# 1 SUPPLEMENTAL MATERIALS

# 2 APPENDIX A

#### **3** Detailed methodology for the assessment of the neuromuscular function

4 A.1 Countermovement jumps height

5 For each trial of the CMJ, participants performed a set of 3 jumps (separated by ~10 s) to assess jump height.

6 CMJs were performed with hands on the hips. The highest value recorded for each set was retained for

- 7 further analyses.
- 8

#### 9 A.2 Maximal torque, voluntary activation and contractile function

For all maximal contractions, participants were instructed to extend the knee "as hard as possible". During 10 11 the contraction, the twitch interpolation technique was used to measure the voluntary activation and 12 contractile function (except after the third match). The interpolated twitch technique included a first 13 supramaximal 100-Hz doublet that was superimposed when torque reached a plateau (superimposed 14 doublet), and a second doublet (potentiated doublet) that was delivered at rest two seconds after the maximal 15 contraction. Stimulation intensity was determined at rest by increasing the stimulation intensity by 10 mA starting from 30 mA until the twitch torque response plateaued. The 130% of the intensity producing the 16 17 greatest peak torque response was used to ensure supramaximal twitch response (average stimulation 18 intensity =  $181\pm 28$  mA). For the analysis, the maximal torque achieved during the maximal contraction was determined as the highest peak torque recorded before the superimposed doublet. Voluntary activation was 19 20 calculated using the formula of Strojnik and Komi (1998):

21

$$Voluntary activation (\%) = 100 - \left[ \frac{Superimposed doublet \times \left( \frac{Torque_{prestim}}{Maximal Torque} \right) \times 100}{Potentiated doublet} \right]$$

22

where Torque<sub>pre stim</sub> is the voluntary torque level just before the stimulation. To minimize measurement error,
all participants that showed <70% of voluntary activation at baseline were discarded (Place et al. 2007).</li>
Changes in potentiated doublet were used as an index of changes in contractile function.

26

#### 27 A.3 Rate of torque development

Participants were instructed to extend the knee "as fast and strong as possible" with a focus on fast for allrapid contractions. The contraction was repeated in case of countermovement or pre-tension, determined by a

30 torque  $\leq 2$  N·m and  $\geq 2$  N·m right before the rapid contraction onset, respectively. The rapid contractions 31 were also repeated if the force level was <70% of the maximal torque measured during the maximal 32 isometric contraction that preceded the series of rapid contractions (Varesco et al. 2019, 2022). Visual 33 feedback of torque responses was provided on a computer monitor. The analysis for the rate of torque 34 development was performed as previously described (Varesco et al. 2019, 2022). Briefly, the best five rapid contractions were determined based on the peak rate of torque development, i.e. the steepest 10-ms segment 35 36 on the force-time curve. These five contractions were averaged for further analysis, while the others were 37 discarded. For each contraction, a nonlinear least-square model was fitted on the first 200 ms torque data 38 from the onset. The slope coefficient calculated from the model was used to quantify the rate of torque 39 development, indicating the rapid force production ability. The onset was automatically defined as the point 40 at which force raised over the average resting baseline by 2 N·m. This onset was also checked visually by an 41 experienced investigator.

#### 42 A.4 Experimental apparatus

Participants sat upright on an isometric chair with the knee and the torque meter rotational axes aligned 43 44 (ARS dynamometry, SP2, Ltd., Ljubljana, Slovenia), the hip and the knee positioned at 90° and 120° 45 extension, respectively (180°=full extension), and the leg attached just above to the malleoli using a non-46 compliant strap. Hips and chest were securely strapped to maintain the position during contractions. The 47 position was recorded and reproduced for each athlete between days. Torque data were sampled at 2 kHz 48 using a PowerLab system (16/30-ML880/P, ADInstruments, Bella Vista, Australia), and transmitted to the 49 computer through Labchart 8 interface (ADInstruments). For the electrical stimulations, two self-adhesive 50 surface electrodes (80×130 mm, Stimex electrodes, Pierenkemper, Wetzlar, Germany) were placed over the 51 rectus femoris and over the vastus medialis portions to allow the stimulation of the quadriceps femoris muscle (Zarkou et al. 2017). Electrical stimuli of 2-ms duration and 400 V output voltage were delivered via 52 53 a constant-current stimulator (DS7A; Digitimer, Welwyn Garden City, Hertfordshire, UK), similarly to what 54 was previously reported to improve accuracy in the measured outcomes (Zarkou et al. 2017). The position of each participant on the chair was registered and reproduced between neuromuscular function evaluations. 55 56 The electrodes position was marked on the skin with a permanent marker to be kept consistent between tests. 57 The performance during CMJs, i.e. jump height, was measured using an optoelectronic system 58 (OptojumpNEXT, Microgate, Bolzano, Italy) and exported using its native software (Optojump v.1.12.23, Microgate). The knee-extensors neuromuscular data were analyzed offline using Labchart 8 and exported to 59 60 excel (Excel v.2206, Microsoft, Redmond, Washington) and R-studio (v. 2022.07.1.554, Boston, MA) to 61 perform calculations and statistical analysis.

62

#### 63 Detailed methodology for the assessment of the subjective variables

64 A.5 Perception of Fatigue and Effort

The question for the visual analog scale for fatigue was 'How fatigued are you right now?', and the anchors were 'Not fatigued at all' and 'extremely fatigued'. The question of the visual analog scale for effort was 'How much effort did you put into performing in the round you just completed?', and the anchors were 'no effort' and 'maximal effort'. Data from the visual analog scales were manually measured (from 0 to 10 cm).

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#### 70 A.6 Perceived workload

71 Administering the NASA<sub>TLX</sub> questionnaire involves participants rating each of the six dimensions (Mental demand, Physical demand, Temporal demand, Effort, Performance and Frustration level) on scales from 72 "Low" to "High", or from "Good" to "Poor" in the case of Performance (Hart 2006). The raw score for each 73 74 of the six items could be multiplied by the weight obtained from an additional questionnaire to generate an overall workload score. However, being highly time-consuming, this procedure has been skipped across 75 several studies.<sup>6</sup> Because participants needed to report to the neuromuscular testing stand as soon as possible 76 77 once the bout was over, we administered only the rating questionnaire. Data were obtained for each 78 dimension of the NASA<sub>TLX</sub> expressed as a scale from 0 to 100, with each one of the 20 squares corresponding to 5-points. For clarity, data for the item "performance" were reversed, so "0" corresponded to 79 the worst performance possible and "100" to a perfect performance. 80

81

#### 82 Detailed methodology for statistics

#### 83 A.7 Statistical analysis: model fitting and assumptions

84 Given the dependence of the data for the participants, a random intercept for participants was built into each model. The empirical test of the model assumptions was performed via model residuals graphical analysis of 85 86 the Q-Q plots, that allowed also the detection of eventual outliers. Shapiro-Wilk test was used to ensure that 87 the assumption of normality was respected for the residuals and random effects. Simulated residuals 88 [DHARMa package (Hartig 2018)] were used when adopting glmmTMB. The build models were reduced 89 when no main effect of time, weapon, sex, or result was observed, accordingly to the Occam razor principle, 90 and compared using Akaike Information Criteria (AIC). Day-by-day reliability and agreement were 91 evaluated for CMJ, maximal torque, rate of torque development, voluntary activation and potentiated doublet 92 (familiarization session data vs. pre-competition data of the testing session; procedures and results are 93 available in Supplemental Materials 2). Finally, to evaluate if maximal torque and rate of torque 94 development were linked to voluntary activation, these variables were modelled in the function of all voluntary activation data obtained (i.e. not filtered for values at rest >70%; detailed analysis and results 95 96 presented in Supplemental Materials 3).

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# 118 APPENDIX B

TOTAL = 29 athletes	Bout 1			Bout 2			Bout 3			Bout 4*			Bout 5 <sup>#</sup>		
Period	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Athletes (n)	29	29	15	29	29	13	29	29	15	28	26	11	25	22	7

119 Simulated competition: number of athletes that disputed each bout.

120 *Notes:* \*one athlete did not dispute the  $4^{th}$  bout due to pain. <sup>#</sup>four athletes did not dispute the  $5^{th}$  bout due to

121 *pain*.

# 123 APPENDIX C

#### 124 Effect of victory or defeat on the NASA<sub>TLX</sub> dimensions



126The estimated density function for each dimension of the NASA<sub>TLX</sub> (all bouts pulled together) separated by127the result of the bouts. Dotted lines represent the mean. \*=difference in density distribution between victory128and defeat (P < 0.05).

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## 131 APPENDIX D

#### 132 Reliability analysis and results

- 133 Day-by-day reliability and agreement were evaluated for CMJ, maximal torque, rate of torque development,
- voluntary activation, and potentiated doublet (familiarization session data vs. pre-competition data of the
- testing session) using intra-class correlation coefficients [ICCs (A,1); Two-way mixed model (Koo and Li,
- 136 2016); irr package (Gamer et al. 2012)] and coefficients of variation (CVs), respectively, both presented with
- 137 95% confidence intervals (95% CI). Based on the ICC estimate, values between 0 and 0.50 were considered
- poor, 0.50–0.75 Moderate, 0.75–0.90 good, and >0.90 excellent (Koo and Li 2016). As a rule of thumb, CVs
- 139 were considered high and low, respectively, for values greater and lower than 10%.
- Agreement and reliability for CMJ were high and excellent, respectively [CV = 3% (2%; 4%); ICCA, 1 =
- 141 0.96 (0.93; 0.98);], being not different between days (P=0.94). Maximal torque was different across days
- 142 (P=0.02), being greater during the familiarization session than the day of the bout at baseline  $(313 \pm 64 \text{ N} \cdot \text{m})$
- 143 vs.  $275 \pm 61$  N·m), consequently, the agreement was on average low [CV = 11% (8%; 14%)] and reliability
- 144 moderate [ICCA, 1 = 0.68 (0.07; 0.88)]. The rate of torque development was not different between days
- 145 (P=0.05), with low agreement [CV = 15% (10%, 20%)] and moderate reliability [ICCA, 1 = 0.50 (0.14;
- 146 (0.73)]. Agreement and reliability for potentiated doublet were high and good, respectively [CV = 9% (5%;
- 147 12%); ICCA, 1 = 0.83 (0.66; 0.92);], being not different between days (P=0.88). Voluntary activation was
- different across days (91  $\pm$  5% vs. 84  $\pm$  6%; P=0.008), the agreement was high [CV = 6% (3%, 9%)] and
- 149 reliability was poor [ICCA, 1 = 0.05 (0; 0.45)].
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### 158 APPENDIX E

#### 159 Association between maximal torque, rate of torque development and voluntary activation

160 To evaluate if maximal torque and rate of torque development were linked to low voluntary activation,

- 161 maximal torque and rate of torque development obtained were modelled in the function of all voluntary
- activation data obtained (*i.e.* not filtered for values at rest >70%). We found a significant exponential
- 163 function for both maximal torque (P<0.001) and rate of torque development (all P=0.002), and a Pearson'R
- 164 coefficients (obtained on log-transformed data) of 0.57 ( $t_{(0.53)} = 5.1$ , P < 0.001; strong correlation) and 0.37
- 165  $(t_{(0.53)} = 2.9, P = 0.006; moderate correlation)$  respectively (figure below). Additionally, potentiated doublet
- and voluntary activation were not correlated (R = 0.09;  $t_{(0,53)} = 0.6$ , P = 0.52). This would indicate that low
- 167 reliability in maximal torque and rate of torque development (see Supplemental Materials 2) was associated
- 168 with low voluntary activation in participants, which could be either due to measurement error such as
- submaximal volitional effort during contractions (in the case of values <70%, for example) or to a real deficit
- in voluntary activation.

