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1 **Original article**

2 **Maturation-related changes in the development and etiology of**
3 **neuromuscular fatigue**

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21 **ABSTRACT**

22 **Purpose:** The aim of the present study was to investigate the role of maturation on the etiology
23 of neuromuscular fatigue induced by repeated maximal voluntary isometric contractions
24 (MVIC).

25 **Methods:** Nine prepubertal boys (9.9 ± 1.3 yr.), eight male adolescents (13.6 ± 1.3 yr.) and
26 eleven men (23.4 ± 3.0 yr.) performed a series of repeated isometric MVICs of the knee
27 extensors until the MVIC torque reached 60% of its initial value. Magnetic stimulations were
28 delivered to the femoral nerve every five MVICs to follow the course of voluntary activation
29 level (VA) and the potentiated twitch torque ($Q_{tw_{pot}}$).

30 **Results:** Task failure was reached after 52.9 ± 12.7 , 42.6 ± 12.5 and 26.6 ± 6.3 repetitions in
31 boys, adolescents and men, respectively. VA remained unchanged in men whereas it decreased
32 significantly and similarly in boys and adolescents ($p < 0.001$). In contrast, $Q_{tw_{pot}}$ remained
33 unchanged in boys and decreased significantly less in adolescents than adults ($p < 0.05$).

34 **Conclusions:** Children and adolescents experience less peripheral and more central fatigue than
35 adults. However, adolescents experience more peripheral fatigue than children for a comparable
36 amount of central fatigue. This finding supports the idea that the tolerance of the central nervous
37 system to peripheral fatigue could increase during maturation.

38 **Keywords:** Adolescent, central fatigue, peripheral fatigue, electromyography, peripheral
39 magnetic stimulation

40 **Abbreviations:**

41 %CoAct: Level of antagonist co-activation

42 %REP: Percentage of the number of repetitions

43 η^2 : Partial eta-squared

44 ANOVA: Analysis of variance

45 APHV: Age from peak height velocity

46 BF: Biceps femoris

47 CI: Confidence interval

48 EMG: Electromyography

49 KE: Knee extensors

50 M_{max} : Maximal M-wave amplitude

51 MVIC: Maximal voluntary isometric contractions

52 M-wave: Compound action potential

53 $Q_{tw_{pot}}$: Potentiated twitch torque

54 Q_{tw_s} : Superimposed twitch torque

55 $Q_{tw_{unpot}}$: Unpotentiated twitch torque

56 RF: Rectus femoris

57 RMS: Root Mean Square

58 VA: Voluntary activation level

59 VL: Vastus lateralis

60 INTRODUCTION

61 Neuromuscular fatigue is commonly defined as “any exercise-induced reduction in the ability
62 of skeletal muscle to produce force or power irrespective of task completion” (Bigland-Ritchie
63 and Woods 1984). Historically, potential factors involved in neuromuscular fatigue were
64 classified into two categories, (i) central factors involving the central nervous system and neural
65 pathways, and (ii) peripheral factors occurring within the muscle, beyond the neuromuscular
66 junction (Gandevia 2001). As a result, it is possible to distinguish both central, *i.e.* neural, and
67 peripheral, *i.e.* muscular fatigue. As the neuromuscular system is highly adaptable (Enoka and
68 Stuart 1992), neuromuscular fatigue varies in response to different conditions. For instance,
69 numerous studies examined the differential effect of age (Streckis et al. 2007), ageing (Kent-
70 Braun et al. 2002), sex (Hunter et al. 2004), training status (Mira et al. 2018) and the mode of
71 contraction (Souron et al. 2018) on neuromuscular fatigue. However, less attention has been
72 paid to the impact of maturation on the development and etiology of neuromuscular fatigue.

73 Current knowledge suggests that fatigue increases progressively from childhood to adulthood
74 during intermittent whole-body dynamic activities or repeated maximal voluntary contractions.
75 For instance, during repeated cycling sprints, the decline of peak power output is higher in
76 adolescents than children and lower than adults (Ratel et al. 2002). This finding has been
77 confirmed during a series of isokinetic maximal voluntary contractions of the knee extensors
78 (KE) and knee flexors (Zafeiridis et al. 2005; Dipla et al. 2009). Similarly, when muscle
79 contractions include an eccentric phase (*i.e.* a stretch of the active muscle), the decline of peak
80 torque during exercise is higher in adolescents than children and lower than adults (Chen et al.
81 2014). Moreover, the magnitude of symptoms appearing during the days following a series of
82 eccentric contractions (*i.e.* stiffness, oedema, decreased range of motion) is higher in adults
83 than adolescents, and higher in adolescents than children (Chen et al. 2014).

84 However, it is still unclear whether the contribution of central and peripheral factors to
85 neuromuscular fatigue may differ throughout maturation since no objective comparison of
86 central *vs.* peripheral fatigue has been performed between prepubertal children, pubertal
87 children (*i.e.* adolescents) and adults. To date, only Streckis et al. (2007) reported a lower
88 peripheral fatigue and a higher central fatigue during a sustained 2-min maximal voluntary
89 isometric contraction (MVIC) of the KE muscles in 12-14-year old boys and girls than men and
90 women. However, in that study, no objective measurement of maturity status (*i.e.* biological
91 age) was done, hence limiting our knowledge regarding the role of maturation on the etiology
92 of neuromuscular fatigue. Despite this lack of information, peripheral fatigue could be higher
93 in adolescent individuals than children, but lower in adolescents than adults because of (i) the
94 progressive increase in the strength/power-generating capacity (Tonson et al. 2008) and (ii)
95 potential changes in the metabolic profile throughout maturation, e.g. transformation of slow-
96 twitch fibers into fast-twitch fibers (Lexell et al. 1992) and progressive increase in the capacity
97 of the anaerobic energy turnover over maturation (Ratel and Blazevich 2017). Indeed, it has
98 been shown that the higher neuromuscular fatigue of healthy men *vs.* women was no longer
99 observed when subjects were matched for absolute MVIC force (Hunter et al. 2004) suggesting
100 that the higher the MVIC force, the higher the muscle fatigue. In addition, the amount of
101 peripheral fatigue was found to be greater in individuals with predominantly fast-twitch fibers
102 (Hamada et al. 2003) and relying more on anaerobic than aerobic energy turnover as in
103 explosive power-trained athletes (Garrandes et al. 2007). Taken together, these factors could
104 promote a faster and/or greater development of peripheral fatigue during repeated maximal
105 contractions in adolescents than children but a lower peripheral fatigue in adolescents than
106 adults. Consequently, the development of strength/power and the transformation of slow-twitch
107 into fast-twitch fibers over maturation could be associated with a progressive reduction of time
108 to task failure from childhood to adulthood. As central fatigue is mainly promoted by prolonged

109 exercise duration (Thomas et al. 2015), this could translate into a lower central fatigue in
110 adolescents than children as well as in adults than adolescents.

111 Therefore, the purpose of the present study was to verify these assumptions by examining the
112 consequences of maturation on the development and etiology of neuromuscular fatigue induced
113 by repeated voluntary maximal contractions.

114 **MATERIAL AND METHODS**

115 **Participants**

116 A total of nine 8-11 years old boys (Tanner stage 1-2), eight 13-16 years old male adolescents
117 (Tanner stage 3-4) and eleven 18-25 years old healthy men were recruited. All the participants
118 were involved in different physical activities such as rugby, basketball, swimming, etc. To be
119 included, participants had to exercise less than 4 hours per week and to be free of any medical
120 contra-indication to physical activity. The present study was approved by the local Ethics
121 Committee (Protection Committee of People for Biomedical Research South East VI;
122 Authorization Number AU929). All the participants were fully informed of the experimental
123 procedures and gave their written consent before any testing was conducted. In addition, the
124 written consent of the parents/guardians was also obtained for the children and adolescents.

125 **Experimental procedure (design)**

126 All participants were tested on three experimental sessions separated by at least 1 week. The
127 first session was dedicated to collecting participants' physical characteristics, familiarization
128 with the experimental procedures and clinical examination by a medical practitioner or a
129 pediatrician. During the second session, the participants were asked to perform MVIC of the
130 KE muscles at different knee angles (30, 40, 50, 60, 70, 75, 80, 85, 90, 100, 110°; 0° = full
131 extension) in a randomized order to determine the optimal angle for maximal torque production.
132 Participants performed two MVICs at each knee angle. Finally, during the third session, all the
133 participants performed the intermittent voluntary fatigue protocol at the optimal knee angle. All
134 sessions were done in a temperate room (18-22°C).

135 **Anthropometric measurements**

136 Body mass was measured to the nearest 0.1 kg using a calibrated scale and height was
137 determined to the nearest 0.01 m using a standing stadiometer. Height and body mass were
138 measured without shoes and while wearing underwear only. Sitting height was also measured
139 while the participants sat on the floor against a wall, using the same stadiometer. Body mass
140 index was calculated using a standard formula, mass divided by height squared ($\text{kg}\cdot\text{m}^{-2}$). Body
141 fat and fat-free mass were determined using dual-energy X-ray absorptiometry (HOLOGIC,
142 QDR-4500, Hologic Inc, Bedford, Massachusetts, USA).

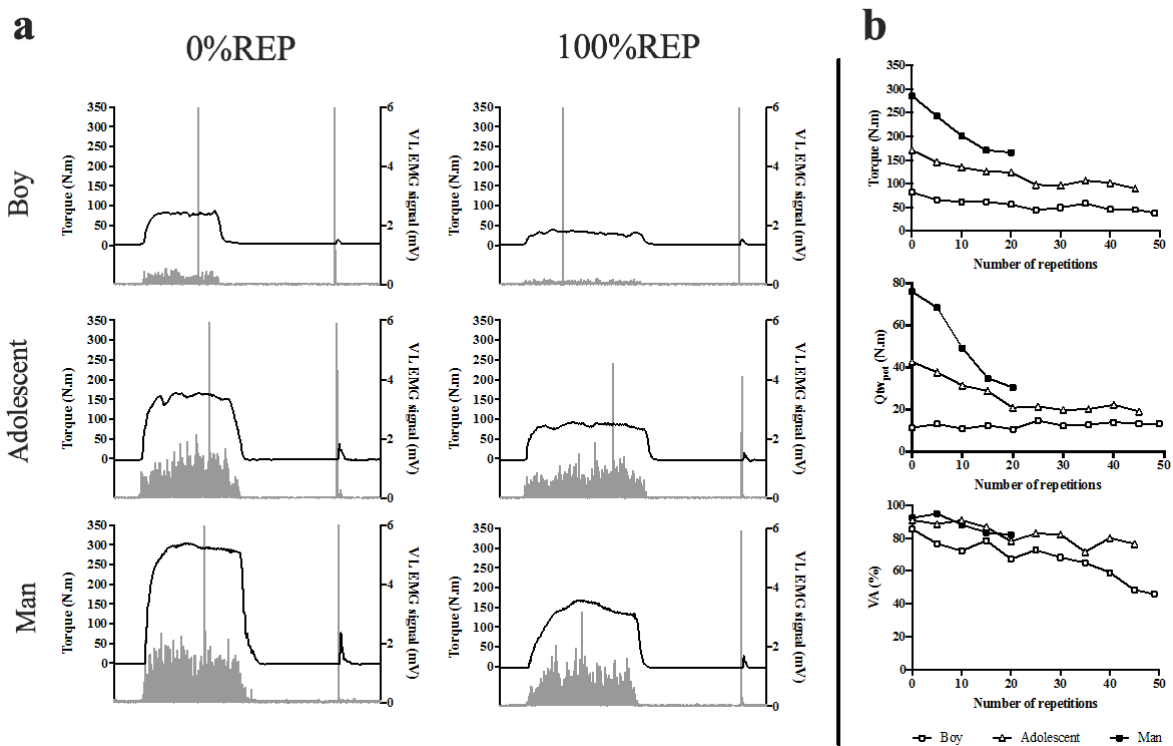
143 **Maturity status assessment**

144 Two methods were used to assess children and adolescents' maturity status: 1) Tanner stages
145 were determined from self-reported assessment based on pubic hair and testicular/penis
146 development (Tanner and Whitehouse 1976), the children and adolescents being assisted by
147 their parents while completing the questionnaire; 2) Age from peak height velocity (APHV)
148 was used to assess somatic maturity and determined by using height, sitting height and body
149 mass (Mirwald et al. 2002).

150 **Intermittent voluntary fatigue protocol**

151 Participants performed an intermittent voluntary fatigue protocol consisting of a repetition of
152 isometric 5-s MVICs of the KE muscles interspersed with 5-s passive recovery periods until
153 the voluntary torque failed to reach a target value of 60% of its initial value over three
154 consecutive MVICs. The participants had no visual feedback of torque output during the

155 exercise. However, they were strongly encouraged by the investigators during the entire
 156 fatiguing task. During the fatigue protocol, all participants were allowed to drink water ad
 157 libitum. The knee joint was fixed at the optimal angle for maximal torque production ($75.6 \pm$
 158 5.3° , $90.0 \pm 0.0^\circ$ and $81.8 \pm 7.5^\circ$ in boys, adolescents and men, respectively; $p < 0.001$), which
 159 was determined from the torque-angle relationship during the second visit. The number of
 160 repetitions was considered as the performance criteria to quantify neuromuscular fatigue. Single
 161 magnetic stimulations were delivered to the femoral nerve every five MVICs to determine the
 162 maximal level of voluntary activation (VA) by using the twitch interpolation technique (see
 163 below for further details). The electromyographic (EMG) activity of the *vastus lateralis* (VL)
 164 and *rectus femoris* (RF) muscles was recorded during the entire fatigue protocol. The amplitude
 165 of the potentiated twitch torque ($Q_{tw_{pot}}$; see below) and VL and RF concomitant compound
 166 action potential amplitudes (maximal M-wave; M_{max}) were considered as indicators of
 167 peripheral fatigue. The time-course of VA and normalized EMG (see below) of the VL and RF
 168 muscles throughout the protocol were considered as indexes of central fatigue. Examples of
 169 raw data (MVIC torque and VL EMG; panel A) and time-courses of MVIC torque, $Q_{tw_{pot}}$ and
 170 VA during the fatigue protocol in a typical boy, adolescent and man are presented in Figure 1.



171 **Fig. 1:** (a) Example of raw data for MVIC torque and VL EMG obtained at 0%REP and
 172 100%REP in a typical boy (top), adolescent (middle), and man (bottom). (b) Example of time-
 173 courses of MVIC torque (top), $Q_{tw_{pot}}$ (middle), and VA (bottom) during the intermittent fatigue
 174 protocol in a typical boy, adolescent and man.

175 Torque measurements

176 Voluntary and evoked contractions were assessed in isometric condition with an isokinetic
 177 dynamometer (Cybex Norm, Lumex, Ronkonkoma, NY, USA). Participants were comfortably
 178 positioned on an adjustable chair with the hip joint flexed at 30° ($0^\circ =$ neutral position). The
 179 dynamometer lever arm was attached 1-2 cm above the lateral malleolus with a Velcro strap.
 180 The axis of rotation of the dynamometer was aligned with the lateral femoral condyle of the
 181 right femur. Torque data were corrected for gravity using the Cybex software and were digitized

182 to an external A/D converter (Powerlab 8/35, ADInstruments, New South Wales, Australia)
183 driven by the Labchart 7.3 Pro software (ADInstruments, Australia).

184 **Femoral nerve stimulation**

185 Evoked contractions of the KE muscles were triggered by a single magnetic stimulus, delivered
186 to the femoral nerve using a 45-mm figure-of-eight coil connected to a magnetic stimulator
187 (Magstim 2002, peak magnetic field strength 2.34 T, stimulation duration 0.1 ms; MagstimCo,
188 Whiteland, Dyfed, UK). The coil was placed high in the femoral triangle in regard of the
189 femoral nerve. Small spatial adjustments were initially performed to determine the optimal
190 position where the greatest unpotentiated KE twitch amplitude ($Q_{tw_{unpot}}$) and the greatest VL
191 and RF M_{max} were evoked. The optimal stimulation intensity, *i.e.* the intensity where maximal
192 twitch and concomitant M-waves amplitudes started to plateau, was determined from a
193 recruitment curve. $Q_{tw_{unpot}}$ and M_{max} plateaued at $85.0 \pm 4.3\%$, $84.4 \pm 7.8\%$ and $85.0 \pm 6.7\%$
194 of the stimulator power output for prepubertal boys, adolescents and men, respectively. In order
195 to overcome the potential confounding effect of axonal hyperpolarization (Burke 2002), the
196 stimulation intensity was set to 100% of the stimulator output during the subsequent testing
197 procedures (*i.e.* voluntary intermittent fatigue protocol). This intensity corresponded to $117.9 \pm$
198 6.0% , $119.5 \pm 11.7\%$ and $118.4 \pm 10.1\%$ of the optimal intensity in the boys, adolescents and
199 men, respectively, and was not significantly different between groups. This supramaximal
200 intensity of stimulation ($\approx 120\%$) has been demonstrated as being optimal for an adequate
201 assessment of central and peripheral fatigue of the KE muscles (Neyroud et al. 2014).

202 **Voluntary activation level**

203 To determine VA, the twitch interpolation was used. Briefly, a superimposed (Q_{tw_s}) and a
204 potentiated ($Q_{tw_{pot}}$) single twitch were delivered during MVIC after the torque had reached a
205 plateau, and 3-s after the cessation of the contraction, respectively. These superimposed and
206 potentiated mechanical amplitudes allowed the quantification of VA (%VA) as proposed by
207 Merton (Merton 1954) (Equation 1):

$$208 \quad VA = \left(1 - \frac{Q_{tw_s}}{Q_{tw_{pot}}}\right) \cdot 100 \quad (\text{Equation 1})$$

209 **EMG recordings**

210 The EMG signals of the VL, RF and *biceps femoris* (BF) muscles were recorded using bipolar
211 silver chloride surface electrodes (Blue Sensor N-00-S, 30 x 22 mm, Ambu, Denmark) during
212 voluntary and evoked contractions. The recording electrodes were taped lengthwise on the skin
213 over the muscle belly, as recommended by SENIAM (Surface ElectroMyoGraphy for the Non-
214 Invasive Assessment of Muscles) (Hermens et al. 2000) with an inter-electrode distance of 20
215 mm. The reference electrode was attached to the patella. Low impedance ($Z < 5 \text{ k}\Omega$) at the skin-
216 electrode surface was obtained by shaving, gently abrading the skin with thin sand paper and
217 cleaning with alcohol. EMG signals were amplified (Dual Bio Amp ML 135, ADInstruments,
218 Australia) with a bandwidth frequency ranging from 10 Hz to 500 Hz (common mode rejection
219 ratio $> 85 \text{ dB}$, gain = 1,000) and simultaneously digitized together with the torque signals (2
220 kHz). During the course of the fatigue protocol, Root Mean Square (RMS) values of the VL
221 and RF EMG activity were calculated during the MVIC trials over a 0.5-s period before the
222 superimposed stimulation. This RMS value was then normalized to the maximal peak-to-peak
223 amplitude of the potentiated VL and RF M-waves ($\text{RMS} \cdot M_{max-1}$).

224 **Antagonist co-activation**

225 The level of antagonist co-activation (%CoAct_{BF}) of the BF muscle was computed as the BF
226 EMG activity during knee extensions, normalized to the maximal BF EMG activity recorded
227 during a maximal knee flexion at the optimal angle for maximal KE torque production
228 (Equation 2). To record this maximal BF RMS value, the participants were asked to perform 3-

229 s MVICs of the knee flexors before the fatigue protocol. This measurement was repeated twice,
230 and the best trial was used for subsequent analysis.

$$231 \quad \%CoAct = \frac{RMS_{anta}}{RMS_{ago}} \cdot 100 \quad (Equation 2)$$

232 Where RMS_{anta} is the RMS value of BF during intermittent contractions, and RMS_{ago} is the RMS
233 value of BF during maximal voluntary knee flexion, recorded before the fatigue protocol.

234 **Statistical analysis**

235 The participants were categorized according to their age and maturity level. There were three
236 groups (prepubertal boys, adolescents and men). Therefore, we considered the group as an
237 independent categorical variable while the other variables (i.e. neuromuscular parameters) were
238 dependent variables.

239 All variables measured during the intermittent fatigue protocols were linearly interpolated
240 between the nearest values at 20%, 40%, 60%, and 80% of number of repetitions (%REP) to
241 fairly compare the different groups. Values at 0%REP and 100%REP corresponded to pre- and
242 post-fatigue values, respectively.

243 Data were screened for normality of distribution and homogeneity of variances using a Shapiro-
244 Wilk normality test and the Barlett's test, respectively. All the data were normally distributed,
245 and variances were homogeneous. One-way analysis of variance (ANOVA) were used to
246 compare age and anthropometric characteristics between groups. When ANOVA revealed
247 significant effects, Tukey HSD *post hoc* tests were applied to test the discrimination between
248 groups. Differences in absolute values were analyzed using a two-way (group \times %REP)
249 ANOVA with repeated measures. When significant differences in initial values were identified
250 between groups, two-way (group \times %REP) ANOVA were applied to test differences in percent
251 changes relative to the pre-fatigue values. The effect size and statistical power were reported
252 when significant main or interaction effects were detected. When the ANOVA revealed
253 significant effects or interactions between factors, a Tukey HSD *post hoc* test was applied to
254 test the discrimination between means. The effect size was assessed using the partial eta-
255 squared (η^2) and ranked as follows: ~ 0.01 = small effect, ~ 0.06 = moderate effect, and ≥ 0.14
256 = large effect (Cohen 1969).

257 Moreover, to discriminate the effect of MVIC torque on the etiology of neuromuscular fatigue,
258 we used a mixed general linear model: the initial MVIC torque was used as a continuous
259 predictor variable (= co-variable), the group as a categorical independent variable and the
260 Qtw_{pot} or VA as dependent variables. Pearson's correlation coefficients were used to determine
261 linear correlations between the initial MVIC torque, the total number of repetitions and Qtw_{pot}
262 and VA variations over the fatigue protocol. The limit for statistical significance was set at
263 $p < 0.05$. Statistical procedures were performed using the Statistica 12.0 software (Statsoft, Inc,
264 USA).

265 **RESULTS**

266 **Participants' characteristics**

267 Participants' characteristics are described in Table 1. The boys were prepubertal (Tanner stages
268 I and II and far from their peak height velocity: -3.8 ± 0.7 yr.) while the adolescents were circa-
269 pubertal (Tanner stages III and IV and around their peak height velocity: -1.1 ± 1.3 yr.).

270 **Number of repetitions**

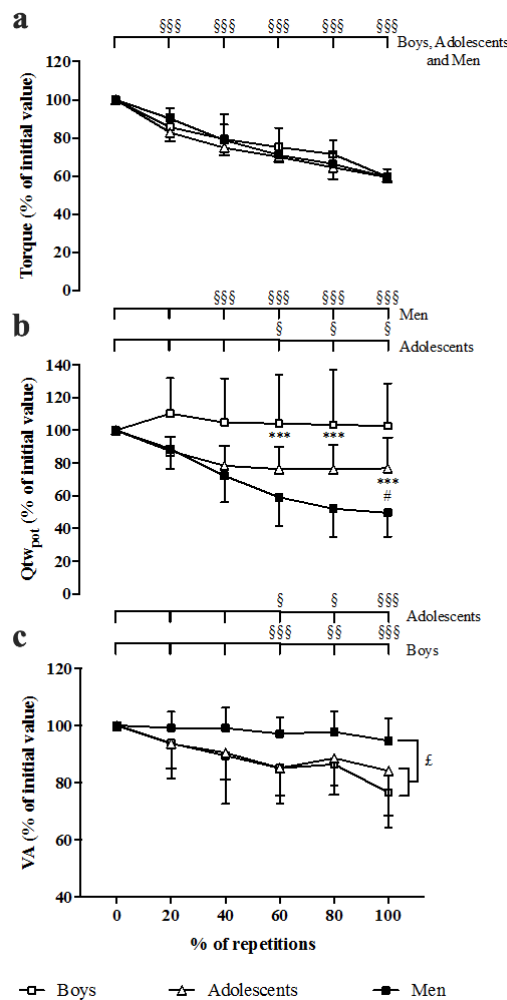
271 Task failure, corresponding to the predetermined 60% decrement of MVIC, was reached after
272 52.9 ± 12.7 [CI 95% = 43.1-62.6], 42.6 ± 12.5 [CI 95% = 32.2-53.0] and 26.6 ± 6.3 [CI 95% =

273 22.4-30.9] repetitions in boys, adolescents and men, respectively. ANOVA revealed a
 274 significant group effect for the total number of repetitions [F(2;25) = 15.80, p<0.001, η^2 = 0.56,
 275 power = 1.0]. The total number of repetitions was significantly higher in boys and adolescents
 276 than in men (p<0.001). The number of repetitions tended to be higher in boys than adolescents
 277 (p = 0.056, η^2 = 0.81, power = 0.39).

278 **MVIC torque**

279 Significant interactions (group \times %REP) were found for the absolute MVIC torque [F(10;125)
 280 = 14.78, p<0.001, η^2 = 0.55, power = 1.0]. As expected, initial MVIC torque values were
 281 significantly lower in boys than adolescents and men, and in adolescents than men (87.4 \pm 31.2
 282 [CI 95% = 63.4-111.3], 184.2 \pm 47.9 [CI 95% = 144.2-224.3] and 298.4 \pm 50.5 [CI 95% =
 283 264.4-332.3] N.m in boys, adolescents and men, respectively; p<0.001).

284 A %REP effect was found for MVIC torque changes, i.e. in percentage of initial values
 285 [F(4;100) = 89.96, p<0.001, η^2 = 0.78, power = 1.0]. MVIC torque significantly and
 286 progressively decreased throughout the fatigue test regardless of group (Fig. 2a).



287 **Fig 2:** Time-courses of (a) MVIC torque of the knee extensor muscles, (b) potentiated twitch
 288 torque (Qtw_{pot}) and (c) voluntary activation level (VA), expressed as a percentage of the initial
 289 values, during the fatigue protocol in prepubertal boys, male adolescents and men. ***:
 290 Significantly different between boys and men at p<0.001 (identified from statistical analysis on
 291 the relative values); #: Significantly different between adolescents and men at p<0.05
 292 (identified from statistical analysis on the relative values); £: Significantly different over the

293 fatigue protocol at $p < 0.05$ (identified from statistical analysis on the absolute values);” §, §§
294 and §§§ significantly different from the initial value at $p < 0.05$, $p < 0.01$ and $p < 0.001$,
295 respectively (identified from statistical analysis on the absolute values).

296 **Potentiated twitch torque**

297 During the fatigue protocol, ANOVA revealed a significant interaction of group \times %REP for
298 absolute $Q_{tw_{pot}}$ values [$F(10;125) = 16.72$, $p < 0.001$, $\eta^2 = 0.57$, power = 1.0]. Initial absolute
299 $Q_{tw_{pot}}$ values tended to be lower in boys than in adolescents ($p = 0.07$) and were lower in boys
300 than in men (23.2 ± 9.3 [CI 95% = 16.0-30.3], 56.8 ± 18.9 [CI 95% = 38.8-71.3] and $79.3 \pm$
301 28.0 [CI 95% = 60.5-98.0] N.m in boys, adolescents and men, respectively; $p < 0.001$). No
302 significant difference was found between adolescents and men for initial $Q_{tw_{pot}}$ values.
303 Adolescents and men showed a significant decrement of $Q_{tw_{pot}}$ during the fatigue exercise,
304 while it remained unchanged in boys (Fig. 2b).

305 Furthermore, a significant interaction of group \times %REP was observed for relative $Q_{tw_{pot}}$, i.e.
306 in percentage of initial values [$F(8;100) = 8.19$, $p < 0.001$, $\eta^2 = 0.40$, power = 0.99]. The $Q_{tw_{pot}}$
307 decreased respectively up to 20% and 60%REP and then remained unchanged until the end of
308 the fatigue protocol in adolescents and men. At the end of the fatigue protocol, men showed a
309 greater relative decrement of $Q_{tw_{pot}}$ than adolescents ($-50.5 \pm 14.5\%$ and $-23.2 \pm 18.9\%$,
310 respectively; Fig. 2b).

311 When the initial MVIC torque value was used as co-variable, a significant interaction of group
312 \times %REP on the course of $Q_{tw_{pot}}$ was observed ($p < 0.05$). Post-hoc analyses revealed that the
313 decrement of $Q_{tw_{pot}}$ was higher in men than adolescents ($p < 0.01$) and higher in adolescents
314 than boys ($p < 0.05$).

315 **Voluntary activation level**

316 ANOVA revealed a significant interaction of group \times %REP for absolute VA values [$F(10;125)$
317 $= 2.20$, $p < 0.05$, $\eta^2 = 0.15$, power = 0.90]. Before the fatigue test, the VA values were not
318 significantly different between groups ($87.1 \pm 7.6\%$ [CI 95% = 81.2-92.9], $84.4 \pm 5.5\%$ [CI
319 95% = 75.5-91.8], $92.2 \pm 3.2\%$ [CI 95% = 90.0-94.3] in boys, adolescents and men,
320 respectively). Boys and adolescents showed a significant decrement in VA during the fatigue
321 protocol, whereas it remained unchanged in men (Fig. 2c). At the end of the fatigue test, VA
322 decrements were $-23.4 \pm 12.2\%$ and $-15.8 \pm 15.7\%$ in boys and adolescents, respectively.

323 However, when the MVIC torque was used as co-variable, no significant interaction or main
324 effect on the time-course of VA was observed.

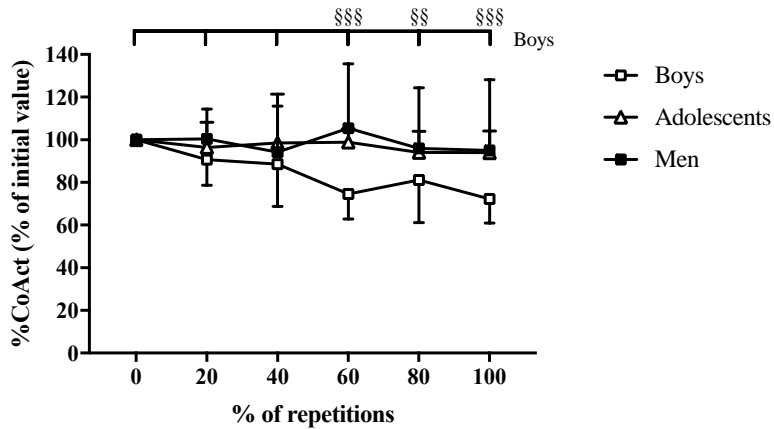
325 **EMG activity**

326 No significant main or interaction effect was found for the VL and RF M_{max} values.

327 However, there were significant interaction (group \times %REP) effects for VL and RF absolute
328 $RMS.M_{max-1}$ [$F(10;125) = 2.01$, $p < 0.05$, $\eta^2 = 0.14$, power = 0.86 and $F(10;125) = 2.56$,
329 $p < 0.01$, $\eta^2 = 0.18$, power = 0.94, respectively]. No difference was found for the initial values of
330 VL and RF $RMS.M_{max-1}$ between age groups. *Post hoc* tests only revealed a decrement of VL
331 and RF $RMS.M_{max-1}$ in boys (at least $p < 0.01$).

332 **Antagonist co-activation**

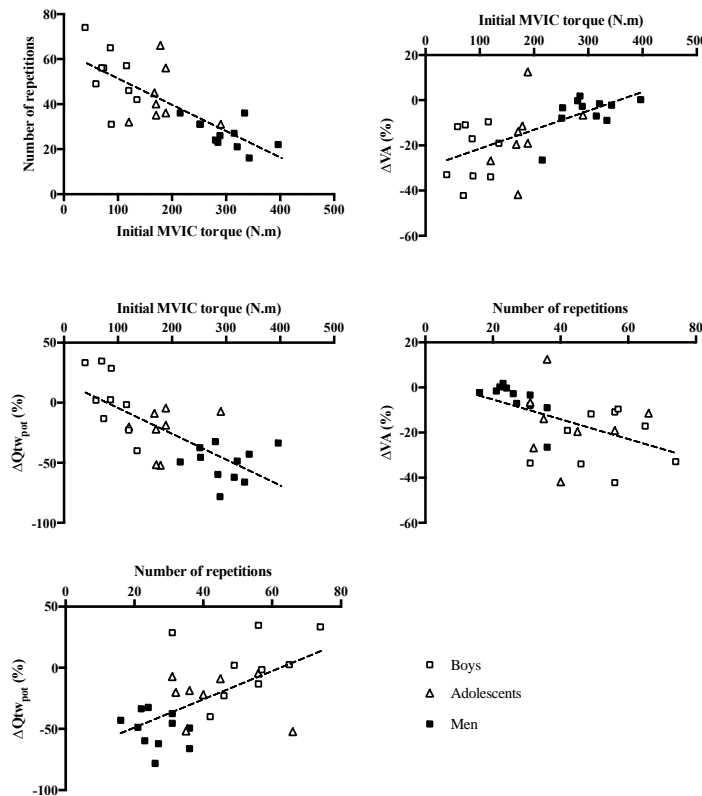
333 ANOVA revealed a significant interaction (group \times %REP) regarding absolute %CoAct
334 [$F(10;125) = 2.72$, $p < 0.01$, $\eta^2 = 0.18$, power = 0.96]. No difference was found for the initial
335 value of %CoAct between age groups. %CoAct values remained unchanged in adolescents and
336 men during the entire fatigue protocol whereas in boys, it decreased significantly at 60%REP,
337 80%REP and 100%REP compared to 0%REP (Fig. 3).



338 **Fig. 3:** Time-course of the coactivation level of the *biceps femoris* (%CoAct) during the fatigue
 339 protocol in boys, adolescents and men. §§ and §§§: significantly different from the initial value
 340 at $p < 0.01$ and $p < 0.001$, respectively (identified from statistical analysis on the absolute values).
 341

Correlations

342 When the three groups were pooled in the analysis, the first MVIC torque of the fatigue protocol
 343 was correlated to the number of repetitions ($r_2=0.59$, $p < 0.001$) and relative $Q_{tw_{pot}}$ changes
 344 ($r_2=0.52$, $p < 0.001$) (Fig. 4); the higher the MVIC torque, the lower the number of repetitions
 345 and the greater the decrement of $Q_{tw_{pot}}$. In contrast, a significant positive relationship was found
 346 between the first MVIC torque and the relative VA decrement ($r_2=0.41$, $p < 0.001$); the higher
 347 the MVIC torque, the lesser the decrement of VA. Furthermore, the number of repetitions was
 348 correlated to the relative VA decrement ($r_2=0.23$, $p < 0.05$) and to the relative $Q_{tw_{pot}}$ decrement
 349 ($r_2=0.33$, $p < 0.01$) (Fig. 4).



350 **Fig. 4:** Correlations between initial maximal voluntary isometric contraction (MVIC) torque,
351 number of repetitions, change in potentiated twitch torque ($Q_{tw_{pot}}$) and change in voluntary
352 activation level (VA). Boys, adolescents and men were pooled in the analysis.

353 **DISCUSSION**

354 The purpose of the present study was to investigate the effect of maturation on the development
355 and etiology of neuromuscular fatigue induced by repeated MVIC of the KE muscles. The main
356 results showed that adolescents tended to fatigue faster than prepubertal boys but slower than
357 men during repeated maximal voluntary contractions. The greater fatigability in adolescents
358 than prepubertal boys was associated with a greater peripheral fatigue and a similar central
359 fatigue. In addition, the lower fatigability in adolescents than adults was associated with a lesser
360 peripheral fatigue and a greater central fatigue. Finally, when MVIC was used as co-variable in
361 the statistical analysis, differences of peripheral fatigue between groups persisted, suggesting
362 that factors other than initial MVIC torque may account for the differences of peripheral fatigue
363 during maturation.

364 The results of the present study confirm that adolescents fatigue faster than prepubertal children
365 and slower than adults during repeated isometric maximal voluntary contractions of the knee
366 extensors. The number of repetitions to task failure tended to be lower in adolescents than
367 prepubertal boys and significantly higher in adolescents than men. Our results agree with the
368 data published by Ratel et al. (2002) showing a higher decline of peak power output in
369 adolescents than children or in adults than adolescents during ten repeated 10-s cycling sprints
370 separated by 30 s of passive recovery. Similarly, it has been reported that during 4 series of 18
371 concentric knee flexions and extensions, the decline of peak torque is lower in prepubertal boys
372 than male adolescents and higher in men than adolescents (Zafeiridis et al. 2005; Dipla et al.
373 2009). Finally, during 5 series of 6 maximal eccentric contractions of the elbow flexors, the
374 decline of concentric maximal torque was found to be higher in adolescents than children and
375 higher in adults than adolescents (Chen et al. 2014).

376 Although scientific evidence supports a higher fatigability in adolescents than children and a
377 lower fatigability in adolescents than adults during high-intensity intermittent exercise, the
378 mechanisms explaining this phenomenon still remain unclear.

379 **Peripheral mechanisms**

380 In prepubertal boys, no significant alteration of $Q_{tw_{pot}}$ was observed during the fatigue protocol.
381 In contrast, in adolescents and men, $Q_{tw_{pot}}$ decreased significantly throughout the fatigue
382 protocol and the decrement was significantly higher in men than adolescents. This suggests that
383 contractile properties and/or excitation-contraction coupling were preserved in prepubertal boys
384 and more strongly altered in men than adolescents. In contrast, the time-course of M-wave did
385 not differ between groups, suggesting that no maturation-related change in the excitability of
386 the sarcolemma was associated with fatigue. These results agree with previous studies, showing
387 a lower alteration of the potentiated twitch torque during fatigue in prepubertal children than
388 adults (Gorianovas et al. 2013; Murphy et al. 2014; Hatzikotoulas et al. 2014; Ratel et al. 2015;
389 Piponnier et al. 2018). Furthermore, they concur with those published by Streckis et al. (2007)
390 showing a lower alteration of twitch torque during a sustained 2-min maximal voluntary
391 contraction of the knee extensors in 13.6-year-old girls and 13.9-year-old boys than adults.
392 However, the results of the present study reveal for the first time an effect of maturation.

393 **Central mechanisms**

394 Regarding neural factors, prepubertal boys and male adolescents showed a significant VA and
395 $RMS.M_{max-1}$ (only in boys) decrement during the fatigue test, whereas no change of VA and
396 $RMS.M_{max-1}$ was observed in men. Moreover, the decrement of VA was similar in prepubertal

397 boys and male adolescents. The interplay of central and peripheral mechanisms of fatigue
398 through maturation remains to be elucidated; however, on the basis of these results, it could be
399 suggested that the greater central fatigue in children and adolescents accounted for their lower
400 peripheral fatigue than adults. Indeed, according to the central governor theory, the central
401 nervous system could limit the recruitment of motor units to prevent any extensive homeostasis
402 disturbance, muscle damage, and biological harm (Noakes et al. 2005). As such, some studies
403 proposed that the central nervous system may “tolerate” a given peripheral fatigue level
404 (Amann and Dempsey 2008; Millet 2011; Zghal et al. 2015). It is currently unknown if this
405 tolerance is different in children, adolescents and adults, but the lower peripheral and the higher
406 central fatigue reported here in children and adolescents suggest that this tolerance could be
407 centrally set at a lower level than adults. Furthermore, the greater peripheral fatigue found for
408 a comparable amount of central fatigue in adolescents than children suggests that the tolerance
409 could be set at higher level in adolescents. This finding could support the idea that (i) the central
410 nervous system could not tolerate the development of an extensive peripheral fatigue in children
411 (Piponnier et al. 2018), contrary to adults, and (ii) the tolerance of the central nervous system
412 to peripheral fatigue could increase during puberty.

413 Regarding to the central regulation of the antagonist coactivation, the results of the present
414 study showed different patterns between groups, since it decreased in prepubertal boys whereas
415 it remained unchanged in male adolescents and men. The progressive decrease of the co-
416 activation level in prepubertal boys may have contributed to limit the loss of torque, and
417 therefore to delay the level of fatigue, as previously reported (Piponnier et al. 2018). This
418 potential mechanism could withdraw through maturation since no significant change of the
419 antagonist coactivation during the fatigue protocol was observed in adolescents. However, these
420 results should be confirmed since, to our knowledge, no other studies have investigated the
421 effect of maturation on the antagonist coactivation associated with fatigue. Furthermore, other
422 studies have reported opposite results when comparing prepubertal children and adults
423 (Paraschos et al. 2007; Armatas et al. 2010; Murphy et al. 2014). Therefore, further studies are
424 required to clarify this issue.

425 **Factors underpinning differences in neuromuscular fatigue**

426 Among the factors that may account for the differences of peripheral fatigue between
427 prepubertal boys, male adolescents and men, is the absolute torque level. Indeed, when the three
428 groups were pooled in the analysis, the first MVIC torque of the fatigue test was correlated to
429 the twitch torque decrement. This suggestion is consistent with other studies that showed that
430 the greater fatigue observed in men *vs.* women was eliminated if subjects were matched for
431 absolute strength (Hunter et al. 2004). However, other factors may account for the differences
432 of peripheral fatigue between prepubertal children, adolescents and adults. Indeed, the
433 differential time-course of the potentiated twitch torque over repeated maximal voluntary
434 contractions persisted between groups when the initial MVIC torque was used as co-variable.
435 This result is consistent with recent reports (Piponnier et al. in press), showing that difference
436 in torque level could not fully account for difference in neuromuscular fatigue at different
437 muscle-tendon lengths between prepubertal children and adults. Thus, other factors could be
438 involved in the development and etiology of neuromuscular fatigue during growth and
439 maturation, such as the metabolic profile (Bontemps et al. 2019). Specifically, the greater
440 proportion of slow-twitch fibers (Lexell et al. 1992) and the greater reliance on oxidative
441 metabolism (Tonson et al. 2010) in prepubertal children may also could account for their
442 reduced peripheral fatigue.

443 The higher implication of central factors in the development of fatigue in children and
444 adolescents could also be ascribed to their lower absolute MVIC torque and as a result, to the
445 concurrent longer exercise duration than adults. Indeed, in the present study, the number of

446 repetitions and the associated exercise duration were clearly higher in prepubertal boys and
447 male adolescents than adults. Furthermore, the relative VA loss was correlated to the first MVIC
448 of the fatigue test and to the number of repetitions. These findings are consistent with another
449 study (Thomas et al. 2015), reporting that central fatigue increases with exercise duration. Also,
450 the results of the present study reveal no significant difference in the time-course of VA between
451 groups when the MVIC torque was used as co-variable. These results agree with those
452 published by Russ (2009), which reported similar changes in MVIC decrements and central
453 activation in strength-matched men and women.

454 **CONCLUSIONS**

455 The results of the present study show that male adolescents fatigue faster than prepubertal
456 children but slower than men during repeated maximal voluntary isometric contractions of the
457 knee extensors. Furthermore, the contribution of central and peripheral mechanisms to
458 neuromuscular fatigue differs between groups. Children and adolescents experience less
459 peripheral and more central fatigue than adults. Moreover, adolescents experience more
460 peripheral fatigue than children for a comparable amount of central fatigue. This could support
461 the idea that (i) the central nervous system could not tolerate the development of an extensive
462 peripheral fatigue in children, contrary to adults, and (ii) the tolerance of the central nervous
463 system to peripheral fatigue could increase throughout maturation. However, further studies are
464 required to better understand the origin of these central regulations and the interplay between
465 peripheral and central mechanisms of fatigue throughout maturation.

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469 **CONFLICT OF INTEREST**

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472 This work is known to and agreed by the co-authors identified on the manuscript's title page.
473 This work required more than six people, because of clinical examination (physician or
474 pediatrician), recruitment of volunteers, experimental procedures, statistical analysis and data
475 analysis.

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