Bampouras, Theodoros, Reeves, Neil D., Baltzopoulos, Vasilios and Maganaris, Constantinos N. (2006) Muscle activation assessment: effects of method, stimulus number, and joint angle. Muscle & Nerve, 34 (6). pp. 740-746.

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Title:

MUSCLE ACTIVATION ASSESSMENT: EFFECTS OF METHOD, STIMULI NUMBER AND JOINT ANGLE

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Running title:

Muscle activation assessment

Abbreviations

ANOVA, analysis of variance; CAR, central activation ratio; ITT, Interpolated twitch technique; MVC, maximal effort voluntary contraction; SEC, series elastic component

MUSCLE ACTIVATION ASSESSMENT: EFFECTS OF METHOD, STIMULI NUMBER AND JOINT ANGLE

Abstract

The aim of this study was to compare and assess the sensitivity of the interpolated twitch technique (ITT) and central activation ratio (CAR) to potential errors introduced by 1) evoking inadequate force, by manipulating the number of stimuli and 2) neglecting differences in series elasticity between conditions, by manipulating joint angle. Ten subjects performed knee extension contractions at 30 and 90 deg knee joint angles during which the ITT [(1-superimposed stimulus torque/resting stimulus torque) x 100] and CAR [voluntary torque/voluntary torque+superimposed stimulus torque] methods were applied using 1, 2, 4 and 8 electrical stimuli. Joint angle influenced the ITT outcome with higher values taken at 90 deg ($P<0.05$), while stimuli number influenced the CAR outcome with a higher number of stimuli yielding lower values ($P<0.05$). For any given joint angle and stimuli number, the CAR method produced higher activation values than the ITT method by 8-16%. Therefore, it is suggested that in the quantification of voluntary drive with the ITT and CAR methods consideration be given not only to the number of stimuli applied but also to the effect of series elasticity due to joint angle differences, since these factors may affect differently the outcome of the calculation, depending on the approach followed.

Keywords: maximum voluntary contraction**,** electrical stimulation, interpolated twitch technique, central activation ratio, series elastic component

INTRODUCTION

The measurement of isometric torque produced by maximal effort voluntary contraction (MVC) has been routinely used for assessing muscle function in different populations, such as a) patients with fibromyalgia and anterior knee pain[32,41], elderly[18,36] and athletes[13,25], and after b) acute (e.g., fatigue[16,31]) and chronic interventions (e.g., exercise training [7,26]). One of the main factors that affect MVC torque generation is the degree to which the agonist muscles tested are activated by volition. This functional parameter shows physiological variation in clinical situations such as motor neuron disorders[17,37] and joint pathologies[32,41], but it may also be subject to methodological variation[2,4,34]. To assess activation capacity, two methods have traditionally been employed: The interpolated twitch technique (ITT)[1,3,4,5,8,13,26,34,38,42] and the central activation ratio (CAR)[18,29,36].

The ITT method involves the application to the muscle or parent nerve of an electrical stimulus (or series of stimuli at frequencies allowing a fused contractile response) during an MVC (superimposed stimulation) and the application of an identical electrical stimulus at rest. Activation capacity with this method is calculated as ITT = (1- superimposed stimulus torque / resting stimulus torque) x 100 [equation 1]. The CAR method involves only the application of a superimposed stimulus and the activation capacity is calculated as CAR = MVC torque / MVC torque + superimposed stimulus torque [equation 2].

Despite the fact that both methods are based on the principle that activation is incomplete if the superimposed stimulation causes any further torque increase above the level of MVC, the quantitative agreement of the two methods and the physiological mechanisms underpinning any possible differences have not been fully elucidated. To date, only Behm et al[4] have compared the two methods and found that the CAR method yielded higher activation values than the ITT method when applied at the same joint angle. Surprisingly, however, superimposing two stimuli and a tetanus at 100 Hz produced similar CAR values. Increasing the number of stimuli would be expected to increase the extra contractile torque produced by the superimposed stimulus due to summation of twitch contractile responses, and should thus result in reduced activation capacity values using the CAR method. In contrast, this would not be the case for the ITT method, since this method encompasses also the torque produced by applying the same stimulus at rest. Errors of different magnitude might also be introduced in the two methods when comparative measurements are taken across a range of joint positions. Changes in joint angle would alter the passive stiffness of the series elastic component (SEC) of the muscle. This alteration may result in changes in the effectiveness of the SEC to transmit the force evoked by the application of an electrical stimulus to the muscle, thus potentially affecting the magnitude of the resting twitch and consequently the calculation of activation capacity using the ITT method. In contrast, the lack of resting twitch in the CAR method would render this method insensitive to errors associated with changes in SEC stiffness with joint angle.

To gain insight into the above methodological issues and their impact on the estimation of activation capacity, we have designed a study with the aim of comparing the ITT and CAR methods when manipulating the number of electrical stimuli and joint angle. We studied the quadriceps muscle group and hypothesized that, for a given level of volitional effort during knee extension contraction, a) the CAR method would be more sensitive than the ITT method to differences in the number of applied stimuli, and b) the ITT method would be more sensitive than the CAR method to knee joint angle changes for any given number of stimuli.

MATERIALS AND METHODS

Subjects. Ten healthy, physically active males (age: 29 ± 7 years, height: 178 ± 6 cm, body mass: 78 ± 10 kg; mean ± SD) provided written informed consent to participate in this study, which was approved by the Institutional Ethics Committee. All subjects were tested in the laboratory on a single occasion, but had previously visited the laboratory on at least another one occasion to become familiar with the experimental procedures involved.

Maximal contractile torque. Knee extension MVC torque was measured on the right leg at knee joint angles of 30 and 90 deg (full knee extension = 0 deg) with the hip joint at 85 deg (supine position = 0 deg), using an isokinetic dynamometer (Cybex NORM, Ronkonkoma, NY). The knee joint angles tested were selected in order to represent positions where the passive stretch applied to the SEC varied considerably, i.e., the SEC is stretched at 90 deg and slacker at 30 deg. The centre of rotation of the knee was aligned with the dynamometer axis. Straps were positioned at the hip, shoulders and over the tested thigh to prevent extraneous movement. The subjects were instructed to perform all contractions by increasing their effort gradually in ~2-3 s and maintain the maximum torque produced for an additional ~1 s. A rest period of 2-3 minutes separated the contractions.

Quantification of voluntary activation. The quantification of voluntary activation during the MVCs was based on the application of electrical stimulation. Femoral nerve stimulation proved to cause major discomfort in some subjects, especially when applying trains of stimuli; hence we opted for percutaneous muscle stimulation. Two 7 x 12.5-cm self-adhesive electrodes were placed on the proximal and distal regions of the quadriceps muscle group. The size and location of the stimulating electrodes was determined in preliminary experiments, with the criterion being the generation of the highest possible knee extension torque by applying a twitch of a given intensity. Signals of torque and electrical stimuli application were displayed on the screen of a computer (Macintosh, G4, Apple Computer, Cupertino, CA, USA), interfaced with an acquisition system (Acknowledge, Biopac Systems, Santa Barbara, CA, USA) used for analog-to-digital conversion, at a sampling frequency of 2,000 Hz. Stimuli of 200-μs pulse width and 10-ms inter-stimulus gap were generated by an electrical stimulator (model DS7, Digitimer stimulator, Welwyn, Garden City, UK) modified to deliver a maximum of 1,000 mA output. One (singlet), two (doublet) and four stimuli (quadruplet) were applied in a randomized order in all ten subjects. Six of the ten subjects were capable of tolerating discomfort levels caused by application of eight stimuli (octuplet); hence, these data were also collected and included in the analysis.

The supramaximal stimulation intensity was determined at each joint angle by single twitches applied at rest with increasing current intensity at 300 V. Supramaximality was defined as the level at which a further increase in current of 50 mA did not elicit an increase in twitch torque.

Supramaximal stimulation was applied during the plateau phase of MVC and 3 s after complete relaxation following the MVC (Fig. 1). The latter resting potentiated stimulus was evoked automatically. Two MVCs were performed and the contraction with the highest torque was selected for analysis.

Activation capacity was calculated from equation 1 for the ITT method and equation 2 for the CAR method (see Introduction). The rate of torque development for the resting stimuli was measured to further elucidate the influence of SEC on the ITT method outcome. Rate of torque development for each stimuli number was measured as the gradient of the torque-time curve from rest to peak torque during stimulation.

Statistics. Normality of the data was examined using the Kolmogorov-Smirnov test. In cases where the data were not normally distributed, a transformation was performed using the most appropriate transformation function[44] prior to further analysis and normality was subsequently confirmed. A one-way repeated measures analysis of variance (ANOVA) was used to examine for differences in baseline MVC torque at each joint angle, just before superimposing the singlet, doublet, quadruplet and octuplet. A one-way ANOVA was also used to examine for differences in the torque ratio of superimposed stimulation to resting stimulation between the singlet, doublet, quadruplet and octuplet at each joint angle. A mixed-design 2 x 2 x 4 repeated measures factorial ANOVA was used to examine for differences in activation capacity between methods, knee joint angles and stimuli number. A 2 x 4 repeated measures factorial ANOVA was used to examine for differences in the rate of torque development between stimuli number and joint angle. Simple effects tests were used for post hoc analysis where appropriate. Values are presented as the mean ± SD. Significance was accepted at the level $P < 0.05$.

RESULTS

The torque values produced prior to superimposed stimulation at each joint angle were not different between contractions ($P > 0.05$; Table 1), indicating that the volitional efforts exerted during the

MVCs were similar. The current corresponding to supramaximal stimulation intensity was identical for the two joint angles (731 ± 92 mA).

ITT method. There was no effect of stimuli number on the ITT outcome for either joint angle ($P > 0.05$). In contrast, there was an effect of joint angle ($P < 0.05$). The singlet, doublet and quadruplet at 90 deg yielded higher activation capacity values than the respective stimuli at 30 deg (9-18% difference, $P < 0.05$), while the octuplet yielded no difference ($P > 0.05$) between joint angles. The torque ratio of superimposed stimulation to resting stimulation at each joint angle did not differ between the singlet, doublet, quadruplet and octuplet ($P > 0.05$; Table 1). The rate of torque development for the octuplet did not differ between joint angles ($P > 0.05$; Table 1), while the singlet, doublet and quadruplet yielded higher values ($P < 0.05$; Table 1) at 90 than 30 deg by 131%, 116% and 71%, respectively.

CAR method. The CAR outcome depended on number of stimuli. At the knee joint angle of 30 deg, the singlet yielded higher activation values than the doublet (4% difference, $P < 0.05$), quadruplet (9% difference, $P < 0.05$) and octuplet (12% difference, $P < 0.05$), and the doublet yielded higher activation values than the quadruplet (6% difference, $P < 0.05$) and octuplet (9% difference, $P < 0.05$). At 90 deg the singlet produced higher activation than the quadruplet and octuplet (3% and 4% respectively, $P < 0.05$), but no differences were found in the comparisons involving the doublet and between the quadruplet and octuplet ($P > 0.05$). In contrast to the ITT method, there was no effect of joint angle on the CAR method outcome for any number of stimuli ($P > 0.05$). For any given stimuli number and joint angle, the CAR method produced higher activation capacity values by 8-16% than the ITT method. These differences reached statistical significance ($P < 0.05$) for the singlet, doublet and quadruplet at 30 deg.

Mean activation values for each method, stimuli number and joint angle are presented in Fig. 2.

DISCUSSION

In the present study, we manipulated the number of electrical stimuli and the knee joint angle and we showed that for a given volitional knee extension effort, a) differences in stimuli number have a greater effect on the CAR than ITT method, and b) knee joint angle changes have a greater effect on the ITT than CAR method. These results support our hypotheses.

ITT outcome. The application of a train of maximal intensity stimuli often causes discomfort and limits the applicability of electrical stimulation for the assessment of activation capacity[8,38]. Nonetheless, it has previously been suggested that multiple stimuli rather than single twitches are required to improve the signal-to-noise ratio and increase the sensitivity of the ITT method by reducing the variability of the superimposed force[42] and more effectively overcoming the antidromic effect of stimulation and spinal reflexes[4], especially during nerve stimulation. However, increasing the number of stimuli in the present study did not alter the activation values taken with the ITT method at either knee joint angle. A similar finding has been reported for the level of knee extensor muscle activation obtained by extrapolating the curve describing the relation between the torque ratio of superimposed twitch to resting twitch, and voluntary torque, when using one, two and five stimuli[5]. Similarly, no differences in elbow flexor activation capacity were found by applying the ITT method using one, two and four stimuli, due to a similarity in the magnitude of the superimposed torque evoked by the three stimulations[2]. In the present study, as in other studies[17,30,40], the magnitude of the extra torque generated by superimposing current increased with stimuli number (201% and 98% increase from singlet to octuplet at 30 and 90 deg, respectively) indicating fuller activation due to increases in myofibrillar calcium concentration[9,10]. However, when this extra torque was normalized to the corresponding torque produced by the reference resting stimulus the differences between stimuli number disappeared, thus producing a constant ITT outcome.

Studies on the effect of joint angle on activation capacity assessed using the ITT method are scarce and report inconsistent results, with longer muscle lengths yielding higher[23], lower[43], or similar activation values[31] compared with shorter muscle lengths. Our ITT results suggest higher activations at longer lengths when using one, two and four stimuli, and similar activations at shorter and longer lengths when using eight stimuli. In seeking to address whether the inter-angle variation in the ITT outcome in any given study is a true biological effect, the effect of SEC stiffness differences at different muscle lengths should be considered. Changes in SEC stiffness can affect the magnitude of twitch force [12,24]. One important factor that can affect the SEC stiffness is joint angle. This is supported by recent ultrasound-based findings that passive joint rotation alters not only muscle fascicle length, but also tendon length and therefore its tensile stiffness[11.] At 90 knee angle the quadriceps SEC would be longer and stiffer than at 30 deg, thus being able to more faithfully transmit the resting twitch force to the tibia, as evidenced by the differences in the corresponding rate of torque development for the singlet, doublet and quadruplet. Reducing the sensitivity of the ITT method to changes in resting series elasticity would require application of reference forces that can be transmitted equally faithfully across joint angles. Our ITT and rate of torque development results indicate that this criterion was met by the application of octuplets.

The present ITT activation capacity values at 30 deg knee flexion are lower than the average ITT values reported for quadriceps voluntary activation, which range from ~84 to 95%[4,6,23,31]. However, our ITT values at 90 deg fall within the above range. This difference may be attributed to the fact that the vast majority of published quadriceps ITT values refer to measurements at 90 deg knee joint angle. As explained above, the quadriceps SEC at 90 deg is stiffer than at 30 deg, thus yielding higher resting twitch responses and lower activation estimates.

CAR outcome. In agreement with Behm et al[4], the CAR method yielded higher values than the ITT method. The present difference, however, was found to be joint angle-dependent, reaching the level of 16% at shorter muscle lengths and 8% at longer muscle lengths. Contrary to the similarity in CAR outcome between a doublet and a tetanus reported by Behm et al[4], we obtained lower CAR values at higher stimuli numbers. The increase in superimposed torque with stimuli number indicates the ineffectiveness of low stimuli number to fully activate muscle fibres left inactivated by volition during MVC and explains the corresponding decreasing activation capacity calculated using the CAR method. However, it must be stressed that percutaneous muscle stimulation can activate only those muscle fibres with nerve endings in the vicinity of the electrodes[14]. Direct nerve stimulation is required to evoke the maximum force in all the inactivated muscle fibres during MVC, but this procedure may cause intolerable discomfort raising ethical concerns. In addition, antagonist muscles will co-contract if also innervated by the stimulating nerve, as is the case for the antagonist sartorius muscle during femoral nerve stimulation for quadriceps muscle testing. Nevertheless, our CAR values are likely higher than those that would have been obtained by direct nerve stimulation[17]. In contrast to the CAR method, experimental findings show that the ITT outcome is largely independent of stimulating site (muscle or nerve), indicating a similarity between protocols in the proportion of activated muscle by superimposed stimulation relative to the stimulation at rest[37].

Contrary to the ITT method, there was very small variation in the CAR outcome with joint angle for any given number of stimuli, which substantiates our hypothesis. However, the number of stimuli required to obtain the lowest CAR value was joint angle-dependent: While at 30 deg knee joint angle the lowest CAR output (highest superimposed torque) was taken with the octuplet, at 90 deg the quadruplet and octuplet produced similar CAR values. It is likely that the inter-angle difference in the number of stimuli required to obtain the lowest CAR values may have been caused by an increased sensitivity of the submaximally recruited muscle fibres to changes in myofibrillar calcium concentration at longer lengths (for a review see Stephenson & Wendt[39]).

Conclusions and recommendations. To conclude, the present results show that the ITT method is more sensitive to changes in joint angle and less sensitive to changes in stimuli number than the CAR method. Based on our findings, we recommend that for a valid comparison of ITT results between tests corresponding to different SEC stiffness values, a number of stimuli adequate to similarly stretch and stiffen the resting muscle-tendon unit in all tests be delivered. Apart from tests at different muscle lengths, the above recommendation also applies to tests in different age groups[15,33], and groups with different physical activity histories and lifestyles[21,22,27], tests before and after acute[19,28] and chronic interventions[20,35,36], and generally in all conditions that may alter the mechanical properties of tendon. Measurements of rate of torque development during stimulation may be used as a guide for assessing whether the criterion of similar passive SEC stiffness between conditions is met, especially when muscle fibre composition is similar. Comparisons of CAR results between tests corresponding to different SEC stiffness values are relatively immune to the above problem. However, to obtain a realistic CAR outcome at a given SEC stiffness state (e.g., a given joint angle in a given population at a given point in time) appropriate steps need to be taken to ensure that a substantial part of the inactivated muscle by volition is activated by the superimposed stimulation.

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TABLES

Table 1. Summarized data (mean ± SD) for maximum effort voluntary contraction (MVC), torque ratios of superimposed stimulation to resting stimulation (TR) and rate of torque development (RTD) for both joint angles and all stimuli number.

	Joint angle (deg)	Singlet	Doublet	Quadruplet	Octuplet
MVC (Nm)	30	129.4 ± 41.5	133.2 ± 39.9	135.3 ± 38.2	153.2 ± 49.9
	90	208.6 ± 42.7	293.3 ± 41.5	197.4 ± 37.6	181.0 ± 42.2
TR	30	7.5 ± 5.5	7.5 ± 5.2	4.3 ± 1.7	5.4 ± 3.8
	90	10.9 ± 6.0	8.5 ± 4.1	14.6 ± 16.1	19.8 ± 33.2
RTD (Nm/s)	30	273.4 ± 162.5	437.3 ± 263.6	602.5 ± 318.3	754.8 ± 424.1
	90	578.3 ± 378.5	821.5 ± 569.9	869.3 ± 628.3	805.0 ± 556.1

FIGURES

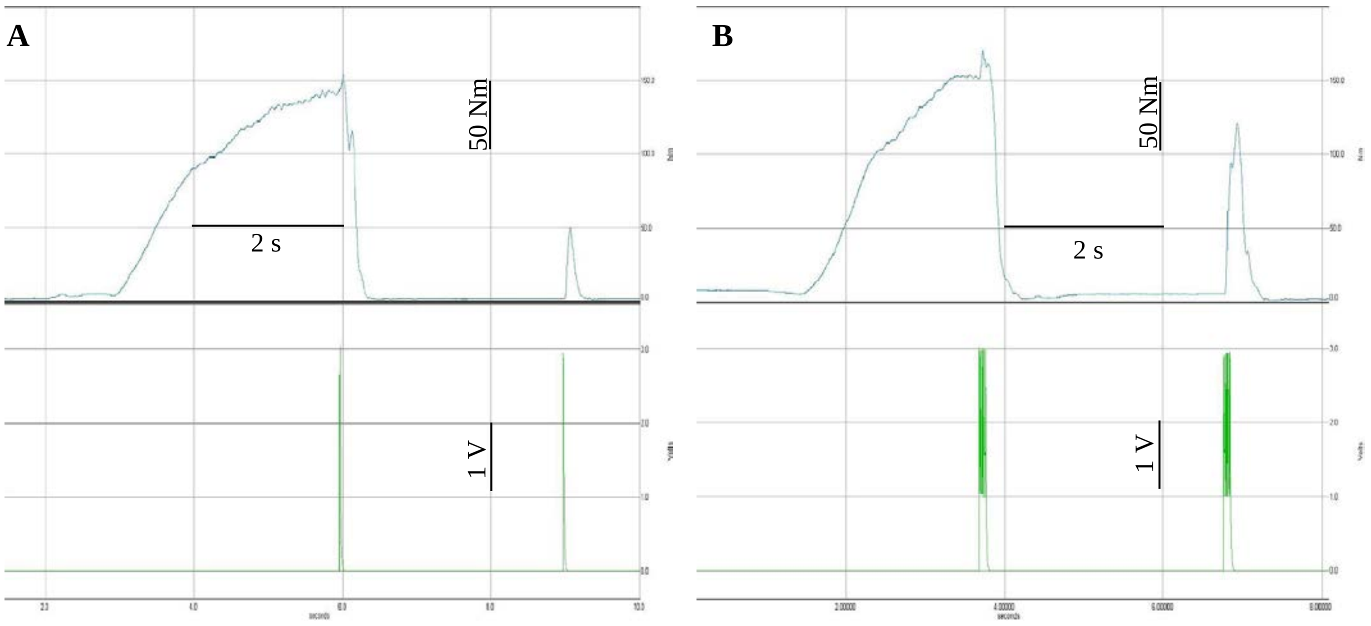

Fig. 1. *Top*: Torque traces for one participant at 90 deg knee joint angle during application of a singlet (A) and an octuplet (B). *Bottom*: Time of stimuli application in the above contractions.

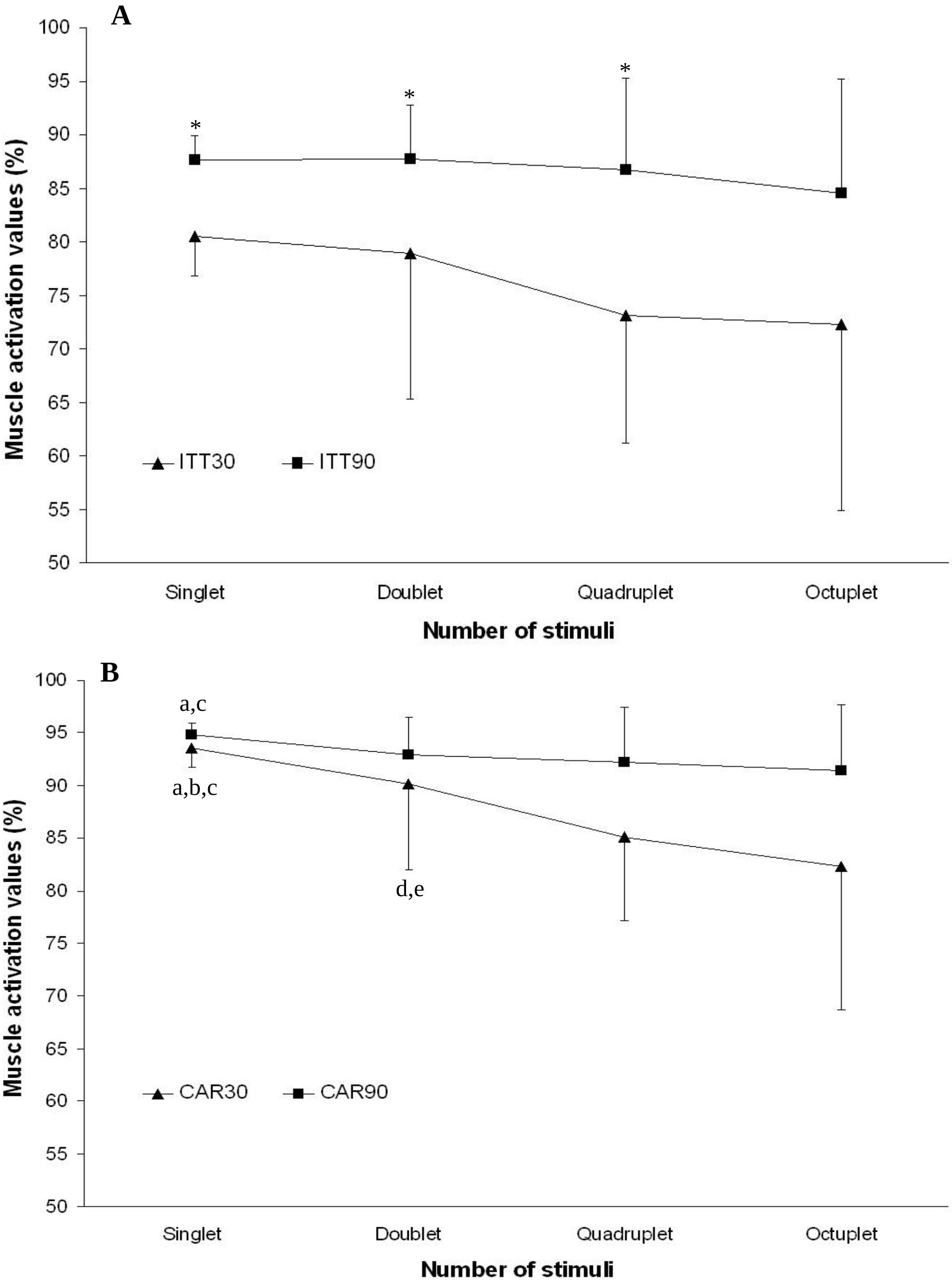

Fig. 2. Muscle activation values for the ITT (A) and CAR (B) methods at 30 and 90 deg knee joint angles using the four different stimuli number in the study. ITT30 and ITT90, activation values at 30 and 90 deg angles, respectively, with the ITT method; CAR30 and CAR90, activation values at 30 and 90 deg angles, respectively, with the CAR method. Significant differences are indicated by *

between joint angles, *a* between singlet and doublet, *b* between singlet and quadruplet, *c* between singlet and quadruplet, *d* between doublet and quadruplet and *e* between doublet and octuplet. Vertical bars denote SD.