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Quadriceps activation during maximal isometric and isokinetic contractions: The minimal real difference and its implications

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Abstract.
BACKGROUND: A method of measurement of voluntary activation (VA, percent of full muscle recruitment) during isometric and isokinetic concentric contractions of the quadriceps femoris (QF) at 60°/s and 120°/s was previously validated.
OBJECTIVE: This study aimed to quantify the test-retest minimal real difference (MRD) of VA during isometric (ISOM) and isokinetic concentric contractions of QF (100°/s, ISOK) in a sample of healthy individuals.
METHODS: VA was measured through the interpolated twitch technique. Pairs of electrical stimuli were delivered to the QF at 40° of knee flexion during maximal voluntary contractions. Twenty-five healthy participants (20–38 years, 12 women, 13 men) completed two testing sessions with a 14-day interval. VA values were linearized through logit transformation (VA_l). The MRD was estimated from intraclass correlation coefficients (model 2.1).
RESULTS: The VA (median, range) was 84.20% (38.2–99.9%) in ISOM and 94.22% (33.8–100%) in ISOK. MRD was 0.78 and 1.12 logit for ISOM and ISOK, respectively. As an example, in terms of percent VA these values correspond to a change from 76% to 95% and from 79% to 98% in ISOM and in ISOK, respectively.
CONCLUSIONS: The provided MRD values allow to detect significant individual changes in VA, as expected after training and rehabilitation programs.

Keywords: Voluntary activation, isokinetic, quadriceps, minimal real difference, rehabilitation

1. Introduction

Voluntary activation (VA) is defined as the degree of recruitment of muscles engaged in a voluntary contraction effort [1]. VA is usually measured as a percentage of the maximum force that could be provided by a muscle if all its fibres contract at tetanic frequency. A 100% VA is rarely achieved for the quadriceps femoris (QF), and for most muscles, even among trained healthy participants [2]. The most commonly used method for VA measurement is the interpolated twitch technique (ITT) which consists of stimulating a representative portion of the muscle belly through an electric shock, causing some initial discomfort but no pain. The stimulus is delivered both at rest and during maximal contraction effort. If the shock does not generate extra force during contraction, all muscle fibres belonging to the sample reached by the electric shock can be claimed to be tetanized [1,3]. Otherwise, the contraction/rest ratio of the peak forces during the "twitches" evoked by the shock provides an estimate of the percentage of mus-

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cle fibres not (or not fully) engaged by the volitional drive: VA is the complement to this ratio. Therefore, the ITT provides an estimation of how voluntary effort translates into muscle force production [3].

A less common procedure, known as the central activation ratio (CAR), implies that the peak moment is evoked with supramaximal stimulation of the femoral nerve, making the resting twitch unnecessary [4–8]. The two methods provide quite superimposable results. In previous research on various muscle groups VA has been measured through ITT during isometric contractions (ISOM) [2,9–11] and during both muscle shortening ("concentric contraction") [9,12] and lengthening ("eccentric contraction") [13–16]. Given that muscle force depends both on muscle length and velocity of shortening (or lengthening), most studies adopted isokinetic dynamometers Here, the activation of the QF during lower limb extension is of concern. The extant literature agrees (despite initial controversies [16–18]) that VA of QF is incomplete during ISOM (around 80–95%) [10,19–21]. In contrast, the debate is ongoing for isokinetic (ISOK) concentric and eccentric contractions. Some authors concluded that maximal ISOK concentric VA did not differ significantly from ISOM VA either at slow (20°/s) or fast (150 and 300°/s) angular velocities [9,10]. Other authors observed that VA during maximal ISOK eccentric and concentric contractions at slow knee angular velocities was significantly lower than VA recorded during maximal ISOM contractions [12]. These divergent results, as discussed in a previous paper [1], may be mainly due to technical difficulties intrinsic to ITT measurements of VA during ISOK contractions. Variation in the instruments adopted and characteristics of the sample may have contributed as well.

In a previous paper [1] a method of measurement of VA during ISOM and ISOK knee extension (velocities of 60 and 120°/s, twitch peak measured at knee joint angle 50°) was validated. In other studies, on isokinetic VA measurement, the electrical stimulus was released at pre-set joint angles. However, a variable delay occurs between the stimulus and the force generation, hence the angle of twitch peak force. In this previous paper [1] the electric shock was delivered at a pre-determined time, allowing the twitch to peak always at the desired knee angle. The applicability of this method to the assessment of changes among individual participants is presented here.

A key property of measurement is its test-retest reliability. This parameter for VA has been evaluated in isometric conditions at 90° knee flexion (0° = full extension) among patients with knee osteoarthritis, and the resulting minimal statistically detectable change (see below) was 6.60% [22]. To the authors' knowledge, in isokinetic conditions test-retest reliability was only assessed at 25°/s at 70° knee angle among healthy participants. The intraclass correlation coefficient (ICC, unspecified model) of VA was 0.87 (95% confidence interval, CI: 0.71–0.95) and 0.86 (CI: 0.63–0.95) in concentric and eccentric contractions, respectively [23]. Conventional reliability tests currently focus on the stability of mean (or median) values across time points or raters (for an overview, see [24]). However, this approach neglects the need for assessing individual changes in clinical and sports training practice. Measures of individual changes are more subject to error than sample means and medians. A valid approach is to estimate the minimal real difference (MRD) [25] (also referred to as the minimal detectable change) [26]. This is the minimal test-retest difference surpassing the amount of change that can be observed by chance (at a given level of significance). If the MRD is reached or trespassed, an association between treatment and outcome can be claimed. The MRD is an intrinsic property of the entire measurement process (i.e., instruments and test procedures). The estimate of MRD requires a dedicated study in which appropriate statistical models and algorithms are applied [1,25]. To the authors' knowledge, only one study tested the MRD of VA of the QF during ISOM [22] and only one study tested the test-retest reliability of the method (based on the stability of mean values) during ISOK concentric and ISOK eccentric at slow angular velocity (25°/s) [23]. No studies of the test-retest MRD, however, have been conducted on ISOK concentric contractions among healthy participants.

Another neglected issue was the nonlinearity of percentage measures, flawed by floor and/or ceiling effects. In the present study, measures of VA and their changes were analysed both as raw percentages (VA) and as values transformed (VA_l) after linearization (logit transformation).

The primary endpoint of the present study was to determine the MRD of VA_l of QF during ISOM and ISOK concentric contractions at 100°/s. Secondary endpoints were to elucidate the dependence of VA on lower limb dominance and sex.

2. Methods

The trial was conducted from September 2019 to April 2020 in a research laboratory on human move-

ment, within a hospital department of neurorehabilitation.

2.1. Participants

Twenty-five healthy participants (18–45 years old, 12 women) took part in the experiments. The inclusion criteria were: i) ability to sign the informed consent form; ii) ability to understand the instructions and to complete the motor task; and iii) to be recreationally active.

Exclusion criteria were: i) pregnancy; ii) a history of epilepsy (to avoid the risk of seizures triggered by the stimuli); iii) implanted electro-sensitive devices; iv) any neurologic or orthopaedic condition limiting the articular mobility or muscular strength of the lower limbs; and v) current treatment with oral anticoagulant or antiplatelet therapy (to avoid the risk of muscle haemorrhage). None of the participants were familiar with the testing method.

2.2. Ethics

All participants gave written informed consent before participating. The study was approved by the ethics committee of the institution (project code 24C721_2017 date of approval 14th November 2017). The study conformed to the Code of Ethics of the World Medical Association (Declaration of Helsinki 2013) for medical research involving human participants.

2.3. Procedures

2.3.1. Test sequencing

Participants took part in the experiment during two sessions, test (T0) and retest (T1), with a 14-day interval. The two sessions took place at about the same time of the day and participants were asked to maintain the same lifestyle habits between the sessions. The 14-day interval was chosen as it is close to the time frame usually adopted for re-testing after rehabilitation or sports training. Both lower limbs were tested. The order in which the lower limbs were tested was randomised (see below).

2.3.2. Trial sequencing

In a separate way for both men and women, for the first enrolled participant the limb to be tested first (dominant vs. non-dominant) was randomly selected. For subsequent participants, the sequence in limb testing was alternated. Then, those participants who had their dominant limb tested as first at T0 started retest with the non-dominant limb, and vice-versa.

2.3.3. Anthropometric measurements

All anthropometric parameters were measured at T0 and at T1 at the beginning of the testing session. Participants were tested for foot dominance using the Waterloo footedness questionnaire-revised [27]. Height and weight were measured.

2.3.4. Instruments

All tests were performed using a Cybex Humac Norm® 2014 isokinetic dynamometer (CSMi-Computer Sports Medicine, Inc.; Stoughton, MA-USA). The participants sat in an upright position with the hip flexed at approximately 90° and grasped ad-hoc seat handles. The lateral epicondyle of the femur was aligned with the main horizontal axis of rotation of the dynamometer, and the lower limb was secured to a Johnson anti-shear device [28].

2.3.5. Range of knee motion and rotation speeds

Isometric tests were conducted with the knee held at 40° flexion (0° = full extension). In ISOK, extension-flexion testing was initiated with the knee at approximately 105° flexion and then reaching full anatomic extension. The isokinetic rotation speed was set to 100°/s. Knee rotations were performed either actively under voluntary contractions or passively through the isokinetic lever (continuous passive motion, CPM).

2.3.6. Electrical stimulation of quadriceps muscle

VA levels were determined according to the ITT method. Electric shocks were delivered through large percutaneous rubber electrodes (120 × 220 mm). The anode and the cathode were positioned medially on the anterior aspect of the upper and lower thigh respectively. Through a constant current high-voltage stimulator (Digitimer® DS7A, Hertfordshire, UK) a doublet of single square-wave stimuli was delivered (interstimulus interval 10 ms) [29–31]. Each stimulus had an amplitude of approximately 300–600 mA and a duration of 50–100 μs. The exact procedure is described elsewhere [1]. The amplitude of the electrical stimuli was tailored for each participant. The same amplitude was used for each limb, during all tests at both T0 and T1. The current amplitude corresponded to the one providing an isometric peak moment at rest of at least 25% of the maximal voluntary contraction (MVC) [32–34] on the first limb tested.

2.3.7. Voluntary efforts and electrical stimulation

Joint moments were displayed online on a PC-screen. The participant was instructed to neglect the screen and to focus on perceiving her/his maximal effort. Verbal

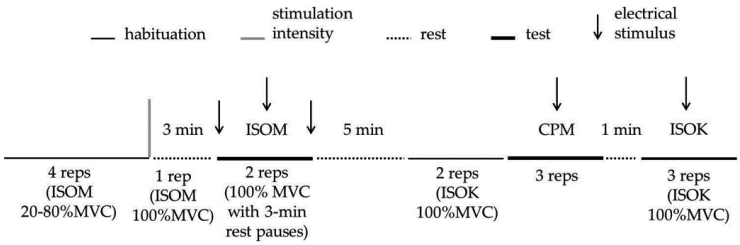

Fig. 1. Schematic representation of the experimental protocol for one limb. The experimental session starts with isometric (ISOM) habituation in which 4 submaximal repetitions were requested. Then, one ISOM repetition at 100%MVC was asked for, in order to calculate the parameters of the electrical doublets to be delivered in the test (represented by the vertical gray bar). After 3-min rest, the participants performed two ISOM repetitions separated by a 3-min rest. In each ISOM repetition, three electrical doublets (represented by arrows) were delivered: the first one was delivered at rest, the second one was delivered 3–4 s after the beginning of the effort initiation, and the third one was delivered at rest 2–3 s after the contraction ended. After the ISOM test, a 5-min rest separated the habituation trials, made by two isokinetic knee extension-flexion contractions at 100°/s (ISOK) at 100% MVC. Then, three repetitions of Continuous Passive Motion (CPM) at 100°/s were made, each with a superimposed doublet. After a 1-min rest, three ISOK at 100% MVC were performed, each with a superimposed doublet.

encouragement was provided to the participants to elicit maximal effort.

To achieve habituation at the beginning of each session participants performed 2–4 submaximal isometric contractions at increasing effort at the pre-determined joint angle (40° knee flexion). Then, the participants were asked to perform a single MVC, which was used to define the amplitude of the electrical stimuli (see above).

After a 3-minute pause, ISOM contractions were requested. During ISOM (knee immobilised at 40° flexion), three electric paired shocks (doublets, interstimulus interval 10 ms) were delivered. The first doublet was delivered at rest before contraction. The second one was delivered 3–4 s after the beginning of the effort initiation when a steady plateau could be appreciated by visual inspection of the joint moment tracings. The third doublet was delivered at rest 2–3 s after the contraction had ended. Two ISOM contractions were performed for each limb, with a 3-minute break.

A 5-minute pause separated the end of ISOM testing from the beginning of isokinetic habituation: two extension-flexion repetitions at maximal effort at the chosen angular velocity (100°/s). Care was taken to ensure that on visual inspection a constant effort was provided within and between repetitions, as revealed by bell-shaped and reproducible moment tracings. Continuous passive motion (CPM) of knee extension-flexion (range 105-0°, angular velocity 100°/s) followed. Three consecutive CPMs were performed for each limb. After a 1-minute pause, ISOK was performed. During both CPM and ISOK a doublet was delivered during each repetition. Through a customised software routine, the instant of stimulation was computed in order to make interpolated twitch (IT) moments to peak at exactly 40° of knee flexion during extension [1]. Three consecutive ISOK procedures were performed for each limb. The same procedure was then administered to the contralateral limb. Figure 1 summarizes the experimental protocol for one lower limb.

2.3.8. Surface electromyography (sEMG) recording

The sEMG was recorded at rest to ascertain the fully relaxed state of the vastus lateralis and the homolateral biceps femoris muscle. Two pairs of silver chloride surface electrodes were applied to each muscle according to the SENIAM guidelines [35]. Low impedance (< 10 kΩ) of the skin-electrode interface was achieved by gently abrading the skin. The centre-to-centre interelectrode distance was 2 cm. The reference electrode was applied over the contralateral patella. EMG signals were recorded simultaneously with the joint moment signal and were used to evaluate the participant's relaxed state. EMG data were sampled at 1000 Hz. The EMG signal was amplified ($\times$ 1000) and filtered (bandpass 50–1000 Hz) using a CED 1902 amplifier (Cambridge Electronic Design, Cambridge, UK).

2.3.9.1. Data acquisition and analysis

A signal acquisition system (CED 1401, Cambridge Electronic Designed Limited-Ced, Cambridge) was used to simultaneously record knee moment, angle, and angular velocity through the isokinetic dynamometer. These variables were sampled at 500 Hz. A customised software (Spike 2, version 8, Cambridge Electronic Design Limited-CED, Cambridge) connected to the acquisition system controlled the electric stimulator in eliciting the stimulus at the desired knee angle. The average moment applied to the transducer when the lower limb was fully extended was used to offset the gravitational moment at all angles by proper computation. Moment signals were off-line filtered (low-pass FIR filter, 1.3 Hz).

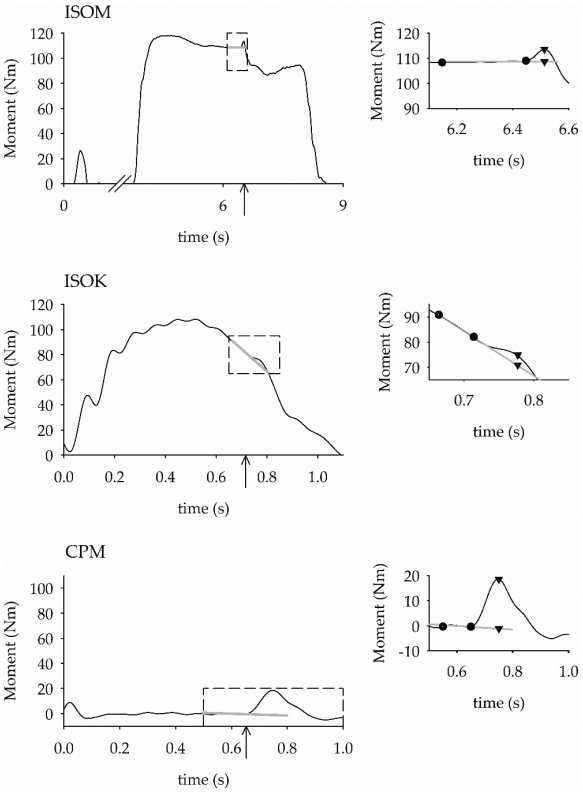

Fig. 2. Tracings from a representative participant (woman, 35 years, 53 kg, 163 m). The gravity-corrected moment (Nm), on the ordinate, is given as a function of time (s), on the abscissa. From top to bottom, in the left column the panels refer to a maximal isometric contraction at 40° knee angle (0° = full extension) (ISOM), a maximal isokinetic contraction at 100°/s (ISOK), and a continuous passive motion (CPM) at 100°/s, respectively. In the top panel the first moment peak (resting twitch) anticipates the beginning of the voluntary effort by a variable interval of 2–3 s (see the break on the abscissa). Arrows mark the delivery of the doublet. The dashed rectangles delimit the events occurring in a short time frame encasing the superimposed twitch. The straight gray segments represent the regression line built on the moment values observed just before the doublet: 300 ms in ISOM, 50 ms in ISOK, and 100 ms in CPM, respectively. In the right column, events in the rectangles are zoomed-in. Circles delimit the regression time windows. The upper and lower black triangles give the peak of the increment following the doublet, and the moment that would have occurred, in the same instant of time, without stimulation, respectively. The difference between the observed peak and the synchronously estimated moment gives the IT value [1].

The VA was determined by the following formula:

$$VA = \left[1 - \frac{IT}{RT}\right] \%$$

where IT and RT are the peak moments caused by the electric doublets during the voluntary contraction and at rest, respectively. During ISOM contractions, pre-contraction RT was adopted in the computation of VA. Indeed, like the twitch during contraction the post-contraction RT may exhibit some potentiation, but it may also be diminished by fatigue [36,37]. The pre-contraction twitch is preferred for consistency with ISOK computation of VA [1]. During ISOK contractions, the time course of the moments was visually analysed off-line to detect any gross abnormalities. In particular, IT had to occur during the descending part of the bell-shaped moment curve (see ref. 1 for details) (Fig. 2).

2.3.9.2. Algebraic considerations

The goal of this study was the computation of the minimal real difference (MRD) of VA, at two time points. The MRD is the minimal change that cannot be attributed to random error, only (usually, at $p < 0.05$). Here, MRD is based on the test-retest reliability estimated through the ICC. Both the ICC and the MRD formulations assume linearity of the measures [25]. This is not the case for VA, which is a proportion bounded between 0 and 1 (0 to 100 percentage scores) and thus flawed by non-linearity. Therefore, VA raw percentages were converted into their logit (log-odd ratio) counterparts to account, here, for the expected ceiling effect [38]. The logit formulation of VA (VA_l) is

$$VA_l = \text{logit} = \log \frac{P}{(1-P)}$$

where P is the observed proportion, i.e., the raw VA percent measure. It is of note that logits are negative for ratios < 1 (hence, for VA $< 50\%$). However, proportions equal to 1 (which are far from exceptional in VA studies) provide infinite logit values, thus preventing a valid estimate of change of VA_l both at an aggregate and individual level. Neglecting VA values equal to 100%, in this study the observed VA_l values ranged between $VA_l = 0.29$ (VA = 34%) and $VA_l = 3.54$ (VA = 99.97%). Whether a "real change" is achieved can be deducted also from percentage measures, if these are logit-transformed and their difference compared with the logit-based MRD provided in this study.

2.3.10. Statistical analyses

For each participant, the best repetition out of two ISOM contractions and out of three ISOK contractions were selected for statistical analysis [39–41]. The normality of data distribution was assessed using the Shapiro-Wilk's test. When applicable, means (SD) were computed and Student's t-test and repeated ANOVA (rANOVA) models were applied. For non-normal data, medians (2^{nd}–3^{rd} interquartile range-IQR) were computed, and the Wilcoxon signed-rank test was adopted.

Table 1
Means, medians, first lower quartiles (Q1) and third upper quartiles (Q3) for age, weight, height, body mass index (BMI) of the dominant and the non-dominant limbs of the twenty-five participants

	Mean	Median	Q1	Q3
Age (years)	27.3	26.0	24.0	30.0
Weight (kg)	70.4	71.0	59.0	78.0
Height (cm)	174.8	176.0	166.0	183.0
BMI (kg m^{-2})	22.9	22.1	21.0	24.0

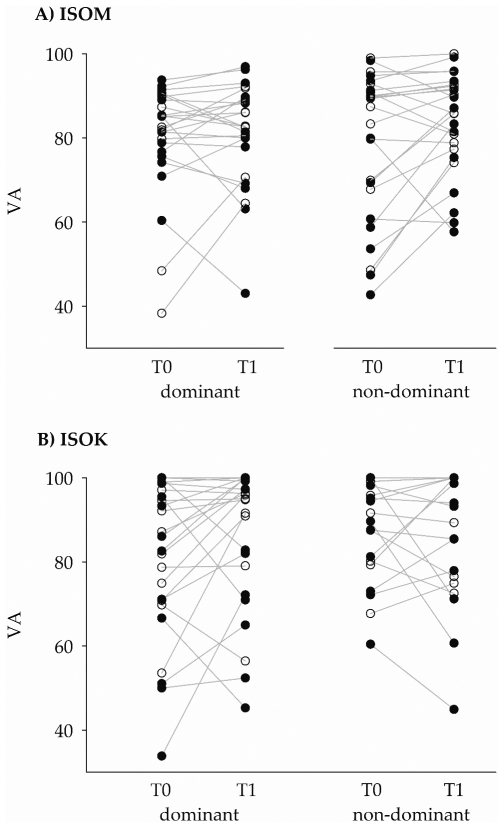

Fig. 3. The ordinate gives the voluntary activation (VA) values of the participants ($n = 25$). Panel A) refers to isometric contractions (ISOM); panel B) refers to isokinetic knee extension contractions at 100°/s (ISOK). Values recorded at baseline (T0) and retest (T1) are given separately for the dominant and the nondominant lower limbs. Each dot represents the best performance of a single participant. Individual values at T0 and T1 are connected by straight segments ("spaghetti" graph). Filled dots refer to men; empty dots refer to women.

As an index of test-retest reliability the ICC was computed on the VA_l transformed values. The $ICC_{2,1}$ model was adopted (all participants with test-retest measures; participants considered as a random sample of a general population; measures refer to one observation, only). The MRDs at a 95% confidence level were then computed (Eq. 10 in ref. 25).

The absolute agreement between VA_l values at T0

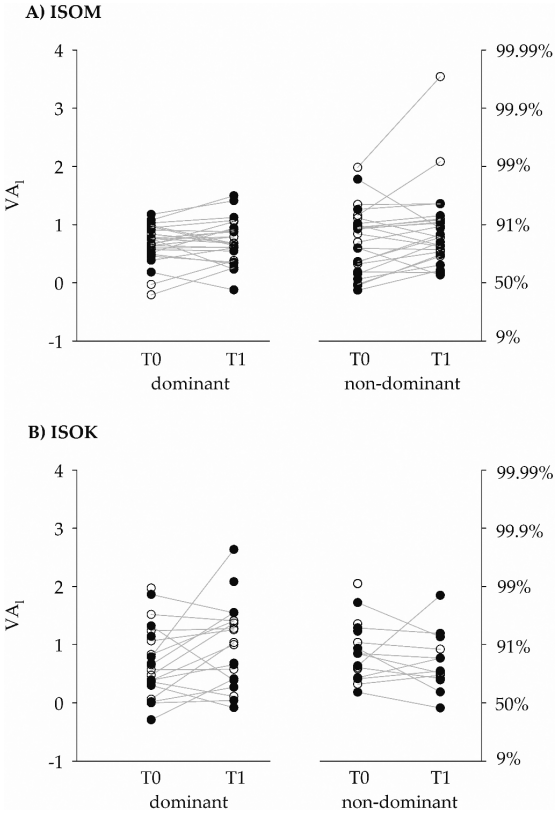

Fig. 4. The left ordinate gives the voluntary activation levels after logit transformation (VA_l). The right ordinate gives the corresponding measures of VA (% values). Only values of VA $< 100\%$ are represented. Other indications as in Fig. 3.

and T1 was assessed through the Bland-Altman plot, computing the difference and the mean between the VA_l values at T0 and T1, its SD, 95% tolerance limits, and 95% confidence limits. At variance with confidence limits, applied to estimated means, tolerance limits can be applied to individual observations [42] and are, therefore, wider [43,44]. The significance level was set at 0.05.

2.3.11. Software

Computations, statistics, and graphic representations were performed using MATLAB™ (MathWorks Inc., version 8, Natick, MA, USA), STATA™ (STATA Corp., version 14.0, College Station, TX, USA), and SigmaPlot™ (Systat software Inc., version 14.0, San José, CA, USA) software.

3. Results

Twenty-five subjects (12 women), median age 27.3 (24.0 ÷ 30.0) years, median weight 71.0 (59.0 ÷ 78.0)

Table 2
Means, medians, 5th and 95th percentiles of the voluntary activation (VA) (%) and the moment exerted at 40° knee flexion (Nm) for the 25 study participants. The values are reported for isometric condition (ISOM) and isokinetic concentric contractions at 100°/s (ISOK) for the dominant and the non-dominant limb at T0 (test) and T1 (retest)

	T0 ($n = 25$)				T1 ($n = 25$)			
	Mean	Median	5th–95th percentile		Mean	Median	5th–95th percentile	
VA (voluntary activation, %)								
ISOM_dominant men	81.52	81.39	60.36	93.75	78.20	80.00	43.03	96.91
ISOM_dominant women	79.97	83.81	38.30	90.66	83.47	84.37	64.42	92.19
ISOM_non-dominant men	74.68	79.92	42.70	98.36	80.24	83.29	57.64	95.84
ISOM_non-dominant women	82.77	88.39	48.55	98.97	87.11	88.06	74.10	99.97
ISOK_dominant men	79.12	86.30	33.83	100.00	82.05	82.79	45.28	100.00
ISOK_dominant women	83.28	84.49	53.55	100.00	91.10	95.44	56.43	100.00
ISOK_non-dominant men	88.59	94.40	60.41	100.00	85.49	93.17	44.94	100.00
ISOK_non-dominant women	91.68	97.44	67.70	100.00	92.76	100.00	72.52	100.00
Moment (Nm)								
ISOM_dominant men	184.85	176.15	135.15	221.08	196.62	206.30	127.00	240.77
ISOM_dominant women	107.78	110.26	69.74	210.54	113.82	108.15	70.98	223.31
ISOM_non-dominant men	168.99	167.33	139.08	213.42	163.69	174.92	103.54	191.85
ISOM_non-dominant women	102.53	105.48	45.86	179.23	109.90	105.19	64.41	181.50
ISOK_dominant men	130.51	141.03	69.32	174.62	121.97	126.30	74.22	164.05
ISOK_dominant women	74.64	72.60	42.48	111.83	80.61	78.30	54.03	130.98
ISOK_non-dominant men	132.42	131.42	88.15	163.42	118.82	122.75	69.19	144.08
ISOK_non-dominant women	81.45	78.48	55.52	140.68	76.91	65.36	47.69	111.31

Table 3
From left to right, the columns report the values of intraclass correlation coefficients ($ICC_{2.1}$), the 95% confidence intervals (C.I.) of the ICC, and the minimal real difference (MRD) of logit-transformed voluntary activation values (VA_l). Observations (obs.) from both sides were merged. No 100% VA values were recorded in ISOM testing (see text). In ISOK testing, test and retest observations of the same side were ignored when a 100% VA was detected. The upper and lower rows refer to isometric contractions (ISOM) and isokinetic concentric contractions at 100°/s (ISOK), respectively

VA_l	$ICC_{2.1}$	95% C.I.	MRD
ISOM (obs.; $n = 50$)	0.63	0.42–0.77	0.78
ISOK (obs.; $n = 30$)	0.63	0.12–0.7	1.12

kg and median height 176.0 (166.0 ÷ 183.0) cm participated in the study (Table 1). In 1 man and 1 woman the left lower limb was dominant.

In agreement with the classic muscle force-velocity relationship [45], higher moment peaks were recorded during ISOM with respect to ISOK, both at T0 and T1. Means, medians, and 5th and 95th percentiles of VA and gravity-adjusted moment at 40° knee flexion are reported in Table 2 for each test at T0 and T1. The distribution of VA values between T0 and T1 in the two different test conditions, ISOM and ISOK is shown in Fig. 3. The individual trend is highlighted by the superimposed "spaghetti" graph.

Tests on raw VA data are given in the Appendix, Table A1.

In the present healthy sample, complete activation was never achieved during ISOM, either on the dominant or on the nondominant limb by any participant. During ISOK at T0 one woman and three men reached VA 100% with the dominant limb, while four women and four men reached VA 100% with the nondominant limb. During ISOK at T1 two women and three men reached VA 100% with the dominant limb, while nine women and four men reached VA 100% with the nondominant limb. Among these participants, one woman and one man, only, performed 100% VA during all isokinetic tests.

The distribution of VA_l values between T0 and T1 in the two different test conditions, excluding observations with VA = 100%, is shown in Fig. 4.

It can be seen that the logit transformation provided a wider spread of measures (hence, a higher discrimination), compared to the original VA observations. The distribution of the data was tested with Shapiro Wilk's test (Appendix: Table A2). The repeated ANOVA model on VA_l revealed significant between-sex differences ($p = 0.001$). For this reason, different aggregate data are provided for men and women. For completeness of information, Table 2 also provides peak moments during the various tests.

In contrast, no differences were recorded depending on side dominance ($p = 0.29$), time points ($p = 0.27$) test conditions ($p = 0.18$) and, of special interest here, on the time point#sex interaction ($p = 0.89$) (Appendix: Table A3). For this reason, the MRD was calculated jointly for both women and men.

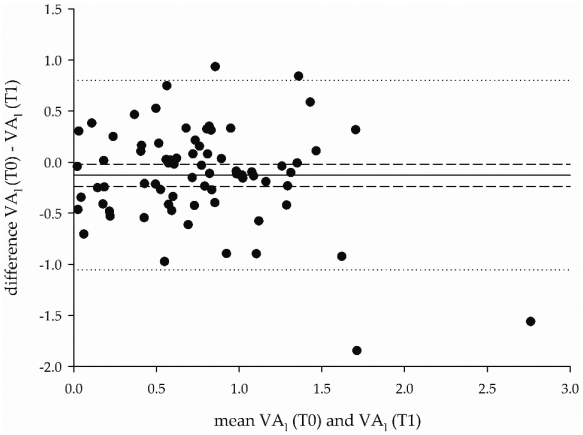

Fig. 5. Bland-Altman plot of the differences between the VA_I values at T0 and T1 (y-axis) versus their mean (x-axis). Solid line reports the mean of the differences; dashed lines mark the 95% confidence limits; dotted lines mark the 95% tolerance limits.

The values of $ICC_{2.1}$ and MRD for VA_I in ISOM and ISOK are reported in Table 3. The MRD for the VA_I was 0.78 and 1.12 in ISOM and ISOK respectively.

Figure 5 shows a Bland-Altman plot on VA_I test-retest differences, as a function of their combined means, thus illustrating the agreement of absolute values and the absence of substantial heteroschedasticity.

4. Discussion

The results indicate that the proposed method of isokinetic measurement of VA on a sample of healthy participants through the ITT method may be reliable and clinically acceptable. To the best of the authors' knowledge, this is the first study proposing aggregate data and test-retest reliability evaluation of quadriceps muscle VA under isokinetic conditions.

4.1. Amount and reliability of activation

These results confirm that VA of the quadriceps muscle is incomplete during maximal ISOM contractions [2,4,19,20,23]. In contrast, some participants in this study displayed complete activation (i.e. VA 100%) during maximal ISOK at 40° knee angle. Moreover the value of VA 100% represents about the 5% of raw data for both men and women in the dominant and non-dominant limbs. To the best of the authors' knowledge, no other studies found complete activation levels during maximal isokinetic concentric contractions, although some studies reported results approaching full activation (e.g. 96.9%) [12].

4.2. Comparison with previous studies; reasons for discrepancies

In this sample VA levels did not differ significantly between ISOK and ISOM, although mean and median values were slightly higher in isokinetic conditions (see Table 2). Previous findings by Babault reported slightly higher VA levels in isokinetic concentric contractions at 120°/s compared to isometric contractions [88.2 (6.6)% vs. 87.9 (5.1)%, respectively] while VA levels were significantly lower in isokinetic concentric contraction at 60°/s [80.9 (8.8)%] compared to both isometric and isokinetic concentric contractions at 120°/s [46]. In the same study, stimuli (not exactly peak moments) occurred at 55° knee angle in both isometric and isokinetic conditions. In agreement with these results, another study from the same author reported lower VA levels for slow isokinetic concentric contractions (20°/s) when compared to isometric contractions [89.7 (1.4)% vs 95.2 (1.2)%, respectively] [12]. In this last article, stimuli were triggered at 50° knee angle, and the sample was entirely composed of physical education students. On the contrary, Newham reported results of VA values for quadriceps that were similar between isometric and isokinetic contractions both at high (150°/s) and low (20°/s) angular velocities [10]. In this case, the onset of electric stimulation occurred at 75° of knee angle.

A possible explanation for these discrepancies lies in the higher VA levels reported for QF at joint angles closer to 90° knee flexion [47,48]. As already stated by Pietrosimone et al. for VA assessment (based on the CAR method) during isometric contractions [48], normative VA values may depend on the joint angle selected. At lower flexion angles, where VA levels are also lower, the knee displays a higher articular instability [49,50]. Therefore, the lower VA levels can be the result of an inhibitory mechanism designed to guaranty stability to the knee joint, particularly to the patellofemoral complex. In the present study, no significant differences were observed in the VA_I levels between sides and between the two contraction modalities. Of course, muscle activation in isometric and isokinetic conditions might be differently affected in pathologic conditions.

In this sample, as far as between-limb differences are concerned, the dominant and non-dominant limbs displayed superimposable VA_I levels. These findings are consistent with the results of a previous study by Pietrosimone et al., in which no significant side-to-side differences in VA were observed during isometric testing at 30°, 70°, and 90° knee flexion angles [48].

The present study revealed statistically significant differences between women and men, with women demonstrating higher VA_l levels. To the best of the authors' knowledge, no studies have reported significant between-sex differences in VA for either ISOM, isotonic [51] or ISOK results. Krishnan et al. observed higher mean VA levels for women compared to men, during ISOM testing at 60° knee flexion with different quantification methods (ITT and CAR), but these differences between sexes were not statistically significant [8]. However, their statistical analysis was performed on raw VA data.

Of relevance here, in the above cited studies and in most currently used isokinetic VA measurement techniques, the electrical stimulus was triggered when the joint reaches a certain angle during its movement. This technique of stimulation introduces an element of variability in the time of elicitation of the interpolated twitch along the curve because its latency depends on many factors: type of contraction (isometric vs. isokinetic), joint angular velocity, muscle conduction velocity, electrode positioning, time for excitation-contraction coupling, and tendon stiffness [1]. Therefore, comparisons are difficult when different angular velocities and knee joint angles are involved. In contrast, the stimulation technique performed in the present study is characterised by the computation of the instant of stimulation in order to have the peak of the interpolated twitch to occur consistently at exactly the desired knee angle [1].

4.3. Advantages of logit linearisation

In the present study, only the analysis performed on transformed data was able to detect a sex-related difference in VA_l levels. The analysis of raw data was flawed by ceiling effects and non-normal distributions The observed sex-related difference may reflect:

a) a difference in muscle fibre structure. Although the overall distribution of fast and slow fibre types in the vastus lateralis muscle is similar between women and men, a significant difference exists when the total area occupied by each fibre type is considered. In a study by Staron et al. [52], slow fibres were observed to occupy a greater area in the vastus lateralis muscle of women, whereas fast IIA fibres occupied a greater area in the vastus lateralis muscle of men. According to Henneman's size principle of motor unit recruitment, the fast motor units also have the highest recruitment thresholds [53]. As already hypothesised by Behm et al. [2], fast-twitch predominant muscles may be more difficult to fully activate during voluntary contractions. Therefore, when compared to women, the quadriceps femoris muscle of men might display lower mean and median values in voluntary activation levels.
b) The results may reflect a (hardly avoidable) mechanical artefact. Since VA is calculated as the complement to the ratio of IT and RT, the modification of one of these two parameters would alter the resulting VA value. In particular, an anterior tilt of the pelvis during knee extension would imply a shortening of the rectus femoris muscle and, as a consequence, a loss of knee extensor moment. The subsequent reduction of IT would lead to an increase in VA. Anatomical resting pelvic tilt does not display significant betweensex differences [54,55]. Nevertheless, women are known to have significantly lower abdominal muscle thickness than men [56], hence a potentially lower capacity for preventing pelvic tilt during maximal knee-extension effort. In addition, women might demonstrate later onsets in anticipatory postural adjustments of trunk muscles compared to men in single lower limb lift movements [57]. Again, these differences may eventually lead to a pelvic tilt causing lower IT peaks and higher VA estimates in women.

4.4. The need for a logit transformation

The suggestion of using logit units, unfamiliar to most clinicians, may appear a major limitation of the method. However, the simple computation of the MRD (second Equation in the Methods) can be easily performed on any spreadsheet or hand calculator. This value represents a threshold that is easy to interpret for decisions on individuals. Its critical advantage on percentage scores is that the measure of change becomes independent of the baseline values. In other words, a 1-logit change means the same amount of change of "activation" at whatever level of the 0–100% scale. It is not so for raw percent changes, which are "compressed" (and therefore, underestimated) when the VA values are approaching the extremes of the acceptable range [46]. The linearization problem, often overlooked in the biomedical literature, arises with VA but not with moments: these are intrinsically linear (i.e., a 1-moment change means the same change whatever was the baseline level). Moments, however, were not the topic of the present article.

The provided MRD values found here can be used to exemplify the logit-to-percentages transformation of change values. The MRD was 0.78 and 1.12 logits for ISOM and ISOK, respectively. A 0.78 logit change might reflect a change in VA from 28% to 70% (see the second Equation in the Methods) and from 76% to 95%. Likewise, a 1.12 logit change might reflect a change in VA from 79% to 98% and from 30% to 85%. In clinical practice, mirror reasoning may apply. The typical question may be checking whether a given observed percentage change does reveal or not a "real" change. In the latter example above, baseline and retest ISOK VA values of 30% and 85% correspond to -0.37 and 0.75 logits, respectively. The change is $[0.75 - (-0.37)] = 1.12$ logits: it may be concluded that the MRD threshold is attained (Table 3) so that the change is "real". Suppose, however, that the same percent change (55%) occurred between a baseline value of 20% and a retest value of 75%. In logit terms, this equals a increase of change of $[-0.12 - (-0.69)] = 0.57$ logit, below the threshold for a "real" change.

4.5. On the size of the observed MRD

In clinical terms, the MRD values of activation, for both ISOM and ISOK contexts, seem rather large. Stated otherwise, a large change must be observed before it can be considered as "real". This may reflect a) limitations of the present study as well as b) difficulties intrinsic to the measurement of this variable.

a) In this particular study, some test-retest instability (thus increasing the MRD) may be due to the small sample size and to the presence of outliers. On the other hand, there were no previously available data allowing for a-priori estimation of sample size. In addition, even smaller sample sizes are common in the literature on isokinetic quadriceps dynamometry [10,12,46]. Some instability might also stem from the absence of substantial habituation-learning processes, prevented by the experimental design. On the other hand, the design aimed to reproduce clinical contexts, in which no substantial practice is usually allowed.
b) With respect to activation itself, this seems to be intrinsically unstable. In fact, VA is a psychological (not a biological) variable which may be influenced by numerous extraneous factors (learning, fatigue, fear, motivation, attention, etc.). In addition, in human physiology the maximum voluntary muscle activation is close to its limit (i.e., 100%). The "crowding" of values hinders the precision of measurement, hence reliability studies.

Last, a "real" change, i.e., a statistically significant one, is not necessarily a clinically important one. In general, clinical "significance" requires statistical significance, but the cut-off is higher and based on external criteria (e.g., mortality risk, the incidence of a given disease, success probability in an examination, or whatever) [58]. In the case of VA_l, however, clinical experience may suggest a more lenient attitude: consistent increments across subsequent retest sessions may increase the confidence that some improvement is ongoing, although "significance" was not reached within pairs of subsequent assessments.

4.6. Further limitations and conclusions

The study limits cannot be overemphasised. The results refer to isometric contraction and to one isokinetic velocity (100°/s), only, thus limiting the generalizability of the estimated MRD to other conditions. More importantly, the MRD values provided here can be validly applied only if the whole measurement procedure is replicated, like for any other reliability-based index [25,59]. Lastly, in case full activation is recorded, the assessment of change becomes problematic. Possibly, reaching full activation might be considered as a favourable outcome irrespective of the observed change. All considered, the proposed method and values seem to represent useful tools for the assessment of change after rehabilitation or sports training programs, refining the assessments based on raw VA percentages.

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Ethical considerations

All participants gave written informed consent before participating. The study was approved by the ethics committee of the institution (project code 24C721_2017, date of approval 14th November 2017). The study conformed to the Code of Ethics of the World Medical Association (Declaration of Helsinki 2013) for medical research involving human participants.

Conflict of interest

The authors have no conflicts of interest to report.

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Appendix

Table A1
Results from A) Shapiro-Wilk's, B) and C) Wilcoxon signed rank tests on percent voluntary activation (VA) data for the isometric contractions (ISOM) and the isokinetic concentric contractions (ISOK) for both limb sides (dominant and non-dominant) during the test (T0) and the retest (T1). *Significant at $p < 0.05$ after Bonferroni correction

VA	
Shapiro-Wilk's test	Bonferroni corrected $p < 0.00625$
ISOM_T0	0.000*
ISOM_T1	0.005*
ISOK_T0	0.000*
ISOK_T1	0.000*
ISOM_dominant	0.000*
ISOM_non-dominant	0.000*
ISOK_dominant	0.000*
ISOK_non-dominant	0.000*

Table A2
Results from the Shapiro-Wilk test on logit-transformed voluntary activation (percent voluntary activation, VA, converted to the logit of the raw values, VA_l) data for the isometric contractions (ISOM) and the isokinetic concentric contractions (ISOK) during the baseline test (T0) and the retest (T1), and for limb sides (dominant and non-dominant). Only values with VA lower than 100% were considered. *Significant at $p < 0.05$ after Bonferroni correction

VA_l	
Shapiro-Wilk test	Bonferroni corrected $p < 0.00625$
ISOM_T0	0.30
ISOM_T1	0.00*
ISOK_T0	0.24
ISOK_T1	0.05
ISOM_dominant	0.54
ISOM_non-dominant	0.00*
ISOK_dominant	0.11
ISOK_non-dominant	0.10

Table A3
Results from the repeated ANOVA model for VA_l on sex, test condition (ISOM and ISOK), limb dominance, time-point, side dominance#time point interaction, and sex#time point interaction. Other indications as in Table A2. *: $p < 0.05$

VA_l	
Repeated ANOVA model	
R^2	0.06
	P
Model	0.08
Sex	0.01*
Test (ISOM vs. ISOK)	0.18
Lower limb side dominance	0.29
Time point	0.27
Dominance#Time point	0.49
Sex#Time point	0.89