THE ETIOLOGY OF NEUROMUSCULAR FATIGUE INDUCED BY THE 5-M SHUTTLE RUN TEST IN ADULT SOCCER PLAYERS

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Abstract:

This study investigated the etiology of neuromuscular fatigue induced by a 5-m shuttle run test (5MSRT) in soccer players. Nineteen adult male amateur soccer players (age: 20.0 ± 2.9 years) participated in the present study. Before and after the 5MSRT, they were instructed to complete a maximal voluntary isometric contraction (MVIC) of the knee extensors (KE) during and after which two electrical stimulations were applied at the femoral nerve. Voluntary activation level (VAL), surface electromyography recordings (sEMG), electrophysiological (M_{max}) and potentiated resting twitch (Ptw) responses of the KE were compared between pre- and post-5MSRT. Rating of perception exertion (RPE) was also assessed before, during the test immediately following each sprint repetition and after the test. The distance covered during each sprint significantly decreased as the number of trials performed increased ($p<.05$). The RPE reported following each sprint significantly increased throughout the test. In addition, MVIC (-9%), sEMG (-23%), VAL (-15%), Ptw (-26%) and M_{max} (~22%) of the KE were lowered from pre-to-post 5MSRT ($.001 < p < .01$). The 5MSRT induced a decrease of repeated-sprint running performance and MVIC of the KE. These decrements were accompanied by lowered VAL, sEMG, Ptw and M_{max} values of the KE reflecting the involvement of both the central and peripheral origins in the 5MSRT-induced fatigue. Given the important muscle stress induced by 5MSRT, this strenuous test must be applied with caution, after an inevitable familiarization phase, and not during the competition period to avoid the risk of serious injury.

Key words: *knee extensors, electrical stimulation, electromyography, repeated-sprint ability, perceived exertion*

Introduction

The ability to reproduce maximal efforts with incomplete recovery is a good index of performance in intermittent sports such as soccer (Bradley, et al., 2009; Bradley, Mascio, Peart, Olsen, & Sheldon, 2010; Rampinini, et al., 2007, 2009). Due to the unpredictable nature of the actions in soccer, a myriad of repeated-sprint tests has been designed and used to evaluate the performance of soccer players. Repeated-sprint tests lasting less than 10s with a recovery period not exceeding 60s were the most commonly used during the last two decades (Aziz, Mukherjee, Chia, & The, 2008; Impellizzeri, et al., 2008; Rampinini, et al., 2007, 2009). During these brief repeated-sprint tests, it has been shown that athletes were unable to maintain the same performance level from the beginning to the end of the exercise (Goodall, Charlton, Howatson, & Thomas, 2015; Townsend, Brocherie, Millet, & Girard, 2021). For example, impairment in maximal power output during cycling exercises (Girard, Bishop, & Racinais, 2013; Girard, Mendez-Villanueva, & Bishop, 2011; Kerhervé, Stewart, McLellan, & Lovell, 2020; Tomazin, Morin, & Millet, 2017), an increase in time to cover a given distance (Goodall, et al., 2015; Perrey, Racinais, Saimouaa, & Girard, 2010) or a decrease in distance for a given duration (Townsend, et al., 2021) during running were reported. This performance decrement is commonly related to a combination of multiple factors leading to the development of neuromuscular fatigue (Collins, Pearcey, Buckle, Power, & Button, 2018; Girard, et al., 2011). Hence, maximal voluntary isometric contraction (MVIC) was lowered up to 15% (Girard, et al., 2011, 2013; Tomazin, et al., 2017) following repeated-sprint cycling and between 8 to 20% following repeated-sprint running (Goodall, et al., 2015; Perrey, et al., 2010; Tomazin, et al., 2017; Townsend, et al., 2021). However, the etiology of such impairment differs between studies. While some attributed MVIC decrement mainly to peripheral mechanisms (Girard, et al., 2013; Perrey, et al., 2010), others revealed the involvement of both peripheral and central origins (Goodall, et al., 2015; Tomazin, et al., 2017; Townsend, et al., 2021). Many factors could explain these contradictory results such as the methods used to investigate neuromuscular function, the type of locomotion (Tomazin, et al., 2017; Kerhervé, et al., 2020), the assessed muscular group, the task performed (Collins, et al., 2018; Girard, et al., 2011) or the exercise characteristics (e.g., number of sets and repetitions, duration, distance, recovery period, etc.).

Physical demands in soccer are continually increasing with the evolution of the game. The intense efforts of modern soccer are increasingly longer, more frequent with shorter recovery phases and incorporating more uncertainties into the game requiring more acceleration, blocking, deceleration, and restart actions (Barnes, Archer, Hogg, Bush, & Bradley, 2014; Harper, Carling, & Kiely, 2019). This would lead to higher metabolic demands along with a higher solicitation of eccentric contractions possibly leading to increased peripheral fatigue (Byrne, Twist, & Eston, 2004; Martin, Millet, Lattier, & Perrod, 2005). Indeed, neuromuscular fatigue in response to 90-min and 120-min simulated soccer matches was assessed and demonstrated a greater magnitude (Goodall, et al., 2017; Thomas, Dent, Howatson, & Goodall, 2017) than fatigue reported following the majority of brief repeated-sprint running designs (Kerhervé, et al., 2020; Perrey, et al., 2010; Tomazin, et al., 2017; Townsend, et al., 2021). Thus, the above-mentioned brief repeated-sprint tests might not be representative of real performance in modern soccer. Alternately, one of the least frequently used repeated-sprint tests is the 5-m shuttle run test (5MSRT) which is designed to determine players' match-related-fitness (Pendleton, 1997) in several team sports characterized by intermittent, short duration and high intensity bouts of activity (Boddington, Lambert, Gibson, & Noakes, 2001; Pendleton, 1997). This test, adopted by the Welsh Rugby Union and modified by the Sports Science Institute of South Africa (Boddington, et al., 2001), consists of six 30-second repeated sprints carried out in a shuttle way over distances increased by 5-m each shuttle, interspersed by 35 s of passive rest periods. Some of the interesting features of the 5MSRT that make it quite different from more popular brief repeated-sprint tests are its long-lasting duration per sprint with a near 1:1 exercise/recovery (E/R) ratio and its shuttle activity requiring repeated accelerations, decelerations and changes of direction. Although these characteristics of the 5MSRT makes it more specific to major team sports including soccer, the fatigue induced by such a test has been overlooked. Yet, understanding the neuromuscular etiology associated with the 5MSRT could be very useful to adequately guide the implementation of training aimed at both reducing fatigability and increasing performance during the 5MSRT and consequently physical performance in soccer competition. Thereafter, the study of the neurophysiological mechanisms underlying reduced fatigability / improved performance following a specific training program becomes therefore feasible and its transfer in the physical activity performance could also be investigated. Although perception of fatigue increased after the 5MSRT (Boukhris, et al., 2020), to the best of our knowledge, no previous study investigated the neuromuscular fatigue induced by a 5MSRT.

Hence, the purposes of the present study were: i) to assess the performance of adult soccer players during a 5MSRT and ii) to investigate the etiology of neuromuscular fatigue induced by the 5MSRT. Due to the all-out sprint duration, overall distance covered and shuttle nature of the 5MSRT, we hypothesized that both central and peripheral factors may account for the performance decrement during the 5MSRT.

Materials and methods

Participants

Nineteen amateur adult male soccer players (age: 20.0 ± 2.9 years, body height: 172.8 ± 6.5 cm, body mass: 67.4 ± 6.4 kg) competing in the third division of the Tunisian championship volunteered to contribute to this study. Participants trained two days a week for an average of 2h per training session plus the competition. They were informed about the possible risks and discomforts associated with the experimental procedures and gave their written informed consent. The study was conducted according to the Declaration of Helsinki and the protocol was fully approved by the Institute Research Ethics Committee. The inclusion criteria were the following: no smoking, no alcohol consumption, no injury for at least six months before participation in the study, tolerance to the study protocol and training routine for at least one hour during two days per week.

Experimental design

One week after two prior visits aiming at getting familiar with the experimental procedures (i.e., neuromuscular measures and 5MSRT), participants were enrolled in the experimental session. After a warm-up, an assessment of neuromuscular function was carried out before and following the 5MSRT. During the 5MSRT, perceived exertion was also rated immediately following each of the all-out 30-second repeated sprints (Figure 1).

The warm-up consisted of a 5-min jog at a self-selected comfortable pace followed by a 5-min series of dynamic stretching (e.g., hip flexion/extension, hip abduction/adduction, butt kicks) and five progressive sprints over different distances with and without change of direction. The assessment of neuromuscular function included three and one 3-s maximal voluntary isometric contractions (MVIC) of the knee extensor muscles (KE) performed before (PRE) and immediately after (POST) the 5MSRT, respectively. Superimposed (over the 3-s MVICs) and potentiated (3s after the contraction) twitches were delivered by electrical nerve stimulation. The highest MVIC value among the three trials at PRE and the single trial at POST was taken into account for subsequent analysis. To exclude the confounding effect of fatigue induced by repeated muscular contractions, the MVICs performed at PRE were separated by a 2-minute recovery period. During each MVIC, surface electromyography (sEMG) activity of KE muscles was measured.

MVIC. MVIC was measured using an isometric dynamometer (Good Strength, Metitur, Finland) equipped with a cuff connected to a strain gauge. This cuff was attached to the anterior aspect of the shank ≈2 cm above the lateral malleolus using a Velcro strap. During testing, the participants were seated with the hip and knee angles set at 90° (knee full extension = 0°). Safety belts were strapped across the participants' chest, thighs and hips to avoid lateral, vertical and frontal displacements. All measurements were taken from the right lower limb. Participants were instructed to exert maximal voluntary knee extension against the lever arm. Participants were strongly encouraged while performing the MVIC.

Surface electromyography recordings. The sEMG signals of the *vastus lateralis* (VL), *vastus medialis* (VM) and *rectus femoris* (RF) muscles were recorded using bipolar silver chloride surface electrodes (Blue Sensor N-00-S, Ambu, Denmark) during MVIC and nerve stimulations. The electrodes were taped lengthwise on the skin over the muscle belly following SENIAM recommendations, with an inter-electrode distance of 20 mm. A reference electrode was attached to the patella of the same leg. Low impedance ($Z<5k\Omega$) at the skin-electrode surface was obtained by shaving, abrading the skin with thin sandpaper and cleaning with alcohol. sEMG signals were amplified (Octal Bio Amp ML 138, ADInstruments, Australia) with a bandwidth frequency ranging from 10 Hz to 1 kHz (common mode rejection ratio > 96 dB, gain = 1000) and simultaneously digitized together with the force signals using an acquisition card (Powerlab 16SP, AD Instruments, Australia) and Labchart 7.0 software (AD Instruments, Australia). The sampling frequency was 2 kHz. The root mean square (RMS) values of the VL, VM and RF sEMG recordings were calculated over a 0.5-second period of the MVIC plateau, just before the superimposed stimulation and averaged. The RMS values were normalized to the respective peak-to-peak M-wave amplitudes (M_{max}) of each muscle.

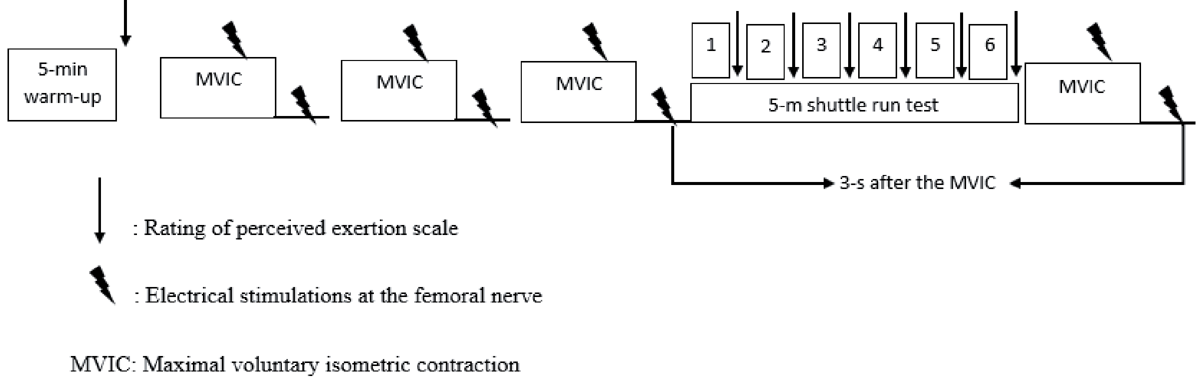

Figure 1. Schematic representation of the experimental design.

Electrical nerve stimulation. The femoral nerve was stimulated percutaneously with a single square-wave stimulus of 1-ms duration with maximal voltage of 400 V delivered by a constant current stimulator (Digitimer DS7A, Hertfordshire, United Kingdom). The cathode (self-adhesive electrode: Ag–AgCl, 10 mm diameter) was positioned in the femoral triangle and pressed firmly into place by an experimenter. The anode, a 10 × 5 cm self-adhesive stimulation electrode (Medicompex SA, Ecublens, Switzerland), was placed midway between the greater trochanter and the iliac crest. Optimal stimulation intensity was determined from M-wave and twitch force measurements before the testing session. Briefly, the stimulation intensity was increased by 5 mA until there was no further increase in either potentiated resting twitch (Ptw) or in concomitant VL, VM and RF M-wave peak-to-peak amplitudes. During the subsequent testing procedures, the intensity was set at 150% of the optimal stimulation intensity to overcome the potential confounding effect of axonal hyperpolarization.

The contractile properties of the KE muscles were evaluated by analyzing the Ptw and the M_{max} peak-to-peak amplitude of VL, VM and RF muscles. VAL was computed using the following corrected formula (Strojnik & Komi, 1998), as the superimposed stimulus was not always delivered over the MVIC plateau:

VAL (%) = [1 – superimposed twitch × (superimposed twitch / MVIC) / potentiated resting twitch] × 100.

5-m shuttle run test (5MSRT)

The 5MSRT consisted of six all-out maximal sprints separated by 35-s of recovery. Each sprint lasted 30 s and consisted of a maximal shuttle run over increasing distances of 5 m, 10 m, 15 m, 20 m, etc. (Boddington, et al., 2001). Participants started the test in line with the first beacon and began sprint shuttling at auditory signal over 5m, 10m, 15m, etc., until finishing the 30s duration. The distance covered (measured by a precision of 5m) during each all-out sprint was registered and the following indices were recorded:

- Higher distance (HD) (m): the greatest distance covered during a 30-s shuttle
- Total distance (TD) (m): the total distance covered over the six 30-s shuttles
- Fatigue index (FI): calculates the drop-off in performance from the best to the worst performance
  FI (%) = [(shuttle$^{(1)}$ 1 + shuttle 2) – (shuttle 5 + shuttle 6) / (shuttle 1 + shuttle 2)] × 100
  $^{(1)}$ Shuttle consists of the covered distance for the corresponding trial (from the first to the sixth trial)
- The percentage of decrement (PD):
  PD (%) = [(HD × number of sprints) – TD / (HD × number of sprints)] × 100

Rating of perceived exertion scale (RPE)

Participants were asked to rate their overall RPE, that is the combination of chest RPE (i.e., breathlessness) and leg RPE (i.e., local perception of effort) before (i.e., after the warm-up), during (i.e., after each all-out sprint) and immediately after the 5MSRT. The validated French version of the RPE scale was used (Haddad, et al., 2013). The RPE is an 11-points scale ranging from “0 (nothing at all)” to “10 (maximal)”. The RPE score increased for higher ratings of perceived exertion.

Statistical analysis

Statistical tests were performed using the STATISTICA software (StatSoft, France; version 10) and data are presented as means ± standard deviation (SD). The Shapiro-Wilk test confirmed that the data were normally disturbed. The six calculated distances and the assessed RPE during the 5MSRT were analyzed using repeated measures ANOVA (6 all-out sprints). MVIC, RPE, RMS, M_{max}, RMS/M_{max}, Ptw and VAL were analyzed by repeated measures ANOVA to investigate differences between before and after the 5MSRT. When appropriate, significant differences between means were tested using the Bonferroni *post-hoc* test. Effect sizes were calculated as partial eta-squared (η^2_p) to estimate the meaningfulness of significant findings. η^2_p values of 0.01, 0.06 and 0.13 represent small, moderate, and large effect sizes, respectively. A Pearson correlation coefficient was used to assess the relation between the performance variables (HD, TD, FI and PD), RPE and delta of neuromuscular parameters (MVIC, Ptw, VAL, M_{max}, RMS and RMS/M_{max}). Significance was accepted for all analyses at the level of $p \leq .05$ and was reported as $p < .05$, $p < .01$ and $p < .001$.

Results

The 5-m shuttle run test (5MSRT) performance and RPE

The values measured for the HD, TD, FI and PD were 119.8 ± 7.7m, 659.8 ± 43.9m, 12.3 ± 4.9% and 8.2 ± 4.1%, respectively, and the distance covered during each all-out sprint is presented in Figure 2. During the 5MSRT performance, a significant effect of the sprints was observed for HD ($F_{(5,90)} = 63.7$, $p < .001$, $\eta^2_p = 0.78$), as well as for RPE ($F_{(5,90)} = 289.8$, $p < .001$, $\eta^2_p = 0.94$). The HD of the 1st sprint was significantly higher than the five other sprints ($p < .001$). Thereafter, the covered distance decreased progressively over sprints (.001 < p <

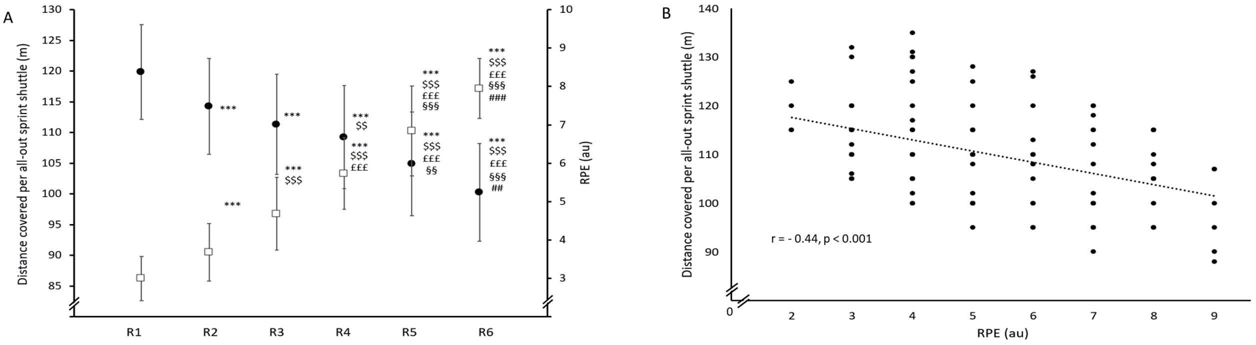

Figure 2.
*(A) Evolution of the covered distance and the rating of perceived exertion (RPE) during each sprint (from S1 to S6) of the 5-m shuttle run test (mean ± SD). *** (p<.001): significantly different from the 1st sprint; $$ (p<.01) and $$$ (p<.001): significantly different from the 2nd sprint; £££ (p<.001): significantly different from the 3rd sprint; §§ (p<.01) and §§§ (p<.001): significantly different from the 4th sprint; ## (p<.01) and ### (p<.001): significantly different from the 5th sprint.*
(B) Correlation between the covered distance by each sprint and the averaged rating of perceived exertion (RPE).

Table 1. Neuromuscular measurement and electromyographic responses recorded before (PRE) and after (POST) the 5-m shuttle run test (mean ± SD)

	PRE	POST	DELTA (%)
MVIC (N)	688.6 ± 137.4	628.3 ± 152.3***	-9.3 ± 9.2
Ptw (N)	5.4 ± 1.7	4.3 ± 1.5***	-26.1 ± 13.3
VAL (%)	5.4 ± 1.7	4.3 ± 1.5***	-14.9 ± 9.3
M_{max} (mV)			
vastus lateralis	5.4 ± 1.7	4.3 ± 1.5***	-21.2 ± 14.4
vastus medialis	3.4 ± 1.4	2.7 ± 1.5***	-22.0 ± 18.0
rectus femoris	3.7 ± 1.6	2.9 ± 1.5***	-21.7 ± 17.8
RMS (mV)			
vastus lateralis	0.38 ± 0.09	0.33 ± 0.10***	-13.5 ± 11.4
vastus medialis	0.37 ± 0.11	0.30 ± 0.09***	-18.0 ± 8.9
rectus femoris	0.47 ± 0.21	0.32 ± 0.08**	-27.2 ± 16.6
RMS/M_{max} (mV)			
vastus lateralis	0.08 ± 0.03	0.09 ± 0.06	14.5 ± 31.4
vastus medialis	0.12 ± 0.05	0.14 ± 0.07	12.7 ± 38.6
rectus femoris	0.17 ± 0.14	0.15 ± 0.11	-0.9 ± 37.8

Note. MVIC: Maximal voluntary isometric contraction; Ptw: potentiated resting twitch; VAL: voluntary activation level; M_{max}: peak-to-peak M-wave amplitude; RMS: Root mean square. ** (p<.001) and *** (p<.001) indicate a significant difference between PRE and POST values.

.01; Figure 2A). In parallel, RPE was significantly increasing progressively from sprint one to the following throughout the test (p<.001; Figure 2A). A significant inverse correlation was found between HD covered and RPE values of each all-out sprint (r = -0.44, p<.001; Figure 2B).

Neuromuscular responses to the 5MSRT

MVIC ($F_{(1,18)} = 26.3$, p<.001, $\eta^2_p = 0.59$, -9.3 ± 9.2%), Ptw ($F_{(1,18)} = 46.4$, p<.001, $\eta^2_p = 0.72$, -26.1 ± 13.3%) and M_{max} values of each KE muscle (VL, $F_{(1,18)} = 40.7$, p<.001, $\eta^2_p = 0.64$, -21.2 ± 14.4%; VM, $F_{(1,18)} = 25.3$, p<.001, $\eta^2_p = 0.58$, -22.0 ± 18.0%; RF, $F_{(1,18)} = 26.0$, p<.001, $\eta^2_p = 0.60$, -21.7 ± 17.8%) significantly decreased after the 5MSRT (Table 1).

VAL ($F_{(1,18)} = 57.8$, p<.001, $\eta^2_p = 0.76$, -14.9 ± 9.3%), RMS values of each KE muscle (VL, $F_{(1,18)} = 27.1$, p<.001, $\eta^2_p = 0.60$, -13.5 ± 11.4%; VM, $F_{(1,18)} = 47.5$, p<.001, $\eta^2_p = 0.73$, -18.0 ± 8.9%; RF, $F_{(1,18)} = 14.2$, p<.01, $\eta^2_p = 0.45$, -27.2 ± 16.6%) were significantly impaired after the 5MSRT, whereas no significant change was noted for the RMS/M_{max} values of each KE muscle (p>.05; Table 1). No significant correlation was found between any of the performance variables and neuromuscular parameters.

Discussion and conclusions

The aim of the present study was twofold: (1) to assess the evolution of the performance during the 5MSRT in adult soccer players and (2) to quantify the degree of peripheral and central factors contributing to neuromuscular fatigue elicited by this all-out repeated-sprint test. The main findings were (*i*) the progressive performance decrement throughout the 5MSRT was accompanied by force production impairment at the termination of the test and (*ii*) both peripheral and central factors accounted for the neuromuscular fatigue observed.

HD and TD covered in the present investigation are in agreement with previous studies (Belkhir, Rekik, Chtourou, & Souissi, 2019; Boddington, et al., 2001; Boukhris, et al., 2020) although the characteristics of the participants differed (i.e., physically active male, amateur male team sports players, female field hockey players, male soccer players). More specifically, we found a progressive performance decrease induced by the 5MSRT when comparing the covered distances throughout the test (i.e., -5% between the first and the second sprint reaching -17% in the last all-out sprint in comparison with the first one, leading to a PD of ~8%). The fatigue index (FI) in our participants reached 12.3%, which was slightly greater than a previous observation in male soccer players (~8%; Belkhir, et al. 2019). Finally, the 5MSRT-performance decrements were accompanied by a progressive increase of RPE indicating the occurrence of greater effort perception throughout the test (Boddington, et al., 2001; Kerhervé, et al., 2020; Townsend, et al., 2021). Originally, a correlation was found between HD decreases and RPE increases (Figure 2B). Taken together, these observations suggest the development of fatigue throughout the test, but no study was previously conducted to investigate neuromuscular fatigue and the associated underpinning mechanisms induced by a 5MSRT.

After the 5MSRT, a 9% decrease of KE MVIC was observed confirming that fatigue occurred. Comparable KE MVIC impairment was reported after short duration (i.e., < 6s) repeated sprint running (Goodall, et al., 2015; Tomazin, et al., 2017; Townsend, et al., 2021). Concomitantly to the decrease of the MVIC, both the resting potentiated twitch (Ptw) and the M_{max} values of the KE muscles were reduced. M_{max} decreases indicate a neuromuscular transmission failure, arising probably from ionic disturbance of the Na^+-K^+-ATPase activity resulting in extracellular K^+ accumulation (Clausen, Nielsen, Harrison, Flatman, & Overgaard, 1998). Such alterations of sarcolemma excitability were highlighted after maximal dynamic contractions of the knee extensors muscles leading to fatigue (Fraser, et al., 2002). After repeated sprint running protocols, inconsistent observations were reported in the literature. To date, M_{max} of the KE muscles was unaffected after short sprint duration (i.e., < 7s; Goodall, et al., 2015; Tomazin, et al., 2017; Townsend, et al., 2021), whereas M_{max} of the *soleus* muscle was impaired for similar short sprint duration (Perrey, et al., 2010). Although many factors (i.e., differences in muscle tested, time spent until assessment, recovery time, fitness level of the participants, etc.) could account for these controversial results, the specificity of the 5MSRT (i.e., shuttle form including acceleration, deceleration and change of direction) may contribute to the M_{max} impairment of the KE muscle in our participants. Future studies are needed to determine the contribution of influencing factors in muscle excitability impairment after all-out repeated sprint running protocols.

An alteration of sarcolemma excitability is often associated with diminished Ca^{2+} release and/or decreased myofibrillar Ca^{2+} sensitivity leading to reduced actin-myosin bridge formation and consequently Ptw impairment (Allen, Lamb, & Westerblad, 2008). Other factors, like accumulations of Pi and H^+ associated with elevated blood lactate concentration, generally observed after repeated-sprint running protocols (Goodall, et al., 2015; Perrey, et al., 2010), may have impeded the excitation-contraction coupling through inhibition of Ca^{2+} handling within the KE muscles. In addition, a recent study showed that immediately after a 5MSRT, important perception of muscle soreness and increased biomarkers of muscle damage and inflammation were present in amateur male sports players (Boukhris, et al. 2020). These observations could be ascribed to the stretch-shortening cycle (SSC) actions and the eccentric contractions due to repeated accelerations, decelerations and changes of direction associated with the shuttle form of the 5MSRT. Indeed, Ptw impairments of the KE muscles are commonly reported after intense exercises involving either SSC (Strojnik & Komi, 2000) or eccentric contractions (Souron, Nosaka, & Jubeau, 2018). Hence, possible muscle damage may have led to changes in sarcoplasmic reticulum structure participating in the reduced Ca^{2+} release and uptake observed after intense exercise (Byrd, McCutcheon, Hodgson, & Gollnick, 1989). The 26% reduction of Ptw after our 5MSRT agrees with twitch impairments of the KE muscles observed in the literature after repeated sprint running (Goodall, et al., 2015; Townsend, et al., 2021) and 90min (23%) and 120min (23%) simulated soccer match (Goodall, et al., 2017), while muscle excitability remained unaffected in these studies. This further reinforced the important specificity of the shuttle form of the 5MSRT associated with the higher metabolic demand induced by the duration of each all-out sprint (i.e., 30s) compared to shorter (i.e., < 7s) sprint durations (Duffield, Dawson, & Goodman, 2004), leading to concomitant muscle excitability and excitation-contraction coupling impairments. Given the great peripheral fatigue and important muscle stress induced by 5MSRT, this strenuous test must be applied with caution, after an inevitable familiarization phase, and not during the competition period to avoid the risk of serious injury.

Central mechanisms also seem to play a determinant role in the KE MVIC reduction. Our findings demonstrated a -15% decrease of voluntary activation between PRE and POST measures. Interestingly, the VAL reduction in the present study was greater than voluntary activation alterations of the KE muscles reported in the literature (~6.5-9%) after repeated sprint running protocols (Goodall, et al., 2015; Tomazin, et al., 2017; Townsend, et al., 2021) but similar to those reported after 90-min (15%) and 120-min (18%) of simulated soccer match (Goodall, et al., 2017). While a single 200-m sprint of performance duration similar (i.e., ~31s) to that of the 5MSRT did not impair voluntary activation (Tomazin, Morin, Strojnik, Podpecan, & Millet, 2012), two short duration sprints (i.e., < 4.4s) led to a decrement in voluntary activation by ~6.5% (Goodall, et al., 2015). A review of the literature indicated that increasing the number of short duration (i.e., < 6s) sprints led to more pronounced impaired voluntary activation by ~7% after eight (Townsend, et al., 2021) or ten (Tomazin, et al., 2017) sprints and up to ~9% after twelve (Goodall, et al., 2015) or twenty sprints (Tomazin, et al., 2017). With six repetitions, the longer duration per all-out sprint might therefore explain the greater voluntary activation impairment observed after our 5MSRT. In fact, the reduction of voluntary activation by up to 30% was observed after a maximal all-out cycling sprint lasting 30 s (Fernandez-del-Olmo, et al., 2013). Although the tested locomotion was different here, one can argue that central fatigue may have occurred after the first all-out 30-s sprint of the 5MSRT. Further studies are needed to ascertain this suggestion since we did not assess the time course of neuromuscular fatigue after each sprint.

Central fatigue was also investigated through the measure of RMS and RMS/M_{max} values of the KE muscles. Like previous repeated sprint protocols (Hader, Mendez-Villanueva, Ahmaidi, Williams, & Buchheit, 2014), KE muscles' RMS values were significantly lowered after the 5MSRT, but RMS/M_{max} values remained unaffected by the exercise. To date, only two studies have reported impaired RMS/M_{max} values of the *vastus lateralis* (Tomazin, et al., 2017) or *rectus femoris* (Townsend, et al., 2021) muscle after repeated sprints of short duration. Heterogeneity of the experimental designs might contribute to the inconsistencies observed but caution is required when interpreting RMS/M_{max} values due to its lower reproducibility than voluntary activation measures during fatigue (Place, Maffiuletti, Martin, & Lepers, 2007) and amplitude cancellation phenomena in sEMG signals. Indeed, the concomitant decrease of both RMS and M_{max} values could explain, at least in part, the unchanged RMS/M_{max} observed in our study. This could explain why the evolution of RMS/M_{max} values and VAL differed after our 5MSRT.

Notwithstanding these concerns, the altered VAL after our 5MSRT indicates that the MVIC decrease may in part be due to neural drive failure leading to inhibitory effects onto motoneurons. As discussed above, repeated sprint running provokes metabolic by-product accumulation (Goodall, et al., 2015; Perrey, et al., 2010), which is involved in the enhanced inhibitory feedback from group III and IV afferences (Pollak, et al., 2014) that could occur at both the spinal and supraspinal levels. Although motor nerve stimulation does not allow to differentiate between the spinal and supraspinal inhibition, supraspinal fatigue was recently evidenced through the use of transcranial magnetic stimulation after a 30-s maximal all-out cycling sprint (Fernandez-del-Olmo, et al., 2013), repeated sprint running (Goodall, et al., 2015) and 90min and 120min simulated soccer match (Goodall, et al., 2017). Therefore, it is likely that supraspinal factors are involved in the VAL impairment observed here.

As recently observed after a 5MSRT (Boukhris, et al. 2020), important muscle soreness and inflammation suggesting muscle damage may also participate in the elevated inhibitory influence of group III and IV afferents (Hoheisel, Unger, & Mense, 2005). Both the duration of each maximal all-out sprint and the shuttle form of the 5MSRT requiring accelerations, decelerations and changes of direction could account for this possible muscle damage. Albeit muscle soreness was not investigated here, a progressive increase in perceived exertion was found. In addition, we have highlighted that perceived exertion increases were correlated to performance decrements. Perception of muscle soreness, or pain (Pollak, et al., 2014) and perception of exertion (de Morree, Klein, & Marcora, 2012) have different neurophysiology constructs; nonetheless, both may have contributed to unpleasant sensations impairing the 5MSRT performance.

From a practical point of view, this information on the neuromuscular fatigue etiology related to the 5MSRT as a specific repeated-sprint test characterized by its long duration with a near 1:1 E/R ratio and its shuttle form could better orientate the coaches and/or physical trainers in their evaluation and training choice. The similar magnitude of peripheral and central fatigue development after 5MSRT and simulated soccer matches could reflect a better representativeness of real efforts and fatigue induced in modern soccer by this test in comparison with the brief repeated-sprint protocols classically used. Further investigations should thus be carried out to evaluate the relationship between fatigability and performance exerted during 5MSRT and a simulated soccer match. Then, adequate training protocols could be implemented aimed at reducing both central and peripheral fatigability and therefore improving the performance during the 5MSRT and consequently in competition.

One important limitation of the present study was the use of a single stimulus rather than two stimuli to investigate neuromuscular function. The use of a single stimulus may have underestimated the voluntary activation level (Place, et al., 2007; Shield & Zhou, 2004;). However, Goodall et al. (2015) found similar central and peripheral impairments after short duration repeated-sprint running with the use of a single twitch. Nevertheless, future investigations of neuromuscular function after fatigue-induced 5MSRT should use doublet stimuli to confirm our observations. Second, the distinction between the contribution of supraspinal and spinal aspects of the amount of fatigue and 5MSRT-induced performance impairment is limited with the voluntary activation measure. Future transcranial magnetic stimulation and/or reflex studies could assess this issue more precisely. Third, we cannot exclude a possible alteration of antagonist muscles' coactivation to performance decrements with fatigue, especially when changes of direction are part of the exercise (Hader, et al., 2014). Finally, the initial level of the tested athletes may have influenced the obtained results. Thus, their generalization to other populations with different physical levels should be done with caution.

The present study originally studied the neuromuscular fatigue and underlying mechanisms following the 5MSRT. Although no correlation was observed, there were similar performance (i.e., 8%) and force production (i.e., 9%) decreases after the 5MSRT. We originally demonstrated that the 5MSRT-performance impairment was associated with neuromuscular fatigue involving both peripheral and central factors. Such findings have practical implications for scientists, coaches and/or physical trainers for training and testing purposes. Adequate training protocols such as endurance training (Zghal, et al., 2015), concurrent strength and endurance training (Eklund, et al., 2014) or high-intensity interval training (Lee, Hsu, & Cheng, 2017; Milioni, et al., 2019), which have already been shown to be effective in limiting the development of central and peripheral fatigue, could be implemented to reduce the 5MSRT-induced neuromuscular fatigue and to improve the physical performance of athletes during competitions.

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