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EFFECT OF STIMULATION INTENSITY ON ASSESSMENT OF VOLUNTARY ACTIVATION

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ABSTRACT: Introduction: The interpolated twitch technique is often used to assess voluntary activation (VA) of skeletal muscles. We investigated VA and the voluntary torque-superimposed torque relationship using either supramaximal nerve stimulation or better tolerated submaximal muscle stimulation, which is often used with patients. Methods: Thirteen healthy subjects performed maximal and submaximal isometric knee extensions with superimposed maximal or submaximal doublets (100 Hz). Results: Superimposed torque relative to potentiated resting doublets was smaller with maximal nerve than with submaximal muscle stimulation. Maximal VA was 87 \pm 7% and 93 \pm 5% for submaximal muscle and maximal nerve stimulation, respectively. The individual voluntary torque-superimposed torque relationships were more linear for submaximal muscle stimulation, possibly leading to less overestimation of VA. Conclusions: Submaximal muscle stimulation can be used to estimate VA in the knee extensors. It is less painful, and overestimation of VA may be less compared with maximal nerve stimulation.

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The interpolated twitch technique was first used by Merton¹ to assess muscle inactivation in the adductor pollicis. When a muscle is not fully activated during a voluntary contraction and a (supra) maximal electrical pulse is applied, this will lead to an increase in torque (superimposed torque, e.g., see Fig. 1). This technique is reliable² and has been applied in different muscle groups. It has become the standard technique to assess voluntary muscle activation (VA).²⁻⁴ The quadriceps has been studied frequently with superimposed stimulation,^{2–7} because it is a large muscle group with important contributions during sports and locomotion in daily life. The electrical stimulation is typically applied over either the nerve trunk (nerve stimulation) or the muscle belly (muscle stimulation).⁸ In patients, superimposed electrical stimulation is used to assess voluntary activation^{9,10} or to assess changes in neural activation due to training or disuse.^{11–13} With patients however, submaximal muscle stimulation is used frequently to calculate VA,^{8,10,14,15} because submaximal currents are bet-

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ter tolerated.^{10,16} Muscle stimulation is also easier to apply than nerve stimulation because of the location of the femoral nerve in the femoral triangle. Disadvantages of maximal nerve stimulation are shifting of the femoral nerve during voluntary contractions and unwanted stimulation of the sartorius muscle.¹⁶ The disadvantages of submaximal muscle stimulation are incomplete¹⁶ and random recruitment¹⁷ and possible antagonist stimulation,¹⁸ although antagonist stimulation is less likely with submaximal stimulation compared with maximal stimulation.¹⁸ Previously, voluntary activation was found to be similar when it was assessed with maximal percutaneous or maximal nerve stimulation for the plantar flexors.⁶ Recently, Place et al.¹⁶ showed that submaximal quadriceps muscle stimulation resulted in equal superimposed torques compared with maximal nerve stimulation, but VA was not calculated in that study. In this study, we elaborate on these observations by investigating the effects of stimulation type on actual VA, which in most studies that use superimposed stimulation is the primary parameter of interest.^{10–14}

It is assumed that there is a linear relationship between voluntary torque of the stimulated muscle and superimposed torque. This indicates that VA is also related linearly to voluntary torque. While the relationship between voluntary torque and superimposed torque indeed was reported to be linear,^{5,19} there is growing evidence that this relationship is curvilinear for the knee extensors^{2,3,6,7} and also for other muscles.^{6,20,21} It is time consuming and difficult to obtain a good and complete relationship between superimposed and voluntary torque. Therefore, in most studies VA has been calculated with the superimposed responses upon the highest of a few maximal voluntary contractions.^{9,22–24} However, if the relationship indeed is curvilinear, VA is overestimated for lower contrac-tion intensities,^{2,3,7,25} such as those observed in patients.^{2,9,10,12} For maximal contractions VA may also be overestimated, but without a gold standard for the maximal torque capacity (MTC), the extent of overestimation cannot be assessed.

The aim of this study was to investigate if less painful submaximal muscle stimulation results in similar voluntary torque-superimposed torque relationships and voluntary activation as obtained with maximal nerve stimulation. It was expected that submaximal muscle stimulation would result in

Abbreviations: ANOVA, analysis of variance; MTC, maximal torque capacity; $MTC_{60-100\%}$, MTC calculated from voluntary torque between 60 and 100% MVC; $MTC_{100\%}$, MTC calculated at MVT; MVC, maximal voluntary torque between 40 tary contraction; MVT, maximum voluntary torque; VA, voluntary activation; $VA_{60-100\%}$, voluntary activation, calculated from voluntary torque between 60 and 100% MVT; $VA_{100\%}$, maximal voluntary activation, assessed at MVT

Key words: electrical stimulation, interpolated twitch technique, maximal torque capacity, quadriceps, voluntary activation Correspondence to: D. M. Van Leeuwen; e-mail: d.m.van.leeuwen@vu.

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FIGURE 1. Typical torque traces during maximal nerve (black) and submaximal muscle stimulation trials (gray) for almost equal torque levels (target torque was 80% MVT \sim 156 Nm). Torque traces are aligned to the onset of superimposed stimulation (vertical line at t = 0.0 s). The inset shows an enlarged graph of the superimposed response. Arrows indicate the size of the superimposed response and the potentiated resting doublet for maximal nerve stimulation.

similar voluntary torque-superimposed torque relationships and similar estimations of voluntary activation. These experiments assess whether a practical modification of the interpolated twitch technique to make it less painful for subjects would result in similar levels of VA. A less stressful stimulation technique is important, because superimposed stimulation is the gold standard for measuring maximal voluntary activation in frail elderly subjects and subjects with musculoskeletal disorders.^{8,10,14,15}

MATERIALS AND METHODS

Subjects. The participants were 13 healthy volunteers (9 male, 4 female) aged 26.0 ± 3.6 years. Their body weight was 69.5 ± 7.8 kg, height was 1.80 ± 0.08 m), and they were unfamiliar with electrical stimulation. All subjects gave written informed consent, and the study was approved by the local ethics committee.

Torque Measurements. Measurement of the contractile properties of the knee extensor muscles took place on a custom made adjustable dynamometer which recorded the exerted torque at its axis of rotation. All measurements were performed on the right leg at a knee angle of 60° (0° is full extension) during isometric contraction. Subjects sat in the dynamometer with a hip angle of 80° (0° is full extension) and were firmly attached to the seat with straps at the pelvis to prevent extension of the hip during contraction and a strap at the chest. The axis of rotation of the dynamometer was visually aligned to the axis of rotation of the knee joint. The lower leg was strapped tightly to the arm of the dynamometer. Torque was sampled at 10 kHz, digitized, filtered with a 4th order bidirectional 150 Hz Butterworth low-pass filter, and stored on a PC for offline analysis. Torque signals were corrected for gravity; the average torque applied by the weight of the limb was set at zero.

Electrical Stimulation. Constant current electrical simulation (pulse width 200 μ s) was applied through self-adhesive surface electrodes (Schwa-Medico, Leusden, The Netherlands) by a computer-controlled stimulator (model DS7A, Digitimer Ltd., Welwyn Garden City, UK). For maximal nerve stimulation, the anode $(8 \times 13 \text{ cm})$ was placed over the gluteal fold, and the cathode (5 \times 5 cm) was placed over the femoral nerve in the femoral triangle. For submaximal muscle stimulation, the distal electrode (8 \times 13 cm) was placed over the medial part of the quadriceps muscle just above the patella, and the proximal electrode (8 \times 13 cm) was placed over the lateral portion of the muscle to prevent inadvertent stimulation of the adductors. The skin in the area of the electrodes was shaved before the electrodes were applied. The stimulation current was increased until torque in response to doublet stimulation (two pulses at 100 Hz) leveled off. Subsequently, to ensure maximal stimulation, stimulation current was increased a further 50 mA for nerve stimulation (range, 200-400 mA). For submaximal muscle stimulation the stimulation was increased until a plateau was observed, and it was then lowered to produce 50% of the maximum doublet torque (range, 80-125 mA). This ensured that a substantial amount of muscle mass was stimulated, but it significantly reduced stimulation related discomfort. Doublet stimulation was chosen to increase the signal-to-noise ratio² and to decrease effects of potentiation.²⁶

Experimental Protocol. After a warm-up of 10 submaximal isometric extensions of increasing intensity, subjects performed one MVC for the knee extensors to determine target levels for the subsequent submaximal contractions with superimposed electrical stimulation. For each stimulation method, contractions of 30, 50, 70, 80, and 90 %MVT and two MVCs were performed in random order. Thus a total of 14 contractions were performed $(2 \times 7 + 1 \text{ MVC to estimate torque levels})$. Of these contractions, 7 were near maximal (>90%). To avoid possible effects of fatigue, the number of near-maximal contractions was limited to these 7 attempts. Three minutes of rest were taken between contractions. For all superimposed contractions, torque was displayed in real time for the subjects, and they were verbally encouraged to

exceed their maximum value during MVCs. When torque was stable and close to the target line, a superimposed doublet was delivered to the muscle. Two seconds after each contraction, a (potentiated) doublet was delivered to the relaxed muscle. The order of the type of stimulation (nerve or muscle) was randomized among the subjects, but the measurements of one stimulation type were fully completed before the measurements of the other type were made, for convenience. There was no familiarization, because in practice, particularly with patients, it is often difficult to include a familiarization session.

Data analysis. Electromechanical delay was taken into account when voluntary torque and superimposed torque were calculated.²⁶ Maximal voluntary torque (MVT) was defined as the highest torque recorded at the onset of stimulation, because this torque was expected to have to closest link with the superimposed torque response. Maximal voluntary activation (VA_{100\%}) was calculated with use of the following equation: $VA_{100\%} = MVT/MTC_{100\%}$ \times 100%.^{7,27} MTC_{100%} is the theoretical maximum torque estimated from MVT with the following equation: $MTC_{100\%} = 1 / [1 - (superimposed tor$ que/potentiated resting doublet)] \times MVT.⁷ In addition, we calculated VA in an alternative way $(VA_{60-100\%})$ as suggested by others,^{7,27} by dividing MVT over $MTC_{60-100\%}$, which was obtained by extrapolation of the linear regression line fitted on the superimposed torques obtained for voluntary torques greater than 60% MVC. Figure 2A illustrates the calculation of $MTC_{100\%}$ and $MTC_{60-100\%}$. The range of 60-100% MVT was chosen, because inclusion of lower torque levels tends to increase the errors of MTC estimation.^{2,3,7,28}

Best fits for superimposed torque data as a function of voluntary torque for each individual subject were calculated using a least squares algorithm. Linear, quadratic, cubic and exponential (two and three variables) fits were calculated. Akaikes Information Criterion with a second order correction for small sample sizes was used to determine the best fit.²⁹ Because actually produced torque was not exactly equal to the target percentages of MVT, values for 30, 50, 70, 80, 90, and 100% MVT were subsequently obtained from the individual fitted curves to statistically compare stimulation types. The best fits were not used for estimations of MTC, because such relations in many cases did not cross the x-axis or did so at unrealistically high values.

Statistics. Differences between stimulation types regarding the superimposed-voluntary torque data were analyzed using analysis of variance (ANOVA) repeated measures with a Bonferroni *post-hoc*



FIGURE 2. Superimposed torque data (A) for one subject as a function of voluntary torque for maximal nerve (black circles) and submaximal muscle stimulation (open squares). MTC_{100%} was calculated by linear extrapolation of the data point obtained at MVT assuming a linear relation between superimposed torque and resting doublet (crossing of the dotted lines with the x-axis). Alternatively, when more data points are available, MTC can be estimated by linear extrapolation of the regression line on data points with torques above 60% MVT torque (solid lines, MTC₆₀₋ 100%). Both (B) relative and (C) absolute superimposed torques for maximal nerve and submaximal stimulation. Individual data points with averages $(\pm SD)$ for the stimulation types are displayed. Because actually produced torque was not exactly equal to the target percentages of MVT, torque values from the individual best fits were used to calculate mean group values. * indicates a significant difference between stimulation types.

	Table 1. MVT, doublet torque, voluntary activation, and MTC.					
	MVT (Nm)	Doublet (Nm)	VA _{100%} (%)	VA _{60-100%} (%)	MTC _{100%} (% MVT)	MTC _{60-100%} (% MVT)
Maximal Submaximal	244 ± 64* 252 ± 64	86 ± 17* 43 ± 10	$93 \pm 5^{*^{\dagger}}$ 87 ± 7	90 ± 7* 84 ± 8	108 ± 5* 115 ± 9	111 ± 9* 120 ± 12

MVT (highest plateau torque), (potentiated) doublet torque, maximal voluntary activation (VA_{100%}), VA calculated from torque between 60 and 100% MVT (VA_{60-100%}), MTC calculated at MVT (MTC_{100%}), and MTC calculated from torque between 60 and 100% MVT (MTC_{60-100%}) for maximal nerve and submaximal muscle stimulation. All values are means \pm SD. An asterisk indicates a significant difference (P < 0.05) between stimulation types. The dagger indicates a significant difference between VA_{100%} and VA_{60-100%} (P < 0.05).

correction. The Pearson correlation was used to investigate relationships between variables. The level of significance for all tests was set at 0.05 (two-tailed).

RESULTS

Superimposed Torque Relationship. Figure 1 shows typical torque traces for both stimulation types. There were no significant differences in time to peak for potentiated doublets and time to peak superimposed torque between stimulation types, although a more pronounced drop in torque was seen following maximal nerve compared with submaximal muscle stimulation for contraction intensities greater than ~80% MVT.

Figure 2A shows a typical superimposed torque-voluntary torque relationship for 1 subject. Curve fitting of the individual data points $(r^2 ranged between 0.92 and 1.00)$ showed that relationships for superimposed maximal nerve stimulation were best fitted (lowest Akaikes Information Criterion) with an exponential function for twelve subjects and a linear function for only one subject. For submaximal muscle stimulation, the superimposed torque relationship was best fitted with an exponential function for eight subjects and a linear function for five subjects. Figure 2B shows relative superimposed torques for maximal nerve and submaximal muscle stimulation for all subjects together with group averages. Because actually produced torque was not precisely equal to the target percentages of MVT, values from the individual best fits were used to calculate group averages and for statistical comparison. Submaximal muscle stimulation during voluntary contractions resulted in greater relative superimposed torques than maximal nerve stimulation. There was a main effect of stimulation type on normalized (to resting doublet) superimposed torque, with a near significant interaction effect (P = 0.06) between stimulation type and torque. Post-hoc tests revealed significant differences, indicating that relative superimposed torque at 50, 70, 80, and 90% of MVT was lower with maximal nerve compared with submaximal muscle stimulation (see Fig. 2B). Figure 2C shows absolute torque increments for maximal nerve and submaximal muscle stimulation. An interesting finding was that the absolute superimposed response upon MVT with submaximal muscle stimulation (5.7 \pm 3.5 Nm) was similar (P = 0.28) to that obtained with maximal nerve stimulation (6.4 \pm 3.8 Nm), even though MVT was significantly higher (P < 0.05) just before submaximal muscle stimulation (252 \pm 64 Nm) compared with submaximal muscle stimulation (244 \pm 64 Nm, see Table 1). This is surprising, because potentiated resting doublets for submaximal muscle and maximal nerve stimulation were 43 \pm 10 and 86 \pm 17 Nm, respectively, suggesting a twofold difference in activated muscle mass between stimulation types.

In pilot experiments, additional superimposed measurements were done with *maximal muscle stimulation* for some subjects. Figure 3 shows absolute (A) and normalized (B) superimposed torques for



FIGURE 3. Both (A) absolute and (B) relative superimposed torque relationship for a single subject during pilot experiments established with *submaximal* muscle (open squares), *maximal* muscle (gray triangles) and maximal nerve stimulation (black circles).

one of our subjects with maximal nerve stimulation, *submaximal* muscle stimulation and *maximal* muscle stimulation. The voluntary torque-superimposed torque relationships for maximal nerve and maximal muscle stimulation were quite similar, whereas submaximal muscle stimulation resulted in higher normalized superimposed torques for voluntary torques near MVT (note that in this subject, the absolute torque increments were even larger for submaximal muscle stimulation compared with maximal muscle and maximal nerve stimulation for contraction intensities near MVT).

Voluntary Activation. Average values for voluntary activation calculated at MVT (VA_{100%}) were higher with maximal nerve (93 ± 5%) than with submaximal muscle stimulation (87 ± 7%; P < 0.05, Table 1), although voluntary torque was 3% lower just before maximal nerve stimulation (P < 0.05). At somewhat lower levels of activation such as could be expected in patients, calculated differences in VA between stimulation methods were even larger. At 70% of MVT, VA was 77 ± 7% for maximal nerve and 68 ± 9% for submaximal muscle stimulation (see Fig. 2B).

As indicated before, VA was also calculated by expressing MVT as a percentage of $MTC_{60-100\%}$. MTC_{60-100%} was estimated by extrapolation of the linear regression line fitted on the superimposed torques obtained for voluntary torques greater than 60% MVT. The average r^2 values for these regression lines were 0.94 (range, 0.89-0.99) for maximal and 0.84 (range, 0.51-0.97) for submaximal muscle stimulation. Although this alternative manner of calculating VA led to slight reductions of calculated maximal VA, the difference between both stimulation methods remained. For maximal nerve and submaximal muscle stimulation, maximal VA was reduced from 93 \pm 5% to 90 \pm 7% (P < 0.05) and from 87 ± 7% to 84 ± 8% (P =0.06), respectively.

It is important to note that we used voluntary torque at stimulation onset for our calculations of VA, because this torque was expected to have the closest link with the superimposed torque response. However, when instead the highest voluntary torque observed at any time during any of the contractions (259 ± 63 Nm) was used to calculate VA (by dividing this maximum value by MTC_{100%}), maximal VA would increase from $93 \pm 5\%$ to $99 \pm 6\%$ for maximal nerve (P < 0.05) and from $87 \pm 7\%$ to $90 \pm 7\%$ (P < 0.05) for submaximal muscle stimulation. For several subjects, the highest voluntary torque observed was higher than MTC_{100%} estimated with maximal nerve stimulation, resulting in VA levels above 100%. This sug-

gests underestimation of MTC and consequently overestimation of maximal VA.

DISCUSSION

Superimposed Torque Relationship. An important finding of this study is that superimposed submaximal muscle stimulation during voluntary contractions resulted in higher superimposed torques when expressed relative to the resting doublet and resulted in more linear superimposed torque relationships compared with maximal nerve stimulation (Fig. 2B).

The difference in relative superimposed torque could have been caused by the difference in stimulation site and/or by the stimulation intensity between both methods. The pilot data shown in Figure 3 indicate that the voluntary torque-superimposed torque relationships for maximal nerve and maximal muscle stimulation were quite similar, whereas submaximal muscle stimulation resulted in higher normalized superimposed torques for voluntary torques near MVT. Therefore, it is more likely that the difference in stimulation intensity rather than stimulation site accounted for the higher relative amplitude of the superimposed torque during submaximal muscle stimulation compared with maximal nerve stimulation for all our subjects.

The lower relative response during maximal nerve stimulation could have been caused by unwanted stimulation of antagonist muscles using high current intensities, such as the sartorius muscle (by means of the femoral nerve) and/or hamstrings as suggested by others,^{16,18} but there are several other possible explanations. First, tendon slack can reduce resting doublet torque to a greater extent with submaximal muscle stimulation compared with maximal nerve stimulation and lead to a relatively high superimposed torque during submaximal stimulation.³⁰ However, the absolute torque increments upon MVT were high during submaximal muscle stimulation (5.4 Nm) and were not statistically different from those obtained with maximal nerve stimulation (6.3 Nm). Moreover, for some subjects the absolute torque increments were even systematically larger for submaximal muscle stimulation upon MVT compared with maximal activation (e.g., Fig. 3A). Slack can increase *relative* superimposed torque because of relatively low resting doublet torque with submaximal muscle stimulation, but it cannot increase abso*lute* superimposed torque. This indicates that slack cannot (fully) explain the differences between the superimposed responses during maximal nerve and submaximal muscle stimulation. Spinal effects such as recurrent inhibition, hyperpolarization of the motoneuron, or inhibitory effects of muscle

afferent stimulation could also affect superimposed torque.³¹ The shorter distance of the electrodes to the spinal cord and higher stimulation currents used with maximal nerve compared with submaximal muscle stimulation, could lead to greater inhibition or hyperpolarization of the motoneuron and therefore relatively smaller superimposed torques during maximal nerve stimulation. In addition, if motor axons are in a refractory state at the instant of stimulus application, this can suppress superimposed torque, particularly at higher values of MVT.³² Furthermore, there is a nonrandom distribution of fiber types in the quadriceps, with relatively more type II fibers³³ and more larger motor units^{34,35} in superficial layers. Percutaneous stimulation, with lower stimulation currents will not reach deep into the muscle tissue,¹⁸ and, therefore, potentially more type II fibers may be activated with submaximal muscle compared with maximal nerve stimulation. This affects the superimposed torque response more than the resting doublet torque, because during MVT all type I fibers are probably already recruited and are (close to) maximally activated. However, because these differences in fiber type localization are rather subtle in human muscles,^{34,35} it seems unlikely that preferential activation of superficial fibers with submaximal muscle stimulation can fully account for the presented differences between stimulation methods. A final explanation for differences in relative superimposed torque could be related to the occurrence of antidromic collisions. Antidromic collisions take place when stimulation pulses collide with voluntary action potentials. This will reduce the rate of motoneuron discharge immediately after the stimulus and can reduce the superimposed response.³¹ Because these collisions can only occur in axons of muscle fibers that are voluntarily active and electrically stimulated at the same time, these collisions are expected to occur more often during maximal nerve stimulation This results in a lower absolute superimposed torque and lower relative superimposed torque, because the resting doublet is unaffected by antidromic collisions.

Voluntary Activation. Irrespective of the method used to calculate VA, submaximal muscle stimulation resulted in lower values for VA compared with maximal nerve stimulation. This is in line with a very recent study,¹⁶ where similar absolute superimposed torque increments were observed for maximal nerve and submaximal muscle stimulation. Although VA was not calculated in that study, submaximal muscle stimulation would have led to lower calculated VA, because significantly lower resting doublets were observed for submaximal



FIGURE 4. Effect of curvilinearity of superimposed torque relationships on the calculation of MTC for submaximal muscle (white squares) and maximal nerve stimulation (black circles). The average superimposed torques for 70 and 100% MVT are shown. MTC was calculated by linear extrapolation and can be visualized by the intercept with the x-axis of the line between the resting doublet and the superimposed torque. For maximal nerve stimulation, MTC estimated from 70% MVT was approximately 84% of the estimation using 100% MVT (thick solid lines), whereas for submaximal muscle stimulation this was \sim 90% (dashed lines). As a result, a decrease of voluntary torque from 100 to 70% MVT results in a relatively larger decrease of the estimated MTC for maximal nerve stimulation compared with submaximal muscle stimulation.

muscle stimulation. 16 $\rm VA_{60-100\%}$ was significantly lower than $\rm VA_{100\%}$ for maximal nerve and almost for submaximal muscle stimulation. The r² values of the regression lines for submaximal muscle stimulation were quite low for some subjects (range, 0.51-0.97). Therefore, calculation of VA_{60-100%} may be less accurate for submaximal muscle stimulation. However, in common practice, when VA is usually determined by the superimposed responses upon the highest of a few maximal voluntary contractions, $^{9,22-24}$ there are indications that VA may be overestimated with maximal nerve stimulation. Several subjects were able to elicit voluntary torques above $MTC_{100\%}$, and consequently $VA_{100\%}$ was calculated to be above 100%. At 70% MVT, which corresponds better to activation levels of patients,^{2,9,12} differences in VA were larger between the two methods (Fig. 2B).

With submaximal stimulation, an overestimation of VA seems less likely to occur for two reasons. First, the higher the exerted volitional torque is, the more accurate the estimation of voluntary activation becomes.^{2,3,7,25} MVT just before the instant of application of the superimposed doublet was significantly higher with submaximal muscle (252 ± 64 Nm) than with maximal nerve stimulation (244 ± 64 Nm). This could be related to stimulus anticipation.³⁶ Second, the relationships between voluntary and superimposed torque were more linear with submaximal muscle stimulation than with maximal nerve stimulation (Fig. 2). Because calculations of VA are usually based on linear relationships, and curvilinear relationships tend to overestimate VA,^{2,3,7,25} overestimation of VA is less likely with submaximal muscle stimulation.

It is important to note that these curvilinear relationships can have consequences for repeated measurements of MTC and VA. This is demonstrated in Figure 4, where average superimposed torques for 70 and 100% MVT are displayed, and MTCs are visualized by the intercept with the x-axis of the lines between the resting doublet and the superimposed torque. For maximal nerve stimulation, using a voluntary torque of 70% MVT resulted in an estimation of MTC, which was $\sim 84\%$ of the estimation using 100% MVT, whereas for submaximal muscle stimulation this was $\sim 90\%$. Differences in voluntary torque will thus be less properly reflected in calculated VA values when it is assessed with maximal nerve stimulation, because VA is inversely related to MTC. This is especially important for repeated measurement of VA in patients with neuromuscular disorders after disuse or an intervention.

This study only compared the assessment of VA between one submaximal muscle stimulation intensity and maximal nerve stimulation. Further research into effects of stimulation intensity is needed to confirm these findings.

CONCLUSIONS

Submaximal muscle stimulation upon voluntary isometric knee extension resulted in higher relative superimposed torques compared with maximal nerve stimulation. Calculations of voluntary activation from MVTs with superimposed stimulation provided lower values with submaximal muscle than with maximal stimulation. Submaximal muscle stimulation with superimposed doublets can be used to estimate VA in knee extensors. It is not painful, and overestimation of maximal VA may be less compared with maximal nerve stimulation, particularly for subjects with lower levels of voluntary muscle activation, such as patients with neuromuscular disorders. Submaximal muscle stimulation seems to be a good alternative for maximal nerve stimulation.

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