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Reliability and agreement of a dynamic quadriceps incremental test for the assessment of neuromuscular function.

Giorgio Varesco^{1,*}, Nicolas Royer¹, Benjamin Singh¹, Audrey Parent², Léonard Féasson^{1,3}, Thomas Lapole¹, Guillaume Y Millet^{1,4} & Vianney Rozand¹

¹Université de Lyon, UJM-Saint-Etienne, Laboratoire Interuniversitaire de Biologie de la Motricité, EA 7424, F-42023, Saint-Etienne, France.

²Université du Québec à Montréal (UQAM), Montréal, Canada.

³Unité de Myologie, Service de Physiologie Clinique et de l'Exercice, Centre Référent Maladies Neuromusculaires Euro-NmD, CHU de Saint-Etienne, France

⁴Institut Universitaire de France (IUF)

*Corresponding author at:

Giorgio Varesco

Laboratoire Interuniversitaire de Biologie de la Motricité,

Campus Santé Innovations–IRMIS,

10 rue de la Marandière,

42270 Saint-Priest-en-Jarez, France.

giorgio.varesco@univ-st-etienne.fr

Abstract (200 words)

The quadriceps-intermittent-fatigue (QIF) test assesses knee extensors strength, endurance and **performance fatigability** in isometric condition. We aimed to **assess reliability and agreement** for this test in dynamic conditions and with the use of transcranial magnetic stimulation. On two separate sessions, 20 young adults (25±4 yr, 10 women) performed stages of **100 knee extensors concentric** contractions at 120°/s (60° range-of-motion) with 10% increments of the initial maximal concentric torque until exhaustion. **Performance fatigability** across the test was quantified as maximal **isometric and concentric** torque loss, and its mechanisms were **investigated through the** responses to transcranial magnetic and electrical stimulations. Reliability and agreement were assessed using ANOVAs, coefficients of variation (CVs) and intra-class correlation coefficients (ICCs) with 95% CI. Good inter-session reliability and high agreement were found for number of contractions [489±75 vs. 503±95; P=0.20; ICC=0.85 (0.66; 0.94); CV=5% (3; 7)] and total work [11,285±4,932 vs. 11,792±5,838 Nm.s; P=0.20; ICC=0.95 (0.87; 0.98); CV=8% (5; 11)]. **Poor reliability but high agreement** were observed for isometric [-33±6 vs. -31±7%; P=0.13; ICC=0.47 (0.05; 0.75); CV=6% (4;8)] and **concentric** [-20±11% vs. -19±9%; P=0.82; ICC=0.26 (-0.22; 0.63); CV=9% (6; 12)] **torque loss**. **The dynamic QIF test represents a promising tool for neuromuscular evaluation in isokinetic mode.**

Keywords: evaluation; fatigability; **neuromuscular fatigue**; isokinetic; transcranial magnetic stimulation.

1 **Introduction**

2 Strength, power, endurance and **performance fatigability** can be considered as the main components of the
3 neuromuscular function and are important to understand how sex, age, or different clinical conditions affects the
4 neuromuscular system (Hunter 2018). In 2013, Bachasson et al. (2013b) validated the “quadriceps intermittent
5 fatigue” (QIF) test that evaluates the neuromuscular function **and the kinetic of impairments in contractile
6 function and muscle activation in the knee extensors (KE)**. The test consists of sets of intermittent isometric
7 contractions at increasing intensity until task failure. **Contractile function and muscle voluntary activation** are
8 assessed during the QIF test using the interpolated twitch technique (ITT) with peripheral magnetic or electrical
9 stimulation. This test has been shown to be sensitive to detect differences in strength, endurance and
10 **performance fatigability** among sex (Bachasson et al. 2013b), age, level of training (Bachasson et al. 2016) and
11 the effect of pathologies (Bachasson et al. 2013a).

12 **Bachasson et al. (2013b) evaluated agreement and reliability for the performance at the QIF test and the
13 neuromuscular variables prior, during and after the fatiguing incremental task. Specifically, high agreement
14 [quantified through coefficient of variation (CV)] was found for number of contractions (5.6%) and total
15 strength-time product (4.1%; calculated at 50% of the test). Performance fatigability showed high agreement
16 along the test both in terms of maximal force loss produced by the KE (CVs <7.5%) and evoked responses to
17 femoral nerve magnetic stimulations (CVs <6.6%). For the same variables, good reliability was found using
18 intra-class correlation coefficients (ICCs; >81) and visual inspection of Bland-Altman plots.**

19 **One limitation of the original QIF test study is the isometric mode of contraction. Most of the daily activities are
20 performed using dynamic contractions, and the study of both isometric and dynamic conditions is important to
21 specifically understand and evaluate neuromuscular function across different populations (e.g. athletes vs.
22 sedentary adults, young vs. old adults). For instance, a recent review from our research group (Krüger et al.
23 2018) highlighted that after an isometric fatiguing task, the percentage of isometric torque loss was greater in
24 young adults compared to older adults. However, when performing dynamic tasks, the percentage of isometric
25 torque loss was similar between groups, while the percentage of power loss was more pronounced in the elderly
26 (Krüger et al. 2018). Thus, a similar QIF test involving dynamic contractions would **better represent contractions
27 involved in daily life activities and associated with functional task’s performance (Severijns et al. 2017). Lower-
28 limbs concentric fatigability protocols better reflect some functional capacities and perception of fatigue
29 compared to isometric protocols in different clinical populations such as multiple sclerosis patients (Taul-
30 Madsen et al. 2020) and older adults (Senefeld et al. 2017). Furthermore, because peripheral and central factors
31 contribute differently to performance fatigability in isometric vs. dynamic contractions (Hunter 2018), assuming
32 similar reliability and agreement for an isometric and dynamic QIF test could be problematic and needs to be
33 investigated.****

34 To evaluate the mechanisms of **performance fatigability**, the original ITT has been adapted to the use of
35 peripheral femoral nerve magnetic stimulation (FNMS; Verges et al. 2008) and transcranial magnetic stimulation
36 over the motor cortex (TMS; Todd et al. 2016). The use of FNMS for the assessment of **contractile function and**
37 **muscle activation** proposed in the original QIF test (Bachasson et al. 2013b) is more comfortable for participants
38 than classic high frequency percutaneous electrical stimulation (Verges et al. 2008). However, we have shown
39 that subcutaneous fat thickness could affect the effectiveness of the magnetic stimulation to activate the femoral
40 nerve (Tomazin et al. 2011), making this measure less suitable for specific populations like overweight
41 individuals. Another possibility to evaluate **the mechanisms of performance fatigability** is represented by single
42 pulse TMS that, associated with peripheral nerve electrical stimulation (PNS), enables to further discern the
43 involved mechanisms [e.g. corticospinal excitability/inhibition, muscle relaxation rate (Todd et al. 2016)] along
44 the fatiguing task.

45 Hence, the aim of this study was to **assess** the reliability and **agreement** of the QIF test performed with
46 **concentric** (isokinetic) contractions, using TMS and PNS to evaluate **contractile function and muscle voluntary**
47 **activation** throughout the test. It was hypothesized that the dynamic QIF test would induce a progressive
48 decrease in both isometric and dynamic torques, which would **be due to the decline in both contractile function**
49 **and muscle voluntary activation**.

50

51 **Methods**

52 *Participants*

53 Twenty young adults (age: 25±4 yr; body mass: 65±11 kg; height: 172±9 cm; 10 females) **recruited from the**
54 **Jean-Monnet University's department of kinesiology** participated in the experiment and were clearly informed
55 on the modalities of the test prior giving their written consent. **The sample size was chosen based on the previous**
56 **QIF test validation study of Bachasson et al. (2013b)**. All participants were free from musculoskeletal or
57 neurological disorders **and familiar with isometric and isokinetic force assessment**. Participants visited the
58 laboratory twice, separated by at least 48h, in which they performed two identical dynamic QIF tests (DYN1 and
59 DYN2). Participants were asked to avoid strenuous exercise for 24h and alcohol consumption for 12h prior
60 testing, **and to keep their caffeine consumption habits consistent for the duration of the study**. All the
61 experimental procedures were approved by the institutional ethics committee and performed in accordance with
62 the *Declaration of Helsinki* (except for registration in a database).

63 *Mechanical recording*

64 Participants were seated upright on an isokinetic dynamometer (Con-Trex MJ; CMV-AG, Dubendorf,
65 Switzerland) (Maffiuletti et al. 2007) and were tested on the right leg. For isometric measurements, hip angle

66 was set at 95° and knee angle at 90° (180°= full extension; Figure 1B). For all **concentric isokinetic**
67 measurements and for the QIF test, the range of motion was set at 60° (from 90° to 150°) and the velocity was
68 120°s⁻¹. Extraneous movements of the upper body were minimized using straps across the thorax and waist
69 integrated in the dynamometer. During the first experimental session, the position of each participant was
70 registered and kept constant throughout the protocol. Torque was acquired at 2 kHz, transmitted without
71 analogical or digital filter using a Power-Lab data system (16/30-ML880/P, ADInstruments, Bella Vista,
72 Australia) and recorded on a computer through Labchart software (v8, ADInstruments). Visual feedback was
73 provided on a screen positioned in front of the participant.

74 *Experimental procedures*

75 Participants performed two maximal voluntary isometric contractions (MVICs) separated by 1-min to assess
76 maximal isometric torque. Subsequently, the hotspot and intensity for TMS were identified (described below).
77 After 1-min of rest, three consecutive maximal voluntary **concentric** contractions (MVDCs) were performed **in**
78 **isokinetic mode (range of motion=60°, angular speed=120°s⁻¹)** for the assessment of **concentric** peak torque. If
79 the difference between the contractions (MVIC or MVDC) was >5%, the procedure was repeated after 1-min of
80 recovery.

81 Then, participants performed a first neuromuscular evaluations (NME) to familiarize with this procedure.
82 Following 1-min of rest, the baseline NME was performed. NME consisted in a 5-s MVIC followed by **~3-s**
83 contractions at 60% and 80% MVIC (Figure 1A). A single-pulse TMS was delivered during the plateau of each
84 contraction (**100%-80%-60% MVIC**) based on the technique originally developed for the elbow flexors (Todd et
85 al. 2003) and later for the KE (Goodall et al. 2009; Sidhu et al. 2009). Single-pulse PNSs were delivered during
86 the MVIC and at rest ~1 s following the MVIC (Figure 1A). Thereafter, participants performed a rapid
87 contraction with the instruction of contracting “as fast as you can” for the assessment of rate of torque
88 development (RTD). NME duration was ~25-s. A schematic representation of the experimental setup is provided
89 in Figure 1B.

90 Following the baseline NME, participants performed the dynamic QIF test **in concentric condition** (Figure 1C).
91 The test consisted of sets of 100 **concentric** contractions (**range of motion=60°, angular speed=120°s⁻¹**) starting at
92 10% of MVDC torque **and increasing by 10% MVDC for each subsequent set until exhaustion**. Participants
93 visualized the target torque level on the screen with a line of 2-Nm thickness (1 Nm under and above). They
94 were instructed to start the concentric phase as soon as the dynamometer passively returned the leg to the starting
95 position (90°), and to reach the target torque level at each contraction. **Once each 100-contractions set was**
96 **completed, the dynamometer was set in isometric mode and** the NME was performed ~3 s after. Following the
97 NME, the dynamometer was set back to isokinetic mode and participants were asked to perform a MVDC
98 (Figure 1C). **Immediately after**, participants started the following 100 contractions set. Exhaustion was defined
99 as five consecutive contractions below the target torque.

100 *Electromyography*

101 Electromyographic (EMG) activity was recorded from the *vastus lateralis* (VL), *rectus femoris* (RF), *vastus*
102 *medialis* (VM) and *biceps femoris* (BF) muscles of the right leg using pairs of self-adhesive Ag/AgCl surface
103 electrodes (recording diameter=10 mm, Kendall MediTrace 130 foam electrode, MA) placed **accordingly to the**
104 **SENIAM recommendations** in bipolar configuration over the muscle belly with a centre-to-centre distance of 30
105 mm. A reference electrode was placed on the patella. Prior to electrode placement, the skin was shaved, abraded
106 and cleaned with alcohol. EMG signals were amplified (gain=500) using an octal bio-amplifier (ML138, AD
107 Instruments) with a bandwidth frequency range of 10–500 Hz obtained through a band-pass filter and digitized
108 simultaneously to the torque signal at the same sampling rate (2 kHz) by Power-Lab System (16/30-ML880/P,
109 AD Instruments).

110 *Transcranial Magnetic Stimulation*

111 TMS was delivered via a concave 110-mm double cone coil connected to a monophasic magnetic stimulator
112 (Magstim 200, Magstim, Whitland, UK) over the hotspot of the motor cortex area for the right **KE** muscles (peak
113 magnetic field output= 2.5T, stimulation duration= 0.1 ms). The orientation of the coil induced a posterior-to-
114 anterior current flow in the underlying cortical tissue. At the beginning of each session, the coil was positioned
115 with the center ~1 cm lateral to the vertex of the left motor cortex. Identification of the hotspot was guided by
116 moving along a 1-cm grid drawn on a swim cap and was determined as the location that elicited the greatest
117 torque response and motor evoked potential (MEP) amplitude in the VL, RF and VM while the participant
118 contracted at 10% MVIC. This position was marked on the swim cap to ensure repeatable placement of the coil
119 throughout the experiment. Optimal stimulation intensity was determined as the intensity eliciting the greatest
120 torque response and MEP amplitude, without an increase in BF MEP amplitude, during brief 10% MVIC
121 contractions (mean intensity= 65±10% of the maximal stimulator output).

122 *Femoral Nerve Electrical Stimulation*

123 Percutaneous electrical stimulation was delivered on the femoral nerve through a constant current stimulator
124 (DS7R, Digitimer, Welwyn Garden City, UK), which delivered a square-wave stimulus of 2 ms of duration (400
125 V) (Zarkou et al. 2017). The cathode electrode (Kendall MediTrace) was placed on the inguinal triangle while
126 the anode, a 50×90 mm electrode (Durastick Plus; DJO Global, Vista, CA) was placed on the gluteal fold.
127 Participants were instructed to fully relax before the stimulation was delivered. The intensity was increased until
128 the torque response reached a plateau. The 130% of the plateau intensity was chosen to ensure supramaximality.

129 *Data analysis*

130 All data were analysed offline using Labchart software (v8, ADInstruments). MVIC torque was defined as the
131 average of the highest 0.5-s window for each MVIC. Root-mean-square (RMS) EMG of the VL, RF and VM

132 were assessed during the same time window, and then normalized to the compound muscle action potential
133 (M_{sup}) superimposed via PNS during the same MVIC. MVDC was defined as the maximal value reached at each
134 maximal **concentric** contraction. RTD was calculated as the averaged slope over a 150-ms time window from the
135 torque onset, defined as a rise from the baseline >2 Nm (Maffiuletti et al. 2016). Total work was assessed
136 through the sum of torque–time integral over each contraction of each stage. **Performance fatigability was**
137 **defined as the percentage loss in both MVIC and MVDC. Muscle** voluntary activation was calculated for both
138 TMS (VA_{TMS}) and PNS (VA_{PNS}) responses using the following equations (Todd et al. 2007, 2016):

139
$$VA_{PNS} (\%) = (1 - \frac{SIT_{PNS}}{Pt}) \times 100 \quad (\text{Equation 1})$$

140 where SIT_{PNS} is the amplitude of the superimposed twitch evoked by PNS, and Pt is the amplitude of the resting
141 twitch evoked by PNS. When the stimulations were not delivered at the MVIC torque value, a correction was
142 applied to the equation (Strojnik and Komi 1998).

143
$$VA_{TMS} (\%) = (1 - \frac{SIT_{TMS}}{ERT}) \times 100 \quad (\text{Equation 2})$$

144 where SIT_{TMS} is the amplitude of the superimposed twitch evoked by TMS during MVIC, and ERT is the
145 estimated resting twitch, corresponding to the y-intercept obtained from the linear regression of the TMS-evoked
146 twitch during the contractions at 100%, 60% and 80% MVIC. If the regression showed a Pearson's $R < 0.9$,
147 VA_{TMS} was discarded (Todd et al. 2016). Pt was analysed as the amplitude of the resting twitch evoked by PNS.
148 **To evaluate neuromuscular propagation**, amplitude and area for VL, VM and RF of M-waves (M_{max}) elicited
149 during SIT_{PNS} were analysed. **To evaluate changes in corticospinal excitability**, MEP amplitude and area elicited
150 during SIT_{TMS} were analysed. MEPs were normalized to M_{sup} elicited during the same MVIC (MEP/M_{sup}).

151 **To evaluate changes in corticospinal inhibition**, SIT_{TMS} -evoked silent period (SP) was quantified as the duration
152 (ms) from the stimulus artefact to the return of muscle activity on EMG signal by visual inspection (Damron et
153 al. 2008) and performed by two investigators. Peak relaxation rate (pRR_{norm}) was calculated as the averaged
154 10-ms interval around the steepest negative slope (5-ms each side) after the SIT_{TMS} and normalized to MVIC
155 torque (Todd et al. 2007; Vernillo et al. 2020). **Changes in Pt and pRR_{norm} were used to identify impairments in**
156 **the muscle contractile function.**

157

158 *Statistical Analysis*

159 All variables are reported as mean \pm standard deviation. Assumption of normality for all variables was
160 investigated using the Shapiro-Wilk test. Development of **performance fatigability** throughout the dynamic QIF
161 test was analysed using two-way [stage (stages 1, 2, 3, 4 and exhaustion) \times session (DYN1 and DYN2)]
162 repeated-measures analysis of variance (ANOVA) for all variables. Paired sample T-test was carried out to
163 compare the percentage of loss in MVIC vs. MVDC torque at exhaustion. For VA_{TMS} , Generalized Estimating

164 Equations (GEE) analysis using an autoregressive (AR-1) structure were performed to detect changes along the
165 protocol and between sessions (stage × session), because ANOVA would automatically exclude the participants
166 with missing values (discarded due to invalid ERT),
167 Paired-sample T-tests or Mann-Whitney U-tests, **intra-class correlation coefficients [ICCs (A,1); Two-way**
168 **mixed model, absolute agreement definition, single measure coefficient (Koo and Li 2016)]** and coefficients of
169 variation (CVs) with 95% confidence intervals (95% CI) were used to assess the reliability of the dynamic QIF
170 test between DYN1 and DYN2 for the number of contractions and total work, and the neuromuscular
171 parameters, i.e. MVIC, MVDC, RTD, VA_{TMS}, VA_{PNS}, and Pt (Hopkins 2000; Weir 2005). Reliability was
172 calculated at baseline (Pre) on absolute values. For reliability assessment at the last common stage completed by
173 all the participants and at exhaustion, values were expressed as percentage from baseline (%Pre), excluding
174 VA_{TMS} and VA_{PNS} (Bachasson et al. 2013b). Changes in the mean values between the two sessions with 95% CI
175 were also calculated for the detection of systematic bias. **Heteroscedasticity was evaluated through analysis of**
176 **the scatterplot graph of the predictive value of the independent variable with its linear regression residual and**
177 **Glejser's test for heteroscedasticity (Machado and Santos Silva 2000). If data were not heteroscedastic or if ICC**
178 **and CV indicated discordant results, Bland and Altman's plots with 95% limit of agreement (1.96 × standard**
179 **deviation) were used to investigate reliability. ICCs were not calculated for VA_{TMS} and VA_{PNS} due to the ceiling**
180 **effect associated with these variables. Based on the ICC estimate, values between 0-0.50 were considered as**
181 **poor, 0.50-0.75 moderate, 0.75-0.90 good, and >0.90 excellent (Koo and Li 2016). Variability was defined high**
182 **and low, respectively, for CV values greater and lower than 10% referring to the results obtained by Bachasson**
183 **et al. (2013b).**
184 Reliability was also calculated for each sex independently. For all ANOVAs, sphericity was tested with the
185 Mauchly test. If the assumption of sphericity was violated, the correction of Greenhouse-Geisser was applied.
186 When significant main effects were observed, Bonferroni's correction was applied for post-hoc analysis.
187 Significance was set as P<0.05 for all tests. Statistical analysis was conducted using IBM SPSS software
188 (Version 23.0, SPSS Inc., Chicago, IL).

189

190 **Results**

191 *Performance, reliability and agreement of the dynamic QIF test*

192 Participants performed 489±77 submaximal contractions during DYN1 and 507±97 during DYN2 (P=0.20), with
193 a mean difference of 17 contractions (95% CI: -4; 39). **Reliability and agreement were good** between sessions
194 [ICC=0.85 (95% CI: 0.66; 0.94); CV=5% (95% CI: 3; 7)]. The participants performed a total work of
195 11,285±4,933 Nm.s for DYN1 and 11,792±5,838 Nm.s for DYN2 with no significant difference between the
196 two sessions (P=0.21). **Reliability and agreement were excellent** for this parameter [Mean difference=507 Nm.s
197 (95% CI: -311; 1325); ICC=0.95 (95% CI: 0.87; 0.98); CV=8% (95% CI: 5;11)].

198 *Performance fatigability*

199 MVIC, MVDC, RTD, VA, Pt and pRRnorm progressively decreased along the QIF test without significant
200 differences between sessions (session \times stage: $P > 0.05$; Figures 2-3). As results for VL, VM and RF were similar,
201 EMG data are presented only for VL in Figure 4. No session \times stage interaction or difference from baseline were
202 found for normalized RMS-EMG (Figure 4A), MEP/ M_{sup} amplitude and area (Figure 4B-C), M_{max} amplitude and
203 area for all the muscles (VL, VM, RF; $P > 0.05$; Figure 4E-F). SP duration increased starting from the third stage
204 without differences between sessions (Figure 4D). Finally, percentage of isometric torque loss was greater than
205 **concentric** torque loss for both DYN1 ($-33.1 \pm 5.8\%$ vs. $-20.1 \pm 11.5\%$; $P < 0.001$) and DYN2 ($-31.6 \pm 6.9\%$ vs. -
206 $19.4 \pm 8.6\%$; $P < 0.001$).

207 *Neuromuscular function reliability and agreement*

208 Excellent and good **reliability and agreement** were observed for MVIC, MVDC, RTD, VA, Pt and pRRnorm at
209 baseline (Table 1), with ICC ranging from 0.96 to 0.87 and CV from 2.9% to 9.4%. **Only MVDC data at post**
210 **were not heteroscedastic ($P = 0.022$). Analysis of the Bland-Altman Plot revealed a limit of agreement of 36 Nm,**
211 **which was considered acceptable but not excellent based on the dispersion of the MVDC and MVIC at pre.**

212 Reliability **and agreement** of changes in volitional and evoked quadriceps responses at the last common stage
213 (i.e. Stage 4) and at exhaustion are presented in Table 2. Overall, at Stage 4 **reliability and agreement** were poor
214 to **moderate** with ICC ranging from 0.76 to 0.55 and CV ranging from 5.6% to 15.3%. Finally, at exhaustion
215 reliability **and agreement** were poor to good with ICC ranging from 0.26 to 0.68 and CV ranging from 5.6% to
216 16.6%. Bland-Altman plots of differences vs. mean of the two dynamic QIF tests (DYN1 and DYN2) for MVIC
217 and MVDC are presented on Figure 5.

218 For VA_{TMS} , 6 participants presented valid ERT ($R > 0.90$) for all stages for DYN1 and 11 for DYN2, and 4
219 participants showed valid ERT throughout the protocol for both sessions. GEE analysis detected no significant
220 difference between stages ($P = 0.08$), sessions ($P = 0.96$) or stage \times session ($P = 0.84$). For the calculation of
221 **reliability and agreement**, 18 participants showed valid ERT for both sessions at Pre, 13 at Stage 4 and 4 at
222 exhaustion. At Pre, VA_{TMS} was $95 \pm 3\%$ for DYN1 and $95 \pm 4\%$ for DYN2, with a mean difference of 0.3% (95%
223 CI: -2.7; 3.3) and a CV of 2.9% (95% CI: 1.3; 4.6). VA_{TMS} was similar at Stage 4 for both sessions [DYN1:
224 $93 \pm 6\%$ ($n = 14$); DYN2: $93 \pm 6\%$ ($n = 16$)], with a mean difference of 0.5% (95% CI: -2.03; 3) and a CV of 2.8%
225 (95% CI: 1.4; 4.2). At exhaustion, VA_{TMS} was $85 \pm 15\%$ for DYN1 ($n = 11$) and $86 \pm 14\%$ for DYN2 ($n = 12$). Due to
226 the small sample size ($n = 4$), **agreement** as mean difference and CV was not calculated at Exhaustion.

227 Similar results were found for DYN1 and DYN2 when men and women were analysed separately. **Reliability**
228 **and agreement** in the two sexes can be found in the electronic supplementary material.

229 **Discussion**

230 The present study aimed to assess the reliability and agreement of the dynamic (isokinetic) QIF test in healthy
231 young adults. This test allowed to assess isometric and concentric strength, endurance, performance fatigability
232 in one session. The test represents a promising tool for the evaluation of the neuromuscular function in dynamic,
233 i.e. more ecological, condition. As hypothesized, the task induced a progressive decrease in both isometric and
234 concentric torque with impairments in both contractile function and muscle voluntary activation.

235 *Reliability*

236 The participants started the two sessions in a very similar functional state. Indeed, maximal torque (isometric and
237 concentric), RTD, VA_{TMS} , VA_{PNS} and Pt showed good reliability at the beginning of each session, as previously
238 shown (Maffiuletti et al. 2007; Zarkou et al. 2017). Furthermore, participants performed similarly in the two
239 sessions, as good reliability and agreement were found for total number of contractions and total work, indicators
240 of relative and absolute endurance performance, respectively. For both MVIC and MVDC torque loss, intra-
241 subjects agreement (CV) remained low across the test while inter-subjects reliability (ICC) became poor. For
242 these parameters, the difference in mean values between DYN1 and DYN2 was close to zero and analysis of the
243 Bland-Altman's plot excluded the presence of systematic bias (Figure 5). Bland-Altman's plots indicated that
244 these low ICCs for torque loss (isometric and concentric) at exhaustion were mainly due to inter-individual
245 differences. In particular, the 95% CI for MVDC at exhaustion assumed negative values for the lower bound (-
246 0.22). Negative values are usually observed when calculating ICC (A,1) from the sample mean squares of
247 ANOVA, which can result in negative ICC value due to low between-subject variance or high residual variance,
248 even in the case of small inter-individual difference (Bartko 1976; McGraw and Wong 1996).

249 For Pt, agreement decreased along the test: at Stage 4, while relative reliability remained good, low agreement
250 indicated that within-subject variability increased. Because agreement for performance fatigability remained
251 high at stage 4 and exhaustion, this might be due to insufficient intensity of the stimulation that could not elicit a
252 response in all the motor units of the KE for some subjects. Reliability and agreement may be improved by using
253 doublets instead of single pulses (Shield and Zhou 2004). Intra- and inter-subject variability in RTD increased at
254 stage 4 and exhaustion, indicating that a single rapid contraction should not be adopted to evaluate RTD along
255 the QIF test. Variability could be reduced by performing several rapid contractions and averaging the obtained
256 values (Varesco et al. 2019). However, due to time constraint, this is feasible only at baseline and exhaustion,
257 limiting the usefulness of this measure during the task. Regarding VA_{PNS} , intra-subject agreement remained good
258 for both sessions (<6%).

259 Overall, performance at the dynamic QIF test was reproducible between sessions. The neuromuscular variables
260 showed excellent reliability only at pre, while agreement remained high throughout the protocol, indicating high
261 variability in the between-subject response to the fatiguing task, but similar fatigability within-subject. Our
262 results for performance fatigability agreement were similar to those observed by Bachasson et al. (2013b), while
263 contractile function and muscle voluntary activation were consistent only at pre, indicating that improvement in

264 the methodology are needed. Agreement and reliability were similar when evaluated separately on men and
265 women.

266 *Induced fatigue*

267 The dynamic QIF test induced a progressive decline in both MVIC (-33% at exhaustion) and MVDC (-20% at
268 exhaustion). MVDC was assessed at the end of NME, which induced a short recovery that may explain the
269 ~13% difference between MVIC and MVDC torque loss. However, differences in neural pattern between
270 isometric and dynamic contractions exist, such as differences in the motor units recruitment, recruitment
271 threshold or firing rate to the active motor units, which partially depends on the duration of the MVIC vs.
272 MVDC (5-s vs 0.5-s) (Linnamo et al. 2003). Furthermore, the angular position where the maximal torque is
273 recorded differs between contractions mode. For each participant, isokinetic contractions allowed to record
274 torque at different angular positions, including when KE contracted at the optimal length, which is individually
275 defined by the force-length relationship (Rassier et al. 1999). Because the force-length relationship can be
276 influenced by fatigue (MacNaughton et al. 2007), this “optimal” KE length for MVDC could differ between Pre,
277 during and Post dynamic QIF test, whereas the length of the muscle remained constant during the MVIC.

278 The reduction in maximal isometric and concentric torque production capacities were due to both impairments in
279 muscle voluntary activation capacity and contractile function. The large drop in Pt (-50%) indicated that the
280 decrease in torque production capacity was mainly driven by impairments in the contractile function of the KE.
281 More specifically, the decrease in Pt amplitude since the first stage likely indicated a progressive failure in
282 excitation–contraction coupling along the test (Bachasson et al. 2013b). Furthermore, increase in peak relaxation
283 rate suggested that impairments in Ca^{2+} reuptake was also involved in the loss in torque production in the last
284 stages (Todd et al. 2007; Vernillo et al. 2020). Similar amplitude and area of M-waves and MEP/ M_{sup} along the
285 test indicated that mechanisms such as impairment in action potential conduction or change in the corticospinal
286 excitability were not involved, similarly to the original QIF test study (Bachasson et al. 2013b). The increase in
287 the duration of SP starting from the third stage suggested a progressive increase in corticospinal inhibition along
288 the protocol, influencing the ability to activate the quadriceps muscles, as shown by the decrease in VA_{PNS}
289 (Damron et al. 2008). However, decrease in VA_{TMS} was not significant at exhaustion compared to baseline
290 values and this is discordant with the results obtained via PNS. This difference was mostly due to the reduced
291 number of valid ($R > 0.90$ for ERT) samples throughout the test.

292 *Methodological limitations*

293 The main reliability issue in the present protocol is the low number of participants who showed valid ERT
294 ($R > 0.90$) for both sessions throughout the protocol and at exhaustion, making the calculation of VA_{TMS} not
295 possible.

296 A problem in the assessment of valid ERT for the calculation of VA_{TMS} at exhaustion was the difficulty to
297 maintain a stable plateau, probably due to fatigue. Consequently, the stimulation obtained during an ascendant or
298 descendent phase in one of the three contractions could influence the measure of ERT. A possible solution
299 consists in performing TMS twitch interpolation technique at baseline and exhaustion with two evaluations each,
300 or to increase the number of submaximal contractions for the calculation of ERT, rising the likelihood to obtain
301 an $R > 0.90$ for the ERT extrapolation (Dekerle et al. 2019). The use of TMS, compared to FNMS or PNS alone,
302 could provide additional information about the site of failure in the motor command or corticospinal
303 excitability/inhibition. However, this technique is more challenging, as shown in previous studies (Todd et al.
304 2016), and precautions should be taken when evaluating patients. **It is thus advised to provide a familiarization**
305 **session and include a standardized warm-up prior the testing session to reduce a possible risk of injury;**
306 **particularly when testing frail populations. Furthermore, high between-subject variability underlines the possible**
307 **influence for factors such as physical activity level or training background that we did not controlled and that**
308 **possibly influenced variability in our sample.**
309

310 *Conclusions*

311 **The present protocol was able to identify changes in strength, endurance and performance fatigability** in young
312 adults. Changes in **contractile function and muscle voluntary activation** induced by the test were also well
313 detected with the current protocol. The use of TMS for the assessment of voluntary activation was limited, thus
314 the methodology should be **enhanced**. Further studies should be conducted **modifying the methodology to**
315 **improve reproducibility and** evaluate its feasibility in older adults or specific populations. In conclusion, the
316 dynamic QIF test is a promising tool for neuromuscular investigation in isokinetic condition, which is more
317 representative of daily life activities.

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398

399

400 **Table 1.** Between-session reliability of Pre values for volitional and evoked knee extensors responses.

| | DYN1 | DYN2 | P-value | Change in mean (95% CI) | CV (95% CI) | ICC (95% CI) |
|--------------------------------|-------------|-------------|----------------|------------------------------------|--------------------|---------------------|
| Pre | | | | | | |
| MVIC (Nm) | 184±62 | 178±63 | 0.29 | 5.8 (-5.3; 16.9) | 6.7 (4.1; 9.2) | 0.93 (0.82; 0.97) |
| MVDC (Nm) | 144±39 | 142±43 | 0.52 | 1.8 (-3.9; 7.5) | 4.8 (2.8; 6.8) | 0.96 (0.89; 0.98) |
| RTD (Nm.s⁻¹) | 883±296 | 879±259 | 0.84 | 4.3 (-41.4; 50.1) | 6.1 (4.2; 8) | 0.94 (86; 0.98) |
| VAPNS (%) | 95±2 | 93±6 | 0.18 | 2.3 (0.2; 4.9) | 2.9 (1.1; 4.7) | n/a |
| Pt (Nm) | 51±16 | 50±18 | 0.64 | 0.9 (-3.1; 5) | 9.4 (5.5; 13.3) | 0.87 (0.70; 0.95) |

401

402

403 **Table 2.** Between-session reliability of volitional and evoked responses during the QIF tests at the last common
 404 stage performed by all the participants and at exhaustion relatively to pre-values.

| | DYN1 | DYN2 | P-value | Change in mean (95% CI) | CV (95% CI) | ICC (95% CI) |
|-----------------------------|-------------|-------------|----------------|------------------------------------|--------------------|---------------------|
| Stage 4 | | | | | | |
| MVIC (%) | 73.2±8.7 | 72.9±9.6 | 0.86 | 0.3 (-3.7; 4.4) | 6.2 (3.5; 8.9) | 0.56 (0.15; 0.80) |
| MVDC (%) | 84.3±11.6 | 85.3±9.6 | 0.66 | -0.9 (-5.56; 3.6) | 6.5 (4.1; 8.9) | 0.59 (0.21; 0.82) |
| RTD (%) | 78.2±16.2 | 79.6±14.9 | 0.69 | -1.4 (-8.3; 5.6) | 10.1 (5.3; 14.9) | 0.55 (0.14; 0.80) |
| VA_{PNS} (%) | 88.6±7.9 | 89.2±8 | 0.82 | -0.6 (-5.5; 4.3) | 5.6 (2.3; 8.9) | n/a |
| Pt (%) | 54.7±20.5 | 56.3±19.1 | 0.66 | -1.5 (-7.9; 4.9) | 15.3 (9.1; 21.5) | 0.76 (0.49; 0.89) |
| Exhaustion | | | | | | |
| MVIC (%) | 66.9±5.9 | 68.3±6.9 | 0.36 | -1.4 (-4.5; 1.7) | 5.6 (3.6; 7.6) | 0.47 (0.05; 0.75) |
| MVDC (%) | 79.9±11.1 | 80.6±8.6 | 0.81 | -0.6 (-6.4; 5.1) | n/a | 0.26 (-0.22; 0.63) |
| RTD (%) | 74.6±14.8 | 73.3±15.6 | 0.74 | 1.3 (-6.5; 9.1) | 11.7 (5.5; 18) | 0.41 (-0.04; 0.71) |
| VA_{PNS} (%) | 89.7±5.9 | 86.2±6.4 | 0.79 | 3.5 (-0.2; 7.1) | 5.8 (4.1; 7.4) | n/a |
| Pt (%) | 49.2±16.1 | 51.3±14.3 | 0.97 | -2.1 (-7.9; 3.6) | 16.6 (11; 22.2) | 0.68 (0.35; 0.85) |

405

406

407 **Figures Captions**

408 **Figure 1.** Schematic representation of the protocol. A: Typical trace of the torque during the neuromuscular
409 evaluation. B: Representation of the experimental setup. C: Dynamic QIF test protocol. MVIC= maximal
410 voluntary isometric contraction; MVDC= maximal voluntary dynamic contraction; NME= neuromuscular
411 evaluation; SIT= superimposed twitch; TMS= transcranial magnetic stimulation; PNS= peripheral nerve electric
412 stimulation; Pt= resting potentiated twitch; RTD= rate of torque development.

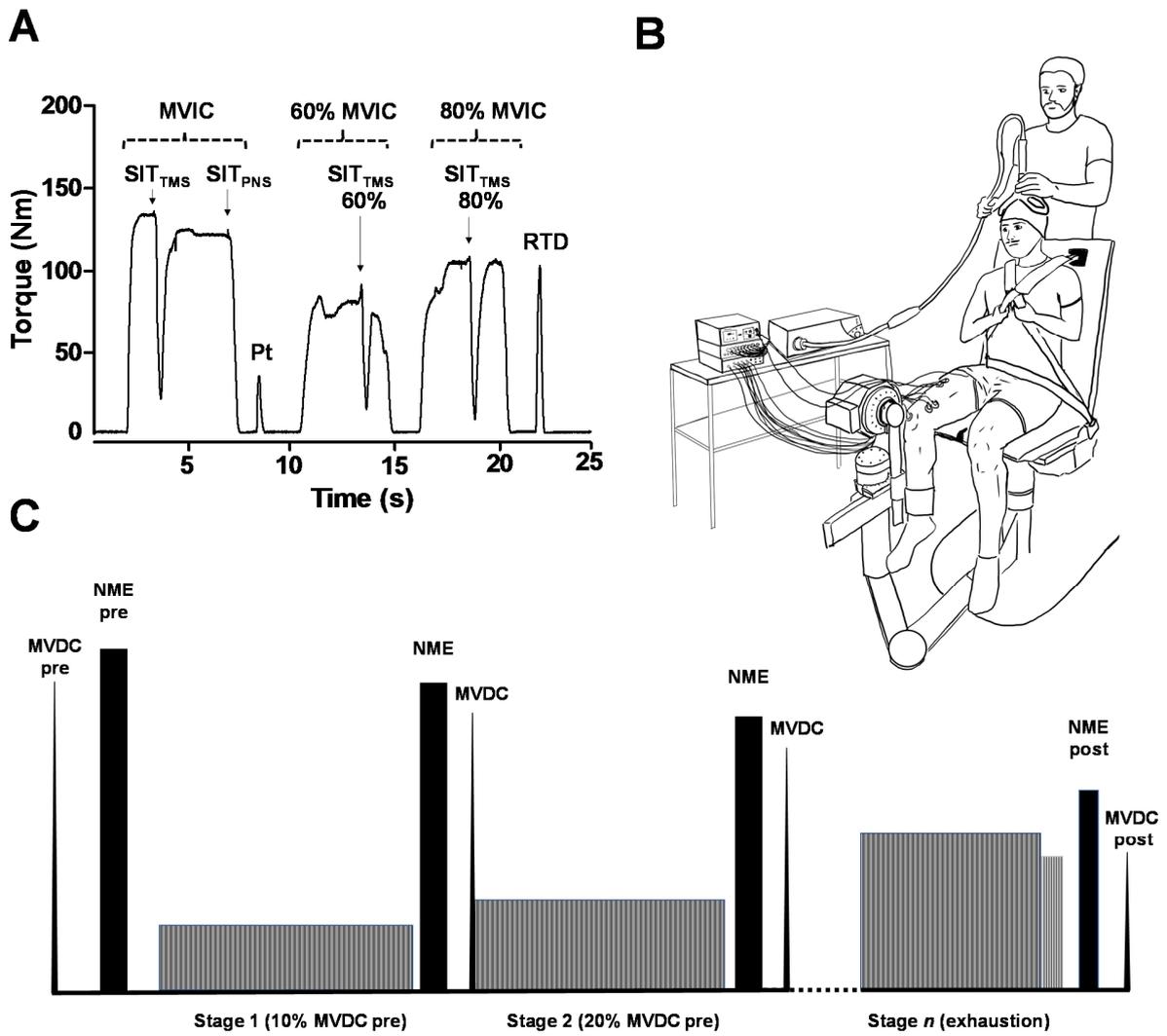
413 **Figure 2.** Comparison between sessions of maximal isometric (MVIC; A) and dynamic (MVDC; B) torque, rate
414 of torque development (RTD; C) and total work (D). *=significant difference from Pre for DYN1 (P<0.05);
415 #=significant difference from Pre for DYN2 (P<0.05).

416 **Figure 3.** Comparison between sessions of voluntary activation assessed with peripheral nerve stimulation
417 (VA_{PNS}; A), resting potentiated peak twitch (Pt; B) and normalized peak relaxation rate (pRRnorm; C).
418 *=significant difference from Pre for DYN1 (P<0.05); #=significant difference from Pre for DYN2 (P<0.05).

419 **Figure 4.** Comparison between sessions of the EMG evoked responses for the *vastus lateralis* (VL). A: Maximal
420 EMG root-mean-square (EMG-RMS) normalized to the amplitude of the M-wave evoked during the same
421 isometric maximal contraction (M_{sup}). B: Peak-to-peak amplitude of the motor evoked potential (MEP)
422 normalized to M_{sup} amplitude. C: Area of the MEP normalized to M_{sup} area. D: Silent period (SP). E: Amplitude
423 of the M-wave evoked at rest (Mmax). F: Area of the Mmax. *=significant difference from Pre for DYN1
424 (P<0.05); #=significant difference from Pre for DYN2 (P<0.05).

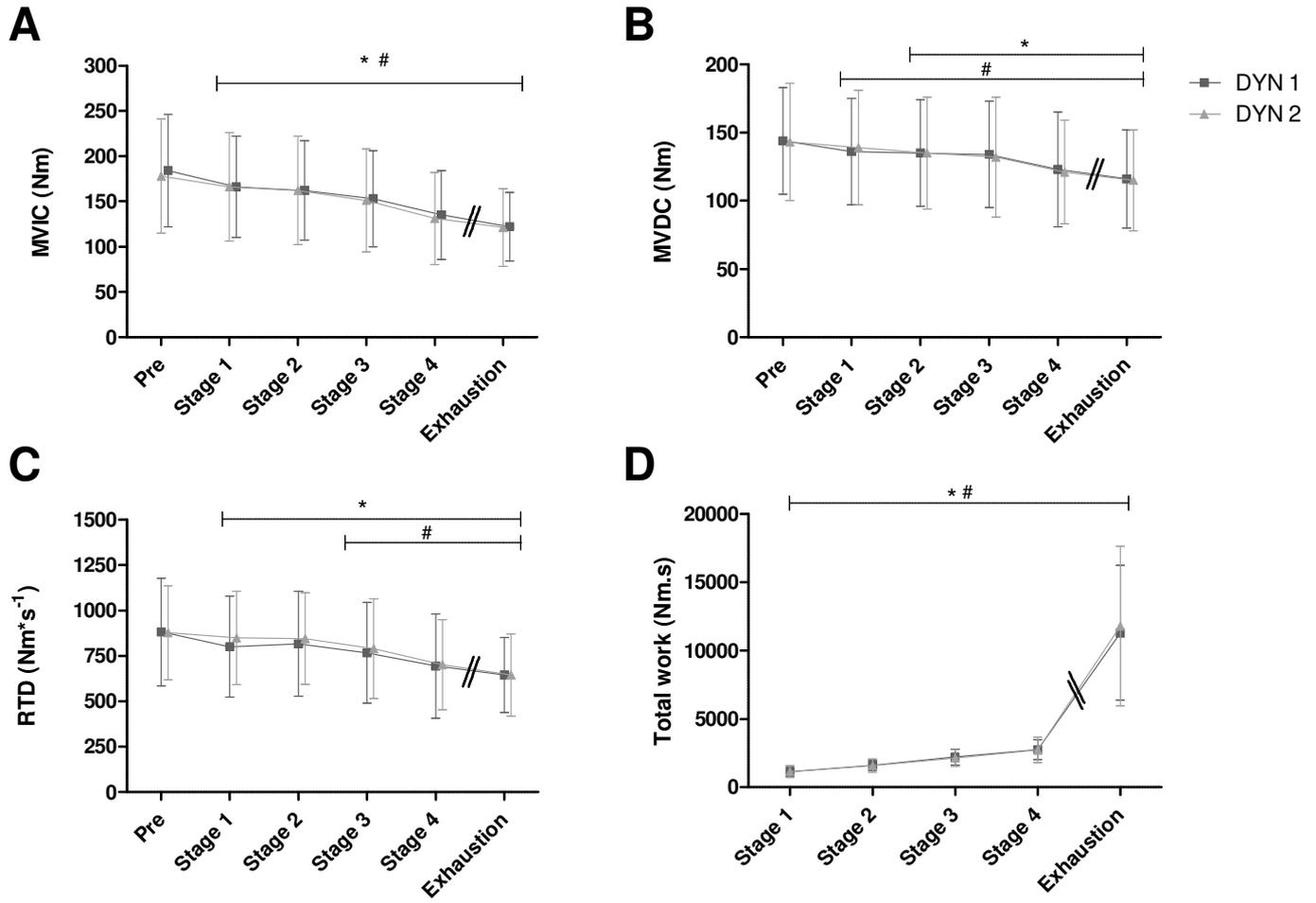
425 **Figure 5.** Bland-Altman plots of differences vs. mean of the two dynamic QIF tests (DYN1 and DYN2) for
426 maximal voluntary isometric contraction torque (MVIC) at the beginning of the test (A) and at task failure (B),
427 and maximal voluntary dynamic contraction torque (MVDC) at the beginning of the test (C) and at task failure
428 (D). Horizontal lines represent mean (Bias) and Upper and Lower Limits of Agreement (U-LoA and L-LoA
429 respectively; set as $\pm 1,96 \times$ standard deviation) $\pm 95\%$ CI.

430



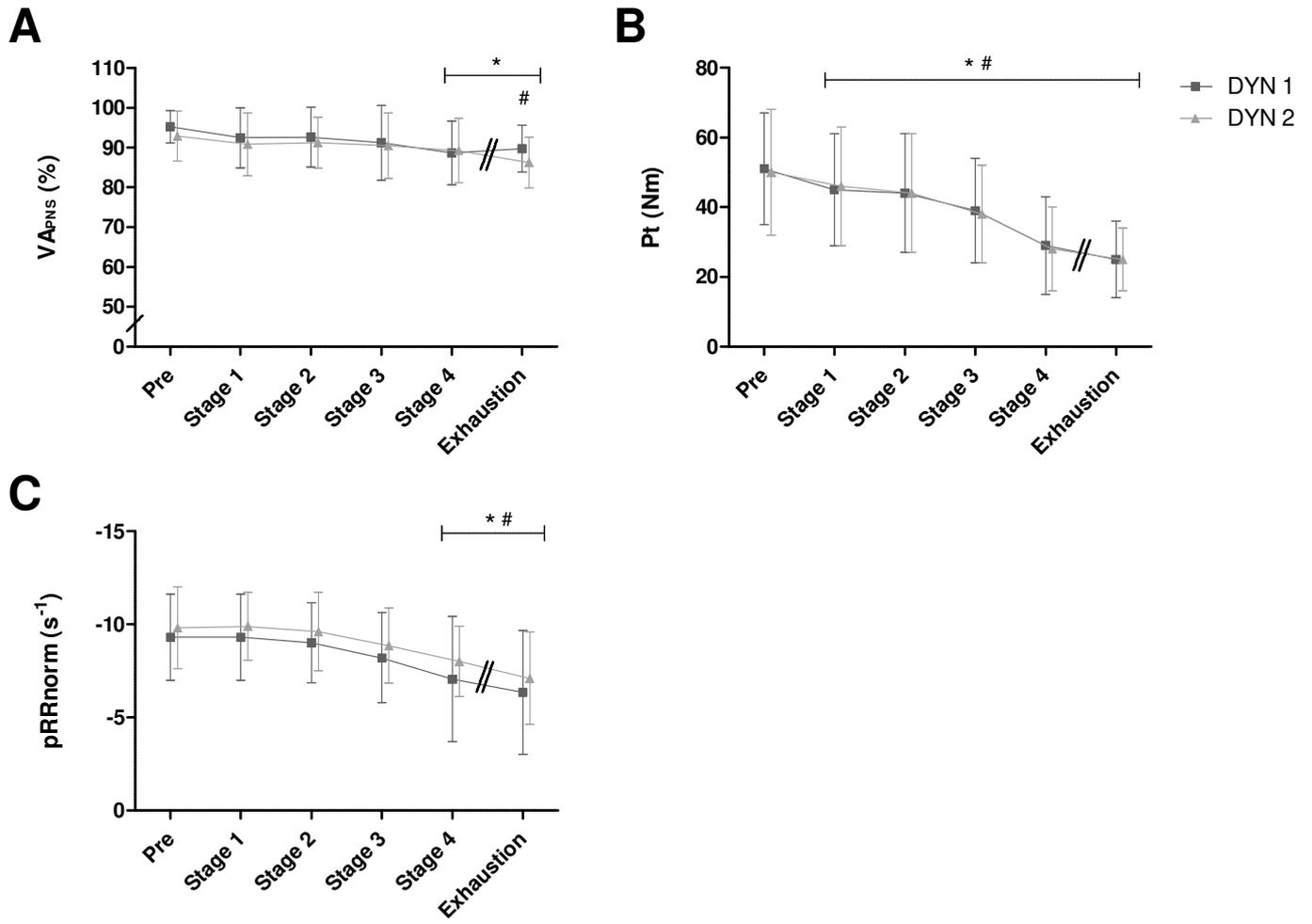
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434 Figure 2.



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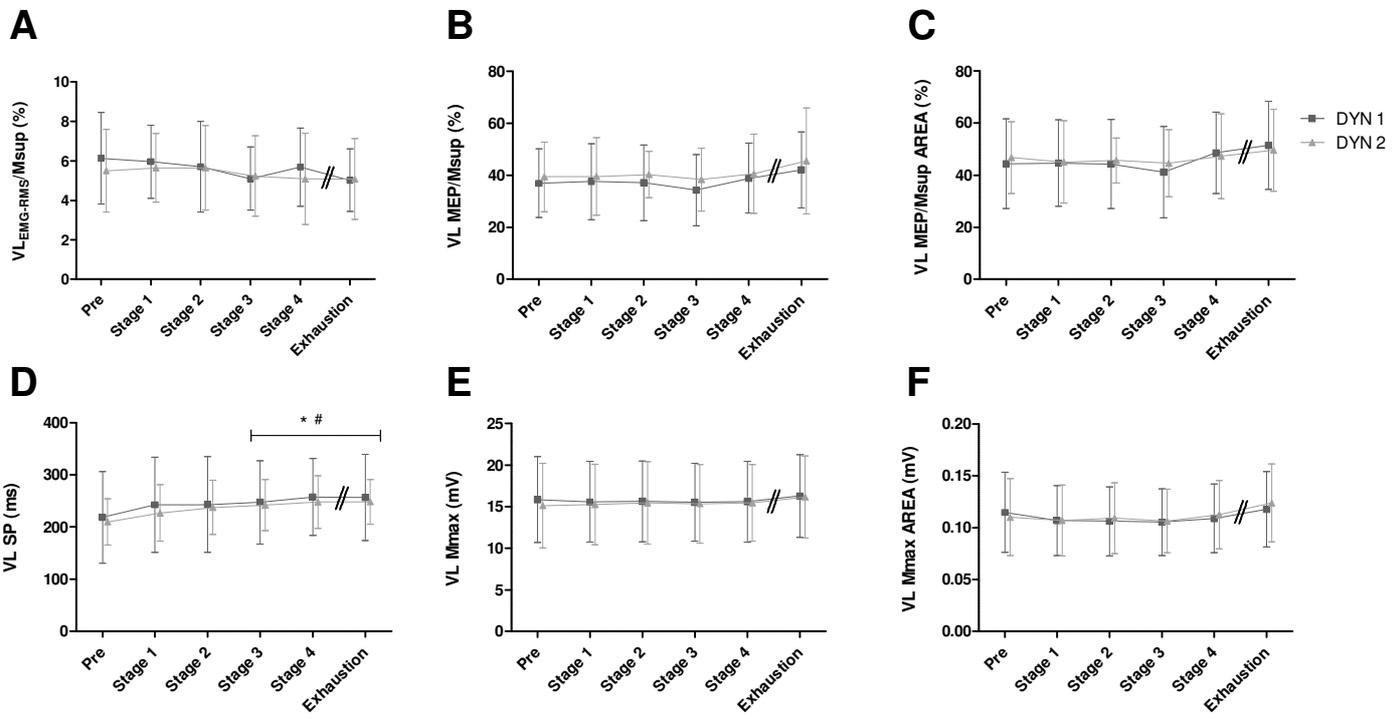
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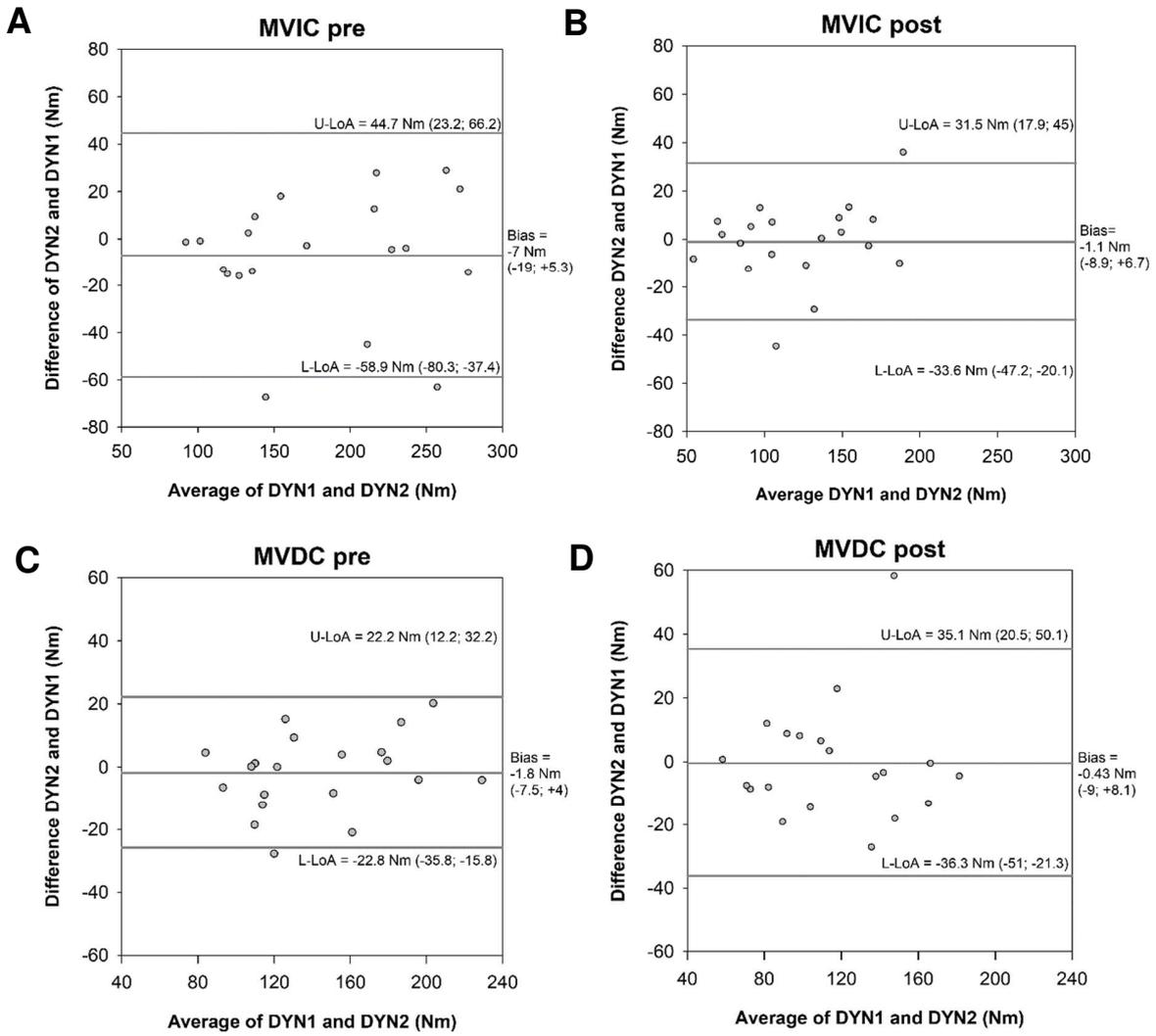


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446 **Electronic supplementary material.**

447 Reliability in men and women

| | Pre | Stage 4 | Exhaustion |
|---------------------|--------------------|--------------------|--------------------|
| MVIC | | | |
| <i>Men</i> | | | |
| <i>ICC (95% CI)</i> | 0.67 (0.10; 0.90) | 0.64 (0.28; 0.90) | 0.52 (0.12; 0.85) |
| <i>CV (95% CI)</i> | 6.6 (2.5; 10) | 5.2 (2; 8.3) | 5 (1.5; 8.4) |
| <i>Women</i> | | | |
| <i>ICC (95% CI)</i> | 0.71 (0.23; 0.91) | 0.46 (0.02; 0.83) | 0.43 (0.03-0.82) |
| <i>CV (95% CI)</i> | 6.8 (2.9; 10.6) | 7.4 (2.4; 12.3) | 6.2 (3.4; 9) |
| MVDC | | | |
| <i>Men</i> | | | |
| <i>ICC (95% CI)</i> | 0.92 (0.72; 0.98) | 0.56 (0.04; 0.87) | 0.08 (-0.65; 0.67) |
| <i>CV (95% CI)</i> | 4.1 (1.8; 6.4) | 6.2 (3.5; 8.9) | 8.3 (3.6; 13) |
| <i>Women</i> | | | |
| <i>ICC (95% CI)</i> | 0.70 (0.16; 0.90) | 0.62 (0.09; 0.89) | 0.29 (-0.44; 0.77) |
| <i>CV (95% CI)</i> | 5.6 (1.8; 9.3) | 6.8 (2.3; 11.5) | 8.8 (4.1; 13.6) |
| RTD | | | |
| <i>Men</i> | | | |
| <i>ICC (95% CI)</i> | 0.88 (0.61; 0.97) | 0.64 (0.04; 0.90) | 0.69 (0.19; 0.91) |
| <i>CV (95% CI)</i> | 6.2 (3; 9.4) | 7 (0.5; 13.5) | 9.3 (3.4; 15.2) |
| <i>Women</i> | | | |
| <i>ICC (95% CI)</i> | 0.85 (0.51; 0.96) | 0.42 (-0.30; 0.82) | 0.19 (-0.44; 0.71) |
| <i>CV (95% CI)</i> | 6 (3.3; 8.7) | 13 (0.5; 21.1) | 14.2 (1.8; 26.6) |
| VAPNS | | | |
| <i>Men</i> | | | |
| <i>CV (95% CI)</i> | 1.4 (0.5; 2.3) | 3.4 (-0.1; 8.5) | 5.1 (2.8; 7.5) |
| <i>Women</i> | | | |
| <i>CV (95% CI)</i> | 4.4 (0.8; 8) | 7.8 (3; 12.6) | 6.4 (3.7; 9.1) |
| Pt | | | |
| <i>Men</i> | | | |
| <i>ICC (95% CI)</i> | 0.87 (0.60; 0.97) | 0.86 (0.52; 0.96) | 0.80 (0.37; 0.95) |
| <i>CV (95% CI)</i> | 6.9 (2.1; 11.7) | 8.4 (2; 14.8) | 13.2 (5.4; 21.1) |
| <i>Women</i> | | | |
| <i>ICC (95% CI)</i> | 0.49 (-0.17; 0.85) | 0.64 (0.11; 0.89) | 0.55 (-0.04; 0.86) |
| <i>CV (95% CI)</i> | 11.8 (5.3; 18.4) | 22.2 (12.4; 31.9) | 20 (11; 29) |

448 MVIC: maximal voluntary isometric contraction torque; MVDC: maximal voluntary dynamic contraction; RTD:
 449 rate of torque development; VAPNS: voluntary activation assessed through peripheral nerve stimulation; Pt:
 450 potentiated resting twitch. CV: coefficient of variation; ICC: intraclass correlation coefficient.

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