Comparisons between automatic force based triggering and manually triggered methods of quantifying quadriceps voluntary activation assessment

by © Xi Hong
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ABSTRACT

The interpolated twitch technique is a widely used method of assessing muscle voluntary activation. The approach involves delivering an electrical stimulus to determine the degree to which a voluntarily activated muscle is activated. If the muscle is not fully activated then additional force will be generated by this stimulus. The amount of additional force generated can be used to quantify the degree of activation. Conventionally, for this technique, stimuli are delivered either manually (when the force reaches a plateau) or after a set time period post onset of contractions. This study examined an approach for interpolated twitch that has recently been suggested to improve precision of the technique. For this method, stimuli were delivered once the force produced by participants reached 97% of their previously recorded maximum voluntary isometric contraction force. This method was used to examine quadriceps activation in 15 male volunteers. Muscle activation was determined using two different methods of stimulus delivery. One method involved the stimulus being automatically triggered when participant's knee extension force reached 97% of their maximum voluntary isometric contraction. The other method involved the stimulus being manually delivered when the force tracing reached a plateau. The purpose of the study was to verify that this automatic force based triggering method improved the precision in delivering stimuli near peak force. Also, the quadriceps voluntary activation determined using both stimulus delivery approaches were compared. The findings indicated that the automatic force based triggering method reduced the stimulus delivery timing errors by 119% and increased voluntary activation levels by 3% on average. In conclusion, automatic force based triggering is suggested as an alternative approach to stimulus delivery when using interpolated twitch to assess voluntary isometric muscle activation.

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In addition, a thank you to Assistant Professor Chandramouli Krishnan (University of Michigan), who introduced me the comparison of automatic torque based method, traditional time-based method and manually triggered method for ITT in his paper (2010). My study is based on his findings and an extension to his research.

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Chapter I: Introduction

People conduct various movements such as walking, jogging and jumping during daily activities. No matter how complex or simple the movements are, they are generated by different levels of forces produced by skeletal muscles. Skeletal muscles in general are soft tissues connected to bones, either directly, or through the intervention of fibrous structures as a supporting structure for humans (Gray, 1985). Muscles are responsible for maintenance and changes in posture, and locomotion as well as all other movements that humans perform (Marieb and Hoehn, 2010). Skeletal muscles, consisting of striated muscle fibers, are linked to two bones and can span one or more joints. Unlike cardiac and smooth muscles, skeletal muscle forces can be controlled consciously during voluntary contraction. All voluntary contractions are controlled by the central nervous system (CNS), consisting of the brain and spinal cord. The CNS sends signals to motor nerves and activates the muscles through electrical impulses. Then the activated skeletal muscles contract and produce force and movement.

A muscle’s ability to produce force is modulated by many factors, such as its size, length, velocity of the contraction, and fatigue level. Also, the capacity to produce maximal force is dependent on the level of voluntary muscle activation. The degree of muscle voluntary activation (VA) reflects the ability of the CNS to recruit motor units and control the frequency of motor units firing rate (Ounjian et al., 1991). Several different methods can be used to quantify the level of voluntary muscle activation. These include the interpolated twitch technique (ITT), central activation ratio (CAR), modified CAR, transcranial magnetic stimulation (TMS), T2-weighted magnetic resonance imaging and electromyography (EMG). However, most researchers advocate ITT as the best measurement of muscle activation due to its convenience and relative accuracy (Krishnan et al., 2009). This technique has been shown to be reliable for use in different muscle groups and has become a standard technique to assess the voluntary muscle activation level (Behm et al., 1997; Behm et al., 2001, Kooistra et al., 2009). Despite the widespread use of ITT to assess muscle VA, it remains a highly variable technique that has numerous critiques (Allen et al., 1998; Enoka and Fuglevand, 2001; Button and Behm, 2008; De Haan et al., 2009). As a result it requires particular diligence in its application (Behm et al., 1997**;** De Haan et al., 2009; Todd et al., 2004). The thesis research was intended to examine a method of potentially reducing the variability of ITT. Prior to describing the study an in-depth review of the literature related to muscle activation and ITT will be presented.

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Chapter II Literature review

2.1 Skeletal muscle contraction and activation

2.1.1 Voluntary muscle force production

While some muscle force can be produced involuntarily (ie. reflex contractions), most force production is done voluntarily. Voluntary muscle contractions take place as a result of conscious effort originating in the brain. In general, the brain sends signals, in the form of action potentials, through the spinal cord to the motor neuron that innervates and activates several muscle fibers (Cacioppo et al., 2007). As reviewed by Kent (2002) an action potential is generated in the motor cortex and transmitted to a motor neuron which sends the action potential down its own axon. Once the action potential reaches the muscle, it activates voltage gated sodium channels at neuromuscular junctions. This causes a large calcium ion influx from the voltage gated calcium channels. The calcium influx causes a widespread diffusion of vesicles containing acetylcholine into the neuromuscular junction, which in turn causes the opening of sodium and potassium channels on the muscle cell membranes. Potassium rushes out and sodium rushes in, and as a result of this, the muscle fiber membrane becomes more positively charged and an action potential is triggered. This muscle fiber action potential causes the sarcoplasmic reticulum to release calcium ions (see Fig 2.1.1a). Tropomyosin covers the myosin binding sites on actin preventing contact between myosin and actin. In Figure 2.1.1b, the released calcium binds to troponin, resulting in a change in troponin configuration, which moves the tropomyosin away from the binding groove. As a result the myosin head can contact the binding sites on actin and form a cross bridge.

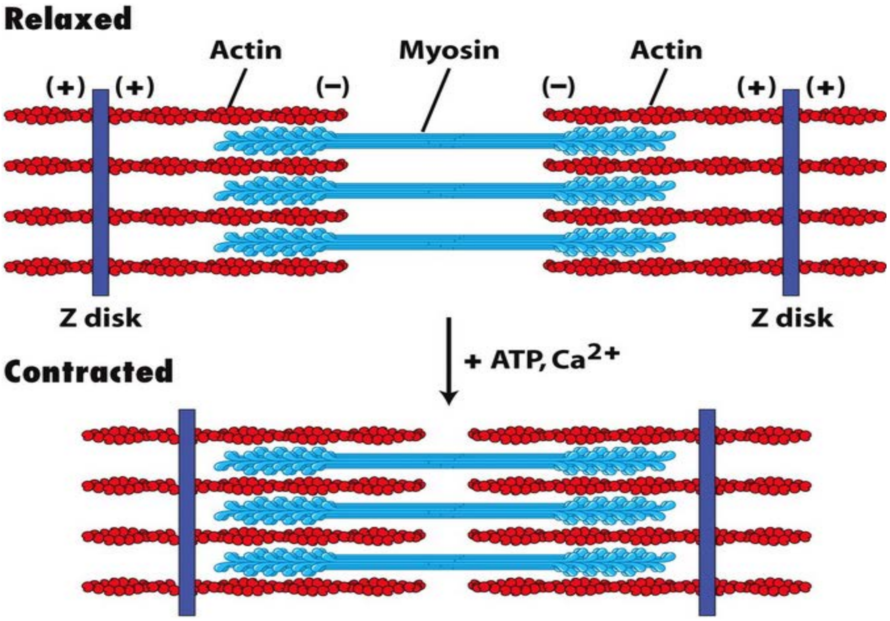


Figure 2.1 Actin and myosin filaments in the relaxed state. Tropomyosin blocks the binding sight on acton so no cross-bridges can form. Following the release of calcium from the t-tubule, it binds with troponin enabling the actin/mysosin cross-bridge to form and contraction to occur (Figure from Lodish et al., 2007)

Cross-bridge binding results in energy release which in turn results in the cross-bridge twisting as the stretched thin filaments slide toward the M line sarcomere center resulting in the shortening of sarcomere and muscle fiber contraction (Brooks et al., 1996). Figure 2.2 shows a brief process of muscle contraction.

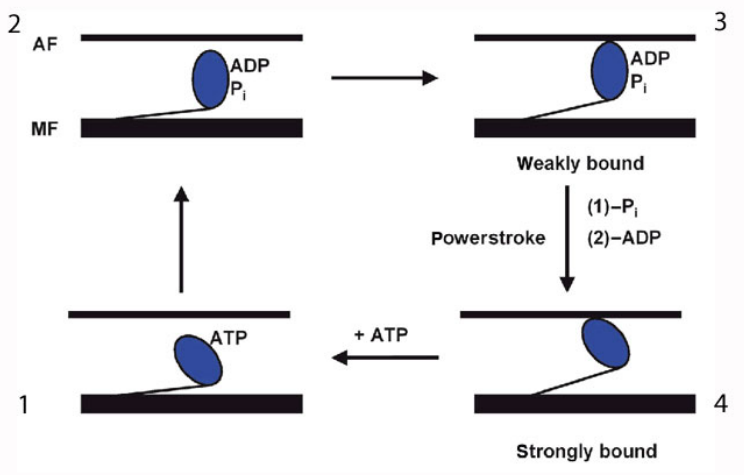


Figure 2.2 (1)→(2) The ATP splits into ADP and Pi, the myosin head is exposed. (2)→(3)The myosin head attaches to the actin filament with cross bridge formed and ADP with Pi. (3)→(4) Myosin head rotates and pulls the actin filament, sliding toward the M line and force is produced. (Figure taken from Goody, 2003)

2.1.2 Factors that influence muscle force production

While the process described above outlines how individual cross bridges are formed it takes multiple cross bridges to produce muscle force, as human movement requires the ability to produce a wide range of muscle forces. The factors listed below can all influence a muscles ability to produce force:

1. The size of the muscle: the more muscle fibers a muscle contains the more force it can produce. The accepted measure of muscle size is physiological cross sectional area (PCSA). For pennate muscles, the PCSA is the total area of the cross-sections perpendicular to the muscle fibers. Comparing to parallel muscles, pennate muscles has one advantage in containing more muscle fibers with more force production (Otten, 1998).

2. Muscle length: Muscles operate with maximal force when close to an ideal length (their resting length) and the force decreases as they are either stretched or shortened (Gordon et al., 1966). Figure 2.3 shows the relationship between muscle force and length. This figure shows that total muscle tension is composed of the passive tension and the active tension. The active tension is from the cross-bridges themselves and the passive tension is from other elastic structures outside the cross-bridge (a protein called titin) and also from the structures within the myofibrils themselves (Magid and Law, 1985). The total tension increases as the number of cross-bridges’ increases and drops at lengths greater than resting length, mainly because the cross-bridges have been separated apart and the number of cross-bridges that form decreases. As the length continues to increase beyond resting length, passive tension is created. The passive tension increases as muscle length increases due to the elastic property of muscle.

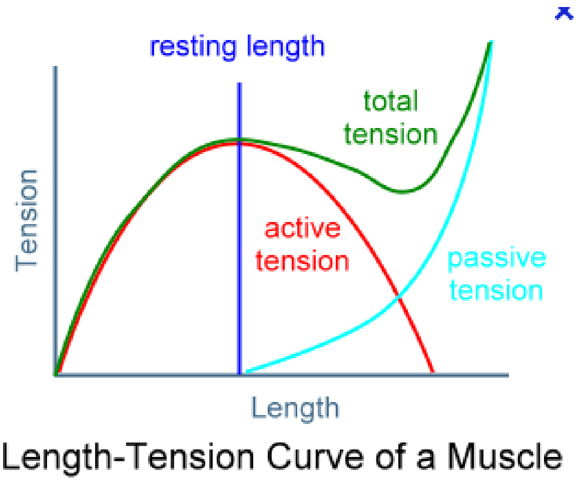


Figure 2.3 Figure illustrating the effects of single muscle fiber length on muscle force production. Total tension is a sum of both active and passive tensions. (Figure taken from Andersson and Chaffin, 1986)

3. Velocity of contraction: As discussed by Callahan et al. (2009), Hill was one of the first researchers to suggest that during a concentric contraction, muscle force decreases as the velocity of contraction increases. Muscle force increases with increased velocity of contraction during eccentric contraction, but it will eventually plateau (Huxley, 1957). When the velocity increases to the maximal velocity, there is no force generated during concentric contraction.

4. Muscle fiber types: There are three major different muscle fiber types: type I, type IIA and type IIx. They are differentiated from one another based on different composition of myosin heavy chain (MHC) (Gardiner, 2001). According to their structural and functional properties, they can be classified into slow-twitch and fast-twitch muscle fibers. At any given velocity of movement, the amount of force produced depends on the fiber type. During a dynamic contraction, when the fiber is either shortening or lengthening, a fast-twitch fiber produces more force than a slow-twitch fiber (Fitts & Widrick, 1996). Table 2.1 shows the relation between MHC divided muscle fiber types and their characteristics in exercise including strength, velocity, endurance and elasticity.

Table 2.1: Muscle fiber properties

Muscle Fiber Types	**Type IA**	**Type IIA**	**Type IIX**
Strength	Low	High	High
Velocity	Low	High	High
Endurance	High	Low	High
Elasticity	High	Low	High

5. Fatigue: Muscle fatigue usually refers to an exercise-induced reduction in maximal voluntary force or power output. Fatigue results in a degradation of the ability of the muscle to generate force. There are primarily two types of fatigue that occur- central and peripheral. Central fatigue force deficits result mainly due to deficits in neural drive to the muscle. Peripheral fatigue on the other hand results due to factors in the muscle itself. Regardless of the type of fatigue that occurs the end result will be similar- the muscles ability to produce force will be diminished (Davis, 1995).

6. Level of muscle activation: Another factor that plays a key role in determining the force a muscle can produce is the level of activation of the muscle. Generally speaking the more fibers that are active the greater the force that will be produced. As this thesis will focus on methods used to quantify the level of voluntary muscle activation a more detailed review of this factor will now be presented.

Many factors would influence the muscle VA. Muscle VA level represents the level of neural drive to a muscle during exercise (Gandevia et al., 1995). Two main mechanisms of force modulation are recognized, 1) recruitment of motor units 2) changing the frequency of motor unit firing rate. When a muscle is activated smaller motor units are recruited first. The recruitment of motor units follows the size principal (Henneman et al., 1965). As more force production is required additional, larger motor units are activated to increase overall force production. In this process, activation of more motor neurons will result in increasing motor unit recruitment with more muscle fibers being activated, therefore producing a stronger muscle contraction. A muscle with a greater number of muscle fibers activated is generally considered to have a higher level of VA.

Another important factor of muscle VA is the motor unit firing rate stimulated by the motor neuron. This frequency, also known as the alpha motor neuron firing rate, can be varied to produce large differences in muscle force (Hulliger, 1984). A slow firing rate will produce a series of single twitch contractions. When the firing rate increases, it produces a series of twitches that accumulate creating a tetanic like contraction and thus higher force production. Once the maximum firing rate is reached there is no further means to increase force production for the active motor unit. Additional force from the muscle must then come from recruitment of non-active motor units (De Luca, 1985). In order to have maximal muscle activation, all motor units must be recruited and must be firing at their maximal firing rates.

When an individual is exerting maximal effort during a muscle contraction it cannot be assumed that their muscle will be maximally activated. This is because there are several factors that can limit a muscles ability to become fully activated. These include muscle inhibition, fatigue, motivation and previous experience with producing maximal contractions. An understanding of these factors is important when trying to examine the accuracy of a technique like ITT as any or all of these factors my effect results.

Muscle inhibition is generally thought of as being a reflex modulated inability to fully activate a muscle, despite the muscle and nerve being normal (Hurley et al., 1997). Muscle inhibition can cause a reduced capability for the muscle to produce maximal force therefore affecting VA level even when maximal effort is being exerted. Several factors contribute to muscle inhibition including injuries, trauma, hydration, over-use or stress (Page et al., 2010).

A second factor that results in reduced VA during maximal level contraction is fatigue. Fatigue is mostly exercise-induced and results in a deficit in force or power production of muscles. As stated previously, both peripheral and central factors can contribute to the development of fatigue (Gandevia et al., 2001).. As fatigue limits muscle force production, it will cause deficits in muscle VA particularly if the fatigue is central (i.e. arising from spinal cord or brain) in origin (Behm and St-Pierre, 1997). This means after a series of maximal or even sub-maximal contractions, reductions in nerve signal frequency and motor unit recruitment cause the force generated by the contraction to diminish (Rasmussen, 2007). No pain or discomfort occurs, but the muscle force and VA gradually drops.

In summary, VA is determined by two main intrinsic factors the motor unit recruitment and the firing rate of motor units. Also, other factors such as muscle inhibition and fatigue can also influence VA.

2.2 Quantifying level of muscle VA

A question of great interest for basic science and clinical or performance based research is can humans fully activate their muscles? This question is a contentious one. Researchers have found that human can fully activate their dorsi-flexors (Belanger et. al, 1989; McComas et al., 1983), and plantar flexors (Behm et. al, 2002) while others have reported a lack of full activation of the quadriceps (Kalmar and Cafarelli, 1999; Behm et al., 2002) and elbow flexors (Lloyd et al., 1991; Behm et al., 2002). In all of the above studies researchers used the interpolated twitch technique (ITT) to investigate the percentage of muscle activation.

In addition to ITT, EMG and central activation ratio (CAR) have been used to quantify VA. Also, scientists have recently begun using approaches like TMS and T2-weighted MRI (magnetic resonance imaging) (Kendall et al., 2006; Todd et al., 2004) to quantify VA. These methods will now each be reviewed in detail.

2.2.1 EMG

EMG is a technique for recording and assessing the electrical activity produced by skeletal muscles (Robertson et al., 2004). It detects the muscle membrane electrical potential generated by muscle cells, when these cells are electrically or neurologically activated. There are many applications for EMG. Apart from clinical use, EMG is applied extensively in research laboratories including those in neuromuscular physiology, motor control, ergonomics, physical therapy and biomechanics. Usually bipolar recording methods are used for surface EMG signals in biomechanics research. Typically EMG is used to quantify the level of VA during a given task by comparing muscle activation level to the activation level during a maximum contraction (Robertson et al., 2004). This approach is not very accurate because there is no guarantee that the EMG recorded during the maximal voluntary contraction (MVC) represents a fully activated muscle. As a result, EMG does not generally provide accurate estimates of %VA.

2.2.2 ITT and CAR

ITT and CAR are both based on the same basic principal – if a muscle is not fully activated and an electrical stimulus is delivered to it then this stimulus will activate inactive fibers resulting in an increase in force. They differ in the specific methods used and the estimation equations they employ.

ITT is a non-invasive method for assessing the completeness of muscle activation in clinical settings as well as scientific research, especially for testing whether a muscle is fully activated during MVC (Huang et al., 2010). To assess VA using ITT a supra-maximal stimulus is delivered to a muscle while an MVC is being performed. If the muscle is not fully activated then the added electrical stimulus will result in recruitment of any inactive fibers. This will result in an increase in force production by the muscle. On the other hand if the muscle is already fully activated no additional force will be observed.

In order for ITT to be performed correctly an appropriate level of stimulus must be delivered to the already contracted muscle. Typically a supra-maximal stimulus (a stimulus that can activate all the muscle fibers) is delivered. To determine the magnitude of this supra-maximal stimulus an increasing current with fixed voltage is typically sent to the subject while they are relaxed. The intensity of this current is increased until the twitch force produced reaches a peak (Shield and Zhou, 2004). Quantification of VA with ITT typically involves comparing the magnitude of the twitch force evoked when muscle is in the rest state (control twitch) with that evoked when the twitch is superimposed upon an MVC (Gandevia et al., 1998). The VA of the stimulated muscle is quantified with the linear equation (Allen et al., 1995):

$$\mathbf{VA\ (\%) = [1-(ST\ /CT)] * 100}$$

Where ST is the twitch superimposed on the MVC and CT is the control twitch. The control twitch is normally evoked 1.5-5 seconds after the MVC (Allen et al., 1998). Although some authors have used pre-contraction twitches (Hamada et al., 2000; Miller et al., 2006), Folland and Willams (2007) recommended that post-contraction potentiated twitches be used, as the superimposed twitch on a high level contraction appears to be potentiated. Therefore, the CT after the MVC is more accurate in assessing the muscle activation, because it will have been potentiated by the MVC. Although ITT can in theory be used with any muscle, it has been used most extensively to quantify VA in biceps brachii (Gandevia et al., 1998), quadriceps (Dowling et al., 1994; Behm et al., 1997; Todd et al., 2004), and gastrocnemius and soleus (Bebault et al., 2002)

An alternative to ITT is central activation ratio or CAR. This approach uses essentially the same methods as ITT with the exception that no potentiated resting twitch is delivered after the superimposed twitch. In the CAR method VA is estimated by comparing the voluntary torque when the stimulus is delivered to the peak force measured during superimposition of electrical pulses (see Figure 2.4, formula 2) (Belanger and Mc Comas 1981). Another calculation approach is the modified CAR. Instead of using the force at the time of stimulus delivery, the modified CAR approach compares the peak value before the stimulus to the peak force during supra-maximal stimulation (see Formula 3, Figure 2.4).

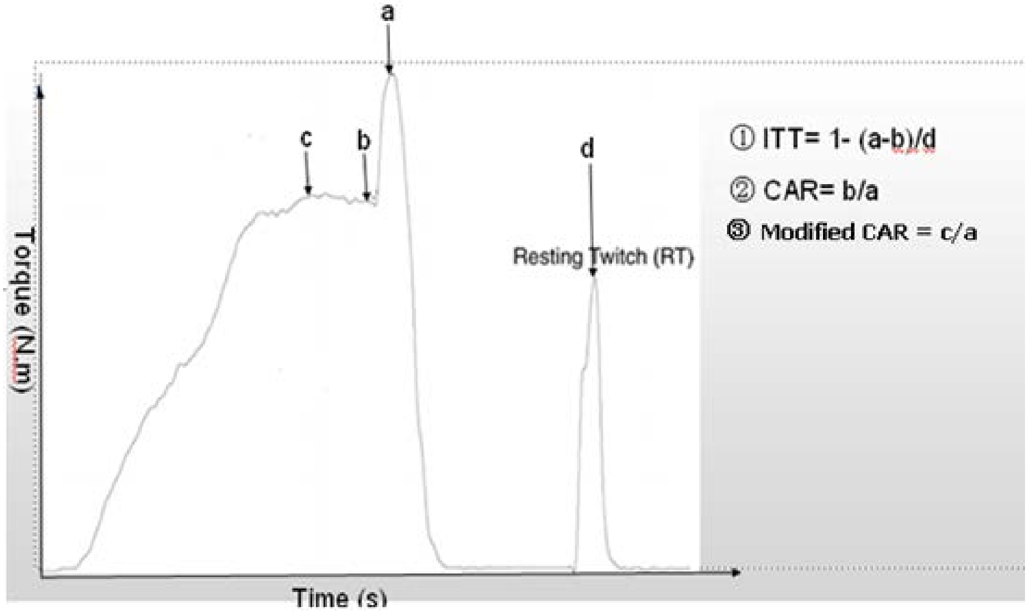


Figure 2.4: Torque measurements recorded during delivery of superimposed (a) and resting twitches (d). CAR = b/a, modified CAR = c/a, where c is the exactly peak before the first twitch (Figure adapted from Krishnan et. al. (2009))

Krishnan and Willams (2010) compared ITT, CAR and modified CAR methods for determining VA. In particular, they used all three methods to find quadriceps VA in 22 participants. They found that the three methods produced significantly different VA results and that there were strong correlations between ITT and CAR. In their work, Krishnan and Willams (2010) also determined a linear equation that related ITT and CAR based predictions of VA [ITT%VA - = 1.661(CAR%VA) -66.260. From this equation, it is clear that if CAR is less than 100%, then ITT is always smaller than CAR. Also, these authors reported that modified CAR tended to overestimate VA levels compared to both CAR and ITT.

Behm and colleagues (2001) compared ITT, CAR and modified CAR. These authors recruited ten healthy male subjects to do three MVCs and a series of contractions at 25%, 50% and 75% MVC. They compared the estimates of muscle inactivation derived from a variety of CAR and ITT methods using either doublet or tetanic forms of stimulation delivered during single MVCs of quadriceps. They also created predictive equations to estimate the VA% by analyzing the ITT based VA% results from different a variety of different contraction intensities ranging from 25% MVC to MVC. They compared the VA estimated using the predictive equations to those determined using the various stimulation protocols (tetanus ITT, CAR, triplet ITT, CAR and doublet ITT, CAR). Their comparisons revealed that the most accurate means of measuring muscle inactivation would be using either polynomial or exponential-regression prediction equations. In order to do this, however, multiple contractions at various contraction intensities are required to create these equations. This number of contractions would not be practical in the case of clinical populations (where pain may be an issue) or in situations where fatigue may be a concern. Because of this the use of regression equations is often not be a viable alternative for many research questions and as a result this approach is rarely used. The second best choice based on the results of Behm et al. (2001) was the use of tetanic stimulation (a high-frequency sequence of individual stimulations over muscle nerves) to create the superimposed and resting twitches. As tetanic stimulation is often very painful it is not usually used. As an alternative Behm et al. (2001) suggested that ITT using doublet stimulations would produce the next most reliable results. Allen and his colleagues (1998) also tested the effect of the number of stimuli by assessing the VA levels in the elbow flexors (biceps brachii and brachiradialis). They found that at high voluntary torque, the muscles have identical responses produced by single, paired and train of four stimuli, which means single stimuli are adequate for VA estimation. This result contradicts the findings of Behm et al. (2001). As Behm et al.'s work examined activation levels in the quadriceps and Allen examined elbow flexors this discrepancy could be partly due to different behaviors of these muscle group when exposed to ITT.

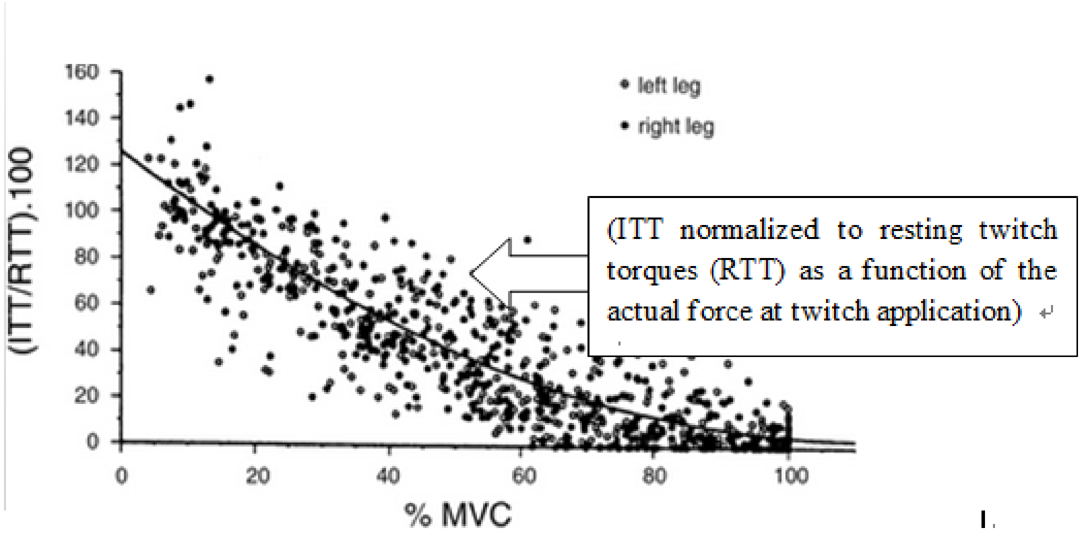
2.3 Reliability and validity of ITT

When using ITT there are always measurements errors present in VA estimates (Oskouei et al., 2003). The validity and reliability of ITT rely on there being a linear relationship between the superimposed twitch and force MVC ratios. Despite this need for a linear relationship, researchers have reported both linear and non-linear relationships between twitch force and activation levels. Behm et al. (2001) compared results of ITT based estimates of VA with those obtained using predictive equations. They found that the precision of these techniques need to be questioned because the superimposed force to voluntary force relation is not linear but actually curvilinear. This is also supported by numerous other researchers (Belanger et al., 1981, Bulow et al., 1993; Dowling et al., 1994; Lloyd et al., 1991; Norregard et al., 1994; Rutherford et al., 1986). However, such a linear relationship has been reported with adductor pollicis (Loring and Hershenson, 1992) and quadriceps femoris (Chapman et al., 1984). If such a linear relationship exists then it helps provide an accurate prediction of the MVC from a single IT ratio. For example, in a perfectly linear relationship, a muscle force equal to 87.5% of MVC would initiate a superimposed twitch to potentiated twitch ratio of 12.5%. However, other researchers have reported non-linear relationships in dorsi-flexors (Belanger and McComas, 1981), quadriceps (Bulow et al., 1993; Norregard et al., 1994; Rutherford et al., 1986, Behm et. Al., 2001) and elbow flexors (Dowling et al., 1994).

Behm et al. (1996) have assessed the reliability of ITT with an investigation of isometric plantar flexor and leg extension contractions. They compared a variety of superimposed responses with potentiated evoked torque with sub-maximal and MVC torque and found a shallow hyperbolic curvilinear relationship for MVC prediction from ITT although the relationship was not perfect. They used second order polynomial equations to estimate the errors in MVC prediction and concluded that ITT was valid and reliable during high intensity contractions. From their point of view, the shallow hyperbolic curve of the ITT-voluntary force relationship was not significantly altered by type of stimulus (i.e. single, doublet, or quintuplet) delivered to the muscle for superimposed and potentiated torque. Place et al. (2007) also tested the reliability of ITT and CAR for estimating quadriceps VA by examining central and peripheral fatigue. Their results suggested that the ITT doublet is more consistent than the CAR, as the resting peak doublet or the potentiated peak doublet was not influenced by the fatiguing contraction along with a fast recovery of evoked peak force. From their findings, the ITT doublet is recommended for both central fatigued and contractile impaired situations.

Although ITT is quite a popular technique and has been used extensively in research for many years, there are several limitations for this technique. One major limitation is that ITT tends to overestimate VA levels. This has been reported by several researches. Yue and her colleagues (2000) performed ITT on the elbow flexors during both dynamic and static contractions. The reported VA of the elbow flexors during static contractions was 98.5%, while 94.5% VA was found during the dynamic contractions. Yue et al. (2000) suggested that this significantly lower muscle activation level observed during the dynamic contractions means the traditional ITT technique is an overestimate of the activation level during isometric contraction. Kooistra and his colleagues (2009) also reported overestimation for the ITT method of assessing VA. In their study, they found that individuals who were able to produce an activation level of 90% or greater, were subsequently observed to be able to produce force that was greater than 10% higher than that recorded during the 90% VA contraction. If the muscle was truly 90% activated then such increases in force should not have been possible. Kooistra et al. (2009) suggested that these results indicate an overestimation of muscle VA using ITT. Other authors (Adams et al., 1993; Kendall et al., 2006) have made similar conclusions using magnetic resonance imaging to predict VA of quadriceps femoris and comparing it to ITT. Gandevia and his colleagues (1991) also found that performing ITT using doublet stimulations overestimated the elbow flexors' VA level. The reason for the overestimate is that the evoked twitch torque cannot be fully developed due to the conflicts of the anti-dromic volley from the electrical stimulation along with spinal reflexes (Upton et al., 1971; Herbert and Gandevia, 1999). The conflicts create a reduction in force and would lower the amplitude of the superimposed force indicating that fewer muscle fibers have been activated by the stimulation.

Also, ITT is highly variable for any given contractile condition (i.e. MVC vs. %MVC contractions). ITT has been shown to estimate VA that differs by as much as 10–15% for tests on the same subject with identical contraction conditions (Allen et al., 1998). To further investigate this issue Oskouei and his colleagues (2003) enrolled sixteen subjects in a sub-maximal knee extensor contraction protocol that involved efforts from 40% to 50% then to 60% and 100% MVC. They found that ITT/RTT (ITT normalized to resting twitch torque) had a variation of scatter when plotted against %MVC force. A similar study testing the ITT under different contractile conditions was completed by Suter and his colleagues in 1996. Twenty healthy subjects performed 20 knee extensor contractions varying from 5% to 100% of the maximal voluntary force. ITT to measured VA levels that ranged from 0 to 100% for contractions around 60% of maximal voluntary force. From this, Suter et al. (1996) concluded that ITT has a significantly positive correlation with the actual force (See Figure 2.5) and that ITT results are very sensitive to small changes in voluntary forces at sub-maximal contraction levels and highly concentrated when force is approaching the MVC level.



(ITT normalized to resting twitch torques (RTT) as a function of the actual force at twitch application)

Figure 2.5: Experimental different ratio of MVCs and their corresponding muscle (quadriceps) voluntary inactivation. A non-linear relation exists (Figure taken from Suter et al., 1996).

2.4 Factors that influence ITT:

Although ITT is widely used in research, its accuracy is still influenced by some factors (Shield and Zhou, 2004).

2.4.1 Experience of the participant:

A key factor that has been shown to influence an individual's ITT measured VA levels is the experience the individual has performing both maximum level contractions and being tested using ITT (Button and Behm, 2008; De Haan et al., 2009). There are two main reasons why lack of experience has been shown to affect results of ITT investigations. The first is related to the anticipation of receiving electrical stimulation that participants experience when undergoing ITT. In individuals with minimal experience this anticipation appears to reduce the MVC force they are able to produce. For example De Haan and colleagues (2009) demonstrated that inexperienced participants (1-2 orientation sessions) averaged 12% and 21% less in force and EMG activity, respectively, when expecting an interpolated twitch during an MVC. Similarly, Button and Behm (2008) reported on two groups, one with prior experience (previously experienced 10 or more series of IT tests) and the second with no experience with ITT. The experienced group exhibited non-significant decreases in MVC during trials where ITT stimulation was expected compared to performing MVCs without any stimulation. In contrast the group with no previous ITT experience significantly reduced their MVC force (12.4%) level compared to their MVC without any stimulation. This resulted in an overall significantly higher VA (10.4%) for experienced group compared to the group with no experience. Based on these studies, prior experience with ITT appears to improve participants' muscle VA level as recorded using ITT. It appears that experience (at least 10 or more previous ITT tests) may help the subjects to overcome their anticipation of the stimulus (Shield and Zhou, 2004). Therefore, as for inexperienced subjects, a suitable orientation period for training is highly recommended in decreasing the anxiety against the electrical stimulus (Button and Behm, 2008).

2.4.2 Site of stimuli

An important issue for consideration in delivering the stimulus is where to place the electrodes. Electrodes can be placed over either the nerve or the muscle belly (Hultman et al., 1983). As discussed by Place et al. (2010), supra-maximal stimulation of the femoral nerve is preferred over quadriceps muscle stimulation for the assessment of knee extensor VA. This is largely due to the fact that muscle stimulation tends to be more superficial, resulting deeper motor units not being activated. Nerve stimulation, on the other hand can theoretically recruit all motor units simultaneously and should therefore result in more accurate estimates of VA. However, there are some limitations for nerve stimulations. First, higher doses of nerve stimulation often result in greater discomfort levels for subjects than does muscle stimulation (Place et al., 2010). Also, according to Place et al. (2010), the electrode may move relative to the nerve during muscle contraction as a result of movement of nearby tendons. Additionally, nerve stimulation may activate the target muscle and the antagonists of this muscle simultaneously (Munsat et al., 1976). For example, when the femoral nerve is stimulated with the purpose to study the quadriceps femoris, the sartorius may also contract. Also, when the peroneal nerve is stimulated, the tibialis anterior along with the plantar flexors would be activated (Belanger and McComas, 1981). As reported by Awiszus and colleagues (1997), such antagonist activation has the potential to reduce the size of the control twitch during an ITT. Such a decrease in control twitch could result in subsequent higher estimation of muscle VA both during maximal or submaximal contractions. In contrast muscle stimulation has been shown to be an effective alternative method for nerve stimulation when the contraction intensity is over 60% of MVC (Place et al., 2010). This is because high levels of force production (⩾ 60% MVC) require the recruitment of the faster motor units located deep in the muscle, so the concern about muscle stimulation not penetrating deep enough to activate this fibers is less important. Despite the findings of Place et al. (2010), care needs to be exercised when using muscle stimulation due to the fact that the use of large stimulation intensities could result in the antagonists activation due to flow of current through the soft tissues (Burke and Gandevia, 1998). As reported above, this could result in overestimation of VA levels.

In conclusion, nerve stimulation is superior because of its ability to simultaneously recruit more motor units than muscle stimulation, but it can bring higher discomfort to the subjects and activate the antagonists. As an alternative method, muscle stimulation is effective and applicable for strong voluntary contraction (⩾60%） and is perhaps preferred due to the lower discomfort levels for participants.

2.4.3 Number of stimuli

When using ITT, the superimposed twitch can be induced by either single or multiple stimuli. Several researchers have compared the effect that the number of stimuli either single, doublet, triplet or tetanus, has on the ITT results. Allen et al. (1998) compared single, paired and trains of four stimuli in assessing the VA of elbow flexors near-maximal efforts. They observed identical stimuli responses concluding that a single stimulus is adequate for VA assessment. As explained by Allen and his colleagues (1998), this phenomenon is as a result of no extra discharging motor units being activated by paired or quadruple stimuli when assessing the elbow flexors. In contrast, Suter and Herzog (2001) suggested that the number of stimuli and timing of twitch application have a variable effect on ITT and increasing number of stimuli can result in less variation and randomness of the ITT. Meanwhile, less variability has also been observed when multiple stimuli are used due to the enhanced signal-to-noise ratio created due to the relatively large evoked force (Allen et al., 1998; De Serres and Enoka, 1998). As the number of stimuli increased, it increased the stimulated response signal creating a greater signal to noise ratio, therefore resulting in bigger observed responses (Bigland et al., 1986; McKenzie et al., 1992; Behm et al., 2001). Also, more stimuli increased the possibility that the superimposed stimuli and the muscle action potential occur simultaneously – a condition that is felt to be required for large evoked responses to be observed (Suter and Herzog, 2001). However, as the number of stimuli increases it can intensify the discomfort experienced by subjects during stimulation (Miller et al., 2006; Suter and Herzog, 2001). An additional criticism of using multiple stimuli is that spinal reflexes have more time to influence the superimposed response, potentially affecting the calculated VA level (Herbert et. al., 1997, 1999). Based on creating a balance between the pros and cons of multiple stimuli, doublet or triplet stimuli are most often used in ITT research (Behm et al., 1996; Sheild and Zhou, 2004).

2.4.4 Stimulus Timing:

The inability to precisely introduce stimuli at peak torque/force is recognized as a source of error for superimposed twitch (Miller et. al., 2006; Shield and Zhou, 2004. Traditional methods of stimulus delivery follow one of two approaches – either time based (Behm et al., 2001; Krishnan and Willams, 2008; Williams et al., 2005) or manually triggered (Bampouras et al., 2006; De Serres and Enoka, 1998). During the manual approach the stimulus is delivered when the researcher perceives that the participant has reached their peak force production. This is usually determined by visually inspecting the force curve until a plateau is reached. The time based method involves the stimulus being delivered automatically at a set time-point following the onset of volitional contraction (Krishnan et al., 2009). An obvious problem with both types of stimulus delivery is that they may result in stimuli being applied to the muscle at a force level that is not the persons' voluntary maximum force. Krishnan et al.'s results (2009) indicated that time based and manually triggered methods resulted in the stimulus being delivered at a force level that was 6.0% to 6.2% less than the maximal voluntary torque a person could produce. Krishnan's findings were also supported by Herda et al. (2011), who detected that peak force from the ITT MVC was 6.7% less than peak force from the MVC without ITT. The work of Button and Behm (2008) discussed previously also agrees with these findings. Such timing of the stimulus would obviously result in underestimates of VA levels as ITT is based on the assumption that the stimulus is delivered at max force levels. Additionally, Allen and his colleagues (1995) tried a different approach in delivering stimulus during ITT of the elbow flexors. The stimuli were delivered automatically 350ms after individuals reached a peak in torque. This was indicated using a computer algorithm to detect when torque levels declined, as indicated by a change in slope, over an interval of 100ms. While the approach used by Allen et al. was designed to try and ensure that the responses were evoked closer to the peak force these authors did not report on how close to MVC torque values the stimulus was actually delivered. Based on previous literature (Herda et al., 2011; Krishnan et al., 2009) it is quite possible that the peak torque reached by participants in Allen's work was not the peak torque individuals were capable of producing.

Krishnan et al. (2009) examined this very issue. Specifically these authors were interested in comparing the two traditional methods of stimulus deliver to a third method that they termed the triggered delivery method. This triggered delivery method involved the stimulus being delivered when the torque level being produced by the participants reached a level that matched the participant previously measured MVC. To perform this study, Krishnan and his colleagues (2009) determined the participants MVCs. They then had participants complete a pilot test which examined the precision of stimulus delivery during traditional time based delivery (the stimulator was triggered approximately 3 seconds after the onset of contraction) and a manually delivered approach (the stimulator was manually triggered by visually inspecting the torque plateaus). They determined the stimulus precision by calculating the percent error associated with the difference between the torque at the point in time when the stimulus was delivered and the peak torque they recorded during participants MVCs using the following equation:

$$① \quad \%\mathbf{Error} = (\mathbf{PT - Tstim})/\ \mathbf{PT} * 100$$

Where Tstim represents the torque when the stimulus was received and PT stands for the peak torque produced during an MVC. Results showed that there were no significant differences in precision of the manually triggered and the traditional time based methods. The precision for these two methods was 2.9% for time based vs. 3.1% for manual. Following the pilot test, they compared the precision of the traditional time based method to the automatic torque based method. Results indicated that the automatic torque-based triggering method resulted in significantly greater precision- the stimulus was delivered nearer participants' peak torque levels than the conventional time-based triggering method. The conventional time-based method delivered the stimulus when the exerted torque was, on average, 5.1% (standard deviation: ±4.9%) less than peak torque. The automatic torque-based triggering method delivered the stimulus when the exerted torque was, on average, 1.2% (±0.8%) less than peak torque. Krishnan and his colleagues concluded that the automatic torque based triggering method appeared much better than the conventional time based stimulus delivery as it significantly improved the precision of stimulus delivery. As the inability to precisely introduce stimuli at peak torque/force is recognized as a source of error for superimposed twitch of the ITT in testing VA (Miller et al., 2006; Shield and Zhou, 2004), the results of Krishnan et al. (2009) suggest that an automatic torque based triggering stimulus delivery may help reduce error in ITT results.

Despite the promising nature of Krishnan et al's work (2009), one major weakness of the study was that these authors did not measure the effects of the different stimulus delivery methods on estimates of %VA. This was due to the fact they did not actually deliver supra-maximal stimuli to muscles during their testing – they simply measured that force levels at which stimuli would have been delivered using their automatic method. As a result they could not quantify the effect of the triggered stimulus delivery approach on VA levels. The research that was carried out for this thesis addressed this weakness of Krishnan et al.'s work by using the triggered stimulus method to calculate VA levels in the quadriceps femoris. The specific research question that was asked was:

Does an automatic force or torque based triggering method results in more precise stimulus delivery and higher estimates of VA compared to the traditional manually triggered method when doing ITT?

Hypothesis:

Different ITT triggered methods, automatic force based triggering and manually triggered ITT methods do significantly affect the results of VA. Automatic triggering method will significantly improve the precision of stimulus delivery and will result in increased VA estimates.

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Chapter III Methodology

Methodology:

Subjects: Fifteen young individuals (mean age 26.06 years, range 18-34 years) participated in the study. All subjects were healthy and had no history of lower limb surgery or fractures. The experimental procedures were approved by the ethics board of Memorial University of Newfoundland. All subjects gave their informed consent by signing an informed consent form prior to participation in the study.

Experimental Protocol:

The experimental protocol was divided into two days of testing. The first session consisted of MVC testing and ITT familiarization while the experimental testing was carried out on day two.

During day 1 of testing participants were first asked to do a 5 minute warm up on a cycle-ergometer (50 RPM, 0.5 KP). Following this, participants were seated on a custom designed bench with their back straight against a back rest and the knees hanging down and feet not touching the floor. A padded strap was placed around the ankle of subjects' dominant leg. Dominant leg was determined by asking subjects which leg they would kick a ball with. To ensure reproducibility of force measurements the resting place of the ankle strap on the leg was marked so it could be replicated on day 2 of testing. The ankle strap was attached, via a cable, to a load cell (Omegadyne Inc, Sunbury, OH, USA) mounted on the chair, where the participant was seated. Straps placed over the shoulders and thighs were securely fastened and tightened to ensure participants remained stable during all contractions. They were then asked to produce 2-3 maximum contractions of the knee extensors. Verbal encouragement was provided to all participants during these contractions. Ten minutes rest was given following each MVC. If the difference between maximum forces of the first two trials was greater than 5%, then a third trial was conducted, if not then only two trials were completed. Force data was recorded and stored for future reference using Acknowledge 4.11. The collection rate was 2000Hz. The maximum force levels produced during these contractions were recorded for use on day 2 of testing. Because of the technical requirements of the software that was used to trigger stimulus delivery during day 2 all force measures were kept in their raw voltage format rather than being expressed in newtons of force.

Following these maximal contractions, participants who were not familiar with ITT were introduced to the apparatus and stimulated three times with a small amount of current (20mA). To enable stimulus delivery to the quadriceps two 2cm x 4 cm carbon rubber electrodes (Diamond Athletic Medical Supplies Inc., Winnipeg, MB, Canada) were attached on the muscle belly of subjects' quadriceps. One electrode was placed 5 cm above the patella and the other 2-3 cm below the inguinal space Voltage for all stimulations was set at 400V and pulse width was maintained at 100 micro-seconds. The stimulus was delivered using Digitimer DS7AH constant current stimulator (Digitimer Ltd, Hertfordshire, UK).

On the second testing day participants were asked to sit in the testing chair using the same set-up as day 1. Stimulating electrodes were attached using the configuration described above. In addition surface EMG electrodes were also placed over vasuts lateralis (with ground on the fibular head). Before doing ITT testing the supra-maximal stimulus intensity was determined. This was determined by increasing stimulus intensity until twitch force reached a peak. This was the stimulation intensity used for the remainder of the study. Two ITT trials were then run – one using manual stimulus delivery, the other using triggered delivery. The order of the two tests was randomized and individuals were given a 10 minute rest period between protocols. Regardless of the ITT protocol used, participants were asked to rest for 10 minutes following supra-maximal intensity determination before any further testing was carried out. All force and EMG data for this portion of the study were collected at a rate of 2000Hz.

MVC with automatically triggering ITT stimulation

The automatic stimulus triggering protocol used custom designed software to ensure that the twitch stimulus was not delivered until participants reached at least 97% of the MVC force level that produced on day 1 of testing. This protocol was modified from the work of Krishnan et al. (2009). Participants were asked to contract their quadriceps maximally as verbal encouragement was provided. Five-hundred milliseconds after the target force was reached, doublet stimuli (100Hz) were delivered. Following stimulus delivery the participant was encouraged to continue with force production for one - two seconds. They were then asked to relax fully. Three seconds following the force reaching zero a second set of doublets of the same intensity and duration were delivered.

MVC with manually triggered ITT stimulation

For this protocol all stimulation parameters were identical to those used in the automatic protocol. The only difference was in the method used to initiate the stimulus delivery during the MVC contraction. In this case the stimulus was delivered once the force level was observed to have plateaued. This method is one of the more commonly used methods of timing stimulus delivery (Sheild and Zhou, 2004). Participants were asked to produce a maximum contraction while verbal encouragement was provided. When the force level plateaued (as judged by a researcher who was blind to the participants actual MVC force level) a doublet stimulus was delivered. Following stimulus delivery the participant was encouraged to continue contracting for one-two seconds. After this period of contraction they were asked to fully relax. Following 3 seconds of relaxation a resting doublet stimulus was delivered. Data collection for both protocols lasted for 15 seconds.

Stimulus software

The software used to control both the timing of stimuli delivery during the automatic force based triggering method and the delivery of the resting twitch in both ITT methods was programmed using AcqKnowlege 4.1 software. This software, developed by technical support personnel from Biopac, used a combination of control and calculations channels to detect when the required force level was met and to trigger stimulus delivery. Similar combinations of channels were used to detect when post-MVC muscle relaxation had occurred and to deliver the stimuli 3 seconds following this time. Due to constraints of the system two computers were required to properly deliver ITT.

Data analysis:

VA was estimated using the ITT-based percent activation ① and the CAR method ②, using the following formula:

$$① \quad \%\text{Activation} = [\ 1- (\mathbf{PF\text{-}Fstim})/\mathbf{RestF}]\ {*}100$$

$$② \quad \%\text{Activation} = \mathbf{Fstim}\ /\mathbf{PF}\ {*}100$$

Fstim represents the force level at the point in time the first stimulus was delivered; **PF** is the peak force that resulted due to the first stimulus being delivered and **RestF** represents the force that resulted due to the second stimulus (i.e. the resting twitch). All analysis was done using a combination of AcqKnowledge 4.1 software and Microsoft Excel. See Figure 3.1 for further details.

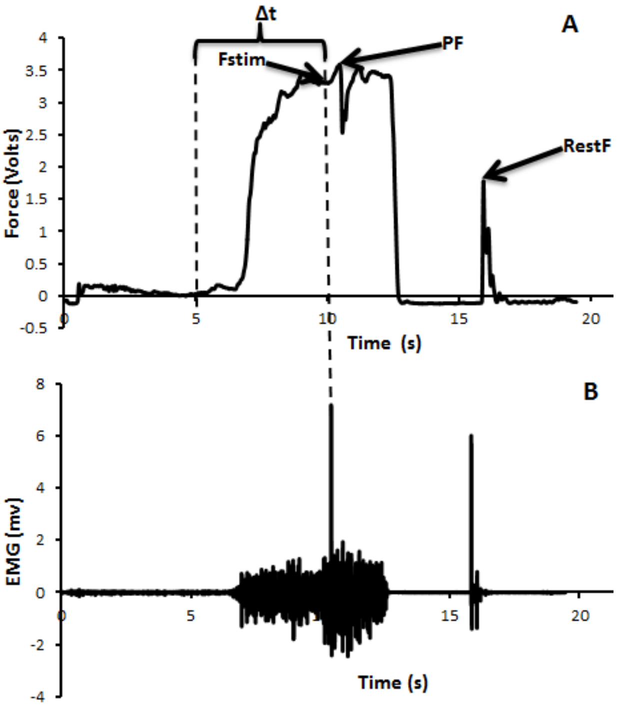


Figure 3.1 **A** illustrates a sample from a test with ITT stimulation and **B** is a simultaneous **EMG**. Δt represents for the time from the beginning of the force to the time when stimulus has been delivered. **Fstim** stands for the force when stimulus delivered and **PF** is the peak force. **RestF** is the resting twitch force.

Meanwhile, the stimulus delivery precision of the two triggering methods was assessed by calculating the percent error associated with the difference between the force when first twitch received and the MVC recorded in the first day trials as the following formula (as per Krishnan et al. 2009):

① $$\%\mathrm{Error} = (\mathbf{MVC} - \mathbf{Fstim}) / \mathbf{MVC} * 100$$

MVC is the peak force recorded on day 1 of testing and Fstim has been defined above. A paired t-test was used to determine if the differences in precision using the two different triggering approaches were significant. Also a paired t-test was used to assess the differences between the ITT and CAR based VA values for the automatic force based triggering method and the manually triggered method. The aim of the comparison was to determine if automatic triggering method significantly improved the quadriceps VA percentage estimation.

Chapter IV Results

Fifteen male subjects participated in the experiment with average age 26.06, average height 177.07cm, 76.69kg.

The VA assessed by automatic triggering ITT was significantly different compared to the manually triggered based VA (p= 0.038). The average automatic triggering VA for ITT was 93.50% (SD= 4.83%) and the average manually triggered based VA for ITT was 90.69% (SD= 8.36%). (See Figure 4.1),

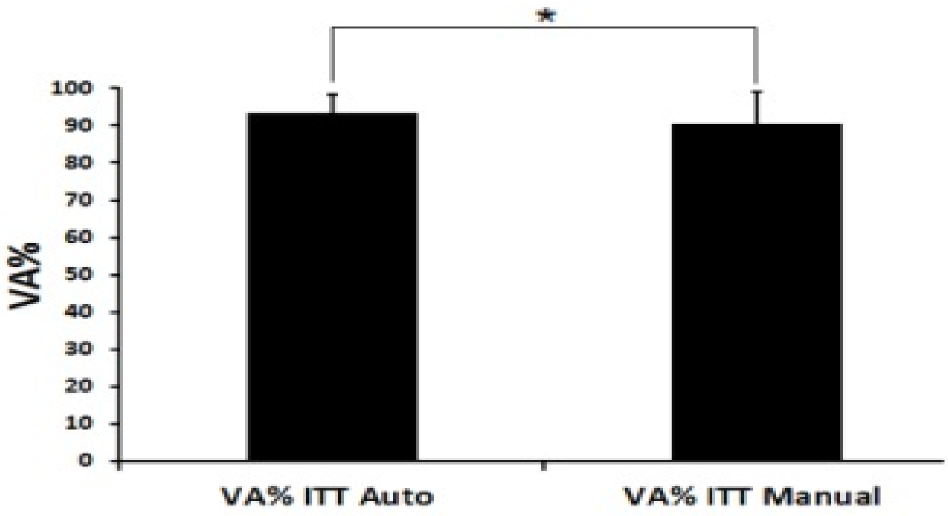


Figure 4.1 illustrates the average VA% based on automatic method and the VA% based on manual method using ITT. Auto triggering resulted in significantly higher VA% than the manual approach.

The VA assessed by automatic triggering CAR was significantly different from the manually triggered based VA using CAR (p= 0.026). The average automatic triggering based VA for CAR was 96.92% (SD= 2.37%) and the average manually triggered based VA for CAR was 95.36% (SD= 4.16%). (See Figure 4.2)

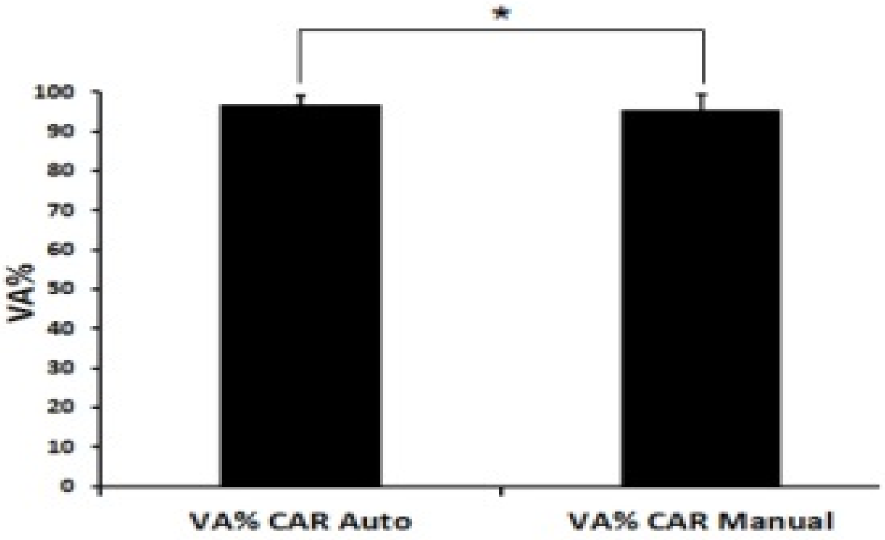


Figure 4.2 illustrates the average VA% based on automatic triggering method and the VA% based on manual method using CAR.

Percentage error during automated and manual trials differed significantly (p= 0.00027) (See Figure 4.3). When the automated triggering approach was used it reduced percentage error by approximately 119.30% (%$\text{Error}_{\text{Manual}}$ -%$\text{Error}_{\text{Auto}}$)/ %$\text{Error}_{\text{Manual}}$).

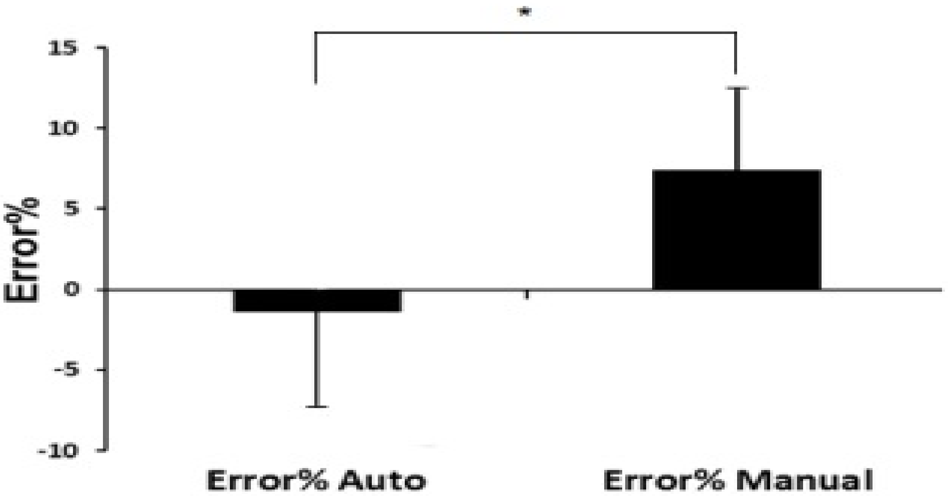
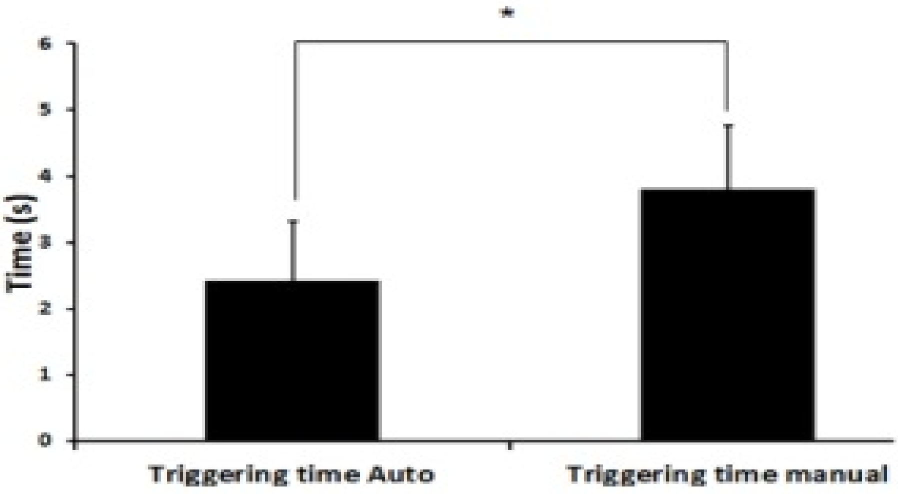


Figure 4.3 illustrates the average Error% based on automatic triggering method and Error% based on manual method using both ITT and CAR. Negative values mean that

stimulus was delivered at a force that was higher than the 97% MVC value.

In addition to the variables described above it became obvious during the data collection process that a difference that seemed to exist between the two methods was the time it took from the initiation of voluntary contraction until the stimulus was delivered. To quantify this fact the time from subjects' initiating force production to stimulus delivery was determined. When these results were compared using a paired t-test, it was found that the two methods resulted in significantly different contraction times (p= 0.0005). The average time for the automated method was 2.42s (SD= 0.89s) while the stimulus was delivered at 3.81s (SD= 0.96s) for the manually triggered method. (See Figure 4.4)



4.4 illustrates the average time from the initiation of the MVC until the delivery of the electrical stimulus for automatic and manual methods

Chapter V: Discussion and Summary

Manually triggered and traditional time based triggering have been extensively used when assessing muscle VA using ITT (Sheild and Zhou, 2004; Gandevia et al., 1995). The present study examined an alternative method of initiating stimulus delivery. This method uses software to detect when a previously determined maximum force level has been reached before delivering the stimulus. One of the most important findings of the research was that the automatic force based triggering method significantly improved the precision in delivery timing and resulted in significantly greater muscle VA estimates. The results supported the study hypothesis that the automatic force based triggering method would increase VA estimates and enhance the precision of ITT and CAR. Also, the calculated CAR is greater than the ITT based VA% which agrees with previous literature (Krishnan and Willams, 2010; Behm et al., 2001).

This research is based on the work of Krishnan et al. (2009). These authors examined different methods of timing the delivery of muscle stimuli during ITT. However, they did not directly compare the automatic torque based triggering and manually triggered methods. Instead, these authors initially did a pilot test that compared traditional time based and manually triggered methods finding no significant difference. Then they analyzed the automatic torque based and traditional time based methods detecting a significant improvement in the precision of delivery when the automatic torque based triggering method was used (automatic error was 1.20% vs traditional time based error was 5.10%). Based on these results Krishnan et al. (2009) concluded that the automatic torque based triggering method represented a more precise method of stimulus delivery when doing ITT. The results of the current study agree with those of Krishnan et al. (2009) namely that automatic torque based stimulus delivery resulted in errors of 1.43% vs. error during the manual methods of 7.38%. Additionally, in the present study stimuli were actually delivered to the muscle and as such the effects of this method on VA% were able to be assessed. Results indicated that the improvements in precision of delivery timing lead to relatively higher VA% estimates. This improvement in VA level prediction suggests that the triggered approach is the one that should be considered when doing ITT or CAR.

The calculated quadriceps VA levels in the present study were within the ranges observed in previous research. For healthy, non-fatigued subjects, quadriceps VA levels assessed by manually based ITT have been reported to span from 73% to 100% (Amann et al., 2013; Paillard and Borel, 2013; Bachasson et al., 2013; Neil et al., 2013; Gerrits et al., 2013; Park et al., 2012; Pietrosimone and Saliba, 2012; Skurvydas et al., 2011; Stähli et al., 2010; Krishnan et al., 2009; Vivodtzev et al., 2008; Jubeau et al., 2007; Miller et al., 2006; Urbach et al., 2006). The manually based VA levels examined in our study ranged from 75.98% to 98.35%. Also, the VA levels assessed by automatic force based triggering method ranged from 83.89% to 98.85%. Similarly, VA levels assessed by manual and auto CAR methods were within the range reported in the literature from 82% to 100% (Campbell et al., 2013; Paillard and Borel, 2013; Poulsen et al., 2013; Park and Hopkins, 2013; Pietrosimone and Saliba, 2012; Stackhouse et al., 2010; Petterson et al., 2011; Zory et al., 2010; O'Brien et al., 2008; Tammik et al., 2008; Place et al., 2005). It is important that my tested VA% either by ITT or CAR agree with the values in the literature.

The results of the present study showed a 3% average increase in ITT based VA% when the automatic force based triggering method was used. Though the average increase was relatively small, the differences for some individual subjects were large. When individual participant results were examined four subjects had more than a 5% increase in VA% when the automatic force based triggering method was used. However, five subjects had less than 1% change in VA% using these two methods. However, for others there was a clear difference between the VA assessed by the two approaches. So while the average change in activation may appear small at 3%, for some individuals the differences were very large and could have the potential to substantially impact findings in research examining VA of the quadriceps. Based on this it is the recommendation of the current authors that the triggered approach be the approach of choice when doing ITT based research.

Research uses of the automatic force based triggering method

Although the results of this study suggest that automatic force based triggering approach may be a more precise method for determining %VA, it cannot be used in all situations where ITT or CAR are required. In general any experimental intervention that has the potential to affect muscle force production may make the triggered ITT or CAR methods inappropriate due to the fact that individuals will not be able to reach the target force required for the stimulus to be delivered. One such intervention would be fatigue based studies which require pre/post fatigue assessments of VA. In such situations once the subject becomes fatigued, he or she will not be able to reach the set threshold (97% of the MVC), and therefore no stimuli will be delivered. To assess the VA% when fatigue is a factor the manually triggered method is highly recommended (Stackhouse et al., 2001, 2005). Even in situations where there is no fatigue protocol being carried out researchers need to be aware of the potential effect of multiple contractions on muscle force production ability, as even minor reductions in force can mean the triggered approach will not work. In the present study fatigue was minimized by ensuring adequate rests (10 minutes rest between all MVCs performed on both days of testing).

Besides fatigue, any other experimental designs where pre/post ITT and CAR are done with interventions that can reduce force output would also be inappropriate for used of the automatic force based triggering stimulus delivery. These would include things such as muscle vibration (Bosco et al., 1999), sustained aerobic activity (Ullrich and Brüggemann, 2008), muscle cooling (Robinson et al., 2013; Kovac et al.,

2010) or circulation occlusion (Sumide et al., 2009).

In addition to the types of experimental designs described above a second issue with implementing the automatic triggering stimulus delivery approach relates directly to the complexity of implementing the protocol. The software available for use with this project had to be programmed with approximately 47 equations and functions in order to automatically deliver the stimuli both once the 97% force level was reached and during the resting twitch. To accomplish this, two computers had to be used. While some of the complexity of the set-up was in part due to limitations of the hardware and software available for use in the project, performing ITT and CAR with this set-up was very equipment intensive. Now that the usefulness of this technique has been confirmed, more streamlined and cost-effective methods of applying the idea will be sought.

In conclusion, under appropriate testing circumstances, the automatic force based triggering method is the recommended choice to analyze muscle VA level with higher precision. While it is not appropriate for use in experimental protocols that while reduce force production, in instances where researchers want only to quantify the VA of a muscle, without looking at the effects of some variable on VA, the automatic method would appear to be a better option.

Limitations of the study

The present study examined the use of triggered ITT and CAR for estimating the %VA of the quadriceps. The results cannot therefore be generalized to all muscles. However, given that the automatic force based triggering method appears to improve the precision of stimulus delivery it is anticipated that this affect would be observed regardless of the muscle being examined.

The first limitation that needs to be considered is the variability of the interpolated twitch technique. On the second day of our study, subjects performed two MVCs using the ITT technique with only one testing contraction under each condition (manual or automatic). As discussed previously, the technique is quite variable, as such the risk of doing just one repetition of each approach is that the results may have varied had more trials been performed. While this is a possibility, one contraction was chosen because of the concern that multiple trials of each condition would result in fatigue, making the triggered approach unusable. Now that the possible increased accuracy of stimulus timing delivery have been confirmed by the present study future research should examine the whether or not variability of ITT is also potentially reduced by using the triggered force approach.

Despite efforts that were made to ensure that both manual and automatic delivery methods were as similar as possible, one major difference between the two was the amount of time that occurred between the onset of the voluntary contraction and the delivery of the stimulus. On average during the manual method individuals contracted for 3.81 sec before the initial stimulus was delivered. This was in contrast to the 2.42 sec it took for the stimulus to be delivered in the automatic triggering approach. It is possible that the difference in contraction times may have led to the increase in voluntary activation levels observed in this study. Work by Vandervoort et al. (1983) would suggest that it is unlikely that the timing differences could have accounted for the increased VA observed during the automatic 2.42s. These authors reported that an MVC had a greater potentiating effect on a twitch the longer it was applied (ranging from 1s – 10s). This would suggest that the longer contraction observed during the manual 3.81s should have resulted in a greater resting twitch. All other things being equal, such an increase in the resting twitch would have resulted in higher activation levels being found for the manual method. Because the work of Vandervoort et al. (1983) examined plantar and dorsi-flexor muscle groups it is hard to draw direct comparisons to the current work, however Vandervoort`s work does at least suggest it is unlikely the results of the present study were due to differences in contraction time length. Further research is needed to confirm this fact.

Although results of the study indicated more precise stimulus delivery and significantly increased activation levels it is important to note that this study does not provide evidence about the accuracy of the triggered method of stimulus delivery. In order to examine the accuracy of this triggered method, it is necessary to know the actual activation level. All that is known is that for the automatic force based triggering method the stimulus was triggered once the force level reached 97% MVC. . This does not, however, mean the activation level was 97%. This is further shown in the present study. The calculated VA levels were not 97% but ranged from 83.89% to 98.85%. This is mainly because either the MVC recorded might not have been the ‘real’ MVC for the subjects or the individuals tested could not maximally activate their muscles even with supra-maximal stimuli. Also, studies show variability in MVCs productions between different days and even within the same day (Sedliak et al., 2011) so perhaps the MVC recorded on day 1 was not the same as the MVC participants could reach on day 2. Therefore, when using VA% based on automatic force based triggering method, it cannot be claimed that the results are more accurate or not. Additional research comparing results of this method to those using TMS and MRI (as per Adam et al. 1993 and Kendall et al. 2006) is needed to draw these conclusions.

A final limitation of this work was the time interval (500ms) between the subjects reaching the target force (97% MVC) and when the stimulus was delivered. Initially the time interval between subjects reaching the threshold force level and the actual stimulus delivery (i.e. 500ms) was felt to be a limitation of the study. This time interval was actually erroneously used. The intent was to use 350ms as per Krishnan et al.'s paper (2009). Due to a software glitch it was later realized that the stimulus was delivered at 500ms post force threshold being reached. Further inspection of the data however, suggested that this timing may have actually improved the precision of approach, as is shown in Table 5.1. From the table, for twelve out of the fifteen subjects force continued to rise after the 97% force level was reached. As a result the stimuli was delivered at a higher force value than the 97% threshold and five even were even stimulated over the MVC they produced on the day 1 of testing. For the manually triggered method, only three subjects received the stimulation when the force level was over 97% of MVC. Therefore, the 500ms delay in delivery likely resulted in the forces at the time the stimuli were delivered being higher than they would have been if a shorter time period was used. This resulted in a smaller superimposed response with relative larger muscle VA% estimation. Therefore, this length of time is recommended to be used as a delivery timing delay for automatic force based triggering method. Further research should examine what the optimal level of delay would be to result in peak force values at stimulus delivery. Alternatively, it is also possible that using a more sophisticated software package would enable stimulus delivery timing to be triggered at the peak after a certain force level is reached.

Table 5.1: Summary of force levels at which stimuli were delivered for both automatic triggering and manually triggered methods. The columns represent the number of participants for whom the force at stimulus delivery fell into the three categories indicated. SF represents the force at the time the stimulus was delivered to the muscle. See text for more details.

	SF > 97% MVC	SF < 97% MVC	SF > MVC
Automatic triggering method	12	3	5
Manually triggered method	3	12	1

Summary

The automatic force based triggering method of ITT and CAR significantly improved the precision in timing delivery of VA assessment in quadriceps with higher VA% estimates. Future studies are required to examine implementing this approach in other muscles. Even though VA% was higher, the accuracy of the automatic estimate with respect to the actual VA%, future studies need to be done in this area perhaps by using MRI in determining the VA%.

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