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Child-adult differences in neuromuscular fatigue are muscle-dependent

Authors: Piponnier Enzo¹, Martin Vincent¹, Bontemps Bastien¹, Chalchat Emeric¹, Julian Valérie², Bocock Olivia², Duclos Martine², Ratel Sébastien¹.

Authors affiliations: ¹ Clermont-Auvergne University, AME2P, F-63000 Clermont-Ferrand, France, ² Clermont University Hospital, Clermont-Ferrand, France.

Running head: Neuromuscular fatigue in young people

Corresponding author:

Dr. Sébastien RATEL

UFR STAPS - Laboratoire AME2P

Université Clermont Auvergne

Campus Universitaire des Cézeaux

5 impasse Amélie Murat

TSA 60026 - CS 60026

63178 AUBIERE CEDEX

FRANCE

Tel: + 33 (0)4 73 40 54 86

Fax: +33 (0)4 73 40 74 46

E-mail: Sebastien.RATEL@uca.fr

ABSTRACT

The aim of the present study was to compare the development and etiology of neuromuscular fatigue of the knee extensor (KE) and plantar flexor (PF) muscles during repeated maximal voluntary isometric contractions (MVIC) between children and adults. Twenty-one prepubertal boys (9-11 years) and 24 men (18-30 years) performed two fatigue protocols consisting in a repetition of 5-s isometric MVIC of the KE or PF muscles interspersed with 5-s passive recovery periods until MVIC reached 60% of its initial value. The etiology of neuromuscular fatigue of the KE and PF muscles was investigated by means of non-invasive methods such as the surface electromyography, single and doublet magnetic stimulation, twitch interpolation technique and NIRS. The number of repetitions performed was significantly lower in men (15.4 ± 3.8) than boys (38.7 ± 18.8) for the KE fatigue test. In contrast, no significant difference was found for the PF muscles between boys and men (12.1 ± 4.9 and 13.8 ± 4.9 repetitions, respectively). Boys displayed a lower reduction in potentiated twitch torque, low-frequency fatigue and muscle oxygenation than men whatever the muscle group considered. In contrast, voluntary activation level and normalized EMG data decreased to a greater extent in boys than men for both muscle groups. To conclude, boys experienced less peripheral and more central fatigue during repeated MVICs than men whatever the muscle group considered. However, child-adult differences in neuromuscular fatigue were muscle-dependent since boys fatigued similarly to men with the PF muscles and to a lower extent with the KE muscles than men.

Keywords: Growth, Peripheral fatigue, Central fatigue, Knee extensors, Plantar flexors.

New & Noteworthy

Child-adult differences in neuromuscular fatigue during repeated maximal voluntary contractions are specific to the muscle group since children fatigue similarly to adults with the plantar flexor muscles and to a lower extent with the knee extensor muscles than adults.

Children experience less peripheral fatigue and more central fatigue than adults regardless of the muscle group considered.

INTRODUCTION

Neuromuscular fatigue is defined as any change that occurs in the central nervous system and/or muscles from exercise, resulting into a reduction in performance (e.g. decrement in force output, number of repetitions, etc.) (21). Neuromuscular fatigue was found to be lower in prepubertal children than adults during isometric (3, 17, 18, 37) and isokinetic (10, 12, 32, 35) maximal voluntary contractions, or during whole-body dynamic exercises such as hopping (16, 24). The lower neuromuscular fatigue in prepubertal children has been mainly attributed to a lower peripheral (i.e. muscular) fatigue in prepubertal children than adults (18, 32, 37), owing to their greater relative energy contribution derived from oxidative sources (38, 44), and their potentially greater proportion of fatigue-resistant slow-twitch muscle fibers (25). Some studies have also reported a greater central fatigue in children, whose origins are currently unknown (37, 42).

In adults it has been shown that the plantar flexor (PF) muscles have a greater susceptibility to central fatigue than the knee extensor (KE) muscles, while the KE muscles have a greater susceptibility to peripheral fatigue (33). Muscle typology and the absolute force level produced may explain these differences between muscle groups (5, 33). Specifically, peripheral fatigue is more pronounced after KE (high force, mixed typology) contractions tasks, whereas PF (low force, slow-type muscle typology) contractions favor exercise duration, and consequently the development of central fatigue (33). Interestingly, prepubertal children may have a greater percentage of slow twitch muscle fibers in vastus lateralis (25) and a higher absolute force level of the KE muscles than adults (37). However, these differences could be reduced between these two age groups when considering the absolute PF force level (22), and the PF muscles slow twitch fiber proportion (13). This could reduce the fatigue differences at peripheral and neural levels between children and adults on the PF muscles as compared to the KE muscles. However, experimental evidence is currently lacking to support this assumption.

The central and peripheral origins of neuromuscular fatigue are not fully elucidated in children. The contribution of sarcolemmal excitability to the peripheral fatigue in prepubertal children is still debated. Our previous work (37) showed no significant change of maximal M-wave (M_{\max}) throughout repeated maximal voluntary isometric contractions (MVICs) of the KE muscles in boys and men, while other studies reported a similar M_{\max} decrement in the PF muscles in boys and men (18) or an increment in boys and a significant decrease of M_{\max} in the KE muscles in men (32). This inconsistency could be attributed to the muscle group investigated since the alteration in the sarcolemmal excitability could be greater with the PF muscles than the KE muscles during fatiguing isometric contractions (33). A direct comparison of these two muscle groups (KE vs. PF) is therefore required to clarify the role of sarcolemmal excitability into the development of peripheral fatigue in both children and adults.

It has also been shown that after a repetitive stretch-shortening cycle fatigue protocol, prepubertal children displayed a lesser decrement of the low- to high-frequency tetanic force ratio than adults, suggesting a lower alteration in the excitation-contraction (E-C) coupling before puberty (16). However, this comparison could be biased by the greater muscle damage induced by intense plyometric exercises in adults than children (26). These differences in muscle damage may have greatly contributed to the differences in low-frequency fatigue between both populations, thereby blunting any difference in the other E-C uncoupling origins (e.g. Ca^{2+} kinetics). Further studies should be achieved under non-damaging exercise conditions, i.e. under isometric fatiguing contraction conditions, to fairly compare prepubertal children and adults.

Moreover, muscle oxygenation could account for differences in peripheral fatigue between children and adults since it modulates the relative energy contribution derived from non-oxidative pathways and thus the accumulation rate of muscle by-products (i.e. H^+ , Pi) inducing fatigue. However, although muscle O_2 saturation was found to decrease during submaximal sustained contractions in children (31) and adults (39), to our knowledge, no study has still combined NIRS-derived measurements of muscle oxygenation with the peripheral components

of neuromuscular fatigue in children and adults. However, it has been shown that prepubertal children rely more on oxidative than anaerobic sources during high-intensity exercise than their older counterparts (38, 44). Therefore, near-infrared spectroscopy (NIRS)-derived muscle oxygenation could be significantly greater in prepubertal children and associated with a lower peripheral fatigue than adults.

Beyond peripheral factors, neural mechanisms could also explain differences in neuromuscular fatigue between children and adults. However, central factors involved in these child-adult differences are still debated. Recent studies have reported a greater decrement in voluntary activation (VA) of the KE muscles in 12-14 year-old children than adults after a 2-min sustained MVIC (41) or in 8-11 year-old boys than men after repeated KE MVICs (37). This greater central fatigue in children may occur at the supra-spinal level and/or at the spinal level, through the inhibitory action of III-IV afferents (2). However, differences in spinal fatigue between children and adults, estimated using non-invasive methods (15) such as mechanical tendon tap stimulation or H-reflex assessment, still remain to be investigated. Contrary to aforementioned studies, other authors have reported similar central fatigue after sustained MVIC of the PF muscles in boys and men (18). These inconsistent results could be attributed to differences in the type of exercise and muscle groups investigated. As mentioned before, some studies showed in men that neuromuscular fatigue induced by sustained (33) or intermittent (5) submaximal contractions is mainly from neural origins for the PF muscles, whereas it seems more from muscular origins for the KE muscles. If this holds true in adults, then, it may be suggested that the differences in neural fatigue between children and adults are increased for the KE muscles (37, 42) and reduced for the PF muscles. This assumption could explain the results obtained by Hatzikotoulas et al. (18), who reported no difference of central fatigue for the PF muscles between children and adults. A direct comparison between KE and PF is required to investigate the effect of muscle specificity on the contribution of central mechanisms to the neuromuscular fatigue differences between children and adults.

The aim of the present study was to investigate the development and etiology of neuromuscular fatigue of the KE and PF muscles during repeated MVICs in children and adults by means of non-invasive methods such as the surface electromyography (EMG), single and doublet magnetic stimulation, twitch interpolation technique and NIRS. We expected that prepubertal children would (i) fatigue less; (ii) develop less peripheral fatigue, possibly because of a lower alteration in sarcolemmal excitability, E-C coupling and contractile properties, and a greater capacity to supply energy from oxidative metabolism; (iii) experience greater central fatigue than adults. We nevertheless hypothesized that (iv) the fatigue difference between children and adults would be reduced for the PF muscles than for the KE muscles, because of a reduced peripheral and central fatigue difference between children and adults.

MATERIAL AND METHODS

Subjects

Twenty-one prepubertal boys (9-11 years) and 24 men (18-30 years) volunteered to participate in the present study. The sample size was estimated from the expected number of repetitions for children and adults, based on our previous study results (37). All the subjects were involved in different physical activities such as rugby, soccer, judo, etc. To be included, they had to perform recreational physical activity for ≤ 4 h per week and be free of any medical contra-indication to physical activity. The local ethics committee (Protection Committee of People for Biomedical Research South-East 6; authorization number, AU 1268) approved the present study. All subjects were fully informed of the experimental procedures and gave their written consent before any testing was conducted. The written consent of the parents/guardians was also obtained for the children.

Experimental procedure (design)

All subjects were tested in three experimental sessions separated by at least one week. During the first session, subjects' physical characteristics (anthropometric measurements, maturation status) were collected; a medical practitioner (pediatrician for the children) performed a clinical examination and the subjects were familiarized with the experimental procedures. Furthermore, at the end of this session, subjects performed a series of MVICs of the KE or PF muscles. They had to maximally contract their muscles at different knee (30° , 50° , 70° , 75° , 80° , 85° , 90° , 100° ; 0° = full extension) and ankle (20° in plantar-flexion, 0° , 10° and 15° in dorsi-flexion; 0° = neutral position) angles in a randomized order, in order to determine the optimal angle for maximal KE and PF torque production. During the second session, all the subjects performed an intermittent voluntary fatigue protocol with the KE muscles at the optimal angle (see below for further details). Finally, during the third session, they had to perform the same fatigue protocol with the PF muscles at the optimal angle. The two fatigue sessions were achieved in the same way, as illustrated in Figure 1.

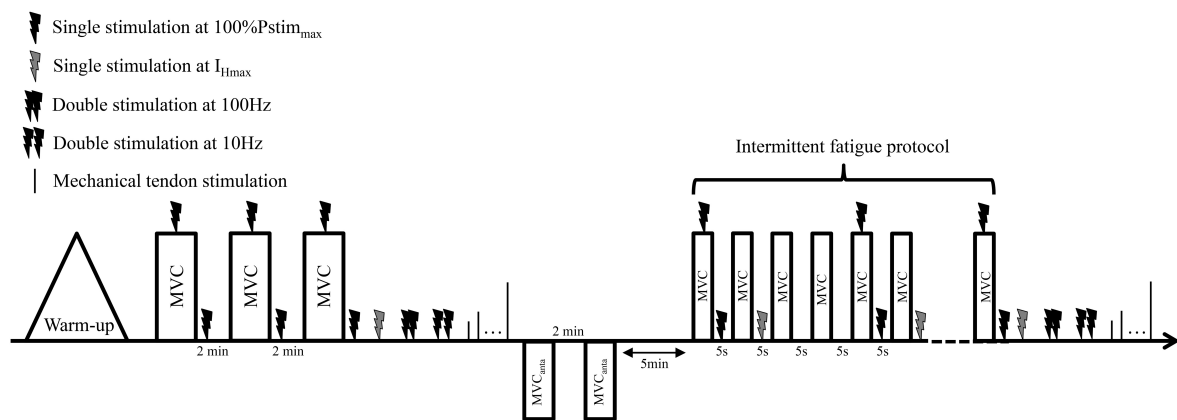


Figure 1: Overview of the fatigue test sessions. All steps of these sessions are commented in the text. Single stimulations at I_{Hmax} (intensity corresponding to maximal H-reflex amplitude) were only triggered during PF fatigue testing. MVIC and MVICanta: maximal

Before any exercise, subjects were equipped and performed a progressive warm-up (4 contractions up to $\sim 50\%$ MVIC, 4 contractions up to $\sim 80\%$ MVIC and 2 contractions up to $\sim 100\%$ MVIC with a rest of 30 s between each contraction). Then, they performed three MVICs of the agonist muscles (i.e. KE or PF) and two MVICs of the respective antagonist muscles (i.e. knee flexors or ankle dorsal flexors) with a rest of 2 min between each contraction. In order to prevent any extensive fatigue, an additional 5-min rest period was allowed prior to each fatigue test.

The intermittent voluntary fatigue protocol consisted in a repetition of 5-s isometric MVIC of the KE or PF muscles interspersed with 5-s passive recovery periods until the voluntary torque reached the target value of 60% of its initial value over three consecutive MVICs. The subjects were not informed of this criterion of task failure and had no visual feedback of torque output throughout the exercise. However, the investigators strongly encouraged the subjects during each maximal effort throughout the experimental protocol. The number of repetitions was considered as the performance criteria to quantify neuromuscular fatigue. Evoked mechanical and EMG responses were measured (see below for further details) in order to quantify the peripheral and central components of fatigue.

Maturation assessment

Two methods were used to assess children's maturation: 1) Tanner stages were determined from self-reported assessment on the basis of pubic hair and testicular/penis development (43), the children being assisted by their parents while completing the questionnaire; 2) Age from peak height velocity was used to assess somatic maturity and determined using height, sitting

height, and body mass. Its calculation was based on sex-specific regression equations according to the method proposed by Mirwald et al. (30).

Torque measurement

Voluntary and evoked torques were measured using a dynamometer (Biodex System 3, Biodex, Shirley, NY). For the KE muscles testing, subjects were comfortably positioned on an adjustable chair with the hip joint flexed at 60° (0° = neutral position). The knee joint was fixed at the optimal angle for maximal torque production, which was determined during the first visit ($77.9 \pm 5.1^\circ$ and $75.4 \pm 4.9^\circ$ in boys and men, respectively). The axis of rotation of the dynamometer was aligned with the lateral femoral condyle of the right femur and the lever arm was attached 1-2 cm above the lateral malleolus with a Velcro strap. During each contraction, the subjects were instructed to grip the lateral handles in order to further stabilize the pelvis. For the PF muscles testing, the subjects laid prone and were strapped on the fully outspread Biodex chair at the hip level to limit upper body contribution to the torque production. Their right foot was positioned in a snowboard binding attached to the Biodex accessory. This setting was aimed at avoiding any movement of the ankle and foot, which is difficult to obtain with the original Biodex accessory. During all PF experiments, hip and knee angles were maintained at 0° (hip neutral position and knee full extension) and ankle angle was set at the optimal angle for maximal torque production (15° in dorsi-flexion for all the subjects). The rotation axis of the dynamometer was aligned with the lateral malleolus. Torque data were corrected for gravity, digitized and exported at a rate of 2 kHz to an external analog-to-digital converter (PowerLab 8/35; ADInstrument, New South Wales, Australia) driven by the LabChart 7.3 Pro software (ADInstrument, New South Wales, Australia).

Magnetic nerve stimulation

KE and PF muscles were stimulated using magnetic stimuli delivered with a 70-mm figure-of-eight coil connected to two Magstim 200² stimulators linked by the Bistim² module (peak magnetic field strength 2.5 T, stimulus duration 115 μ s; Magstim, Witland, Dyfed, UK) to the femoral and tibial nerves, respectively. The coil was placed high in the femoral triangle in regard of the femoral nerve for the KE muscles recruitment and over the posterior tibial nerve in the popliteal fossa for the PF muscles recruitment. Small spatial adjustments were initially performed in order to determine the optimal position where the greatest unpotentiated twitch amplitude ($Q_{tw_{unpot}}$) and the greatest compound muscle action potentials (i.e. maximal M-wave, M_{max}) were evoked. Prior to the testing procedure, the optimal stimulation intensities were determined from recruitment curves (the intensities where $Q_{tw_{unpot}}$ and concomitant M-waves amplitudes of the KE, i.e. *vastus lateralis*: VL, *vastus medialis*: VM and *rectus femoris*: RF, and PF muscles, i.e. *soleus*: SOL and *gastrocnemius medialis*: GM, reached their maximal values and started to plateau, I_{max}). The criterion to determine that the twitch and M-wave amplitudes reached a plateau was no further increase of these amplitudes despite the increase of the stimulation intensity (22). The intensity where SOL H-reflex amplitude was maximal (only for the PF muscles testing; I_{Hmax}) was also determined from its recruitment curve. H-reflex was measured on the SOL muscle because the use of magnetic stimulation for the assessment of the H-reflex has only been validated on the SOL muscle (34, 36). Then, subjects were stimulated at supra-maximal intensity (100% of the magnetic stimulator output) during subsequent testing procedures in order to overcome the potential confounding effect of the axonal hyperpolarization (7). This intensity corresponded to $108.8 \pm 11.2\%$ and $111.9 \pm 15.9\%$ of the optimal intensity in boys with the KE and PF muscles, respectively. The corresponding values for the men were $110.9 \pm 16.1\%$ and $109.7 \pm 14.5\%$ of the optimal intensity. These supra-maximal intensities were statistically higher than the optimal intensities ($p < 0.001$) and were not statistically different between groups.

EMG recordings

In order to achieve low impedance at the skin-electrode interface ($Z < 5 \text{ k}\Omega$), skin was prepared prior to the EMG surface electrodes placement by shaving, lightly abrading with sandpaper and cleaning with alcohol. EMG electrodes (Ag-AgCl, Blue Sensor N-00-S, Ambu, Denmark) were positioned on the right muscle bellies of the VL, VM, RF, *biceps femoris* (BF), SOL, GM and *tibialis anterior* (TA), according to the SENIAM (Surface Electromyography for Non-Invasive Assessment of Muscles) recommendations (19), with an inter-electrode distance of 20 mm. EMG signals were not recorded on the gastrocnemius lateralis because the NIRS probe was placed (see below for further details) on this muscle and there was no further space available to place EMG electrodes (especially in children). EMG signals were amplified (Dual BioAmp, ML 135, ADInstruments, New South Wales, Australia) within a bandwidth frequency ranging from 10 to 500 Hz (common mode rejection ratio $> 85\text{dB}$, gain = 1000) and simultaneously digitized with torque signal by using the external analog-to-digital converter driven by the LabChart 7.3 Pro software. EMG signals were sampled at a frequency of 2 kHz during voluntary and evoked contractions.

Tendon mechanical stimulation

To determine tendon-tap reflex (T-reflex), mechanical stimulations were applied on the patellar or Achilles tendons with an instrumented hammer (Tendon hammer, ADInstruments, New South Wales, Australia) connected to the external analog-to-digital converter. The optimal point of stimulation, i.e. the point where muscle responses were the greatest, was marked with an indelible pen. Before and after the fatigue test (Fig. 1), approximately 10 stimulations were delivered every 5-s at different tap-force intensities in order to determine myotatic reflex gain (see below for further details). Stimulations were always delivered by the same investigator.

Muscle oxygenation

Muscle oxygenation in the VL and *gastrocnemius lateralis* (GL) was recorded 5 min before and during the intermittent fatigue protocol by means of a three-channel, portable continuous-wave NIRS device (PORTAMON, peak wavelengths of 750 nm and 850 nm, Artinis Medical System, Zetten, The Netherlands). Oxygenated (O_2Hb), deoxygenated (HHb) and total hemoglobin (tHb) concentration expressed in μMol were calculated from changes in optical density by using a modified Lambert-Beer law, for which a differential path length factor is used to correct photon scattering within the tissue (4). The tissue saturation index (TSI) expressed in % was also calculated. The TSI corresponds to the O_2Hb proportion of tHb and is derived from the relative absorption coefficients obtained from the slopes of light attenuation at three inter-optode distances and by taking the diffusion scattering law into account (6). The NIRS probe was positioned on the VL midway between the lateral epicondyle and the great trochanter of the femur for the KE muscles testing and on the belly of GL for the PF muscles testing. It was securely strapped in order to ensure that the probe did not move during the experimental session. An opaque black fabric was placed and fixed over the probe to prevent signal interference by ambient light. The subcutaneous fat layer thicknesses, where the probe was placed, were measured using a B-mode ultrasound (Echo Blaster 128 CEXT-1Z; Telemed Ltd., Vilnius, Lithuania) with a 7.5 MHz linear array transducer. Adipose tissue thicknesses over the VL muscle were 6.7 ± 2.5 and 5.8 ± 2.2 mm in boys and men, respectively ($p = 0.25$). The corresponding values for the GL muscle were 4.4 ± 1.9 and 2.9 ± 0.8 mm ($p < 0.01$). Considering that the adipose tissue thickness was relatively low and the penetration depth of the NIRS signal is more or less half of the emitter-detector distance (4 cm), the changes in signals reflected the muscle hemodynamic changes (28). NIRS signals were recorded and sampled at 10 Hz using the Oxysoft software (Artinis Medical System, Zetten, The Netherlands).

For data analysis, TSI signals were expressed as the magnitude of change from the baseline (i.e. the mean value over 30 s before the onset of the first contraction). For all the contractions performed, the minimum TSI value reached during the contraction (ΔTSI) was measured.

Important Δ TSI decreases indicate greater O_2 demand relative to O_2 supply (14). TSI desaturation slope (TSI_{slope}) was also determined as the negative slope of the least-squares regression line of TSI during each contraction. Higher TSI_{slope} indicates an important muscle O_2 demand (14).

Peripheral fatigue indicators

To examine the time course of peripheral fatigue, potentiated single twitches (Qtw_{pot} ; evoked 3 s after the cessation of a MVIC) were measured before the fatiguing protocol, every five MVIC and after the last MVIC of the intermittent fatigue protocol by stimulating the motor nerve (femoral nerve for KE testing and tibial nerve for PF testing; Fig. 1). Concomitant peak-to-peak M-wave amplitudes (M_{max}) were also measured on VL, VM and RF or on SOL and GM. Furthermore, before and after each fatigue protocol (Fig. 1), doublets at 10 Hz and 100 Hz (Dt_{10Hz} and Dt_{100Hz}) were evoked. Dt_{100Hz} is usually considered as an indicator of muscle contractile properties (45). Intensity of double stimulations was set to 60% of the maximal magnetic stimulator output since higher intensities were painful especially in children. The Dt_{10Hz} -to- Dt_{100Hz} ratio (Dt_{10Hz}/Dt_{100Hz}) obtained from magnetic stimulation was calculated and used to assess low-frequency fatigue (46).

Central fatigue indicators

Twitch interpolation technique was used to determine VA. Superimposed single twitch (Qtw_s) was evoked during MVIC after the torque had reached a plateau. Then, Qtw_s and Qtw_{pot} were used to quantify VA before, every five MVIC and during the last MVIC (Fig. 1) as proposed by Merton (29) in equation 1:

$$[1] VA (\%) = [1 - (Qtw_s \cdot Qtw_{pot}^{-1})] \cdot 100$$

The root mean square (RMS) values of the instrumented muscles were calculated during MVICs over a 0.5-s period after the torque had reached a plateau and before the superimposed stimulation was evoked. These RMS values were normalized to respective M_{max} ($RMS \cdot M_{max}^{-1}$) to account for differences in muscle mass and potential changes/differences in sarcolemmal excitability.

Before and after the fatigue protocol, myotatic reflex gain (T_{slope}) was determined as the positive slope of the least-squares regression line of the relationship between tap-force intensity and VL or SOL T-reflex amplitude during KE and PF testing, respectively. In addition, SOL maximal H-reflex (H_{max}) was evoked at I_{Hmax} before, during and after the intermittent fatigue protocol (Fig. 1). We also measured the SOL M-wave evoked at I_{Hmax} (M_{Hmax}) to ensure stimulation intensity consistency throughout the fatigue protocol. The H_{max}/M_{max} ratio was used as a valid parameter (36) to assess and compare spinal excitability between groups.

Finally, the level of antagonist co-activation (%CoAct) was determined every five MVIC using the equation 2:

$$[2] \%CoAct = (RMS_{ago} \cdot RMS_{anta}^{-1}) \cdot 100$$

Where RMS_{ago} is the RMS value of BF or TA during intermittent contractions, and RMS_{anta} is the RMS value of BF or TA during maximal voluntary knee flexion or dorsi-flexion, respectively, recorded before the fatigue protocol.

Statistical analysis

All variables measured during the intermittent fatigue protocols were linearly interpolated between the nearest values at 20%, 40%, 60% and 80% of number of repetitions (%REP) in order to fairly compare the age groups (children vs. adults) and muscle groups (KE vs. PF). Values at 0%REP and 100%REP corresponded to pre- and post-fatigue values, respectively.

Data were screened for normality of distribution and homogeneity of variances using Shapiro-Wilk normality test and the Bartlett test, respectively. The total number of repetitions was compared between age groups and muscle groups using a two-way ANOVA (age group \times muscle group). Differences in absolute MVIC torque, $Q_{tw_{pot}}$, Dt_{10Hz}/Dt_{100Hz} and VA values and their relative changes from respective initial values were separately analyzed using a three-way (age group \times muscle group \times %REP) ANOVA with repeated measures. Furthermore, changes in EMG (M_{max} , RMS/M_{max} , H_{max}/M_{max} , %CoAct), T_{slope} and NIRS (ΔTSI , TSI_{slope}) variables over time were analyzed using a two-way (age group \times %REP) ANOVA with repeated measures, considering each muscle separately. When ANOVA revealed significant effects or interactions between factors, a Tukey HSD *post hoc* test was applied to test the differences between means. The effect size and statistical power have also been reported when significant main or interaction effects were detected. The effect size was assessed using the partial eta-squared (η^2) and ranked as follows: ~ 0.01 = small effect, ~ 0.06 = moderate effect, ≥ 0.14 = large effect (9). Linear regression models were used to determine correlations between VA and %CoAct changes over the fatigue protocol. Statistical tests were performed using the Statistica 8.0 software (StatSoft, Inc, USA). Data are reported as mean \pm standard deviation (SD). The α -level for statistical significance was set at $p < 0.05$. Data presented in figures were expressed as percentage of their initial values.

RESULTS

Subjects' characteristics

The mean age, stature, body mass and body mass index in boys were 10.4 ± 0.7 years, 1.40 ± 0.07 m, 33.9 ± 5.4 kg and 17.3 ± 1.6 kg.m⁻² respectively. The respective values for men were 21.4 ± 3.2 years, 1.79 ± 0.07 m, 72.1 ± 8.1 kg and 22.5 ± 2.1 kg.m⁻². All the boys were prepubertal (Tanner stages I and II). The maturity onset was -3.3 ± 0.3 years and their age at the peak height velocity was 13.4 ± 0.4 years.

KE muscles fatigue test

Global fatigue

Number of repetitions and MVIC torque. ANOVA revealed a significant interaction effect (age group \times muscle group) regarding the number of repetitions [$F(1;43) = 39.03$, $P < 0.001$, $\eta^2 = 0.48$, power = 0.99]. As concerned KE muscles fatigue test, the total number of repetitions was significantly higher in boys than men (38.7 ± 18.8 vs. 15.4 ± 3.8 repetitions, $p < 0.001$). Significant interaction effect (age group \times muscle group \times %REP) was found for the absolute and relative MVIC torque [$F(5;215) = 25.39$, $P < 0.001$, $\eta^2 = 0.37$, power = 1.0 and $F(4;172) = 3.65$, $P < 0.01$, $\eta^2 = 0.08$, power = 0.87, respectively]. In boys, KE MVIC torque, expressed as percentage of the initial value, remained unchanged from 20%REP to 60%REP, while in men MVIC torque significantly and progressively decreased throughout the fatigue test ($p < 0.05$; Fig. 2A).

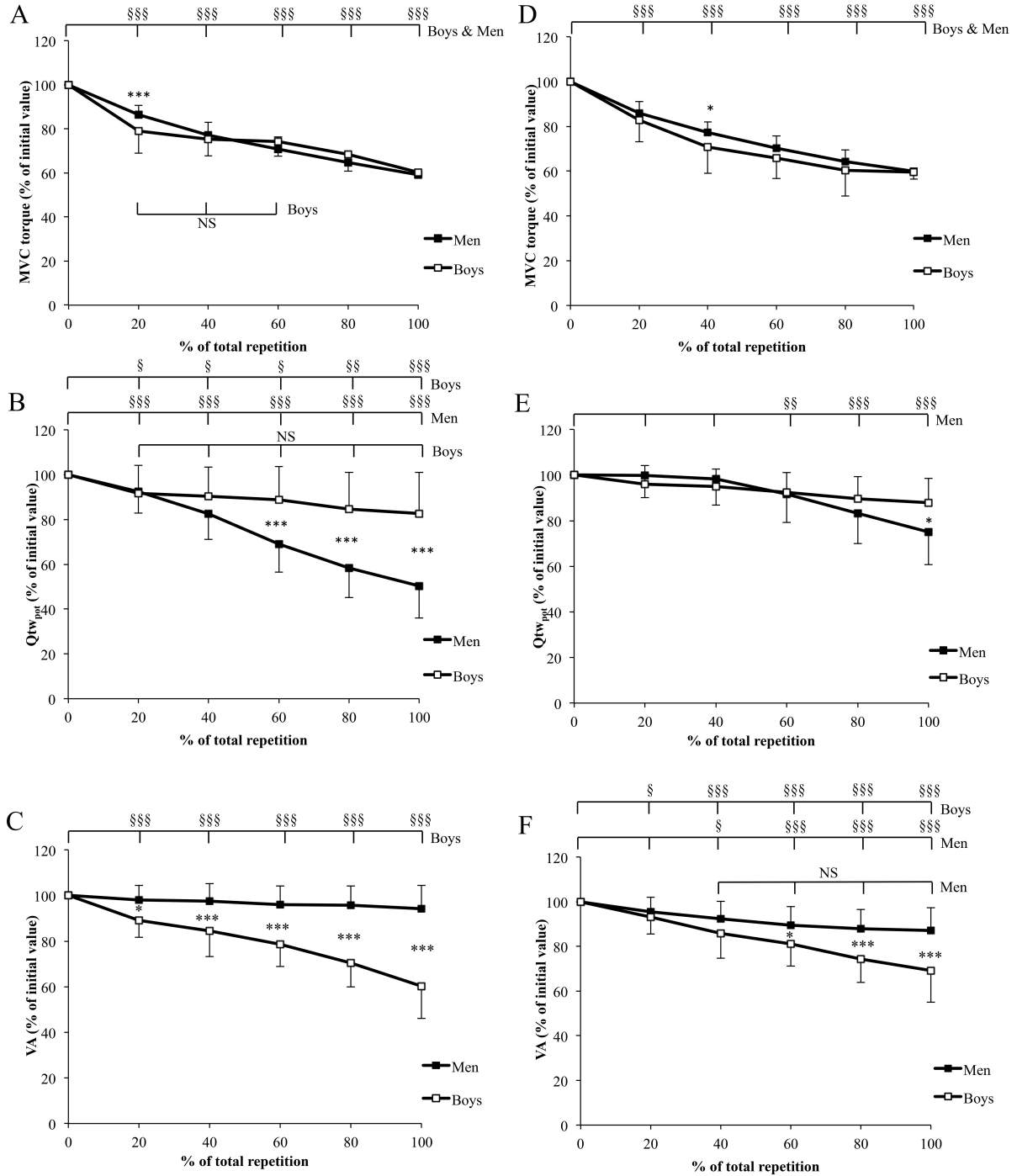


Figure 2: Time course of maximal voluntary isometric contraction (MVIC) torque, potentiated twitch torque amplitude (Qtw_{pot}) and voluntary activation level (VA) of the KE muscles (A, B and C, respectively) and PF muscles (D, E and F, respectively) (expressed as percentage of the initial value) during fatigue protocols in boys (open square) and men (closed square). * and ***: significant difference between boys and men at $p < 0.05$ and $p < 0.001$, respectively (identified from statistical analysis on the relative values); §, §§ and §§§: significantly different from the initial value at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively (identified from statistical analysis on the absolute values).

Peripheral fatigue

Twitch and doublet torque. ANOVA showed a significant interaction effect (age group \times muscle group \times %REP) for the Qtw_{pot} when expressed in absolute values and as percentage of initial value [$F(5;215) = 50.50$, $P < 0.001$, $\eta^2 = 0.54$, power = 1.0 and $F(4;172) = 6.86$, $P < 0.001$, $\eta^2 = 0.14$, power = 0.99, respectively]. As concerned KE muscles fatigue test, men displayed a significantly greater Qtw_{pot} decrement than boys (-49.6 ± 14.3 vs. $-17.3 \pm 18.5\%$,

respectively; Fig. 2B). Furthermore, ANOVA revealed a significant interaction effect (age group \times muscle group \times %REP) regarding the absolute Dt_{100Hz} values [$F(1;43) = 9.93$, $P < 0.01$, $\eta^2 = 0.23$, power = 0.86]. Dt_{100Hz} decreased only in men over the entire fatigue test ($-36.6 \pm 20.5\%$, $p < 0.001$). A significant %REP effect was found for the absolute Dt_{10Hz}/Dt_{100Hz} ratio [$F(1;43) = 66.06$, $P < 0.001$, $\eta^2 = 0.67$, power = 1.0]. ANOVA also revealed a significant age group effect regarding the relative Dt_{10Hz}/Dt_{100Hz} values [$F(1;43) = 12.47$, $P < 0.01$, $\eta^2 = 0.27$, power = 0.93]. Dt_{10Hz}/Dt_{100Hz} ratio decreased to a greater extent in men than boys over the fatigue test (-21.6 ± 18.6 vs. $-9.1 \pm 11.9\%$, respectively; Fig. 3).

M wave. No significant main or interaction effect was found for the VL, VM and RF M_{max} values. Then, no significant change was found for VL, VM and RF muscles in boys and men.

NIRS-derived data. Concerning muscle oxygenation, ANOVA showed a significant interaction effect (age group \times %REP) for the VL ΔTSI [$F(4;172) = 2.85$, $P < 0.05$, $\eta^2 = 0.08$, power = 0.76]. Men displayed a greater ΔTSI than boys in the VL muscle throughout the fatigue test ($p < 0.05$; Fig. 4A). In contrast, ANOVA revealed no significant main or interaction effect for the VL TSI_{slope} . Then, TSI_{slope} remained unchanged in the VL muscle in boys and men.

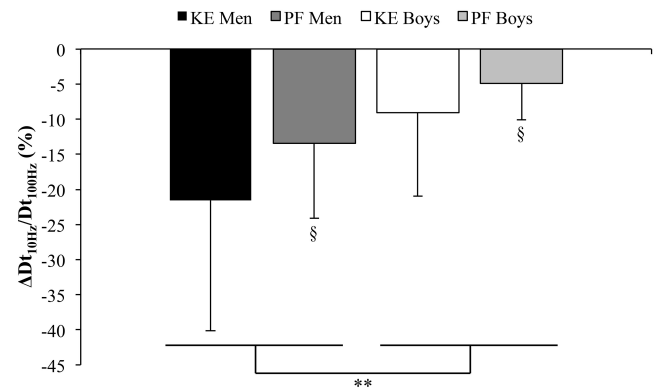


Figure 3: Low- to high-frequency torque ratio variation ($\Delta Dt_{10Hz}/Dt_{100Hz}$) in boys and men after fatigue protocols with the knee extensor (KE) and plantar flexor (PF) muscles. **: significantly different at $p < 0.01$.

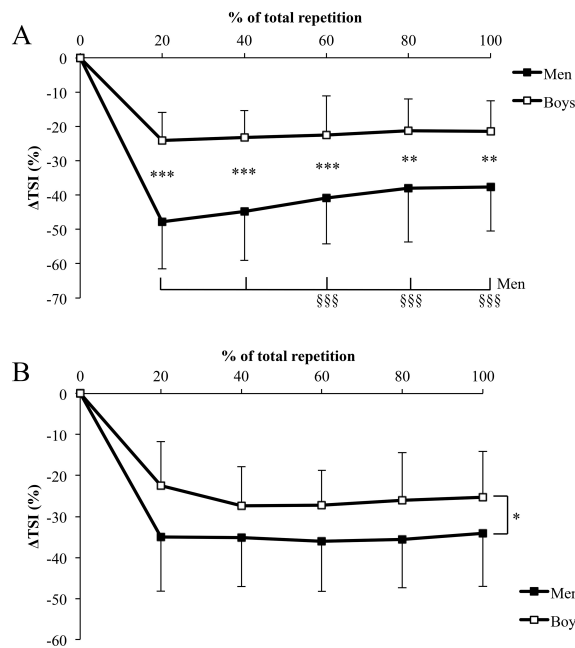


Figure 4: Time course of tissue saturation index variation (ΔTSI) torque of (A) the vastus lateralis and (B) the gastrocnemius lateralis during fatigue protocols in boys (open square) and men (closed square). ** and ***: significant difference between boys and men at $p < 0.01$ and $p < 0.001$, respectively; #: significant difference between boys and men over the fatigue protocol at $p < 0.05$; §§§: significantly different from the 20%REP value at $p < 0.001$.

Central fatigue

VA and normalized EMG. A significant interaction effect (age group \times muscle group \times %REP) was found for the absolute VA values [$F(5;215) = 2.52$, $P < 0.05$, $\eta^2 = 0.06$, power = 0.77]. As concerned KE muscles fatigue test, initial VA values were not significantly different between boys and men (90.4 ± 6.0 vs. $92.7 \pm 4.1\%$, respectively). Boys showed a significant and progressive VA decrement throughout the fatigue protocol ($-39.7 \pm 21.8\%$, $p < 0.001$), while in men VA remained unchanged from its initial value (Fig. 2C). In addition, ANOVA revealed significant age group effect for the VL, VM and RF RMS/ M_{\max} , expressed as percentage of the initial value [$F(1;43) = 3.91$, $P < 0.05$, $\eta^2 = 0.09$, power = 0.49, $F(1;43) = 12.87$, $P < 0.001$, $\eta^2 = 0.24$, power = 0.94 and $F(1;43) = 37.08$, $P < 0.001$, $\eta^2 = 0.48$, power = 0.99, respectively]. The relative decrease of the RMS/ M_{\max} ratio during the fatigue protocol was greater in boys than men for the VL (-37.8 ± 29.4 vs. $-18.0 \pm 29.5\%$, respectively; $p < 0.001$), VM (-44.9 ± 23.3 vs. $-16.3 \pm 26.6\%$, respectively; $p < 0.01$) and RF muscles (-52.9 ± 29.3 vs. $-15.3 \pm 27.2\%$, respectively; $p < 0.01$).

T-reflex. No significant main or interaction effects were found for the VL T_{slope} values. Then, no significant change in VL T_{slope} was observed throughout the fatigue protocol in boys and men.

Co-activation level. ANOVA showed a significant interaction effect (age group \times %REP) for the absolute BF %CoAct values [$F(5;215) = 8.99$, $P < 0.001$, $\eta^2 = 0.20$, power = 0.99] and an age group effect when the BF %CoAct was expressed as percentage of initial value [$F(1;43) = 25.37$, $P < 0.001$, $\eta^2 = 0.42$, power = 0.99, respectively]. Boys showed a significantly higher %CoAct than men at 0%REP (16.4 ± 6.1 vs. $8.2 \pm 4.7\%$, $p < 0.001$). %CoAct values remained unchanged in men throughout the fatigue protocol, whereas in boys it significantly decreased from 0%REP to 20%REP ($p < 0.001$) and then remained unchanged until the end of exercise. No significant correlation was found between the changes in %CoAct and VA over the fatigue KE test.

PF muscles fatigue test

Global fatigue

Number of repetitions and MVIC torque. ANOVA revealed a significant interaction effect (age group \times muscle group) regarding the number of repetitions [$F(1;43) = 39.03$, $P < 0.001$, $\eta^2 = 0.48$, power = 0.99]. As concerned PF muscles fatigue test, the total number of repetitions was not significantly different between boys and men (12.1 ± 4.9 vs. 13.8 ± 4.9 repetitions, respectively). Significant interaction effect (age group \times muscle group \times %REP) was found for the absolute and relative MVIC torque [$F(5;215) = 25.39$, $P < 0.001$, $\eta^2 = 0.37$, power = 1.0 and $F(4;172) = 3.65$, $P < 0.01$, $\eta^2 = 0.08$, power = 0.87, respectively]. MVIC torque, expressed as percentage of the initial value, progressively decreased throughout the fatigue test in boys and men ($p < 0.05$; Fig. 2D). However, at 40%REP, boys showed a greater MVIC torque decrement than men ($p < 0.05$).

Peripheral fatigue

Twitch and doublet torque. ANOVA showed a significant interaction effect (age group \times muscle group \times %REP) for the $Q_{\text{tw}_{\text{pot}}}$ when expressed as absolute values and percentage of initial value [$F(5;215) = 50.50$, $P < 0.001$, $\eta^2 = 0.54$, power = 1.0 and $F(4;172) = 6.86$, $P < 0.001$, $\eta^2 = 0.14$, power = 0.99, respectively]. As concerned PF muscles fatigue test, men showed a significant decrement of $Q_{\text{tw}_{\text{pot}}}$ ($-24.8 \pm 16.0\%$, $p < 0.01$), whereas it remained unchanged in boys throughout the fatigue protocol (Fig. 2E). Similarly, ANOVA revealed a significant interaction effect (age group \times muscle group \times %REP) regarding the absolute $D_{\text{t}_{100\text{Hz}}}$ values [$F(1;43) = 9.93$, $P < 0.01$, $\eta^2 = 0.23$, power = 0.86]. $D_{\text{t}_{100\text{Hz}}}$ only decreased in men ($-49.3 \pm 22.3\%$, $p < 0.05$). Furthermore, a significant %REP effect was found for the absolute

Dt_{10Hz}/Dt_{100Hz} ratio [F(1;43) = 66.06, $P < 0.001$, $\eta^2 = 0.67$, power = 1.0]. ANOVA also revealed a significant age group effect regarding the relative Dt_{10Hz}/Dt_{100Hz} values [F(1;43) = 12.47, $P < 0.01$, $\eta^2 = 0.27$, power = 0.93]. Boys displayed a lower decrement in the Dt_{10Hz}/Dt_{100Hz} ratio than men (-4.9 ± 5.2 vs. $-13.4 \pm 10.7\%$, respectively; Fig. 3).

M wave. No significant main or interaction effect was found for the SOL M_{max} values. Then, no significant change was found for SOL in boys and men. ANOVA revealed a significant interaction effect (age group \times %REP) regarding the absolute GM M_{max} values [F(5;215) = 7.90, $P < 0.001$, $\eta^2 = 0.18$, power = 0.99]. GM M_{max} increased throughout the fatigue protocol only in men ($+17.0 \pm 20.6\%$, $p < 0.01$).

NIRS-derived data. ANOVA showed a significant age group effect for the GL Δ TSI [F(1;43) = 7.22, $P < 0.05$, $\eta^2 = 0.17$, power = 0.74]. Men displayed a significantly greater Δ TSI in the GL than boys throughout the fatigue test ($p < 0.05$; Fig. 4B). In contrast, ANOVA revealed no significant main or interaction effect for the GL TSI_{slope}. Then, TSI_{slope} remained unchanged in the GL muscle in boys and men.

Central fatigue

VA and normalized EMG. A significant interaction effect (age group \times muscle group \times %REP) was found for the absolute VA values [F(5;215) = 2.52, $P < 0.05$, $\eta^2 = 0.06$, power = 0.77]. At 0%REP of the PF fatigue test, VA was not significantly different between boys and men (95.2 ± 3.9 vs. $95.4 \pm 4.5\%$, respectively). Furthermore, while men only displayed a VA decrease between 0%REP and 40%REP, boys showed a progressive and significant VA decrement throughout the fatigue protocol. At the end of the fatigue test, the decrement in VA was significantly greater in boys than men (-30.8 ± 14.2 vs. $-13.0 \pm 10.3\%$, respectively; $p < 0.001$) (Fig. 2F). Furthermore, ANOVA revealed significant age group effects for SOL and GM RMS/M_{max}, expressed as percentage of the initial value [F(1;43) = 5.28, $P < 0.05$, $\eta^2 = 0.12$, power = 0.61 and F(1;43) = 7.65, $P < 0.01$, $\eta^2 = 0.16$, power = 0.77, respectively]. The relative decrease of the RMS/M_{max} ratio over the fatigue test was greater in boys than men for the SOL (-54.4 ± 20.5 vs. $-31.9 \pm 27.2\%$, respectively; $p < 0.01$) and GM muscles (-50.1 ± 18.7 vs. $-31.5 \pm 19.9\%$, respectively; $p < 0.01$).

T- and H-reflex. ANOVA showed a significant %REP effect for the absolute SOL T_{slope} values [F(1;43) = 11.34, $P < 0.001$, $\eta^2 = 0.28$, power = 0.90] but no age group effect when the SOL T_{slope} was expressed as percentage of initial value. Boys and men displayed similar decrements in the T_{slope} for the SOL ($-29.3 \pm 37.5\%$ and $-31.8 \pm 59.2\%$, respectively; $p < 0.01$) during the fatigue test. Similarly, a significant %REP effect was found for the absolute SOL H_{max}/M_{max} ratio values [F(5;215) = 18.29, $P < 0.001$, $\eta^2 = 0.35$, power = 1.0]. However, no significant age group effect was found for the SOL H_{max}/M_{max}, expressed as percentage of initial value. The H_{max}/M_{max} ratio of the SOL significantly decreased from 0%REP to 20%REP ($p < 0.001$) and then remained unchanged, without any significant difference between both age groups (Fig. 5). Only a significant age group effect was found for SOL M_{Hmax} expressed as percentage of M_{max} [F(5;215) = 5.12, $P < 0.05$, $\eta^2 = 0.16$, power = 0.59]. No change of M_{Hmax} was observed throughout the fatigue protocol in children ($19.5 \pm 7.8\%$ and $17.5 \pm 8.3\%$ at pre- and post-fatigue, respectively) and adults ($33.0 \pm 22.2\%$ and $33.5 \pm 21.5\%$ at pre- and post-fatigue, respectively).

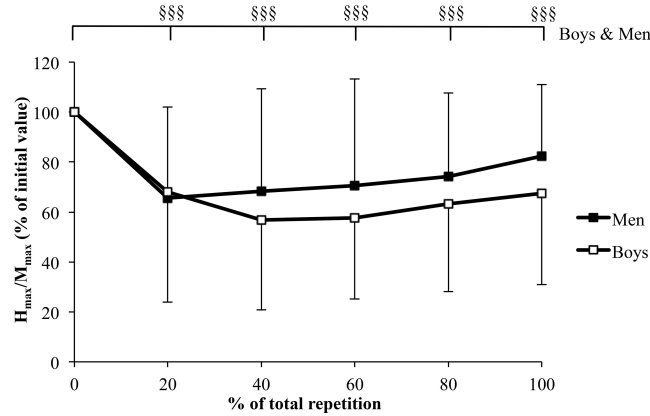


Figure 5: Time course of SOL maximal H-reflex/M-wave ratio (H_{max}/M_{max}), expressed as percentage of the initial value, during the plantar flexor fatigue protocol in boys (open square) and men (closed square). §§§: significantly different from the first MVIC at $p < 0.001$ (identified from statistical analysis on the absolute values).

Co-activation level. ANOVA showed a significant interaction effect (age group \times %REP) for the TA %CoAct when expressed as absolute values and percentage of initial value [$F(5;215) = 9.30$, $P < 0.001$, $\eta^2 = 0.21$, power = 0.99 and $F(4;172) = 5.30$, $P < 0.001$, $\eta^2 = 0.15$, power = 0.97, respectively]. Boys showed a significantly higher %CoAct of the TA than men at 0%REP (19.8 ± 10.1 vs. $11.5 \pm 7.3\%$, $p < 0.05$, respectively). Furthermore, whilst boys displayed a significant and progressive decrement in the %CoAct throughout the fatigue protocol ($p < 0.001$), men showed a significant decrease in the %CoAct only from 60%REP ($p < 0.05$). At the end of exercise, boys showed a higher %CoAct decrement than men ($p < 0.01$). No significant correlation was found between the changes in %CoAct and VA over the fatigue PF test.

Fatigue test: KE vs. PF muscles

Number of repetitions. ANOVA revealed a significant interaction effect (age group \times muscle group) regarding the number of repetitions [$F(1;43) = 39.03$, $P < 0.001$, $\eta^2 = 0.48$, power = 0.99]. The number of repetitions was significantly higher with the KE than the PF in boys ($p < 0.001$), whereas in men no difference was observed between both muscle groups.

Twitch and doublet torque. ANOVA showed a significant interaction effect (age group \times muscle group \times %REP) for the $Q_{tw_{pot}}$ when expressed as absolute values and percentage of initial value [$F(5;215) = 50.50$, $P < 0.001$, $\eta^2 = 0.54$, power = 1.0 and $F(4;172) = 6.86$, $P < 0.001$, $\eta^2 = 0.14$, power = 0.99, respectively]. In boys, $Q_{tw_{pot}}$ significantly decreased with the KE muscles ($p < 0.001$) but not with the PF muscles (Fig. 2B and E). In men, the decrement in $Q_{tw_{pot}}$ was greater for the KE than the PF muscles (-49.6 ± 14.3 vs. $-24.8 \pm 16.0\%$, $p < 0.001$). Furthermore, ANOVA revealed a significant interaction effect (age group \times muscle group \times %REP) regarding the absolute Dt_{100Hz} values [$F(1;43) = 9.93$, $P < 0.01$, $\eta^2 = 0.23$, power = 0.86]. A significant muscle group effect was found for the relative Dt_{100Hz} values [$F(1;43) = 4.84$, $P < 0.05$, $\eta^2 = 0.13$, power = 0.57]. In boys, no significant Dt_{100Hz} decrement was observed in both KE and PF. In contrast, in men, the decrement in Dt_{100Hz} was greater for the KE than the PF muscles (-36.6 ± 20.5 vs. $-18.9 \pm 17.1\%$, $p < 0.01$). Finally, a significant %REP effect was found for the absolute Dt_{10Hz}/Dt_{100Hz} ratio [$F(1;43) = 66.06$, $P < 0.001$, $\eta^2 = 0.67$, power = 1.0]. ANOVA also revealed a significant muscle group effect regarding the relative Dt_{10Hz}/Dt_{100Hz} values [$F(1;43) = 3.76$, $P < 0.05$, $\eta^2 = 0.10$, power = 0.47]. The Dt_{10Hz}/Dt_{100Hz} decrease after the fatigue test was greater with the KE than the PF muscles ($p < 0.05$), regardless the age group.

VA. A significant interaction effect (age group \times muscle group \times %REP) was found for the absolute VA values [$F(5;215) = 2.52$, $P < 0.05$, $\eta^2 = 0.06$, power = 0.77]. At 0%REP, no significant VA difference was observed between the KE and PF muscles in boys and men. In boys, the decrement in VA was greater with the KE than the PF muscles (-39.7 ± 21.8 vs. $-30.8 \pm 14.2\%$, respectively; $p < 0.001$), whereas in men, VA only significantly decreased with the PF muscles ($-13.0 \pm 10.3\%$, $p < 0.001$) (Fig. 2C and F).

DISCUSSION

The main purpose of the present study was to investigate the development and etiology of neuromuscular fatigue of the KE and PF muscles during repeated MVICs in children and adults. We expected that prepubertal children would fatigue less and develop less peripheral and more central fatigue than adults. We nevertheless hypothesized that the neuromuscular fatigue difference between children and adults could be reduced for the PF muscles than for the KE muscles, because of a reduced peripheral and central fatigue difference between children and adults.

The results of the present study partly confirm our assumptions. Boys fatigued less than men during the KE fatigue test, whereas surprisingly, no difference in neuromuscular fatigue of the PF muscles was observed between both populations. The absence of children and adults difference in neuromuscular fatigue has been associated with reduced difference in peripheral and central fatigue between children and adults (Fig. 2). Nevertheless, boys displayed lower peripheral fatigue than men, as illustrated by reduced changes in potentiated twitch torque, in the KE and PF muscles. This lower peripheral fatigue in children could be partly due to a lower alteration in the E-C coupling system and muscular oxygenation in children than adults. Furthermore, in prepubertal boys, neuromuscular fatigue was mainly related to central factors in both muscle groups, as evidenced by the greater voluntary activation level decrement throughout the fatigue protocols.

Global fatigue

In the present study, boys experienced a higher number of repetitions than men for a given level of exhaustion of the KE muscles (i.e. up to 60% of MVIC), which is consistent with previous reports (3, 37). However, we report an unusual result regarding the difference in neuromuscular fatigue of the PF muscles between boys and men. Surprisingly, no difference was found in the total number of repetitions between boys and men. The force level has been proposed as a potential contributor to the difference of neuromuscular fatigue between children and adults on the KE muscles (37). Interestingly, in the present study, the absolute maximal torque difference between boys and men under non-fatiguing conditions was twice lower on the PF than the KE muscles (107.4 vs. 219.1 N.m, respectively). It is possible that this reduced force difference may have contributed to equate neuromuscular fatigue of the PF muscles between boys and men. However, further studies are needed to explore this possibility. While matching children and adults for absolute maximal force is impossible, manipulating muscle length to vary the absolute force differences between boys and men could be a more valuable paradigm (23). This approach would allow investigating the influence of the absolute force on neuromuscular fatigue differences between children and adults. However, other factors, such as muscle typology and task demands (support and stability provided to the fatiguing limb) (21) could also contribute to the neuromuscular fatigue differences observed between the PF and KE muscle groups in children and adults. Nevertheless, these muscle-specific differences in neuromuscular fatigue between boys and men do not seem to have any effect on the etiology of neuromuscular fatigue.

Peripheral mechanisms

The reduced $Q_{tw_{pot}}$ changes in the KE muscles and the absence of change of $Q_{tw_{pot}}$ in the PF muscles in boys suggest lower muscular alterations than men, whatever the muscle group

investigated. Our results are in accordance with previous reports, showing a lower alteration of the potentiated twitch torque of the KE muscles (32, 37, 42) and PF muscles (18) in children than adults. This lower peripheral fatigue has been mainly associated with a potentially greater proportion of fatigue-resistant slow-twitch fibers (25) and a more oxidative metabolism in children (38, 44) than adults. Nevertheless, the relative influence of these different factors is still debated. We also reported that the peripheral fatigue difference between boys and men was reduced with the PF muscles (12.9%; Fig. 2E) as compared to the KE muscles (32.3%; Fig. 2B). The reduced peripheral fatigue difference between children and adults is certainly related to the lower susceptibility of the PF muscles than the KE muscles to peripheral fatigue in adults (5, 33), thereby reducing the peripheral fatigue difference between children and adults.

We also reported no decrement of M_{\max} throughout the maximal intermittent exercise in both populations and within both muscle groups, which is consistent with our previous study regarding the KE muscles (37) but in contrast with other studies reporting changes in M_{\max} of the RF and SOL muscles during different maximal continuous or intermittent fatigue tests (18, 32). In the context of our study, this suggests that differences in peripheral fatigue between children and adults during maximal intermittent exercise could not be explained by an alteration of the excitability of the sarcolemma, whatever the muscle group considered.

In the present study, we measured the high frequency doublet and the low- to high-frequency torque ratio in order to quantify the effect of fatigue on contractile properties and E-C coupling changes, respectively. Decrement in $Dt_{100\text{Hz}}$ was only observed in men for both muscle groups, which is consistent with the results published by Streckis et al. (42). The authors reported a greater decrement in the tetanic torque evoked at 100 Hz in young adults than 12-to-14-year-old children after a 2-min sustained MVIC of the KE muscles. Such results suggest that contrary to men, boys' muscle contractile properties were unaffected by the fatigue test, which could partially account for their reduced peripheral fatigue. However, this finding should be interpreted with caution since $Dt_{100\text{Hz}}$ was evoked with a submaximal intensity to reduce discomfort. Although the use of a submaximal intensity may bias the evaluation of $Dt_{100\text{Hz}}$ (27), this limitation should affect similarly children and adults. As a result, the comparison of $Dt_{100\text{Hz}}$ decrements between age groups should not have been biased.

A lesser impairment in the E-C coupling may also account for the reduced peripheral fatigue in children, as evidenced by the lower decrease of the $Dt_{10\text{Hz}}/Dt_{100\text{Hz}}$ ratio on the PF and KE muscles. This result is consistent with the results of Gorianovas et al. (16), who reported that boys are more resistant to low-frequency fatigue than young men after a damaging exercise. Consequently, beyond the reduced muscle damage in children (8), the present results, observed after a non-damaging exercise, suggest that other mechanisms could account for the lesser alteration of the E-C coupling in children. Such mechanisms may be related to the lower accumulation of metabolic by-products in children, especially inorganic phosphate (44). This mechanisms could help preserving Ca^{2+} release during exercise, and consequently limit the development of low-frequency fatigue in children (1, 20). Nevertheless, direct evidence is still lacking to support this assumption.

The greater ability of children to supply O_2 to the exercising muscles may also have contributed to reduce peripheral fatigue, specifically the low-frequency fatigue. Indeed, our study is the first to demonstrate a lower decrement in the tissue saturation index (TSI) of the VL and GL muscles in boys than in men throughout the intermittent fatigue protocols, suggesting that boys display a lower O_2 demand relative to its supply than men. No difference of $\text{TSI}_{\text{slope}}$ was also found between boys and men, suggesting that O_2 demand, and consequently energy consumption (14), was similar in boys and men. Therefore, the lower changes in TSI in boys could be related to their greater capacity to supply O_2 to the exercising muscles when compared to men. One may nevertheless argue that the lower TSI decrement observed in children could also be attributed to differences of intramuscular pressure. Indeed, it has been suggested that a higher torque is associated with a higher intramuscular pressure (41). However, a higher intramuscular pressure can induce a greater decrease of TSI (11). In the present study, men

developed a higher absolute torque than boys, thereby inducing a higher intramuscular pressure, and potentially a greater TSI decrement. Consequently, our original results on child-adult muscle oxygenation differences should be put into perspective with this limitation of the NIRS technique.

Central mechanisms

A greater central fatigue was observed in boys, as evidenced by their significantly greater decrement in the voluntary activation level and normalized EMG activities throughout the KE and PF fatigue protocols. Our results are consistent with previous studies (16, 32, 37, 42), showing a higher and earlier decrease of the KE activation under fatigue conditions in children than adults. However, they are inconsistent with the findings by Hatzikotoulas et al. (18), which show no significant difference in VA decrement of the PF muscles between children and adults. This discrepancy could be ascribed to muscle specificity since the difference of VA decrease between boys and men is reduced in the PF muscles (17.8%; Fig. 2C) as compared to the KE muscles (39.7%; Fig. 2F) in the current study, supporting the initial hypothesis. Specifically, the greater susceptibility of the PF muscles than the KE muscles to central fatigue in adults (5, 33) could account for the present reduced difference or the absence of difference (18) of central fatigue in the PF muscles between boys and men.

Nevertheless, central fatigue was greater in boys, whatever the muscle group considered. The results of the present study seem to point out to the existence of a specific neural regulation in children during the fatiguing exercises. The approaches used (T-reflex and SOL H-reflex) do not allow explaining the greater central fatigue experienced by the children since no difference was observed between groups. Although, the M-wave amplitude ascribed to the H_{\max} did not change throughout the fatigue protocol (suggesting that the location of the stimulation did not change), the H-reflex assessment suffers from methodological limitations since the SOL muscle does not represent the entire PF muscle group, and H-reflex amplitudes may have been affected by potential changes in Ia afferent excitability occurring during the exercise (40). Further studies are required to definitively conclude on the contribution of spinal mechanisms to central fatigue in children. The contribution of supra-spinal factors, which is currently unknown in children, should also be addressed in future studies.

The greater decrease of the co-activation level in boys than men on the KE and PF muscles is consistent with our previous study on the KE muscles (37). Given that the subjects were fully familiarized with the experimental procedures, a learning effect should not explain this result. The greater co-activation level decrease observed in boys may represent a regulation specific to this age group (37). Such neural modulation may have contributed to preserve the net joint torque production during the fatiguing exercise in boys, and consequently limit the development of peripheral fatigue of the agonist muscles in boys. Together with the greater central fatigue observed in boys, these results point out to specific neural regulations in children during fatiguing tasks. Future research should focus on these neural modulations to identify the underlying regulative mechanisms.

To conclude, child-adult differences in neuromuscular fatigue were specific to the muscle group since children fatigued similarly to adults with the PF muscles and to a lower extent with the KE muscles than adults. However, although peripheral and central fatigue differences between children and adults were reduced with PF muscles compared with KE muscles, the etiology was similar regardless of the muscle group considered. Children experienced less peripheral and more central fatigue during repeated MVICs than adults. The lower peripheral fatigue in children was ascribed to a lower alteration in the E-C coupling and contractile function and to a greater ability to supply O_2 to the exercising muscles. Additional experimental data on spinal and supra-spinal fatigue in children are required to identify the specific neural regulations occurring during fatiguing tasks. A practical implication from these results is that during high-intensity intermittent exercise involving the KE, children may cope better than adults.

Nevertheless, future studies should focus on the consequences of spontaneous and natural forms of exercise involving all the lower limb muscles, since monoarticular isometric exercise has a limited ecological validity.

REFERENCES

1. Allen DG, Lamb GD, Westerblad H. Skeletal muscle fatigue: cellular mechanisms. *Physiol Rev* 88: 287–332, 2008.
2. Amann M, Proctor LT, Sebranek JJ, Pegelow DF, Dempsey JA. Opioid-mediated muscle afferents inhibit central motor drive and limit peripheral muscle fatigue development in humans. *J Physiol* 587: 271–283, 2009.
3. Armatas V, Bassa E, Patikas D, Kitsas I, Zangelidis G, Kotzamanidis C. Neuromuscular differences between men and prepubescent boys during a peak isometric knee extension intermittent fatigue test. *Pediatr Exerc Sci* 22: 205–217, 2010.
4. van Beekvelt MCP, van Engelen BGM, Wevers RA, Colier WNJM. In vivo quantitative near-infrared spectroscopy in skeletal muscle during incremental isometric handgrip exercise. *Clin Physiol Funct Imaging* 22: 210–217, 2002.
5. Bigland-Ritchie B, Furbush F, Woods JJ. Fatigue of intermittent submaximal voluntary contractions: central and peripheral factors. *J Appl Physiol Bethesda Md* 1985 61: 421–429, 1986.
6. Boushel R, Langberg H, Green S, Skovgaard D, Bulow J, Kjaer M. Blood flow and oxygenation in peritendinous tissue and calf muscle during dynamic exercise in humans. *J Physiol* 524 Pt 1: 305–313, 2000.
7. Burke D. Effects of activity on axonal excitability: implications for motor control studies. *Adv Exp Med Biol* 508: 33–37, 2002.
8. Chen TC, Chen H-L, Liu Y-C, Nosaka K. Eccentric exercise-induced muscle damage of pre-adolescent and adolescent boys in comparison to young men. *Eur J Appl Physiol* 114: 1183–1195, 2014.
9. Cohen J. *Statistical power analysis for Behavioral sciences*. Academic Press., 1969.
10. De Ste Croix MBA, Deighan MA, Ratel S, Armstrong N. Age- and sex-associated differences in isokinetic knee muscle endurance between young children and adults. *Appl Physiol Nutr Metab Physiol Appl Nutr Metab* 34: 725–731, 2009.
11. Denis R, Bringard A, Perrey S. Vastus lateralis oxygenation dynamics during maximal fatiguing concentric and eccentric isokinetic muscle actions. *J Electromyogr Kinesiol Off J Int Soc Electrophysiol Kinesiol* 21: 276–282, 2011.
12. Dipla K, Tsirini T, Zafeiridis A, Manou V, Dalamitros A, Kellis E, Kellis S. Fatigue resistance during high-intensity intermittent exercise from childhood to adulthood in males and females. *Eur J Appl Physiol* 106: 645–653, 2009.
13. Edgerton VR, Smith JL, Simpson DR. Muscle fibre type populations of human leg muscles. *Histochem J* 7: 259–266, 1975.
14. Ferrari M, Muthalib M, Quaresima V. The use of near-infrared spectroscopy in understanding skeletal muscle physiology: recent developments. *Philos Transact A Math Phys Eng Sci* 369: 4577–4590, 2011.
15. Gandevia SC. Spinal and supraspinal factors in human muscle fatigue. *Physiol Rev* 81: 1725–1789, 2001.
16. Gorianovas G, Skurvydas A, Streckis V, Brazaitis M, Kamandulis S, McHugh MP. Repeated bout effect was more expressed in young adult males than in elderly males and boys. *BioMed Res Int* 2013: 218970, 2013.
17. Halin R, Germain P, Bercier S, Kapitaniak B, Buttelli O. Neuromuscular response of young boys versus men during sustained maximal contraction. *Med Sci Sports Exerc* 35: 1042–1048, 2003.
18. Hatzikotoulas K, Patikas D, Ratel S, Bassa E, Kotzamanidis C. Central and peripheral fatigability in boys and men during maximal contraction. *Med Sci Sports Exerc* 46: 1326–1333, 2014.
19. Hermens HJ, Freriks B, Disselhorst-Klug C, Rau G. Development of recommendations for SEMG sensors and sensor placement procedures. *J Electromyogr Kinesiol Off J Int Soc Electrophysiol Kinesiol* 10: 361–374, 2000.
20. Hill CA, Thompson MW, Ruell PA, Thom JM, White MJ. Sarcoplasmic reticulum function and muscle contractile character following fatiguing exercise in humans. *J Physiol* 531: 871–878, 2001.
21. Hunter SK. Performance Fatigability: Mechanisms and Task Specificity. *Cold Spring Harb. Perspect. Med.* (May 15, 2017). doi: 10.1101/cshperspect.a029728.
22. Kluka V, Martin V, Vicencio SG, Giustiniani M, Morel C, Morio C, Coudeyre E, Ratel S. Effect of muscle length on voluntary activation of the plantar flexors in boys and men. *Eur J Appl Physiol* 116: 1043–1051, 2016.
23. Kluka V, Martin V, Vicencio SG, Jegu A-G, Cardenoux C, Morio C, Coudeyre E, Ratel S. Effect of muscle length on voluntary activation level in children and adults. *Med Sci Sports Exerc* 47: 718–724, 2015.
24. Lazaridis S, Patikas DA, Bassa E, Tsatalas T, Hatzikotoulas K, Ftikas C, Kotzamanidis C. The acute effects of an intense stretch-shortening cycle fatigue protocol on the neuromechanical parameters of lower limbs in men and prepubescent boys. *J Sports Sci* 36: 131–139, 2018.
25. Lexell J, Sjöström M, Nordlund AS, Taylor CC. Growth and development of human

muscle: a quantitative morphological study of whole vastus lateralis from childhood to adult age. *Muscle Nerve* 15: 404–409, 1992.

26. **Marginson V, Rowlands AV, Gleeson NP, Eston RG.** Comparison of the symptoms of exercise-induced muscle damage after an initial and repeated bout of plyometric exercise in men and boys. *J Appl Physiol Bethesda Md* 1985 99: 1174–1181, 2005.

27. **Martin V, Millet GY, Martin A, Deley G, Lattier G.** Assessment of low-frequency fatigue with two methods of electrical stimulation. *J Appl Physiol Bethesda Md* 1985 97: 1923–1929, 2004.

28. **Matsushita K, Homma S, Okada E.** Influence of adipose tissue on muscle oxygenation measurement with NIRS instrument. *Proc Soc Photo-Opt Instrum Eng* 3194: 159–165, 1998.

29. **Merton PA.** Voluntary strength and fatigue. *J Physiol* 123: 553–564, 1954.

30. **Mirwald RL, Baxter-Jones ADG, Bailey DA, Beunen GP.** An assessment of maturity from anthropometric measurements. *Med Sci Sports Exerc* 34: 689–694, 2002.

31. **Moalla W, Merzouk A, Costes F, Tabka Z, Ahmaidi S.** Muscle oxygenation and EMG activity during isometric exercise in children. *J Sports Sci* 24: 1195–1201, 2006.

32. **Murphy JR, Button DC, Chaouachi A, Behm DG.** Prepubescent males are less susceptible to neuromuscular fatigue following resistance exercise. *Eur J Appl Physiol* 114: 825–835, 2014.

33. **Neyroud D, Rüttimann J, Mannion AF, Millet GY, Maffiuletti NA, Kayser B, Place N.** Comparison of neuromuscular adjustments associated with sustained isometric contractions of four different muscle groups. *J Appl Physiol Bethesda Md* 1985 114: 1426–1434, 2013.

34. **Panizza M, Nilsson J, Roth BJ, Basser PJ, Hallett M.** Relevance of stimulus duration for activation of motor and sensory fibers: implications for the study of H-reflexes and magnetic stimulation. *Electroencephalogr Clin Neurophysiol* 85: 22–29, 1992.

35. **Paraschos I, Hassani A, Bassa E, Hatzikotoulas K, Patikas D, Kotzamanidis C.** Fatigue differences between adults and prepubertal males. *Int J Sports Med* 28: 958–963, 2007.

36. **Piponnier E, Ratel S, François B, Garcia-Vicencio S, Martin V.** Assessment of the H-reflex using two synchronized magnetic stimulators in order to increase stimulus durations: A comparison

with electrical stimulation. *Neurosci Lett* 675: 89–94, 2018.

37. **Ratel S, Kluka V, Vicencio SG, Jegu A-G, Cardenoux C, Morio C, Coudeyre E, Martin V.** Insights into the Mechanisms of Neuromuscular Fatigue in Boys and Men. *Med Sci Sports Exerc* 47: 2319–2328, 2015.

38. **Ratel S, Tonson A, Le Fur Y, Cozzone P, Bendahan D.** Comparative analysis of skeletal muscle oxidative capacity in children and adults: a 31P-MRS study. *Appl Physiol Nutr Metab Physiol Appl Nutr Metab* 33: 720–727, 2008.

39. **de Ruiter CJ, de Boer MD, Spanjaard M, de Haan A.** Knee angle-dependent oxygen consumption during isometric contractions of the knee extensors determined with near-infrared spectroscopy. *J Appl Physiol Bethesda Md* 1985 99: 579–586, 2005.

40. **Rupp T, Girard O, Perrey S.** Redetermination of the optimal stimulation intensity modifies resting H-reflex recovery after a sustained moderate-intensity muscle contraction. *Muscle Nerve* 41: 642–650, 2010.

41. **Sejersted OM, Hargens AR.** Intramuscular pressures for monitoring different tasks and muscle conditions. *Adv Exp Med Biol* 384: 339–350, 1995.

42. **Streckis V, Skurvydas A, Ratkevicius A.** Children are more susceptible to central fatigue than adults. *Muscle Nerve* 36: 357–363, 2007.

43. **Tanner JM, Whitehouse RH.** Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. *Arch Dis Child* 51: 170–179, 1976.

44. **Tonson A, Ratel S, Le Fur Y, Vilmen C, Cozzone PJ, Bendahan D.** Muscle energetics changes throughout maturation: a quantitative 31P-MRS analysis. *J Appl Physiol Bethesda Md* 1985 109: 1769–1778, 2010.

45. **Twomey R, Aboodarda SJ, Kruger R, Culos-Reed SN, Temesi J, Millet GY.** Neuromuscular fatigue during exercise: Methodological considerations, etiology and potential role in chronic fatigue. *Neurophysiol Clin Clin Neurophysiol* 47: 95–110, 2017.

46. **Verges S, Maffiuletti NA, Kerherve H, Decorte N, Wuyam B, Millet GY.** Comparison of electrical and magnetic stimulations to assess quadriceps muscle function. *J Appl Physiol Bethesda Md* 1985 106: 701–710, 2009.

CONTRIBUTIONS

The study was designed by EP, VM, MD, and SR; data were collected and analyzed by EP, VM, BB, EC, VJ, OB, MD and SR; data interpretation and manuscript preparation were undertaken by EP, VM, BB, EC, VJ, OB, MD and SR. All authors approved the final version of the paper.