Neuromuscular fatigability during repeated-sprint exercise in male athletes

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Abstract

Purpose: To determine the pattern of neuromuscular fatigability that manifests during repeated sprint running exercise. **Methods:** Twelve male participants (mean ± SD age, 25 ± 6 yr; stature, 180 ± 7 cm; body mass, 77 ± 7 kg), currently training and competing in intermittent sprint sports, performed a repeated maximal sprint running protocol (12 × 30 m, 30 s rest periods). Pre- and post-exercise twitch responses to transcutaneous motor point stimulation and transcranial magnetic stimulation (TMS) were obtained to assess knee extensor neuromuscular and corticospinal function, respectively. Throughout the protocol, during alternate rest periods, blood lactate samples were taken and a single knee extensor maximal voluntary contraction (MVC) of the knee extensors was performed, with motor point stimulation delivered during and 2 s following, to determine voluntary activation (VA) and peripheral fatigue. **Results:** The repeated-sprint protocol induced significant increases in sprint time and blood [lactate] from the third sprint onwards ($P<0.001$). Furthermore, knee extensor MVC, resting twitch amplitude and VA were all significantly reduced after two sprints, and reached their nadir after sprint ten (Δ12%, Δ24%, Δ8%, $P<0.01$, respectively). In line with a reduction in motor point derived VA, there was also a reduction in VA measured with TMS (Δ9%, $P<0.05$) immediately post-exercise. **Conclusions:** These data are the first to demonstrate the development of neuromuscular fatigability of the knee extensors during and immediately after repeated-sprint exercise. Peripheral and central factors contributing to muscle fatigability were evident after two maximal sprints and over half of the drop in post-exercise MVC was due to supraspinal fatigue. Thus, peripheral, central and supraspinal factors all contribute to the performance decrement and fatigability of the knee extensors following maximal, repeated-sprint activity.

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Introduction

Paragraph 1. The ability to maximally reproduce short sprints (<10 s) with incomplete recovery (<60 s) is an important component of performance in intermittent sports. An inevitable consequence of repeated maximal efforts is fatigability of the exercising musculature which manifests as a reduction in exercise performance and mechanical output (21). To date, numerous studies have demonstrated the presence of fatigue following repeated-sprint exercise, with typical decrements in sprint performance between 5-25% (13, 15, 32, 33); however, the mechanisms underpinning fatigability and the associated performance decrements are not well-understood (13). Understanding the aetiology of fatigability during repeated-sprint exercise could have important implications for athletes competing in intermittent sports.

Paragraph 2. Previous research has attributed fatigue during repeated-sprint exercise to intramuscular mechanisms, such as limitations in energy supply and accumulation of metabolic by-products (7, 14, 18). A limited number of studies have used electrical stimulation at the motor nerve or muscle to assess peripheral (distal to the neuromuscular junction) and central (residing in the central nervous system) contributions to neuromuscular fatigability following repeated-sprint exercise. These studies suggest that peripheral mechanisms predominantly limit performance during such exercise; large reductions in resting twitch force from locomotor muscles have been observed after repeated-sprint running (32) and cycling (13), with small but significant decreases in the voluntary activation of muscle (32, 33). An activation deficit measured using peripheral nerve or muscle stimulation indicates sub-optimal output from lower motoneurons (40). By stimulating the motor cortex it is possible to determine the extent of supraspinal fatigue; that is, fatigability caused by a sub-optimal output from the motor cortex (43). Thus far, only one study has utilised this technique to assess supraspinal fatigue after repeated-sprint exercise. Girard et al. (13) demonstrated reductions in cortical voluntary activation, with no change in corticospinal excitability, during sustained, but not brief, maximal contractions after 10 × 6 s (30 s recovery) all-out sprint cycling. Further research utilising motor cortical and motor nerve or muscle stimulation methods is warranted to provide a greater understanding of the factors contributing to neuromuscular fatigability after repeat-sprint exercise; such understanding could help inform the development of appropriate training and intervention strategies to attenuate fatigue and improve repeat-sprint performance.

Paragraph 3. The aetiology of neuromuscular fatigability *during* repeated-sprint exercise is also not well understood, with previous work limited to pre- vs. post-exercise comparisons. Recent research has demonstrated the potential to study how fatigability manifests over time in self-paced single limb isokinetic exercise (10) and constant load cycling (8, 38). These studies have suggested a duration-dependent contribution of fatigue, with mechanisms of peripheral fatigability largely manifest in the early part of the bout, whereas the contribution from central mechanisms increase in line with the duration of exercise (8, 10). Such a study has not yet been extended to a repeated-sprint paradigm, where the presence of regular recovery periods would allow the uninterrupted assessment of neuromuscular fatigability. Thus, the aim of the present study was twofold; 1) to quantify the degree of peripheral and central factors contributing to neuromuscular fatigability elicited by repeat-sprint exercise and 2) to assess the development of fatigability *during* repeated-sprint exercise.

Methods

Paragraph 4. **Participants**

Following institutional ethical approval and in accordance with the Declaration of Helsinki, written informed consent was obtained from 12 male volunteers (mean ± SD age, 25 ± 6 yr; stature, 180 ± 7 cm; body mass, 77 ± 7 kg) who were currently training and competing in intermittent sprint sports (i.e., association football, rugby and hockey). Participants arrived at the laboratory in a rested and hydrated state, at least 3 h postprandial and having avoided strenuous exercise in the preceding 48 h. Volunteers were also asked to refrain from caffeine for 12 h before each test and alcohol for 24 h prior to each trial. Additionally, we collected data from 9 participants (including 5 of the original experimental cohort) acting as a control group allowing us to assess the repeatability of our neuromuscular measures (see below).

Paragraph 5. **Experimental Design**

Participants completed two trials (practice and experimental) separated by a minimum of 3 and a maximum of 7 days. In both trials participants completed a repeated-sprint protocol consisting of 12 × 30 m sprints with 30 s recovery; measures of neuromuscular function were assessed pre-, during and within 2.5 min post-trial (Figure 1). Specifically, transcranial magnetic stimulation (TMS) of the motor cortex and transcutaneous electrical stimulation of the knee extensors were administered pre- and post-trial to assess exercise-induced fatigability. Transcutaneous muscle stimulation was

administered during and 2 s post a single MVC on alternate repetitions of the repeated-sprint trial during the 30 s recovery period. Eight of the 12 participants completed a control trial without measurement of neuromuscular function, to ascertain whether the neuromuscular assessment itself induced impairments to repeated-sprint performance. All testing took place on an indoor running track where environmental conditions were kept consistent (ambient temperature 18°C).

Paragraph 6. **Neuromuscular Function**

In order to quantify pre- to post-changes in neuromuscular function, force and EMG variables were assessed before and immediately after (within 2.5 min) the repeated-sprint protocol. Maximum voluntary contraction (MVC) force was determined from three, 3 s contractions. Transcutaneous muscle stimulation was delivered during each MVC and then at rest ~2 s after the MVC to determine voluntary activation (VA; 29) and the potentiated knee extensor twitch force ($Q_{tw,pot}$), respectively. Subsequently, TMS was delivered during brief (~5 s) maximal and submaximal voluntary contractions for the determination of VA using TMS (VA_{TMS}; 17, 36). Each set of contractions comprised 100, 75, and 50% MVC efforts separated by ~5 s of rest. The contraction sets were repeated three times, with 15 s rest between each set. Visual feedback of the target force was provided via a computer monitor. To assess the development of neuromuscular fatigability during the repeated-sprint protocol, on alternate repetitions in the 30 s recovery period, participants performed a single MVC with transcutaneous muscle stimulation delivered during and 2 s post-; EMG activity was not measured at this time. Neuromuscular function was tested in a further subset of participants (n = 9) without performance of the repeated-sprint protocol. This allowed repeatability of our methods to be determined and ensured that the neuromuscular assessment itself did not induce a change in neuromuscular function.

Paragraph 7. **Force and EMG Recordings**

A calibrated load cell (MuscleLab force sensor 300, Ergotest technology, Norway) was used to measure knee extensor force (N) during voluntary and evoked contractions. The load cell was fixed to a custom-built chair and connected to a non-compliant cuff attached around the participant's right leg, superior to the malleoli. Participants sat upright in the chair with the hips and knees at 90° of flexion and were encouraged to grasp the handles on the side of the chair for support during contractions. EMG activity was recorded from the vastus lateralis (VL) and biceps femoris (BF). Surface electrodes (Ag/AgCl; Kendall H87PG/F, Covidien, Mansfield, MA, USA) were placed 2 cm

apart over the muscle bellies and a reference electrode was placed over the patella. Electrode placement was marked with indelible ink to ensure consistent placement post-trial; EMG data were not collected during the 30 s rest periods, the EMG electrodes were detached during the protocol. The electrodes were used to record the compound muscle action potential (M-wave) elicited by transcutaneous electrical stimulation of the quadriceps and the motor evoked potential (MEP) elicited by TMS. Signals were amplified (gain ×1000 for EMG and ×300 for force; CED 1902, Cambridge Electronic Design, UK), band-pass filtered (EMG only: 20-2000 Hz), digitised (4 kHz; CED 1401, Cambridge Electronic Design, UK), acquired and later analysed (Spike2 v7.12, Cambridge Electronic Design, UK).

Paragraph 8. **Peripheral Stimulation**

Single, transcutaneous electrical muscle stimuli (200 μs) were delivered to the right knee extensors using a constant-current stimulator (DS7AH, Digitimer Ltd, Welwyn Garden City, Hertfordshire, UK). Rectangular (5 × 13 cm) self-adhesive surface electrodes (Model 895250, Nidd Valley Medical Ltd, North Yorkshire, UK) were placed proximally (over the upper third of the muscle) and distally (just above the patella) over the knee extensors. Single stimuli were delivered to the relaxed muscle beginning at 100 mA and increasing by 20 mA until plateaus occurred in twitch amplitude and M-wave (M_{max}). Supramaximal stimulation was ensured by increasing the final intensity by 30% (mean current 349 ± 34 mA). The positions of the stimulating electrodes were marked with indelible ink to ensure consistent placement throughout and post the repeated-sprint protocol. Pre- and post-exercise muscle contractility was assessed for each peripherally-derived resting twitch as twitch amplitude ($Q_{tw,pot}$: the maximum twitch tension), maximum rate of force development (MRFD: maximum value of the first derivative of the force signal), contraction time (CT: time to peak twitch tension), maximum relaxation rate (MRR: lowest value of the first derivative of the force signal), and one-half relaxation time ($RT_{0.5}$: time taken for twitch force to decay to half of the peak twitch amplitude). Membrane excitability was inferred from the peak-to-peak amplitude and area of the electrically-evoked M_{max} (9).

Paragraph 9. **Transcranial Magnetic Stimulation**

TMS was delivered using a concave double cone coil (110 mm diameter; maximum output 1.4 T), which was powered by a mono-pulse magnetic stimulator (Magstim 200, The Magstim Company Ltd, Whitland, UK). The coil was held over the vertex in order to stimulate the left hemisphere (induced

current = postero-anterior) and was placed in the optimal position to elicit a large MEP in the VL and a small MEP in the antagonist (BF). To ensure a reproducible site of stimulation, the optimal coil position was marked on the scalp with indelible ink. The stimulator output was based on a resting motor threshold (rMT) determined pre-trial. Briefly, TMS was first delivered with the coil placed over the optimal site of stimulation at a sub-threshold intensity of 35% maximum stimulator output. Stimulus intensity was then increased in 5% steps until consistent motor evoked potentials (MEPs) with peak-to-peak amplitudes of more than 50 μV were evoked. Thereafter, stimulus intensity was reduced in 1% steps until an intensity was reached that elicited an MEP of at least 50 μV in 5 out of 10 trials (19). The stimulation intensity that elicited rMT was increased by 30%; thus, the experimental stimulation intensity was 130% of rMT (mean intensity 73 ± 9%). The stimulation intensity elicited a large MEP in the VL (area of 72 ± 18% of M_{max} during MVC); indicating the TMS stimulus activated a high proportion of knee extensor motor units, while causing only a small MEP in the antagonist (BF, 8 ± 6% of VL MEP during knee-extensor contractions).

Paragraph 10. **Repeated-sprint protocol**

After pre-trial assessment of neuromuscular function and a standardised 10 min warm-up, participants completed 12 maximal 30 m sprints separated by 30 s recovery. This protocol has been previously shown to result in significant exercise-induced fatigability, with typical decrements in sprint performance of between 5-25% (15, 16, 32). Sprint times were measured using electronic timing gates (TC Timing System, Brower Timing Systems, Draper, Utah, USA) placed at 0 and 30 m. The timing gates were individually adjusted to align with participants' hip height, and participants started 30 cm behind the start line on each sprint, in a standing start position. Participants were encouraged to perform each sprint maximally. Blood lactate concentration was measured from 20 μL capillary samples taken from the fingertip using sterile techniques, and analysed using an automated analyzer (Biosen C_Line, EKF diagnostic, Barleben, Germany) that was calibrated prior to use with a 12 $mMol \cdot L^{-1}$ standard; intrasample coefficient of variation for this instrument was <1.8%. A baseline blood sample was taken prior to the repeated-sprint protocol. Samples were then taken after alternate repetitions throughout the protocol (when neuromuscular function was not being assessed) and within 3 min post-exercise (Figure 1).

Paragraph 11. **Data Analysis**

Voluntary activation measured through transcutaneous quadriceps stimulation pre-, during and post-exercise was quantified using the twitch interpolation technique (29). Voluntary activation (VA) was quantified by comparing the amplitude of the superimposed twitch force (SIT) during MVC with the amplitude of the potentiated twitch force elicited 2 s post-MVC at rest: motor point VA (%) = (1 – [SIT/$Q_{tw,pot}$] × 100). Pre- and post-exercise VA_{TMS} was assessed by measuring the force responses to motor cortex stimulation during submaximal and maximal contractions. Corticospinal excitability is known to increase during voluntary contraction (35) thus, it was necessary to estimate the amplitude of the resting twitch through linear regression of the SIT force evoked by TMS during the maximal and submaximal contractions (ERT; 17, 36). Regression analysis confirmed the linearity of this relationship both pre- and post-exercise (r^2 = 0.95 and 0.97, respectively). Subsequently, VA_{TMS} (%) was quantified using the equation: (1 – [SIT/ERT] × 100). The peak-to-peak amplitude and area of evoked MEPs and M_{max} were measured offline.

Paragraph 12. **Statistical Analysis**

Data are presented as means ± SD in the text and means ± SE in the figures. Paired samples t-tests were used for pre- to post-trial comparisons. Repeated measures ANOVA was used to assess changes in outcome measures assessed during repeat-sprint exercise. In the event of a significant main effect, Dunnett's multiple comparison procedure was employed with the pre-trial score used as the control category. The assumptions underpinning these statistical procedures were verified as per the guidelines outlined by Newell et al. (31). Statistical significance was assumed at $P < 0.05$. Statistical analyses were conducted using GraphPad Prism (GraphPad Software, Inc. v5, La Jolla, CA, USA).

Results

Paragraph 13. **Repeated-sprint Exercise**

Throughout repeated-sprint activity there was a significant increase in sprint time ($P < 0.001$); specifically, every sprint from number 3 was significantly slower than the first (Figure 2A). The mean (4.45 vs. 4.46 s) and best (4.23 vs. 4.21 s) sprint times recorded in the control and experimental conditions did not differ ($P > 0.05$). In line with the increase in sprint time there was a concomitant increase in blood [lactate] from sprint number 3 ($P < 0.001$; Figure 2B).

Paragraph 14. **Neuromuscular Function**

Our neuromuscular control data demonstrate a consistent, highly repeatable measurement of neuromuscular function (mean ± SD within subject CV = MVC, 2.8 ± 1.1%; $Q_{tw,pot}$, 3.4 ± 0.8%; and VA, 2.4 ± 0.8%; Figure 3). In the experimental trial, after two maximal sprints MVC force had declined significantly by ~9% and as the repeated-sprint exercise ensued further drops in MVC were evident (Figure 4A). Immediately post-exercise the MVC force was reduced ($P < 0.001$) by 12 ± 7%. The reduced MVC was in line with reductions in the $Q_{tw,pot}$ indicative of peripheral fatigability. After two maximal sprints the $Q_{tw,pot}$ was reduced significantly by ~15% and immediately post-exercise by 23 ± 9% ($P < 0.001$) (Figure 4B). The pre- to post-exercise reduction in the $Q_{tw,pot}$ amplitude was accompanied by changes in peripherally derived measures of muscle contractility (Table 1). Specifically, post-exercise MRFD, CT and $RT_{0.5}$ were reduced by 18 ± 22% ($P = 0.02$), 15 ± 11% ($P = 0.002$) and 17 ± 14% ($P = 0.009$), respectively. At baseline, VA measured with motor point stimulation and TMS was high (93 ± 4% and 96 ± 3%, respectively). The repeated-sprint exercise caused significant decreases in motor point VA throughout the exercise and post-exercise was reduced by 9 ± 9% ($P = 0.004$). A similar decrement was observed in VA_{TMS} post-exercise (9 ± 7%, $P = 0.001$; Figure 4C).

Paragraph 15. **EMG responses**

MVC_{RMS} activity was unchanged pre to post the repeated-sprint exercise and similarly, there was no change in M_{max} or MEP amplitudes, areas and/or ratios at any contraction strength.

Discussion

Paragraph 16. The aim of the present study was twofold; 1) to quantify the degree of peripheral and central factors contributing to neuromuscular fatigability elicited by repeat-sprint exercise and 2) to assess the pattern of fatigability *during* repeated-sprint exercise. Our data demonstrate a significant reduction in maximal voluntary force and voluntary activation after just 2 maximal sprints. We provide the first evidence of significant supraspinal fatigue following a period of maximal, repeated-sprint running exercise. These data help to provide a mechanistic insight on why performance decrements are observed during repeated-sprint activity and add to investigations already detailing levels of neuromuscular fatigability that is apparent following repeated-sprint exercise in male games players. Furthermore, we demonstrate that repeatable assessments of

neuromuscular fatigability can be derived throughout repeated-sprint exercise, to provide a mechanistic insight into the performance decrement and factors of muscle fatigability.

Paragraph 17. **Development of fatigue during repeated-sprint exercise**

The design of the present investigation allowed us to study the development of neuromuscular fatigability during repeated-sprint exercise. The repeated-sprint literature, to date, is limited insofar that fatigability measures are only determined pre- and post-exercise; our approach provides the first data to quantify the neuromuscular responses throughout the exercise bout. It is common for declines in power output and performance, throughout a repeat-sprint protocol to be detailed (5, 13, 28, 32, 33), but the necessary apparatus needed to study neuromuscular fatigability in the knee extensors do not permit a straight-forward assessment when using a cycle ergometer. Sidhu et al. (38, 39) have recently demonstrated that excitability of the brain-to-muscle pathway can be studied during cycling exercise; however, the movement associated with maximal sprint efforts would likely disturb such measures. Using a protocol that allowed rapid assessment of neuromuscular function during the recovery period between repetitions, we observed a loss in maximal voluntary strength of the knee extensors and increased peripheral fatigability (reduced $Q_{tw,pot}$) after just two sprints. Furthermore, both of these variables reached their nadir after sprint 10 (Figure 4A & B). The of decay in MVC and the $Q_{tw,pot}$ is similar to that reported by Froyd et al. (10) who studied the development of neuromuscular fatigability during a single limb, isokinetic knee extensor bout, and Decorte et al. (8) who reported the development of fatigability after intermittent bouts of high-intensity cycling exercise.

Paragraph 18. Contrary to previous studies of repeat-sprint exercise, we observed a significant contribution of central fatigability to the decline in MVC, which was evident after two sprints, remaining supressed throughout the protocol and reaching a nadir from sprint ten onwards (Figure 4C). Previous investigations studying the time course of central fatigability during exercise have shown such mechanisms only to become apparent towards the end of the exercise bout. Decorte et al. (8) reported significant reductions in VA only during the last quarter of a high intensity, interval cycling activity, comprising of 5 min intervals at 80% of power at maximum oxygen uptake. Furthermore, when the intensity of exercise is lower, central fatigability only becomes apparent in the final stages of activity (34). It would seem from these data that central fatigability during prolonged endurance type exercise only manifests in the latter stages of exercise. Our data suggest

that central fatigability during maximal, repeated-sprint running exercise follows a different pattern, with an almost immediate decline in VA after just two maximal 30 m sprints (approximately 8 s of activity). The maximal nature of repeated-sprint exercise, which heavily taxes short-term energy pathways and relies on the recruitment of high threshold motor units, might explain the rapid appearance of central fatigability.

Paragraph 19. **Fatigability measured pre- to post-exercise**

The present study design allowed the quantification of neuromuscular fatigability pre- and immediately post-exercise which aligns with the majority of previous research investigating the mechanisms of fatigue following repeated-sprint activity. Interestingly, the reduction in maximal knee extensor strength post-sprint running exercise in the present study (−12 ± 7%) is similar for that observed in the same muscle group following repeated-sprint cycling exercise (4, 13, 33). In line with a reduced MVC, immediately post-exercise reductions were evident in the amplitude of the resting twitch (−23 ± 9%) and associated reductions were seen in peripherally derived measures of muscle contractility (MRFD & CT; Table 1), suggestive of a mechanical change in the elastic components of the knee extensors (32). Specifically, the contractile failure may have been related to disturbances in intramuscular factors affecting excitation-contraction coupling. A reduced free Ca^{2+} concentration, which in turn would lead to less Ca^{2+} release or faster uptake of Ca^{2+} reducing cross bridge formation and ultimately causing a reduction in mechanical output (2, 23). The reduced CT may have been due to post-activation potentiation associated effects or an increased muscle temperature (Davies and Young, 1983). Intuitively, the high glycolytic rate identified by the substantial increase in blood [lactate], would have been associated with the increased accumulation of inorganic phosphate and H^+, which in turn may have interfered with the contractile machinery within the knee extensors (30). Inorganic phosphate is another metabolic by-product that is known to affect excitation-contraction coupling activity via inhibitory effects on Ca^{2+} handling and force development (22). The lack of change in the maximum M-wave pre- to post-exercise (Table 2) is suggestive of a maintained neuromuscular transmission and confirms that the exercise-induced peripheral fatigability was located beyond the sarcolemma. Such changes in the contractile apparatus beyond the sarcolemma would likely have resulted in less propulsive force and reduced sprinting efficiency.

Paragraph 20. To our knowledge, this is the first investigation to assess supraspinal mechanisms of fatigability immediately following a maximal, repeated-sprint running protocol. In parallel with the reduction in motor point VA (−9 ± 9%), immediately post-exercise there was a similar reduction in VA_{TMS} (−9 ± 7%; Figure 4C) suggesting that the mechanisms of central fatigability acted upstream of the motor cortex to impair voluntary descending drive (11, 42). The relationship between force output and VA_{TMS} of the knee-extensor muscles is linear between 50 and 100% MVC (17, 36), thus it is possible to determine the contribution of supraspinal fatigue to the total force loss (42). Post-exercise MVC decreased to 88% of baseline whereas VA_{TMS} dropped by 9%. In the absence of any supraspinal fatigue, post-exercise MVC would have dropped to 96% of the baseline value and the remainder of the drop in voluntary force was due to a reduced VA_{TMS}. Such that, supraspinal fatigue contributed 67% of the overall force loss following the repeated-sprint exercise. Conversely, in a recent review Bishop (6) reported that there is little evidence for changes in neural drive to locomotor muscles following intermittent-sprint activity. Such conclusions were drawn from an article that report no change in peripheral or cortical VA during brief contractions following repeated-sprint cycling (13). This latter investigation used a sprint protocol lasting a similar duration as the present study, but upon completion and after a 6 min rest, participants performed a further bout of exercise and then neuromuscular fatigability was measured within 3 min post-exercise. At this time, central fatigue was absent, but it is unknown whether these responses would have been comparable with the present study if they had been measured immediately following the first bout of activity.

Paragraph 21. Furthermore, in the present study there was a large increase in blood [lactate] (3.1 ± 1.4 $mMol \cdot L^{-1}$ vs. 12.8 ± 3.0, $mMol \cdot L^{-1}$, Figure 2B) immediately following the repeated-sprint exercise, suggesting a disturbance in metabolic homeostasis. Such a disturbance was two-fold greater than after repeated sprint cycling (5) and presumably is due to the greater muscle mass utilised during sprint running vs. cycling. The heightened disturbance in metabolic homeostasis might have contributed to the development of the observed central and specifically supraspinal fatigability. The firing of fatigue-sensitive muscle afferents is known to exert an inhibitory influence on motor cortical cells (24, 25) and multiple ascending afferent pathways have been described that affect higher centres in the brain (3). Moreover, persistent impairments in VA_{TMS} in response to sustained locomotor exercise has been associated with long-term disturbances in metabolic homeostasis (37). Thus, we speculate the increased supraspinal fatigability following maximal, repeated running

exercise might have been due, in part, to the elevated inhibitory influences on central motor drive mediated by metabosensitive muscle afferents.

Paragraph 22. The MEP evoked by TMS during a voluntary contraction is influenced by corticospinal cell and motoneuron responsiveness, and when normalised to the maximal M-wave can be inferred to reflect corticospinal excitability (41). During a maximal contraction the MEP is followed by a period of EMG silence; a suppression in voluntary drive elicited by a profound reduction in motoneuron excitability (27). Further work has demonstrated that rather than being a decrease in muscle spindle discharge, a change in motoneuron excitability is brought about by changes in the intrinsic properties of motoneurons (26). In the present study, maximal, repeated running activity did not alter either the MEP characteristics or the silent period. In the only other study to assess the influence of repeated-sprint exercise on corticospinal excitability, Girard et al. (13) also reported no change MEP and M_{max} amplitudes after repeated-sprint cycling. These authors did, however, find an increased cortical silent period towards the end of a sustained (30 s) contraction performed after the exercise. It is common to observe increases in MEP amplitude and the cortical silent period during a sustained contraction, which reflects an increased corticospinal excitability and inhibition (41). These responses occur without the fatigability induced by repeated-sprint exercise, thus, it is unclear if the data from Girard et al. (13) are the function of the exercise *per se* or the sustained contraction. Our data suggest that repeated-sprint running exercise induces significant supraspinal fatigability but does not impair the responsiveness of the neurons involved in motor cortical output to muscle.

Paragraph 23. **Limitations**

The measures of neuromuscular fatigability derived throughout the repeated-sprint protocol were based from a single MVC performed during the 30 s rest period. Ideally multiple trials should be performed when assessing maximal strength and neuromuscular function (12), but the nature of testing precluded this. Whilst only one contraction was performed, verbal encouragement along with visual feedback was consistently provided during all MVCs for all participants (12). Furthermore, the final, single MVC performed after sprint number 10 was not different from the mean of three performed post-exercise (Figure 4A). Thus, we believe the data captured during the 30 s rest period was not limited, insofar as only a single contraction was performed. Importantly, our method of assessing mechanisms of neuromuscular fatigability during the exercise bout did not

induce additional impairments in repeated-sprint performance; when the protocol was repeated without assessment of neuromuscular function both mean (4.45 vs. 4.46 s) and best (4.23 vs. 4.21 s) sprint times were not different from the experimental session. Furthermore, our control data demonstrate repeatable assessment of MVC, VA and $Q_{tw,pot}$ from a single measurement (Figure 3), thus, we are confident that the testing procedure itself did not induce an impairment in neuromuscular function and we provide a plausible method for future research investigations to employ.

Paragraph 24. It is also important to acknowledge that the EMG data for the knee extensors are only reported for one of the knee extensor muscles, the vastus lateralis. Whilst this is the common choice of knee extensor muscle to investigate during cycling (1), it might not be the most important knee extensor muscle during running. The bi-articular make-up of the rectus femoris, which is important for hip flexion and knee extension, might have been a more suitable muscle to study. However, the location of stimulating pads across the knee extensor muscle in the present study would not have allowed for EMG measurements to be studied in the rectus femoris.

Paragraph 25. A final limitation to highlight is that males only were studied in this investigation. It is well established that the level of muscle fatigability differs between sexes with females generally being less fatigable compared to men (for review 20). It is unknown, however, if differences exist in females compared to males owing to the contribution of factors influencing muscle fatigability during and after repeated-sprint activity; this is an important area that warrants further investigation.

Conclusion

Paragraph 26. A 12 × 30 m repeated-sprint running exercise induces significant peripheral and central knee extensor fatigability which is evident after just 2 maximal sprints, and continues to develop until the end of exercise. The data presented here provide the first evidence of significant supraspinal fatigability following a period of maximal, repeated-sprint, running exercise. The decline in voluntary activation of muscle is evident in the absence of changes in corticospinal and sarcolemmal excitability. These observations help to provide a mechanistic insight on why performance decrements are observed during repeated-sprint activity and further the investigations already detailing levels of fatigue that is apparent following repeated-sprint exercise.

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Table & Figure Legends

Table 1. Neuromuscular function pre- and post- repeated sprint activity. Values are means ± SD for 12 participants.

Table 2. Surface EMG responses during maximum voluntary contraction to transcranial and motor nerve stimulation pre- and post- repeated sprint activity. Values are means ± SD for 12 participants.

Figure 1. Description of the repeated sprint protocol showing when neuromuscular function was measured along with the timing of capillary blood sampling and the delivery of electrical stimulation. The assessment of 'Neuromuscular Function' involved 3 knee extensor MVCs with transcutaneous motor point stimulation delivered to the knee extensors during and 2 s post MVC to determine voluntary activation and peripheral fatigue; then 3 sets of knee extensor contractions (100, 75 & 50% MVC) were performed to determine voluntary activation with TMS. Participants then performed the 12 × 30 m repeated sprint protocol with 30 s rest between each sprint. During the rest period after sprints 1, 3, 5, 7, 9 and 11 capillary fingertip blood samples were taken (🡅); after sprints 2, 4, 6, 8 and 10 participants performed a single knee extensor MVC with transcutaneous motor point stimulation delivered to the knee extensors during and 2 s post the MVC (🡇). Within 2.5 min post-exercise, neuromuscular function measures were repeated and then a final blood sample was obtained.

Figure 2. Sprint time (**A**) and blood lactate (**B**) during the repeated sprint protocol. * = $P < 0.05$ vs. sprint 1 (panel **A**) or pre (panel **B**). Values are means ± SD for 12 participants.

Figure 3. Maximal voluntary contraction (A), potentiated knee-extensor twitch force (B) and voluntary activation (C) for the knee extensors during the control trial. These data emphasise the consistent nature of such measures during a controlled setting. Data are means ± SE for 9 participants.

Figure 4. Maximum voluntary contraction (**A**), potentiated knee extensor twitch force (**B**) and motor point voluntary activation (VA; open bars, panel **c**) pre, during and immediately post the repeated sprint protocol. Data for voluntary activation measured using TMS (VA_{TMS}; closed bars, panel **c**) is

shown pre and immediately post the repeated sprint protocol. * = $P < 0.05$ vs. the pre-exercise value. Values are means ± SEM for 12 participants.

Table 1. Neuromuscular function pre- and post-repeated sprint activity. Values are means ± SD for 12 participants.

	Pre	Post
Global fatigue		
MVC (N)	604 ± 77	529 ± 73*
Peripheral fatigue		
$Q_{tw,pot}$ (N)	212 ± 39	162 ± 29*
ERT (N)	188 ± 60	138 ± 57*
MRFD ($N \cdot s^{-1}$)	7600 ± 2217	5984 ± 1303*
CT (ms)	78 ± 13	66 ± 11*
MRR ($N \cdot s^{-1}$)	−2094 ± 650	−1826 ± 562
$RT_{0.5}$ (ms)	80 ± 9	66 ± 11*
Central fatigue		
Motor point VA	93 ± 4	85 ± 10*
VA_{TMS}	96 ± 3	87 ± 8*

MVC; maximum voluntary contraction, $Q_{tw,pot}$; potentiated twitch, ERT; estimated resting twitch, MRFD; maximum rate of force development, CT; contraction time, MRR; maximum rate of relaxation; $RT_{0.5}$; half relaxation time, VA; voluntary activation, VA_{TMS}; voluntary activation measured using TMS. * $P < 0.05$ pre vs. post.

Table 2. Surface EMG responses during maximum voluntary contraction to transcranial and motor nerve stimulation pre- and post- repeated sprint activity. Values are means ± SD for 12 participants.

	Pre	Post
During MVC		
MVC_{RMS} (mV)	0.33 ± 0.10	0.31 ± 0.11
M_{max} amplitude (mV)	5.50 ± 2.00	4.95 ± 1.09
M_{max} area ($\mu V \cdot s^{-1}$)	43.0 ± 11.8	36.3 ± 10.2
MEP amplitude (mV)	2.76 ± 0.78	2.72 ± 0.62
MEP area ($\mu V \cdot s^{-1}$)	28.3 ± 8.8	26.6 ± 6.2
MEP/M_{max} amplitude (%)	53 ± 16	57 ± 14
MEP/M_{max} area (%)	67 ± 21	76 ± 15

M_{max}; maximum M-wave, MVC_{RMS}; root mean square of EMG during maximum voluntary contraction, MEP; motor evoked potential.

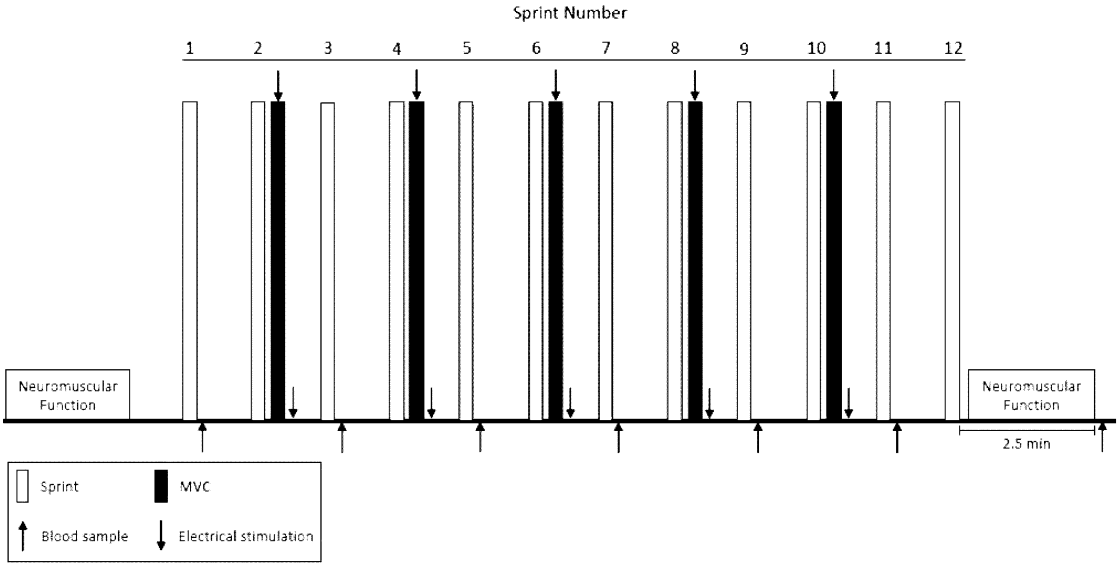
Sprint Number
1
2
3
4
5
6
7
8
9
10
11
12
Neuromuscular Function
Neuromuscular Function
2.5 min
Sprint
MVC
Blood sample
Electrical stimulation

Figure 1

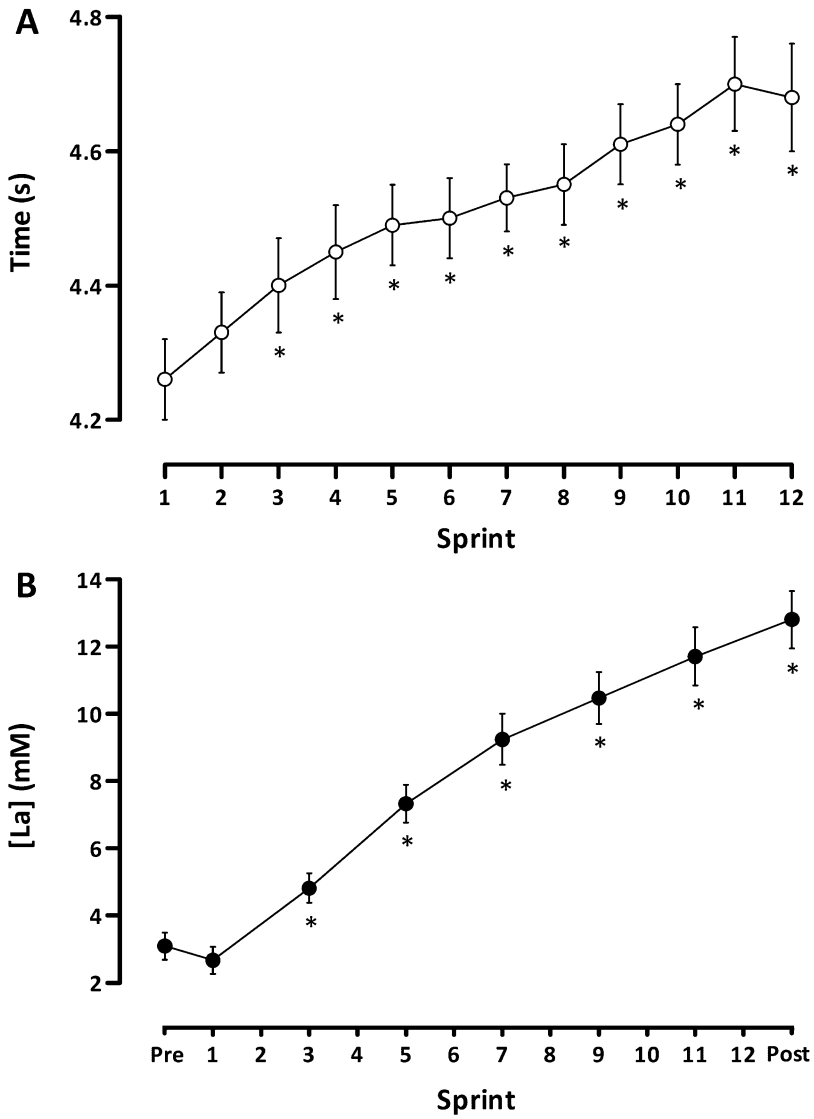
A
Time (s)
4.8
4.6
4.4
4.2
1 2 3 4 5 6 7 8 9 10 11 12
Sprint
B
[La] (mM)
14
12
10
8
6
4
2
Pre 1 2 3 4 5 6 7 8 9 10 11 12 Post
Sprint

Figure 2

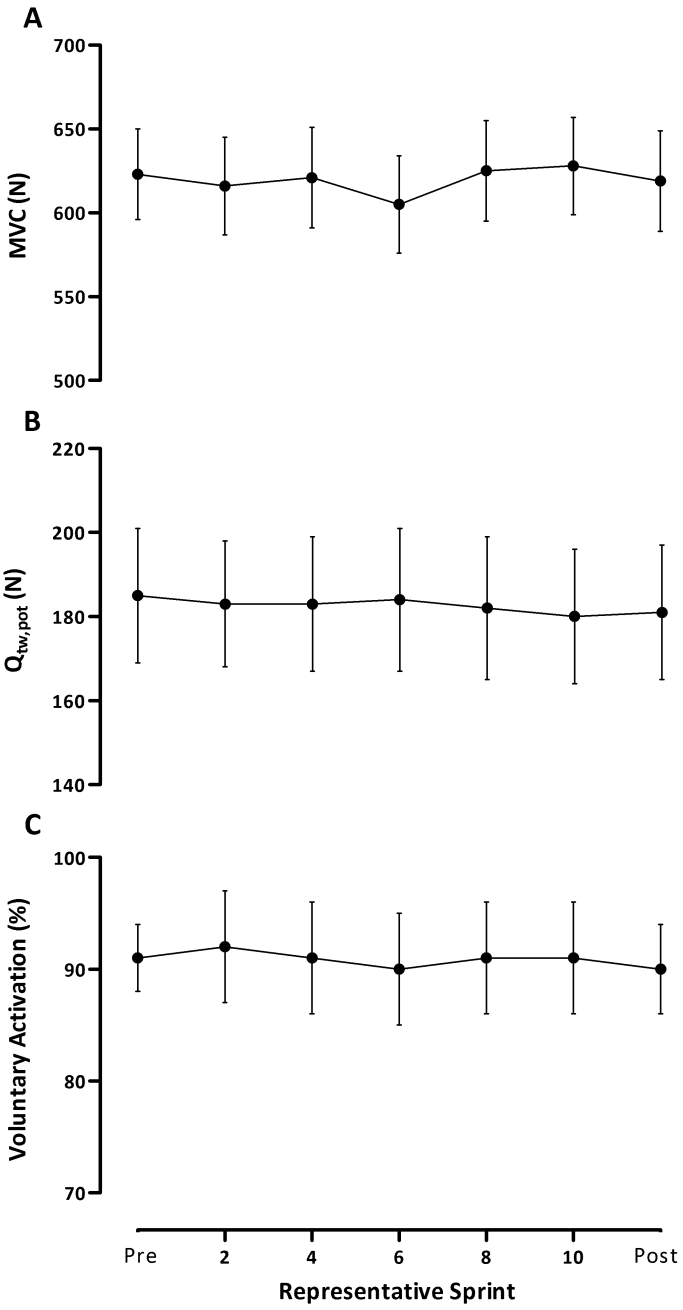
A
700
650
600
550
500
MVC (N)
B
220
200
180
160
140
$Q_{tw,pot}$ (N)
C
100
90
80
70
Voluntary Activation (%)
Pre
2
4
6
8
10
Post
Representative Sprint

Figure 3

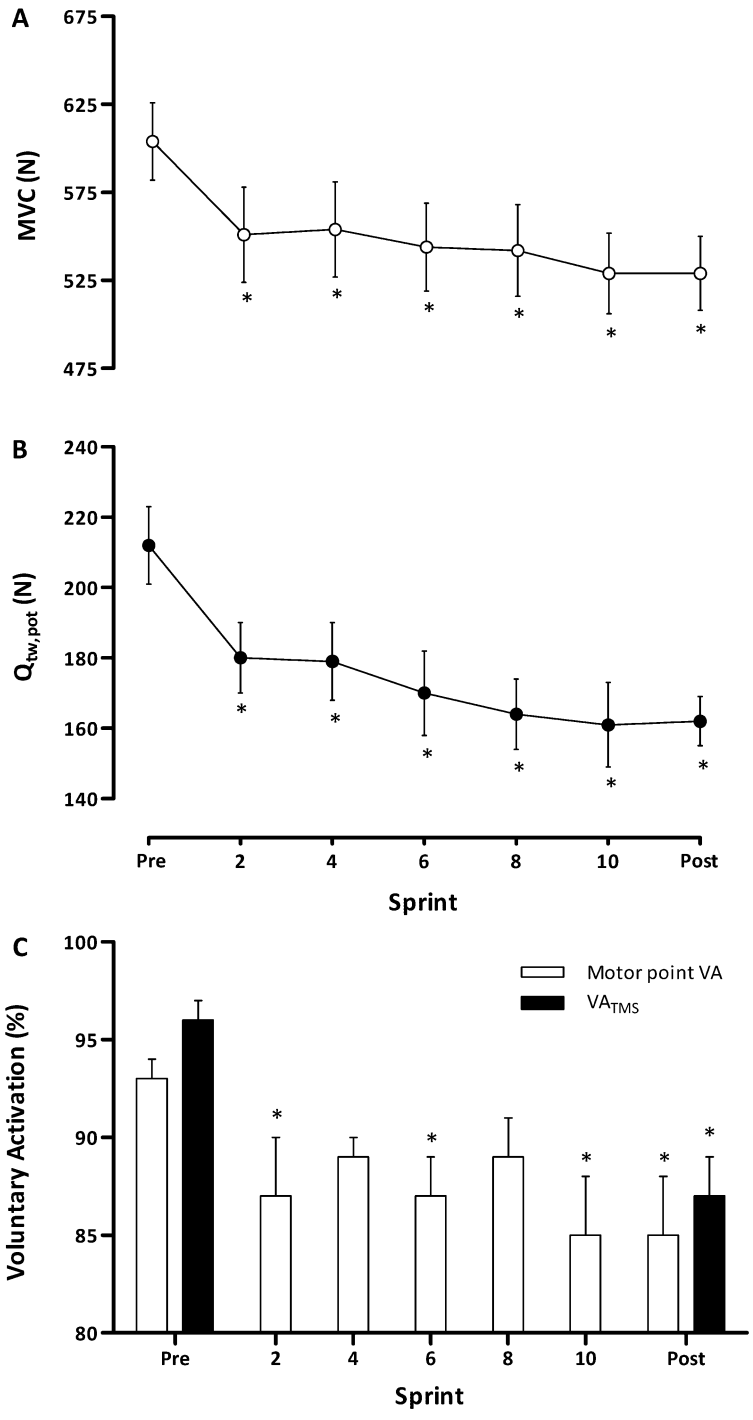
A
675
625
575
525
475
MVC (N)
B
240
220
200
180
160
140
$Q_{tw,pot}$ (N)
Pre
2
4
6
8
10
Post
Sprint
C
100
95
90
85
80
Voluntary Activation (%)
Motor point VA
VA_{TMS}
Pre
2
4
6
8
10
Post
Sprint

Figure 4