and maximal voluntary contractions. *Journal of applied physiology (Bethesda, Md. : 1985)*, *104*(2), 542–50. doi:10.1152/japplphysiol.01053.2007

TESCH, P. A., KOMI, P. V., JACOBS, I., KARLSSON, J., & VIITASALO, J. T. (1983). Influence of lactate accumulation of EMG frequency spectrum during repeated concentric contractions. *Acta Physiologica Scandinavica*, *119*(1), 61–67. doi:10.1111/j.1748-1716.1983.tb07306.x

Thom, J. M., Morse, C. I., Birch, K. M., & Narici, M. V. (2005). Triceps Surae Muscle Power, Volume, and Quality in Older Versus Younger Healthy Men. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, *60*(9), 1111–1117. doi:10.1093/gerona/60.9.1111

Trimble, M. H., & Enoka, R. M. (1991). Mechanisms underlying the training effects associated with neuromuscular electrical stimulation. *Physical therapy*, *71*(4), 273–80; discussion 280–2.

Urbach, D., & Awiszus, F. (2002). Impaired ability of voluntary quadriceps activation bilaterally interferes with function testing after knee injuries. A twitch interpolation study. *International journal of sports medicine*, *23*(4), 231–6. doi:10.1055/s-2002-29074

Urbach, D., Nebelung, W., Becker, R., & Awiszus, F. (2001). Effects of reconstruction of the anterior cruciate ligament on voluntary activation of quadriceps femoris a prospective twitch interpolation study. *The Journal of bone and joint surgery. British volume*, *83*(8), 1104–10.

Van der Hoeven, J. H., Links, T. P., Zwarts, M. J., & van Weerden, T. W. (1994). Muscle fiber conduction velocity in the diagnosis of familial hypokalemic periodic

paralysis—invasive versus surface determination. *Muscle & Nerve, 17*(8), 898–905. doi:10.1002/mus.880170809

Van Lunteren, E., & Sankey, C. B. (2000). Catchlike property of rat diaphragm: subsequent train frequency effects in variable-train stimulation. *Journal of applied physiology (Bethesda, Md. : 1985), 88*(2), 586–98.

Van Schaik, C. S., Hicks, A. L., & McCartney, N. (1994). An Evaluation of the Length-Tension Relationship in Elderly Human Ankle Dorsiflexors. *Journal of gerontology, 49*(3), B121–7. doi:10.1093/geronj/49.3.B121

Vandervoort, A. A., & McComas, A. J. (1986). Contractile changes in opposing muscles of the human ankle joint with aging. *J Appl Physiol, 61*(1), 361–367.

Vivodtzev, I., Flore, P., Lévy, P., & Wuyam, B. (2008). Voluntary activation during knee extensions in severely deconditioned patients with chronic obstructive pulmonary disease: benefit of endurance training. *Muscle & nerve, 37*(1), 27–35. doi:10.1002/mus.20867

Wilder, M. R., & Cannon, J. (2009). Effect of age on muscle activation and twitch properties during static and dynamic actions. *Muscle & nerve, 39*(5), 683–91. doi:10.1002/mus.21233

Winegard, K. J., Hicks, A. L., & Vandervoort, A. A. (1997). An evaluation of the length-tension relationship in elderly human plantarflexor muscles. *The journals of gerontology. Series A, Biological sciences and medical sciences, 52*(6), B337–43.

Yamada, H., Okada, M., Oda, T., Nemoto, S., Shiozaki, T., Kizuka, T., … Masuda, T. (2000). Effects of aging on EMG variables during fatiguing isometric contractions. *Journal of human ergology, 29*(1-2), 7–14.

Yoon, T., Schlinder Delap, B., Griffith, E. E., & Hunter, S. K. (2007). Mechanisms of fatigue differ after low- and high-force fatiguing contractions in men and women. *Muscle & nerve, 36*(4), 515–24. doi:10.1002/mus.20844

Zwarts, M J, & Arendt-Nielsen, L. (1988). The influence of force and circulation on average muscle fibre conduction velocity during local muscle fatigue. *European journal of applied physiology and occupational physiology, 58*(3), 278–83.

Zwarts, M J, Arendt-Nielsen, L., McIntosh, K. C. D., & Gabriel, D. a. (1988). Reliability of a simple method for determining muscle fiber conduction velocity. *Muscle & nerve, 45*(3), 278–83. doi:10.1002/mus.22268

Zwarts, M J, Drost, G., & Stegeman, D. F. (2000). Recent progress in the diagnostic use of surface EMG for neurological diseases. *Journal of electromyography and kinesiology : official journal of the International Society of Electrophysiological Kinesiology, 10*(5), 287–91.

Zwarts, Machiel J. (1989). Evaluation of the estimation of muscle fiber conduction velocity. Surface versus needle method. *Electroencephalography and Clinical Neurophysiology*, *73*(6), 544–548.

Zwarts, Machiel J, & Stegeman, D. F. (2003). Multichannel surface EMG: basic aspects and clinical utility. *Muscle & nerve*, *28*(1), 1–17. doi:10.1002/mus.10358