Greater fatigability in knee-flexors vs knee-extensors after a standardized fatiguing protocol

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Abstract

The present study aimed to investigate the effects of a standardized fatiguing protocol on central and peripheral fatigue in knee-flexors and knee-extensors. Thirteen healthy men (age: 23±3 years; height: 1.78±0.09 m; body-mass: 73.6±9.2 kg) volunteered for the present study. Maximal voluntary contraction (MVC), EMG activity, voluntary activation level (VAL) as an index of central fatigue and twitch potentiation as an index of peripheral fatigue were measured before and after the fatiguing protocol. The fatiguing protocol consisted of a 0.6 duty-cycle to exhaustion (6s isometric contraction, 4s recovery) at 70% MVC. After the fatiguing protocol, MVC decreased in both (ES=1.14) and knee-extensors (ES=1.14), and EMG activity increased in both knee-flexors (ES=2.33) and knee-extensors (ES=1.54). Decreases in VAL occurred in knee-flexors (ES=0.92) but not in knee-extensors (ES=0.04). Decreases in potentiation occurred in both knee-flexors (ES=0.84) and knee-extensors (ES=0.58). The greater central occurrence of fatigue in knee-flexors than in knee-extensors may depend on the different muscle morphology and coupled with a greater tolerance to fatigue in knee-extensors. The present data add further insight to the complicated knee-flexors-to-knee-extensors strength relationship and the mechanisms behind the different occurrence of fatigue.

Key-words: maximal voluntary contraction; voluntary activation level; potentiated twitch; EMG activity.

Introduction

Knee-flexors and knee-extensors are strictly and reciprocally connected since they act as antagonist muscles for both knee and hip joints. Notwithstanding, because of the reported muscle strength imbalance in favour of knee-extensors, possible repercussion on the knee-flexors injury risk exist (Coratella, Bellin, Beato, & Schena, 2015; Coratella, Bellini, & Schena, 2016; Delextrat, Baker, Cohen, & Clarke, 2013; Delextrat, Gregory, & Cohen, 2010; Rahnama, Reilly, Lees, & Graham-Smith, 2003). Knee-flexors and knee-extensors strength was shown to be affected differently by fatigue, since the maximal strength in knee-flexors deteriorates more compared to knee-extensors (Coratella, Bellin, et al., 2015; Coratella et al., 2016; Delextrat et al., 2010; Rahnama et al., 2003). This has practical relevance, given a greater incidence of knee-flexors injuries reported within the last 15 minutes of a soccer match (Hawkins, Hulse, Wilkinson, Hodson, & Gibson, 2001). However, how fatigue affects both knee-flexors and knee-extensors strength is still unclear and the mechanisms behind the occurrence of fatigue need further and deeper investigation.

Overall, fatigue is defined as a “progressive incapacity to maintain a given force continuously or intermittently” (Enoka & Stuart, 1985) or “an exercise-induced reduction in maximal voluntary muscle force” (Gandevia, 2001). The drop in maximal force depends on the neuromuscular capacity to maximally recruit muscle activity, whose impairments can derive from peripheral or central mechanisms (Gandevia, 2001). Such peripheral or central occurrence of fatigue can be investigated using muscular or nervous electrical stimulations (Neyroud, Vallotton, Millet, Kayser, & Place, 2014; Rozand, Grosprêtre, Stapley, & Lepers, 2015). Among the possible markers, peripheral fatigue is characterized by a reduction in resting-twitch elicited from electrical stimulations while central fatigue can be assessed through the voluntary activation level (VAL) using the twitch interpolation technique, which characterizes the ability to voluntary activate most of the available motor units (Gandevia, 2001; Rozand et al., 2015). Previous studies have investigated the mechanisms behind fatigue in knee-extensors or knee-flexors using such a technique. In knee-extensors, inconsistent results have been found, since fatigue was mainly explained by central mechanisms after a rowing fatiguing task (Husmann et al., 2017), while no significant central fatigue was detected after an intermittent isometric exercise (Bachasson, Decorte, Wuyam, Millet, & Verges, 2016). On the contrary, the only study that investigated the knee-flexors occurrence of fatigue using the interpolated-twitch technique after a soccer-match simulation, showed that fatigue was mainly due to central factors (Marshall, Lovell, Jeppesen, Andersen, & Siegler, 2014). However, a direct comparison between knee-flexors and knee-extensors has not yet been performed.

The studies that have previously investigated the effects of fatigue on knee-flexors and knee-extensors have shown some limitations. Consequently, these studies failed to provide an in-depth description of the mechanisms behind the occurrence of fatigue. Firstly, several studies used a soccer match simulation as a fatiguing protocol (Coratella, Bellin, et al., 2015; Delextrat et al., 2010; Marshall et al., 2014; Rahnama et al., 2003). This includes several confounding factors, *i.e.* muscle activation, contraction modality and time under tension that may affect the occurrence of muscle fatigue, precluding a direct knee-flexors *vs* knee-extensors comparison. On the other hand, a study that used a comparable fatiguing task for both knee-flexors and knee-extensors did not investigate the occurrence of central or peripheral fatigue (Coratella et al., 2016). Consequently, although the afore-mentioned studies clearly reported that knee-flexors are more fatigable than knee-extensors, the causes of such a difference have not yet been elucidated. Therefore, the aim of the current study was to investigate the central and peripheral contribution to both knee-flexors and knee-extensors fatigue after a standardized isometric fatiguing task. Since the higher prevalence of type-II fibres in knee-flexors compared to knee-extensors (Garrett, Califf, & Bassett, 1984), it was hypothesized that the knee-flexors would exhibit greater central and peripheral fatigue.

METHODS

Participants

Thirteen healthy men (age: 23(3) yrs; height: 1.78(0.09) m; weight: 73.6(9.2) kg) volunteered for the present study. The participants were recreationally active in several sports but were not engaged in any official competition or regular strenuous physical activity at that time. People with hip, knee or ankle joint or muscular diseases were excluded from the present study. Similarly, the regular use of drugs as well as the presence of any cardio-vascular and pulmonary diseases was considered as exclusion criteria. The participants were instructed about any possible risks related to the present procedures and they signed a written informed consent before their inclusion. The present study was approved by the local Ethical Committee (CPP-Est-I-2016-A00511-50) and it was conducted according to the recommendations of the Declaration of Helsinki (1975) for studies involving human subjects.

Experimental approach to the research question

The current study was designed as a cross-sectional study. The participants were involved in two separate sessions for knee-flexors or knee-extensors respectively. The two sessions were separated by at least two days and their order was randomized. The participants avoided any form of strenuous physical activity at least two days preceding the first session to the end of the study. The fatiguing protocol consisted in a single-limb intermittent isometric exercise until exhaustion, performed by the dominant limb (Figure 1A). The peripheral and central occurrence of fatigue was investigated using electrical stimulations before and after the fatiguing task (Rozand et al., 2015), while the mechanical and EMG signals were recorded throughout the fatiguing task. The study design is depicted in Figure 1B.

Please insert figure 1 here

Strength measurement

The isometric maximal voluntary contraction (MVC) in both knee-flexors and knee-extensors was measured using a customized chair equipped with a strain gauge (Digital Transducer, MIE Medical Research, Leeds, United Kingdom) (Figure 1A). The participants sat on the chair at a hip angle of 80° (0°=full extension) and were secured by two seatbelts. The belts secured the tested limb at 60° of knee flexion (0°=full extension) and the untested limb was immobilized by a fixed lever (Coratella, Milanese, & Schena, 2015). This angle was selected to avoid angles close to the full knee extension or flexion (*i.e.* knee-flexors or knee-extensors long muscle length, respectively), which would have affected the maximal voluntary activation (Doguet et al., 2017; Kluka et al., 2015). The upper-limbs were crossed against the chest. A strap was placed below the knee to avoid side-to-side movement and limit any force dispersion. Before the MVC assessment, the participants were familiarized with the procedures. Thereafter, they performed a warm-up protocol consisting of 20 isometric contractions, separated by 10 s each, starting from a self-selected force and progressively increasing until the maximal volitional force was exerted. Then, two separate MVC trials were performed. If the difference between the two trials exceeded 5%, further trials were performed. Each trial lasted 4 s and was separated by 3 min of passive recovery. Immediately after the fatiguing protocol, one MVC was performed. Lastly, the knee-flexors-to-knee-extensors MVC ratio was calculated and inserted in the data analysis. The operators strongly encouraged the participants to "push" or "pull" as hard as they could to reach their maximal force on each trial. All force signals were recorded at 2 kHz using an AD conversion system (LabChart 8, ADInstruments, Sydney, Australia).

Experimental fatigue protocol

The participants sat on the customized chair used for testing MVC, with the upper-limbs crossed against their chest and the strap placed below the knee. The knee-extensors and knee-flexors MVC was used to individually tailor the fatiguing protocol. This consisted of a duty-cycle of 6 s isometric knee extension or flexion and 4 s of recovery until exhaustion (Conchola, Thiele, Palmer, Smith, &

Thompson, 2015). During the isometric exercise, the participants were required to hold a horizontal line corresponding to 70% of the knee-extensors or knee-flexors MVC on a monitor displaying the force exerted in real time, as previously used (Boccia, Coratella, et al., 2016; Boccia, Dardanello, Coratella, et al., 2015; Boccia, Dardanello, et al., 2016; Boccia, Dardanello, Rinaldo, et al., 2015). The fatiguing protocol ended when the participants were not able to hold the line for two consecutive six-second contractions, despite their maximal efforts. The operators strongly encouraged the participants to resist as long as possible. Rating of perceived exertion was then recorded using a 0-10 Borg scale (Borg & Kaijser, 2006). Similarly, the number of repetitions to exhaustion performed during the fatiguing protocol for knee-flexors or knee-extensors was recorded.

EMG measurements

The activation of knee-flexors and knee-extensors were assessed using surface EMG signals recorded from *vastus lateralis* and the long head of *biceps femoris*, respectively (Conchola et al., 2015). The skin was shaved, abraded and cleaned with isopropyl alcohol to ensure low impedance (< 5 kΩ). The electrodes were placed over each muscle belly following the SENIAM recommendations (Hermens, Freriks, Disselhorst-Klug, & Rau, 2000). The EMG signals were acquired using a wireless system (Trigno, Delsys Inc., USA). Before placing the sensors, an EMG signal-inspection on both knee-extensors and knee-flexors was performed to identify their best position (Beretta Piccoli et al., 2014). The EMG signals were amplified with a bandwidth frequency ranging from 0.3 Hz to 1 kHz at a sampling frequency of 2 kHz using the Powerlab data acquisition system (LabChart 8, ADInstruments, Sydney, Australia). To avoid any possible undesired movement or friction of the electrodes placed on knee-flexors, the participants sat on a tunnel-shaped foam board located under the thigh.

Muscle stimulation

The electrical stimulations were evoked on *biceps femoris* or *vastus lateralis* at baseline, during and 3 s after each MVC. Such a procedure was repeated before and after the fatiguing task. Direct muscle stimulation was applied to *biceps femoris* or *vastus lateralis* using pre-gelled surface electrodes (8 x 6 cm, Medicompex SA, Ecublens, Switzerland). To stimulate *biceps femoris*, the anode was placed above the popliteal fossa and the cathode below the gluteal fold. To stimulate *vastus lateralis*, the cathode was placed 2-to-3 cm above the superior aspect of the patella and the anode 4-to-5 cm below the inguinal fold. Doublet electrical stimulations were delivered by a high-voltage stimulator (Digitimer DS7AH, Welwyn Garden City, UK), which consisted of rectangular pulses (pulse duration: 1 ms) with an inter-pulse duration of 10 ms (100 Hz). Before each MVC, the participants were familiarized with the stimulations. The doublet stimulations were used both at rest and superimposed during each MVC. The stimulation intensity was gradually increased by 10 mA increments until a plateau in single-twitch force was reached. Then, 130% of such a voltage-intensity was used to achieve the supra-maximal intensity during the experimental session. For each MVC, three doublet stimulations at the supra-maximal intensity with an inter-pulse duration of 10 ms (100 Hz) were evoked: at rest before, during (superimposed) and after (potentiated doublet) the MVC. The superimposed doublet was manually triggered once the plateau of the MVC was reached. The potentiated doublet was evoked 3 s after relaxation.

Data analysis

During the MVCs, EMG signal was quantified as root mean square (RMS) amplitude for the 500 ms preceding the doublet stimulation. During the fatiguing protocol, RMS was calculated at 3 s for each isometric contraction during a 500 ms period. The time course of EMG signals during the fatiguing protocol was grouped as 25th, 50th, 75th and 100th percentile of the number of repetitions. The peak force of each mechanical response to doublet stimulations evoked at rest, during (superimposed twitches) and after MVC (potentiated twitches) was measured and analysed. The superimposed doublet was measured as the peak-to-peak mechanical twitch over the force-plateau developed before the stimulation. Each twitch was measured manually after a careful selection and inspection of the time periods before and after the stimulations. Muscle potentiation was estimated through the ratio of the potentiated twitch divided by the resting twitch. The interpolated twitch technique was used to quantify maximal voluntary activation level (VAL) during the MVCs (Allen et al. 1995). The amplitude of each superimposed and potentiated doublet twitch was then used in the following formula: VAL = (1 − (superimposed doublet force / potentiated doublet force)) × 100.

Statistical analysis

The statistical analysis was performed using a statistical software (SPSS 20, IBM, USA). The normality of data was checked using the Kolmogorov-Smirnov test and all distributions were normal. The difference in the number of repetitions and rating of perceived exertion was analysed using a pairwise, two-tailed T-test. A two-way repeated-measures ANOVA was used to calculate differences in MVC, VAL, potentiation and EMG over time (two levels: pre and post) and muscle (two levels: knee-flexors and knee-extensors). Post-hoc analysis was then performed using Bonferroni's correction. A two-way repeated-measures ANOVA was performed to calculate differences in EMG activity during the fatiguing protocol over time (four levels: 25th, 50th, 75th and 100th percentiles) between knee-flexors and knee-extensors. Significance was set at $p < 0.05$. Data are shown as mean(SD). Changes are shown as mean change, with lower and upper bounds of confidence interval (CI95%). Effect-size (ES) was calculated and interpreted according to Cohen's recommendations: < 0.20 = *trivial*; 0.21-0.49 = *small*; 0.50-0.79 = *moderate*; 0.80-1.19 = *large*; ≥ 1.20 = *very large*.

Results

The number of repetitions to exhaustion was higher in knee-extensors than in knee-flexors [49(9) and 22(6) respectively, $p = 0.028$]. No difference was observed in the rating of perceived exertion at the end of both fatiguing protocols [knee-flexors 9.0(1.0), knee-extensors: 9.0(0.6), $p = 1.000$].

Greater MVC was observed in knee-extensors than in knee-flexors, both before [181(41) and 129(28.6) Nm, $p < 0.001$] and after the fatiguing protocol [132(36) and 94(27) Nm, $p = 0.002$]. Similarly, no difference in knee-flexors-to-knee-extensors MVC ratio was found before and after the fatiguing protocol [0.72(0.12) and 0.73(0.21) A.U., $p = 0.735$]. At baseline, greater VAL was found in knee-flexors than in knee-extensors [83(12) and 76(12)%, $p = 0.041$].

No interaction was found for MVC ($p = 0.791$). After the fatiguing protocol, both knee-flexors [-35(20) Nm, CI95% -51. to -17, ES = 1.14] and knee-extensors [-49(25) Nm, CI95% -75 to -23, ES = 1.14] showed decreases in MVC (Figure 2A). No interaction was found for EMG RMS ($p = 0.654$). Increases in EMG RMS were found in both *biceps femoris* [37(12)%, CI95% 22 to 49, ES = 2.33] and *vastus lateralis* [51(33)%, CI95% 38 to 64, ES = 1.54] (Figure 2B). On the contrary, an interaction was found for VAL ($p = 0.026$). After the fatiguing protocol, a decrease in VAL was observed in knee-flexors [-12.8(4.2)%, CI95% -22.1 to -3.8, ES = 0.92] but not in knee-extensors [-3.6(4.4)%, CI95% -13.2 to 6.0, ES = 0.04] (Figure 2C).

A significant decrease in potentiated doublet twitch amplitude was found, with no interaction between each muscle group ($p = 0.792$). The potentiated twitch in knee-extensors was 83(20) Nm and 60(7) Nm before and after the fatiguing protocol ($p = 0.001$). The potentiated twitch in knee flexors was 74(12) Nm and 34(8) Nm, before and after the fatiguing protocol ($p < 0.001$). Regarding potentiation, no interaction was found ($p = 0.138$). Decreases in potentiation were observed both in knee-flexors [-46.9(17.1)%, CI95% -84.2 to -9.6, ES = 0.84] and in knee-extensors [-15.2(6.2)%, CI95% -28.4 to -1.9, ES = 0.59] (Figure 2D).

Please insert figure 2 here

No interaction was found for EMG RMS during the fatiguing protocol ($p = 0.155$). Compared to baseline, increases in EMG RMS were observed in *biceps femoris* after the 25th [34.3(6.5)%, CI95% 11.7 to 56.9, ES = 2.23], 50th [56.6(13.4)%, CI95% 10.7 to 102.6, ES = 2.13], 75th [80.5(15.6)%, CI95% 26.8 to 134.1, ES = 2.60] and 100th [90.5(23.9)%, 8.6 to 172.5, ES = 1.96] percentile of the number of repetitions. Compared to baseline, increases in EMG RMS were observed in *vastus lateralis* after the 50th [33.4(7.2)%, CI95% 8.7 to 58.1, ES = 1.43], 75th [53.0(9.7)%, CI95% 19.7 to 86.3, ES = 1.57] and 100th [71.4(14.5)%, CI95% 21.6 to 121.2, ES = 1.86] but not after the 25th [9.1(4.3)%, CI95% -5.6 to 23.9, ES = 0.27] percentile. Compared to *vastus lateralis*, higher EMG RMS resulted in *biceps femoris* after the 25th [25.1(8.2)%, CI95% 7.2 to 43.1, ES = 1.31] and a strong trend resulted after the 75th percentile [27.4(12.8)%, CI95% -0.4 to 55.4, ES = 0.56]. No difference resulted after the 50th [23.2(12.2)%, CI95% -3.4 to 49.9, ES = 0.63] and the 100th [19.1(24.3)%, -33.8 to 72.0, ES = 0.27] percentile. (Figure 3)

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Discussion

To the best of the authors' knowledge, this is the first study that has investigated the central and peripheral occurrence of fatigue in knee-flexors and knee-extensors after a standardized fatiguing protocol. The current results showed that the reductions in isometric MVC, as well as the changes in EMG RMS and peripheral fatigue, were similar in both knee-flexors and knee-extensors after the fatiguing exercise. However, knee-extensors performed more repetitions before exhaustion than knee-flexors. This difference was accompanied by an evidence of central fatigue observed in knee-flexors only, as highlighted by the decrease in VAL. In addition, EMG activity during the fatiguing protocol increased over time both in *biceps femoris* and in *vastus lateralis*, but the increment occurred earlier in *biceps femoris*.

Very large decreases in isometric MVC were observed after the fatiguing protocol both in knee-flexors and in knee-extensors. While it is well known that fatigue affects the maximum muscle force production (Gandevia, 2001), the testing modality seems to influence the extent of strength loss. Indeed, after different fatiguing protocols, similar strength decrements were observed in knee-flexors and knee-extensors when measured in concentric (Coratella, Bellin, et al., 2015; Delextrat et al., 2010) or isometric (Coratella et al., 2016) modality. Consistently, the present results showed no difference in the isometric knee-flexors-to-knee-extensors MVC ratio, confirming the similar decreases in MVC. In contrast, when force was measured in eccentric modality, greater decrements were reported in knee-flexors compared to knee-extensors (Cohen, Zhao, Okwera, Matthews, & Delextrat, 2014; Coratella, Bellin, et al., 2015; Delextrat et al., 2013, 2010). The unique neural control of the eccentric contraction may account for such a difference. Indeed, earlier type-II fibre recruitment was reported in eccentric compared to concentric contraction (Duchateau & Enoka, 2008; Roger M Enoka, 1996). Therefore, due to the different fibre type distribution in knee-flexors and knee-extensors, it can be argued that the higher type-II fibre prevalence in knee-flexors may result in a greater fatigability when tested in eccentric modality (Coratella, Bellin, et al., 2015). Thus, the similar decreases in MVC shown here can be consistently explained by the current isometric testing modality, which does not account for the muscle fibre type prevalence.

Importantly, the previous studies that investigated the fatigue responses in knee-flexors or knee-extensors used running- or sprinting- or change of direction-based fatiguing protocols (Coratella, Bellin, et al., 2015; Delextrat et al., 2010; Rahnama et al., 2003). Such protocols were not matched for the amount of work or the intensity of effort. Consequently, the knee-flexors or knee-extensors muscle activity and amount of work performed could have been different, possibly leading to greater strength loss in knee-flexors. In the present study, the standardized fatiguing protocol resulted in *very large* decreases in isometric MVC in both knee-flexors and knee-extensors. Lastly, it must be acknowledged that due to the longer duration of the knee-extensors fatiguing task, the participants may have lost motivation and sub-maximally performed the MVCs (Place, Maffiuletti, Ballay, & Lepers, 2005). However, the similar RPE score recorded at the end of the fatiguing protocol in both knee-flexors and knee-extensors might lead to exclude such a confounding factor.

Concurrently with the decreases in MVC, *large* and *very large* increments in EMG RMS were observed in *biceps femoris* and *vastus lateralis* respectively after the fatiguing protocol. The increments in EMG activity reflect the enhancements in the central drive to compensate for the loss of motor unit activation induced by peripheral and spinal mechanisms (Fuglevand, Winter, & Patla, 1993; Garland, Enoka, Serrano, & Robinson, 1994). The current results are in agreement with a previous study that reported increases in EMG activity during an intermittent isometric exercise (Hermans & Spaepen, 1997). However, even if both *biceps femoris* and *vastus lateralis* showed similar increases in EMG activity, an earlier fatigability was observed in *biceps femoris*. Accordingly, during a standardized concentric-only fatiguing exercise, knee-flexors resulted in earlier strength decrements compared to knee-extensors (Coratella et al., 2016). The higher number of repetitions to exhaustion in knee-extensors observed here is also in line with the above-mentioned study. However, since the EMG activity was not measured, a direct comparison cannot be made. Both the earlier increment in EMG activity during the fatiguing protocol and the lower number of repetitions to exhaustion suggest that knee-flexors could be more prone to fatigue compared to knee-extensors. Importantly, knee-extensors were presently tested and fatigued at a shorter muscle length compared to knee-flexors, due to the fixed seated position. In a previous direct comparison, muscles at shorter *vs* longer length were shown to be more fatigue-resistant (Place et al., 2005). Consequently, the longer knee-flexors muscle length may have contributed to decrease their endurance capacity.

Although both knee-flexors and knee-extensors showed decrements in MVC and increments in EMG RMS, different mechanisms seem to be involved. Indeed, *large* decrements in VAL occurred in knee-flexors (Marshall et al., 2014) but not in knee-extensors. The previous studies that evaluated the occurrence of central fatigue in knee-extensors reported inconsistent outcomes. While decrements in central drive were previously shown after repeated all-out sprints (Hureau, Ducrocq, & Blain, 2016) or constant-load cycling (Thomas, Elmeua, Howatson, & Goodall, 2016; Weavil, Sidhu, Mangum, Richardson, & Amann, 2016), other studies did not report any change after intermittent (Bachasson et al., 2016) or continuous isometric knee-extensors fatiguing task (Marshall, Finn, & Siegler, 2015). However, these two studies recruited endurance- (Bachasson et al., 2016) or strength-trained (Marshall et al., 2015) participants. Since trained people could be more fatigue-resistant, a possible greater central tolerance to fatigue may have been developed (Marshall et al., 2015). Similarly, it may be argued that the baseline characteristics (fatigue-resistant muscle fibre phenotype) in knee-extensors and their primary role in endurance daily activities (*e.g.*, standing or walking) compared to knee-flexors may have increased their tolerance to central fatigue (Bachasson et al., 2016). Accordingly, after the same standardized fatiguing task, knee-extensors may have been less affected than knee-flexors. Importantly, the greater VAL shown in knee-flexors suggests that long muscle length may have increased it (Doguet et al., 2017; Kluka et al., 2015). However, this is in contrast with another study that found similar VAL at short *vs* long knee-extensors muscle length (Place et al., 2005). Consequently, it may be speculated that the different morphology in knee-flexors *vs* knee-extensors may have played a major role in muscle fatigue compared to the different muscle length. However, the use of additional measurements (*e.g.* transcranial stimulations) could have added further insight to the occurrence of central fatigue.

In the present study, the decreases in doublet twitch amplitude in both knee- flexors and knee-extensors accounted for a similar global peripheral fatigue (Rozand et al., 2015). A closer look at the mechanisms, such as the change in potentiation, showed that the global fatigue can originate from the impairment in Ca^{2+} releasing process during a MVC (Place et al., 2005). In addition, such a peripheral fatigue can also derive from impairments in the excitation-contraction coupling, changes in cross-bridge properties or metabolic alterations (Allen, Lamb, & Westerblad, 2008). The current study showed that knee-flexors (*very largely*) and knee-extensors (*moderately*) were both affected by peripheral fatigue, contrarily to what was hypothesized previously. Changes in peripheral fatigue were consistently reported in knee-extensors after isometric exercise (Bachasson et al., 2016; Marshall et al., 2015), as well as after repeated all-out sprints (Hureau et al., 2016) or constant load to exhaustion in cycling protocols (Thomas et al., 2016). On the contrary, the only study that measured peripheral fatigue in knee-flexors detected no significant change (Marshall et al., 2014). However, in the latter study, the authors used a fatiguing protocol that simulated a soccer match, which included sprinting and change of direction. During such activities, knee-extensors have a greater role as primary movers compared to knee-flexors and this may result in a preservation of knee-flexors from further peripheral fatigue (Marshall et al., 2014). In contrast, the present procedures used a standardized protocol for both knee-flexors and knee-extensors. Therefore, the similar occurrence of peripheral fatigue (although with a different extent) may have derived from the comparable fatiguing protocol used here.

The present study comes with some acknowledged limitations. Firstly, only two muscles (*biceps femoris* and *vastus lateralis)* were selected to represent the knee-flexors and knee-extensors EMG activity respectively (Conchola et al., 2015). Since the complex morphology in knee-flexors and knee-extensors, further investigations on additional muscles are recommended. Secondly, the spinal contribution was not investigated. However, such a procedure is difficult to assess in both knee-flexors and knee-extensors. Therefore, to be consistent with previous procedures, the spinal contribution was not included here (Bachasson et al., 2016; Marshall et al., 2014), Furthermore, although the same standardized fatiguing protocol and strength measurement were used to avoid any possible confounding factor, different protocols and measurements might have resulted in different outcomes. Additionally, the present fatiguing protocol tested knee-flexors and knee-extensors at different muscle lengths. However, although the greater central activation reported at long *vs* short muscle length (Doguet et al., 2017), the rate of muscle fatigue was not affected (Guex, Degache, Gremion, & Millet, 2013).

In conclusion, a standardized intermittent sub-maximal isometric fatiguing protocol induced similar decreases in isometric MVC in both knee-flexors and knee-extensors. In addition, despite the earlier increase in EMG RMS in *biceps femoris*, both *biceps femoris* and *vastus lateralis* showed *large* increments in EMG RMS after the fatiguing protocol. However, notwithstanding the similar peripheral fatigue, only knee-flexors showed a *large* reduction in central motor commands.

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Figure Captions

Figure 1. The experimental set-up for the knee flexion and the knee extension fatiguing protocols is shown. **A.** Schema of the experimental setup with the participants positioning. **B.** Schematic description of the experimental design used for both knee-flexors and extensors. The vertical arrows depict stimulations used to evoke the twitches.

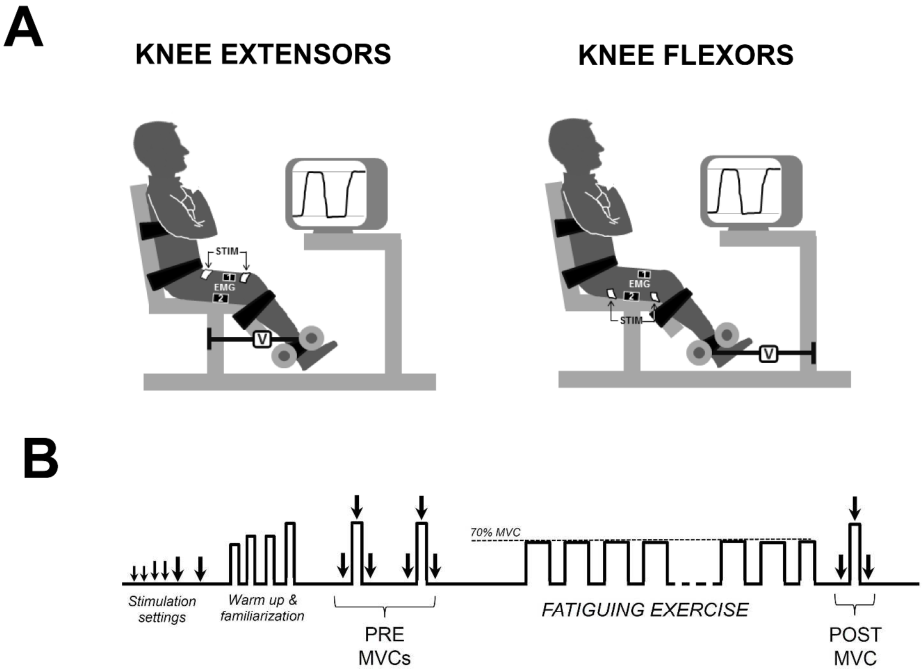
Figure 2. Pre-post changes in MVC (A), EMG (B), VAL (C) and potentiation (D) in knee-extensors and knee-flexors.

* = $p < 0.05$ in pre-post comparisons.

Figure 3. Time course of the EMG activity in *vastus lateralis* and *biceps femoris* during their fatiguing protocols.

* = $p < 0.05$ compared to baseline EMG activity

= $p < 0.05$ compared to *vastus lateralis* EMG activity.



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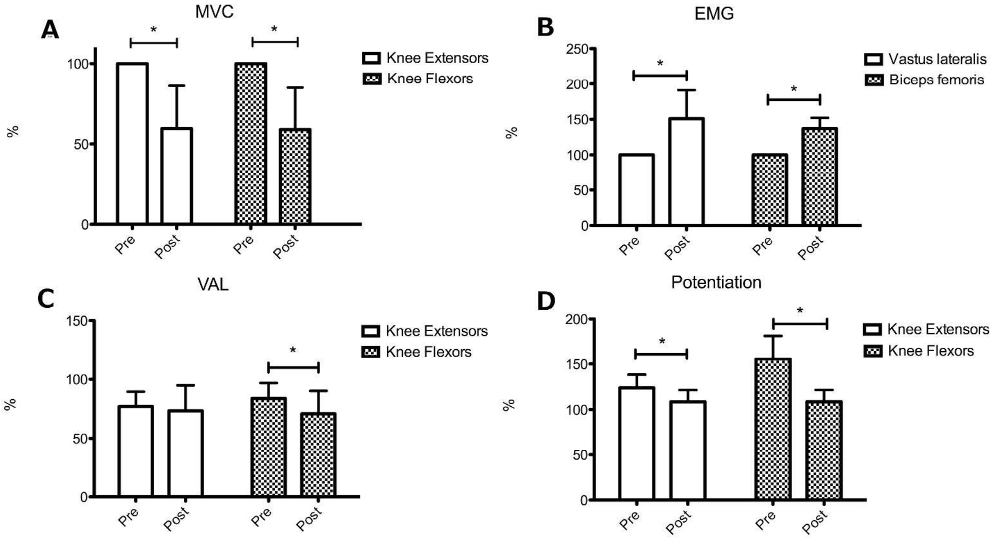

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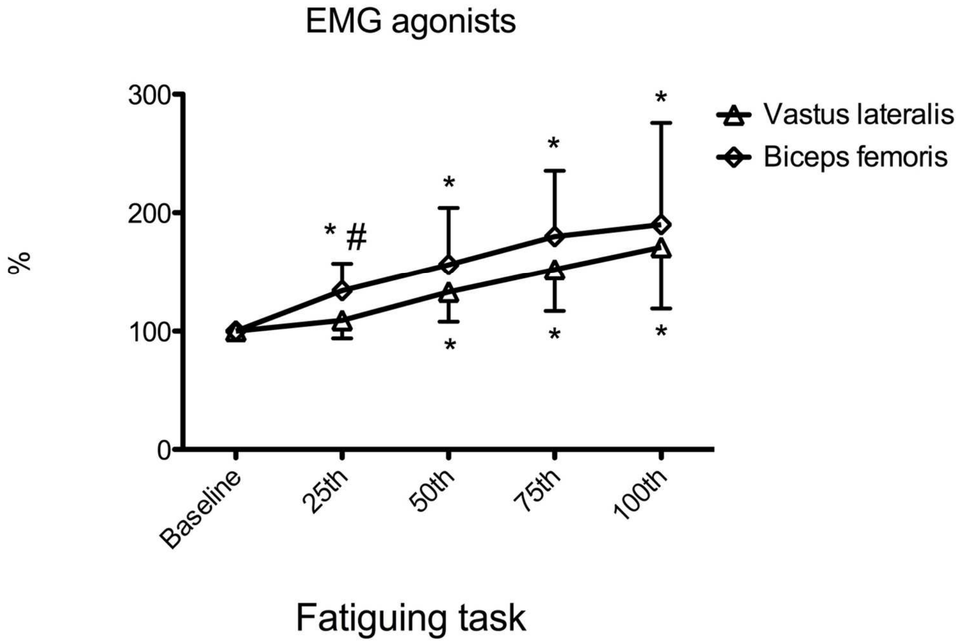

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# = $p < 0.05$ compared to vastus lateralis EMG activity.

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