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.
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:

\[ \text{VA} \% = \left[1 - \left( \frac{T_{\text{interpolated}}}{T_{\text{control}}} \right) \right] \times 100 \]

where \( T_{\text{interpolated}} \) is the superimposed twitch noted during a maximal contraction at the time of stimulation and \( T_{\text{control}} \) 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.

![Torque trace](image)

**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). Bülow 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 \[\text{[normalized evoked force]}^k\], 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](image.png)


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), Shead et al. have argued that fundamental flaws constitutes its notion (Shead & 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, therefore 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, \([\text{Ca}^{++}]_i\)) but only very small increases can be expected when maximal effort is approached.
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\(^{++}\)], 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\(^{++}\)], 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 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
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). Therefore 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^{2+}-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 therefore 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 the 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 electro-
cal 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 per-
formed- 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 mm² (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 produced 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 stimulation 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. ‡: significant 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 resulted 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 interstimulus 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-
agination (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.
REFERENCES:


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


