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Research article

Effect of the Anode Placement on the Antagonist Muscles Recruitment: Implication for the Interpolated Twitch Technique Outcome

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Abstract

The aim of the present study was to compare the recruitment of the antagonist muscles and its effect on the measurement of the voluntary activation level (VA) of the knee extensor (KE) muscles for different anode placements used to stimulate the femoral nerve. We hypothesized that when the anode is positioned over the gluteal fold (GF), the antagonist muscles recruitment would be greater and, thus the VA overestimated, than when the anode is placed midway between the greater trochanter and the lower border of the iliac crest ($Mid_{troc\text{-}iliac}$). Thirteen healthy men (23 ± 4 yr) were tested in both conditions (GF vs. $Mid_{troc\text{-}iliac}$) in a randomized order. Recruitment curves were performed to determine the optimal stimulus intensity (I_{opt}) and quantify antagonist muscles recruitment (i.e. biceps femoris M-wave). Participants performed maximal voluntary isometric contractions (MVIC) and the interpolated twitch technique was used to measured VA. Antagonist muscles recruitment was greater when the anode was placed over the GF than $Mid_{troc\text{-}iliac}$. The I_{opt} was also lower for GF than $Mid_{troc\text{-}iliac}$ placement. However, no significant effect of anode placement was found for the interpolated twitch technique outcome. When the anode was placed over the GF, antagonist muscles recruitment was greater, inducing optimal stimulus intensity underestimation. However, it did not affect VA assessment. To fully avoid this potential limitation, $Mid_{troc\text{-}iliac}$ anode placement should nevertheless be preferred for the KE neuromuscular function assessment, owing to the reduced recruitment of the antagonist muscles.

Key words: Voluntary activation level, electrical nerve stimulation, recruitment curve, knee extensors, knee flexors, electromyography.

Introduction

Electrical nerve stimulation is commonly used to evaluate neuromuscular function in the context of training, fatigue, disuse/pathology or rehabilitation (Millet et al., 2011). This technique produces highly reproducible data and allows a distinction between the peripheral (muscular), and the central (nervous) aspects of force production. The central component is generally evaluated with the interpolated twitch technique, consisting of stimulation of the nerve during a voluntary contraction (Merton, 1954). This technique can offer a valuable approach to evaluate the voluntary activation level providing that methodological issues have been addressed (Taylor, 2009). Indeed, the detection of small activation deficits requires high resolution measurement of force and careful consideration of numerous experimental details such as the site of stimulation, stimulation intensity and the crosstalk contamination of twitches from the stimulation of the antagonist muscles (Gandevia, 2001; Shield and Zhou, 2004). Contamination may result from the placement of the stimulating electrodes close to the antagonist muscles and/or from the use of high intensities of stimulation (Awiszus et al., 1997).

Using cross talk-contaminated twitches severely affects twitch-interpolation results, as these twitches exhibit a nonlinear relationship between twitch amplitude and voluntary torque. Such a nonlinear relationship in turn, complicates estimations of the amount of voluntary activation. Indeed, while the small twitch torques evoked from the antagonist muscles may only slightly reduce the amplitude of control twitches, it may completely mask the small force increments evoked from the agonist muscles during maximal voluntary efforts (Awiszus et al., 1997). From these data, one would then falsely conclude that agonist muscles are fully activated. Furthermore, during the determination of the optimal stimulation intensity, the recruitment of antagonist muscles may contribute to the early plateauing of the evoked torque. This recruitment response suggests that agonist muscles are fully activated, whereas the intensity needed to fully activate the agonist muscles is actually underestimated. Such underestimation of the optimal stimulation intensity may lead to an overestimation of the level of activation, as the stimulation intensity may not be able to evoke maximal superimposed twitches.

To overcome the simultaneous activation of agonist and antagonist muscles during the evaluation of the elbow flexors activation level, Awiszus et al. (1997) suggested the use of a sub- rather than supra-maximal stimulation intensity. However, Burke and Gandevia (1998) argued that using a submaximal stimulation was not a suitable alternative, as it would not allow overcoming the activity-dependent hyperpolarization of motor axons (Burke, 2002). Indeed, the error produced by changes in axonal excitability would result in fewer recruited motor axons, and thereby a smaller evoked twitch, which would be difficult to recognize and measure. Rather, Burke and Gandevia (1998) suggested that the solution to limit the stimulation of the antagonist muscles might be to direct attention toward more selective nerve stimulation techniques. This may be achieved by manipulating the spatial disposition of the

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stimulating electrodes, especially on large muscle groups such as the knee extensor (KE) muscles.

In the literature, two electrode placements have been used for the stimulation of the KE muscles. The anode may be positioned either over the gluteal fold (GF) (Martin et al. 2004) or midway between the greater trochanter and the lower border of the iliac crest ($Mid_{troc\text{-}iliac}$) (Desbrosses et al., 2006). In both cases, the cathode is placed in the femoral triangle, over the femoral nerve. The GF placement could favor the recruitment of the knee flexors (KF), since the anode is situated close to the sciatic nerve. In contrast, the $Mid_{troc\text{-}iliac}$ placement would limit the simultaneous activation of agonist and antagonist muscles, and would then provide a better estimate of the muscle activation level. To date, anode placement comparisons have not been assessed experimentally. Therefore, the purpose of this experiment was to test the effect of the spatial disposition of the anode on the recruitment of the antagonist muscles, and thereby on the outcome of the twitch-interpolation technique on the KE muscles. We hypothesized that when the anode is positioned over the gluteal fold (GF), the antagonist muscles recruitment would be greater and, thus the VA overestimated, than when the anode is placed midway between the greater trochanter and the lower border of the iliac crest ($Mid_{troc\text{-}iliac}$).

Methods

Participants

Thirteen healthy men (age: 23 ± 4 years, height 1.78 ± 0.07 m, body mass 73.1 ± 12.6 kg) volunteered to participate. Participants were either sedentary or active in recreational sports but none had engaged in a specific training. No participant had any orthopedic or neuromuscular disorders. The local ethic committee approved the study (AU 1163) and all procedures were conducted according to the Declaration of Helsinki. Before the experimental session, all participants provided written informed consent.

Experimental design

Each participant was tested for the KE muscles during a single session, after a preliminary familiarization session, separated at least by 48h. During the familiarization session, participants' data on physical characteristics (height and body mass) were collected. The participants were familiarized with the experimental procedures: they were familiarized with electrical stimulation on the resting muscle and then were trained to perform reproducible maximal voluntary isometric contractions (MVIC) with and without superimposed stimulations.

During the testing session, the recruitment curves were randomly and successively acquired for the anode GF and $Mid_{troc\text{-}iliac}$ placements, by progressively increasing the electrical nerve stimulation intensity (minimal intensity: 10 mA; intensity increment: 5 mA, number of trials per intensity: 2; rest: 10 s). The optimal intensity (I_{opt}) was determined from recruitment curves [the intensity where unpotentiated single twitch (Qtw_{unpot}) and concomitant compound muscle action potential (M-wave) amplitudes reached their maximal values and started to plateau]. A supramaximal intensity (I_{sup}), set at 150 % of I_{opt}, was then used during subsequent experimental procedures.

Then, participants performed two sets of two 3-s MVIC of the KE muscles, separated by 2 min of rest. For each set, randomly, one MVIC was tested with the GF and the other with $Mid_{troc\text{-}iliac}$ anode placement. To maximize MVIC, strong verbal encouragement and visual feedback about force development were given to the participants during each MVIC and the best trial within each set was used for further analysis.

Instrumentation

Participants were seated comfortably in a dynamometer equipped with strain gauges (Good Strength, Metitur, Finland) with the trunk-thigh angle set at 90° and the knee flexed at 120° (180° = full extension). Each participant was strapped to the chair via two safety belts across the thorax, one across the hip and the lever arm was attached 2 cm above the lateral malleolus with Velcro straps.

After femoral nerve detection with a ball probe cathode (Medfit, Finland) pressed into the femoral triangle, electrical stimulation was applied percutaneously to the motor nerve via a self-adhesive electrode pressed manually (10-mm diameter, Ag-AgCl, Skintact FS 50, Austria). The anode, a 9 × 5 cm self-adhesive stimulation electrode (Saint Cloud, France), was placed either over the gluteal fold (GF) or midway between the greater trochanter and the lower border of the iliac crest ($Mid_{troc\text{-}iliac}$). A constant current stimulator (DS7A, Digitimer, United Kingdom) was used to deliver a square-wave stimulus of 1000 µs duration with maximal voltage of 400 V.

The detection of the electromyographic signal (EMG) was performed through pairs of silver chloride surface electrodes (N-00-S, Blue Sensor, Denmark) during the MVIC and electrical nerve stimulation. The recording electrodes were taped lengthwise on the skin over the bellies of the vastus lateralis (VL), vastus medialis (VM), rectus femoris (RF) and biceps femoris (BF) muscles, according to SENIAM (Surface ElectroMyoGraphy for the Non-Invasive Assessment of Muscles) recommendations (Hermens et al. 2000), with an interelectrode distance of 20 mm. The reference electrode was attached to the patella. Low impedance ($Z < 5$ kΩ) at the skin-electrode surface was obtained by abrading the skin with thin sand paper and cleaning with alcohol. Myoelectrical signals were amplified with a bandwidth frequency ranging from 10 Hz to 1000 Hz (common mode rejection ratio = 100 dB; impedance = 200 MΩ; gain = 1000; Octal BioAmp, AD Instruments, Australia).

Mechanical and electrical signals were recorded with an A/D board (Power Lab 8/30, AD Instruments, Australia), at a sampling frequency of 2 kHz and analyzed with Labchart 7.2 software (AD Instruments, Australia).

Data analysis

The Qtw_{unpot} and the peak-to-peak M-waves amplitudes of the VL, VM, RF and BF muscles, evoked during the recruitment curves, were measured.

Twitch interpolation technique was used as reliable method (Behm et al., 1996) to determine voluntary activation level (VA). Superimposed single twitch (Qtw_s) was evoked at I_{sup} during MVIC and after the force had reached a plateau. A second stimulation was delivered 3s after the end of the MVIC to evoke a potentiated single twitch (Qtw_{pot}). Then, VA was estimated as proposed by Merton (1954):

$$VA = \left[1 - \left(Qtw_s / Qtw_{pot}\right)\right] \times 100$$

Peak-to-peak M-waves amplitudes of the VL, VM, RF and BF muscles, evoked at I_{sup} were also measured during and after the MVICs.

Statistical analysis

Qtw_{unpot} and M-waves amplitudes were linearly interpolated between the nearest values at 30, 40, 50, 60, 70, 80, 90, 100, 110, 120 and 130% of the I_{opt} to compare the effect of the anode placements (GF vs. $Mid_{troc\text{-}iliac}$) through the recruitment curves.

The normality of data distribution was checked with the Shapiro-Wilk normality test and homogeneity of variances was checked with the Bartlett test. The Qtw_{unpot} and M-waves amplitudes obtained during the recruitment curves were compared between anode placement using a two-way analysis of variance (ANOVA) (anode placement × stimulation intensity). When an ANOVA revealed significant effects or interactions between factors, a Tukey's honestly significant difference post hoc test was applied to test the differences between means. The effect size and statistical power were also reported when significant main or interaction effects were detected. The effect size was assessed using the partial eta-squared (η^2) and ranked as follows: ~0.01 = small effect, ~0.06 = moderate effect, and ≥ 0.14 = large effect (Cohen, 1969). A Student bilateral t-test for paired samples was conducted to identify the effect of the anode placement (GF vs. $Mid_{troc\text{-}iliac}$) on the I_{opt}, I_{sup} and the parameters obtained during MVIC. The effect size was assessed using the Cohen's d and ranked as follows: 0.20 = small effect, 0.50 = moderate effect, and ≥ 0.80 = large effect (Cohen, 1969). For all statistical analyses, a p-value of 0.05 was accepted as the level of significance. The statistical analyses were performed with Statistica 9.0 software (Statsoft, USA). All descriptive statistics presented in the text and figures are mean values ± standard deviation.

Results

Recruitment curves

Student bilateral t-tests revealed that I_{opt} and I_{sup} were significantly lower for GF than $Mid_{troc\text{-}iliac}$ placement (I_{opt}: 40.8 ± 11.5 and 49.6 ± 10.1 mA, respectively, t-value(12) = 2.46, $p < 0.05$, Cohen's d: 0,85; I_{sup}: 62.3 ± 12.2 and 70.0 ± 11.7 mA, respectively, t-value(12) = 2.45, $p < 0.05$, Cohen's d: 0,67).

ANOVA results showed an interaction (anode placement × stimulation intensity) for Qtw_{unpot} values [$F_{(10,120)} = 5.79$, $p < 0.001$, $\eta^2 = 0.33$, power = 0.99]. Qtw_{unpot} values were significantly greater for anode placed $Mid_{troc\text{-}iliac}$ than over the GF at 30 and 40% of the I_{opt} (Figure 1A). No difference was found for Qtw_{unpot} at I_{opt} between GF and $Mid_{troc\text{-}iliac}$ placements. A significant stimulation intensity effect was found for VL, VM and RF M-waves amplitudes [$F_{(10,120)} = 7.32$, $p < 0.001$, $\eta^2 = 0.38$, power = 0.99; $F_{(10,120)} = 13.80$, $p < 0.001$, $\eta^2 = 0.53$, power = 1.0; $F_{(10,120)} = 8.85$, $p < 0.001$, $\eta^2 = 0.42$, power = 1.0, respectively]. No significant difference was reported for VL, VM and RF M-waves amplitudes between GF and $Mid_{troc\text{-}iliac}$ placements (Figure 1B, C and D). In contrast, ANOVA results revealed significant anode placement × stimulation intensity effect for BF M-wave amplitude [$F_{(10,120)} = 23.17$, $p < 0.001$, $\eta^2 = 0.66$, power = 1.0]. Significantly greater BF M-waves were recorded for GF than $Mid_{troc\text{-}iliac}$ anode placement between 60 and 130% of the I_{opt} ($p < 0.001$; Figure 1E).

Maximal voluntary isometric contractions

All parameters obtained during and after MVIC are reported in the Table 1. Difference between the two MVICs was 3.7 ± 3.1% and was not statistically significant. Qtw_{pot}, Qtw_s and VA did not differ between $Mid_{troc\text{-}iliac}$ and GF placements. No significant differences were reported for VL, VM and RF M-wave amplitudes, during and after MVIC, between anode placements. However, BF M-wave amplitudes were greater for GF than $Mid_{troc\text{-}iliac}$ anode placement during superimposed and rest stimulations (at least $p < 0.01$). No significant difference of BF M-wave amplitudes was found between superimposed and rest stimulations, regardless of anode placement (Table 1).

Discussion

The aim of the present study was to compare the recruitment of the antagonist muscles and its effect on the VA when the anode was positioned over the GF or $Mid_{troc\text{-}iliac}$. We hypothesized that for anode placed over the GF, the antagonist muscles would be more recruited, and thereby the VA would be overestimated. The results of the present study partially confirmed this assumption, since the antagonist muscle's recruitment was affected by the anode placement, but not VA.

The anode placement did not affect the recruitment of the agonist muscles, as observed by the lack of difference in VL, VM and RF M-waves amplitudes, obtained during the recruitment curves and during/after MVIC for anode placed over the GF and $Mid_{troc\text{-}iliac}$. However, the recruitment of the antagonist muscles was significantly affected by the anode placement. BF M-wave was greater for GF than $Mid_{troc\text{-}iliac}$ for submaximal, optimal and supramaximal stimulation intensities (60-130% of the I_{opt}, Figure 1E). Greater recruitment of the antagonist muscles for GF than $Mid_{troc\text{-}iliac}$ anode placement was also observed during and after MVIC (stimulus intensity: 150% of the I_{opt}). Nevertheless, it is possible to argue that this increased BF M-wave amplitude may result from cross-talk contamination of the EMG signal from agonist muscles (Koh and Grabiner, 1992, Avrillon et al., 2018). If so, it should also have been observed for $Mid_{troc\text{-}iliac}$ placement. However, this was not the case in the present study. It is then reasonable to consider that the M-wave recorded on the BF muscle reflects recruitment of the antagonist muscles by electrical nerve stimulation when the anode was placed over the GF. These results could be explained by the fact that the GF placement could favor the recruitment of the KF, since the anode is situated close to the sciatic nerve. This pattern of recruitmentb of bantagonist muscles may translate into the

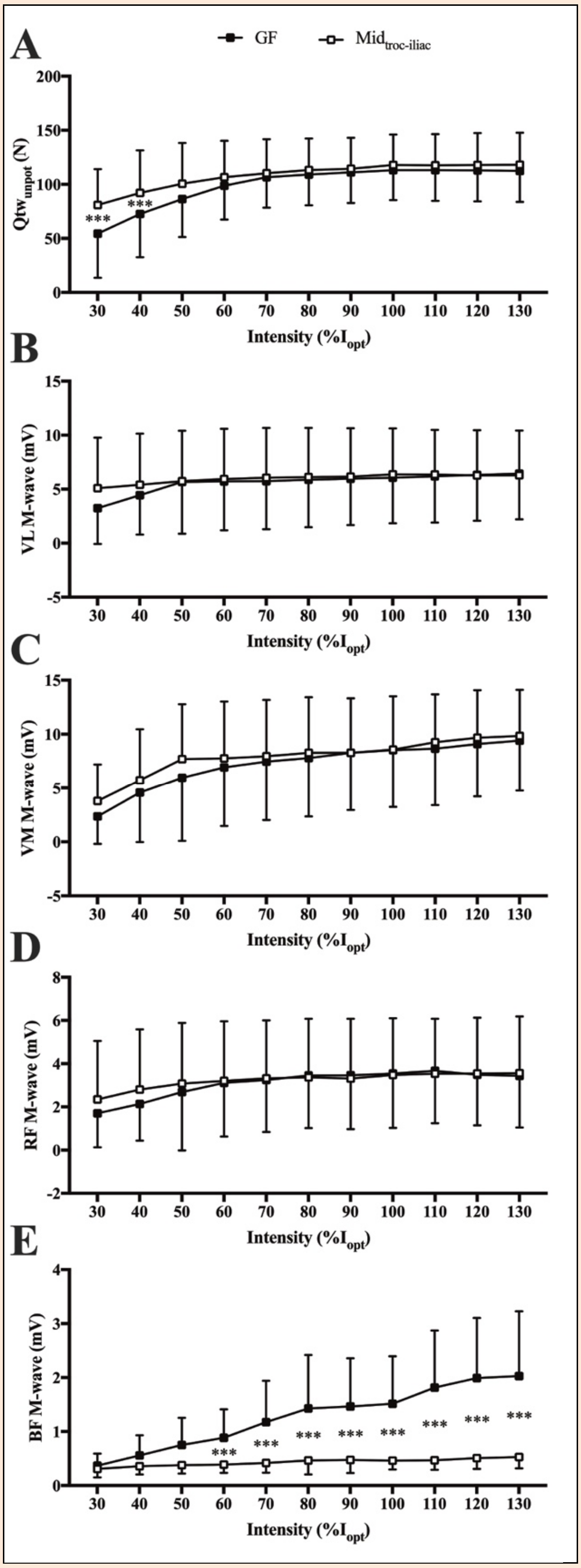

Figure 1. (A) Unpotentiated single twitch (Qtw_{unpot}); (B) vastus lateralis (VL), (C) vastus medialis (VM), (D) rectus femoris (RF), (E) biceps femoris (BF) M-waves amplitudes recruitment curves, obtained with the anode placed over the gluteal fold (GF) and midway between the greater trochanter and the lower border of the iliac crest ($Mid_{troc\text{-}iliac}$). ***: Significant difference at $p < 0.001$.

early plateauing of the evoked force, as observed in the present study. Indeed, I_{opt} was lower for GF than $Mid_{troc\text{-}iliac}$ anode placement, suggesting that agonist muscles were fully activated earlier for GF placement, despite the fact that VL, VM and RF M-wave amplitudes did not differ between anode placements. It is therefore reasonable to suggest that the intensity needed to evoke a maximal twitch force was actually underestimated, since antagonist muscles recruitment may blunt the twitch increment (Awiszus et al., 1997). Furthermore, the lower Qtw_{unpot} for anode GF placement at submaximal intensities (Figure 1A) suggested that agonist muscles M-wave threshold was higher for GF than $Mid_{troc\text{-}iliac}$ anode placement. This higher threshold should translate into late plateauing of the Qtw_{unpot}. However, the opposite was observed in the present study (lower I_{opt} for anode placed over the GF than $Mid_{troc\text{-}iliac}$), suggesting that the greater recruitment of the antagonist muscles impaired the net force production during the recruitment curve.

Alternatively, one may argue that (i) KF are weaker than KE (Szpala et al., 2015) and (ii) the antagonist recruitment was too weak, as evidenced by the small BF M-wave amplitudes (Fig. 1E), to influence the force output during the recruitment curve. The fact that anode placement did not affect Qtw_s, Qtw_{pot} and VA estimation could support this possibility. Additionally, the use of supramaximal stimulus intensity (150% of the I_{opt}) during the interpolated twitch technique may ensure that agonist muscles are fully stimulated by electrical nerve stimulation (Millet et al., 2011). Then, the underestimation of the I_{opt} for GF anode placement could be overcome by the use of supramaximal stimulus intensity when assessing Qtw_s, Qtw_{pot} and VA.

With the current experimental approach, it is thus difficult to draw conclusions on the actual impact of KF recruitment on the reliability of KE VA and contractile properties at rest. However, it could be hypothesized that when KE force output is reduced but not KF, such as after a fatiguing protocol of the KE muscles, the effect of recruitment of the antagonist muscles on twitch amplitudes and VA could be more pronounced. Further studies are needed to confirm this assumption. In the meantime, to avoid the potential confounding effect of antagonist muscles recruitment, $Mid_{troc\text{-}iliac}$ anode placement should be preferred to evaluate neuromuscular function of the KE muscles, owing to the reduced recruitment of the antagonist muscles.

The interpolated twitch technique is a valid and reliable method to estimate the voluntary activation (Behm et al., 1996), however this technique has some limitations. Methodological aspects should be considered, such as timing of the superimposed stimulus, potentiation and type of the superimposed stimulus (single, doublet or multiple pulses) (Folland and Williams, 2007). While no difference in sensitivity of the interpolated twitch technique has been reported between single and doublet pulses (Behm et al., 1996), the signal to noise ratio in the present study could have been increased by using doublet stimulation (Place et al., 2007). In addition, in the present study the VA could be biased by the low sensitivity of the interpolated twitch technique at near maximal contractions intensities (Herbert and Gandevia, 1999) and by the participants' anticipation of the electrical stimulation that could result in lower MVIC level (Button and Behm, 2008). Nevertheless, it is unlikely that these limitations inherent in the interpolated twitch technique would have biased the comparison between the two anode placements or question the present conclusions since measurements were performed in the same conditions.

Table 1. Parameters obtained during and after maximal voluntary isometric contraction (MVIC) when the anode was positioned over the gluteal fold (GF) or midway between the greater trochanter and the lower border of the iliac crest (Mid$_{troc-iliac}$).

		GF	Mid$_{troc-iliac}$	Cohens' d
MVIC force (N)		794 ± 128	780 ± 131	0.11
Qtw$_{pot}$ (N)		183 ± 23	189 ± 22	0.28
Qtw$_{s}$ (N)		16.8 ± 13.6	14.6 ± 13.0	0.17
VA (%)		90.8 ± 7.0	92.3 ± 6.6	0.24
VL M-wave (mV)	**During MVIC**	6.60 ± 5.07	6.60 ± 4.26	0.00
	After MVIC	6.23 ± 4.02	6.19 ± 4.06	0.01
VM M-wave (mV)	**During MVIC**	9.81 ± 3.98	9.22 ± 3.23	0.17
	After MVIC	8.78 ± 5.29	9.11 ± 5.69	0.06
RF M-wave (mV)	**During MVIC**	4.36 ± 1.90	4.04 ± 1.79	0.18
	After MVIC	3.20 ± 2.12	3.24 ± 2.34	0.02
BF M-wave (mV)	**During MVIC**	1.64 ± 1.32	0.43 ± 0.20**	1.33
	After MVIC	1.34 ± 0.63	0.47 ± 0.25***	1.91

Qtw$_{pot}$: Potentiated single twitch; Qtw$_{s}$: Superimposed single twitch; VA: Voluntary activation level; VL: Vastus lateralis; VM: Vastus medialis; RF: Rectus femoris; BF: Biceps femoris; ** and ***: Significant different from values for anode placed over the GF at $p < 0.01$ and $p < 0.001$, respectively.

Conclusion

Anode placement had an effect on the recruitment of antagonist muscles but not on VA during neuromuscular function assessment of the KE by electrical nerve stimulation. When the anode was placed over the GF, antagonist muscles recruitment was significant, inducing optimal intensity underestimation, but this placement did not affect the twitch interpolation outcome. However, to avoid any confounding influence of the recruitment of the antagonist muscles on the KE neuromuscular function assessment, Mid$_{troc-iliac}$ should be preferred to GF anode placement.

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Key points

- Stimulation of the femoral nerve is commonly used to assess the neuromuscular function of the knee extensor muscles.
- Anode placement over the gluteal fold favors the recruitment of the antagonist muscles (knee flexors) as compared to a placement midway between the iliac crest and the greater trochanter.
- Anode placement over the gluteal fold induces an underestimation of the optimal stimulation intensity but as no effect on the determination of the maximal voluntary activation level.

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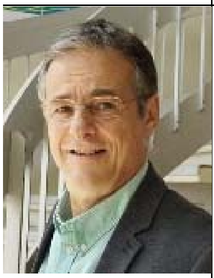

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