Child-adult differences in neuromuscular fatigue are muscle-dependent

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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.
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.) (26). Neuromuscular fatigue was found to be lower in prepubertal children than adults during isometric (3, 17, 18, 38) and isokinetic (10, 12, 33, 36) 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, 33, 38), owing to their greater relative energy contribution derived from oxidative sources (39, 45), 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 (38, 43).

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 (34). Muscle typology and the absolute force level produced may explain these differences between muscle groups (5, 34). 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 (34). Interestingly, prepubertal children may have a greater percentage of slow twitch muscle fibers in vastus lateralis (25) and a lower absolute force level of the KE muscles than adults (38). However, the differences in the absolute force level (22), and slow twitch fiber proportion (13) between these two age groups could be reduced on the PF muscles. 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 (38) showed no significant change of maximal M-wave ($M_{\text{max}}$) throughout repeated maximal voluntary isometric contractions (MVICs) of the KE muscles in boys and men, while other studies reported a similar $M_{\text{max}}$ decrement in the PF muscles in boys and men (18) or an increment in boys and a significant decrease of $M_{\text{max}}$ in the KE muscles in men (33). This inconsistence 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 (34). 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 (27). 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 O₂ saturation was found to decrease during submaximal sustained contractions in children (32) and adults (40), 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 (39, 45). 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 (38). 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 (34) 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 (38, 43) 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 (38). 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.

- Please insert the figure 1 near here -
Before any exercise, subjects were equipped and performed a progressive warm-up (4 contractions up to ~ 50% MVIC, 4 contractions up to ~ 80% MVIC and 2 contractions up to ~ 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 (44), 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. (31).
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 ± 5.1° and 75.4 ± 4.9° 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 μs; 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 (\(Q_{\text{tw unpot}}\)) and the greatest compound muscle action potentials (i.e. maximal M-
wave, \(M_{\text{max}}\)) were evoked. Prior to the testing procedure, the optimal stimulation intensities
were determined from recruitment curves (the intensities where \(Q_{\text{tw unpot}}\) and concomitant M-
waves amplitudes of the KE, i.e. *vastus lateralis*: VL, *vastus medialis*: VM and *rectus
gemoris*: RF, and PF muscles, i.e. *soleus*: SOL and *gastrocnemius medialis*: GM, reached their
maximal values and started to plateau, \(I_{\text{max}}\)). 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; \(I_{\text{Hmax}}\)) 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 (35, 37). 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 ± 11.2% and 111.9 ±
15.9% of the optimal intensity in boys with the KE and PF muscles, respectively. The
| corresponding values for the men were 110.9 ± 16.1% and 109.7 ± 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 > 85dB, 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 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 \(\mu\)Mol 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 \(6.7 \pm 2.5\) and \(5.8 \pm 2.2\) mm in boys and men, respectively \((p = 0.25)\). The corresponding values for the GL muscle were \(4.4 \pm 1.9\) and \(2.9 \pm 0.8\) 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 \((29)\). 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 \(\text{(i.e. the mean value over 30 s before the onset of the first contraction)}\). For all the contractions performed, the minimum TSI value reached during the contraction \((\Delta TSI)\) was measured. Important \(\Delta TSI\) decreases indicate greater \(O_2\) demand relative to \(O_2\) supply \((14)\). TSI
desaturation slope ($\text{TSI}_{\text{slope}}$) was also determined as the negative slope of the least-squares regression line of TSI during each contraction. Higher $\text{TSI}_{\text{slope}}$ indicates an important muscle $\text{O}_2$ demand (14).

**Peripheral fatigue indicators**

To examine the time course of peripheral fatigue, potentiated single twitches ($\text{Qtw}_{\text{pot}}$; 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 the KE testing and tibial nerve for the PF testing; Fig. 1). Concomitant peak-to-peak M-wave amplitudes ($M_{\text{max}}$) 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 ($\text{Dt}_{10\text{Hz}}$ and $\text{Dt}_{100\text{Hz}}$) were evoked. $\text{Dt}_{100\text{Hz}}$ is usually considered as an indicator of muscle contractile properties (46). Intensity of double stimulations was set to 60% of the maximal magnetic stimulator output since higher intensities were painful especially in children. The $\text{Dt}_{10\text{Hz}}$-to-$\text{Dt}_{100\text{Hz}}$ ratio ($\text{Dt}_{10\text{Hz}}/\text{Dt}_{100\text{Hz}}$) obtained from magnetic stimulation was calculated and used to assess low-frequency fatigue (47).

**Central fatigue indicators**

Twitch interpolation technique was used to determine VA. Superimposed single twitch ($\text{Qtw}_s$) was evoked during MVIC after the torque had reached a plateau. Then, $\text{Qtw}_s$ and $\text{Qtw}_{\text{pot}}$ were used to quantify VA before, every five MVIC and during the last MVIC (Fig. 1) as proposed by Merton (30) in equation 1:

$$[1] \text{VA} (\%) = \left[ 1 - \left( \frac{\text{Qtw}_s \cdot \text{Qtw}_{\text{pot}}}{\text{Qtw}_s} \right)^{-1} \right] \cdot 100$$

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 \( M_{\text{max}} \) (RMS\( \cdot M_{\text{max}} \)) to account for differences in muscle mass and potential changes/differences in sarcolemmal excitability.

Before and after the fatigue protocol, myotatic reflex gain \( (T_{\text{slope}}) \) 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 \( (H_{\text{max}}) \) was evoked at \( I_{H_{\text{max}}} \) before, during and after the intermittent fatigue protocol (Fig. 1). We also computed the ratio between SOL M-wave evoked at \( I_{H_{\text{max}}} \) and \( M_{\text{max}} \) (\( M_{H_{\text{max}}} / M_{\text{max}} \)) to ensure stimulation intensity consistency throughout the fatigue protocol. The \( H_{\text{max}} / M_{\text{max}} \) ratio was used as a valid parameter (37) 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:

\[
[2] \quad %CoAct = \left( RMS_{\text{ago}} \cdot RMS_{\text{anta}}^{-1} \right) \cdot 100
\]

Where \( RMS_{\text{ago}} \) is the RMS value of BF or TA during intermittent contractions, and \( RMS_{\text{anta}} \) 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 × 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 × muscle group × %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 × %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 ($\eta^2$) and ranked as follows: $\sim 0.01$ = small effect, $\sim 0.06 = moderate effect, \geq 0.14 = large effect (9)$. 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 ± standard deviation (SD). The $\alpha$–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 (age group × muscle group) for the number of repetitions \[F(1;43) = 39.03, P < 0.001, \eta^2 = 0.48,\] power = 0.99]. For the 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 interactions (age group × muscle group × %REP) were found for the absolute and relative MVIC torque \[F(5;215) = 25.39, P < 0.001, \eta^2 = 0.37,\] power = 1.0; \(F(4;172) = 3.65, P < 0.01, \eta^2 = 0.08,\) 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).

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**Peripheral fatigue**

**Twitch and doublet torque.** ANOVA showed a significant interaction (age group × muscle group × %REP) for the \(Q_{tw_{pot}}\) 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]. After the KE muscles fatigue test, men displayed a significantly greater \(Q_{tw_{pot}}\) decrement than boys \((-49.6 ± 14.3 \text{ vs. } -17.3 ± 18.5\%,\) respectively; Fig. 2B). Furthermore, ANOVA revealed a significant interaction (age group ×
muscle group × %REP) regarding the absolute $D_{100Hz}$ values [$F(1;43) = 9.93$, $P < 0.01$, $\eta^2 = 0.23$, power $= 0.86$]. $D_{100Hz}$ decreased only in men over the entire fatigue test ($-36.6 \pm 20.5\%$, $p < 0.001$). ANOVA also revealed a significant age group effect regarding the relative $D_{10Hz}/D_{100Hz}$ values [$F(1;43) = 12.47$, $P < 0.01$, $\eta^2 = 0.27$, power $= 0.93$]. $D_{10Hz}/D_{100Hz}$ ratio decreased to a greater extent in men than boys over the fatigue test ($-21.6 \pm 18.6$ vs. $-9.1 \pm 11.9\%$, respectively; Fig. 3).

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**M wave.** No significant main effect or interaction 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 (age group × %REP) for the VL $\Delta TSI$ [$F(4;172) = 2.85$, $P < 0.05$, $\eta^2 = 0.08$, power $= 0.76$]. Men displayed a greater $\Delta TSI$ than boys in the VL muscle throughout the fatigue test ($p < 0.05$; Fig. 4A). In contrast, ANOVA revealed no significant main effect or interaction for the VL $TSl_{slope}$. $TSl_{slope}$ remained unchanged in the VL muscle in boys and men.

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**Central fatigue**

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

**T-reflex.** No significant main effect or interaction were found for the VL T\textsubscript{slope} values. No significant change in VL T\textsubscript{slope} was observed throughout the fatigue protocol in boys and men.

**Co-activation level.** ANOVA showed a significant interaction (age group \(\times\) %REP) for the absolute BF %CoAct values \([F(5;215) = 8.99, P < 0.001, \eta^2 = 0.20, \text{power} = 0.99]\). Boys showed a significantly higher %CoAct than men at 0%REP (16.4 ± 6.1 vs. 8.2 ± 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 (age group \(\times\) muscle group) for the number of repetitions \([F(1;43) = 39.03, P < 0.001, \eta^2 = 0.48, \text{power} = 0.99]\). During the 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 interactions (age group \(\times\) muscle group \(\times\) %REP) were found for the absolute and relative MVIC torque \([F(5;215) = 25.39, P < 0.001, \eta^2 = 0.37, \text{power} = 1.0;\]
F(4;172) = 3.65, P < 0.01, $\eta^2 = 0.08$, power = 0.87, respectively. PF 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 (age group $\times$ muscle group $\times$ %REP) for the $Q_{twpot}$ 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; $F(4;172) = 6.86, P < 0.001, \eta^2 = 0.14$, power = 0.99, respectively]. During the PF muscles fatigue test, men showed a significant decrement of $Q_{twpot}$ ($-24.8 \pm 16.0\%$, p < 0.01), whereas it remained unchanged in boys throughout the fatigue protocol (Fig. 2E). Similarly, ANOVA revealed a significant interaction (age group $\times$ muscle group $\times$ %REP) regarding the absolute $D_{t100Hz}$ values [$F(1;43) = 9.93, P < 0.01, \eta^2 = 0.23$, power = 0.86]. $D_{t100Hz}$ only decreased in men ($-49.3 \pm 22.3\%$, p < 0.05). ANOVA also revealed a significant age group effect regarding the relative $D_{t10Hz}/D_{t100Hz}$ values [$F(1;43) = 12.47, P < 0.01, \eta^2 = 0.27$, power = 0.93]. Boys displayed a lower decrement in the $D_{t10Hz}/D_{t100Hz}$ ratio than men ($-4.9 \pm 5.2$ vs. $-13.4 \pm 10.7\%$, respectively; Fig. 3).

**M wave.** No significant main effect or interaction was found for the SOL $M_{max}$ values. No significant change was found for SOL in boys and men. ANOVA revealed a significant interaction (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 $\Delta TSI$ [$F(1;43) = 7.22, P < 0.05, \eta^2 = 0.17$, power = 0.74]. Men displayed a significantly greater $\Delta TSI$ in the
GL than boys throughout the fatigue test (p < 0.05; Fig. 4B). In contrast, ANOVA revealed no significant main effect or interaction for the GL TSI_slope. TSI_slope remained unchanged in the GL muscle in boys and men.

Central fatigue

VA and normalized EMG. A significant interaction (age group × muscle group × %REP) was found for the absolute VA values [F(5;215) = 2.52, P < 0.05, η² = 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 ± 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 ± 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 their initial values [F(1;43) = 5.28, P < 0.05, η² = 0.12, power = 0.61 and F(1;43) = 7.65, P < 0.01, η² = 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 ± 27.2%, respectively; p < 0.01) and GM muscles (-50.1 ± 18.7 vs. -31.5 ± 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, η² = 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 relative decrements in the T_slope for the SOL (-29.3 ± 37.5% and -31.8 ± 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, η² = 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 absolute $H_{\text{max}}/M_{\text{max}}$ 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 $M_{H_{\text{max}}/M_{\text{max}}}$ [F(5;215) = 5.12, $P < 0.05$, $\eta^2 = 0.16$, power = 0.59]. No change of $M_{H_{\text{max}}/M_{\text{max}}}$ was observed throughout the fatigue protocol in children (19.5 ± 7.8% and 17.5 ± 8.3% at pre- and post-fatigue, respectively) and adults (33.0 ± 22.2% and 33.5 ± 21.5% at pre- and post-fatigue, respectively).

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**Co-activation level.** ANOVA showed significant interaction effects (age group × %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; 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 ± 7.3%, $p < 0.05$, respectively). Furthermore, whilst boys displayed a significant and progressive decrement in the absolute %CoAct throughout the fatigue protocol ($p < 0.001$), men showed a significant decrease in the absolute %CoAct only from 60%REP ($p < 0.05$). At the end of exercise, boys showed a higher relative %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**

Significant interaction effects were previously presented. Thus, only main effects (muscle group) and post-hoc test analyses will be described in this section.
**Number of repetitions.** 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 muscle groups.

**Twitch and doublet torque.** In boys, Qtw$_{pot}$ significantly decreased with the KE muscles (p < 0.001) but not with the PF muscles (Fig. 2B and E). In men, the relative decrement in Qtw$_{pot}$ was greater for the KE than the PF muscles (-49.6 ± 14.3 vs. -24.8 ± 16.0%, p < 0.001). Furthermore, 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 ± 17.1%, muscle group effect: F(1;43) = 4.84, P < 0.05, $\eta^2$ = 0.13, power = 0.57). Finally, ANOVA 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 of the age group.

**VA.** 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 ± 14.2%, respectively; p < 0.001), whereas in men, VA only significantly decreased with the PF muscles (-13.0 ± 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 lack of difference in neuromuscular fatigue of the PF muscles between children and adults has been associated with reduced differences in peripheral and central fatigue (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, 38). 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 (38). 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 potentiating twitch torque of the KE muscles (33, 38, 43) 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 (39, 45) 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 difference in peripheral fatigue 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, 34).

We also reported no decrement of $M_{\text{max}}$ throughout the maximal intermittent exercise in both populations and within both muscle groups, which is consistent with our previous study regarding the KE muscles (38) but in contrast with other studies reporting changes in $M_{\text{max}}$ of
the RF and SOL muscles during different maximal continuous or intermittent fatigue tests (18, 33). 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 $D_{t100Hz}$ was only observed in men for both muscle groups, which is consistent with the results published by Streckis et al. (43). 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 $D_{t100Hz}$ was evoked with a submaximal intensity to reduce discomfort. Although the use of a submaximal intensity may bias the evaluation of $D_{t100Hz}$ (28), this limitation should affect similarly children and adults. As a result, the comparison of $D_{t100Hz}$ 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 $D_{t10Hz}/D_{t100Hz}$ 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 (45). This mechanism 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 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 (42). 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, 33, 38, 43), 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, 34) 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 \( M_{H_{\text{max}}} \) did not change throughout the fatigue protocol (suggesting that the stimulation was consistent), 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 (41). 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 (38). 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 (38). 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 O₂ 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.
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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.
FIGURES LEGENDS

Figure 1: Overview of the fatigue test sessions. All steps of these sessions are commented in the text. Single stimulations at $I_{H_{\text{max}}}$ (intensity corresponding to maximal H-reflex amplitude) were only triggered during PF fatigue testing. MVIC and MVIC$_{\text{anta}}$: maximal voluntary isometric contraction of the agonist and antagonist muscles, respectively; $\%P_{\text{stim, max}}$: maximal stimulator power output (i.e. 100%).

Figure 2: Time course of maximal voluntary isometric contraction (MVIC) torque, potentiated twitch torque amplitude ($Q_{\text{tw,pot}}$) 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).

Figure 3: Low- to high-frequency torque ratio variation ($\Delta D_{10\text{Hz}}/D_{100\text{Hz}}$) 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 ($\Delta TSI$) 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 $p < 0.001$.

Figure 5: Time course of SOL maximal H-reflex/M-wave ratio ($H_{\text{max}}/M_{\text{max}}$), 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).