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Isometric knee extensor fatigue following a Wingate test: peripheral and central mechanisms

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Central and peripheral fatigue have been explored during and after running or cycling exercises. However, the fatigue mechanisms associated with a short maximal cycling exercise (30 s Wingate test) have not been investigated. In this study, 10 volunteer subjects performed several isometric voluntary contractions using the leg muscle extensors before and after two bouts of cycling at 25% of maximal power output and two bouts of Wingate tests. Transcranial magnetic stimulation (TMS) and electrical motor nerve stimulation (NM) were applied at rest and during the voluntary contractions. Maximal voluntary contraction (MVC), voluntary activation (VA), twitch amplitude evoked by electrical nerve stimulation, M wave and motor potential evoked by TMS (MEP) were recorded. MVC, VA and twitch amplitude evoked at rest by NM decreased significantly after the first and second Wingate tests, indicating central and peripheral fatigue. MVC and VA, but not the twitch amplitude evoked by NM, recovered before the second Wingate test. These results suggest that the Wingate test results in a decrease in MVC associated with peripheral and central fatigue. While the peripheral fatigue is associated with an intramuscular impairment, the central fatigue seems to be the main reason for the Wingate test-induced impairment of MVC.

Muscle fatigue in human performance can be defined as any exercise-induced decrease in maximal voluntary contraction (MVC) produced by a muscle or a muscle group (Bigland-Ritchie & Woods, 1984). It may arise not only due to peripheral changes in the muscle, but also when the central nervous system fails to drive the motoneurons adequately (Gandevia, 2001). Peripheral fatigue refers to exercise-induced processes that lead to a reduction in force production and that occur at or distal to the neuromuscular junction. It can be demonstrated by a fall in the twitch or tetanic force produced by peripheral nerve stimulation, while the muscle is at rest (Taylor & Gandevia, 2001, 2008). Central fatigue refers to more proximal processes and can be defined as a progressive exercise-induced failure of voluntary activation of the muscle (Taylor & Gandevia, 2001, 2008). It can be demonstrated by an increase in the increment in force evoked by nerve stimulation or by transcranial magnetic stimulation (TMS) during a maximal voluntary effort. The amplitude of the twitch during a MCV, expressed as a fraction of the twitch evoked by the same stimulus in the potentiated relaxed muscle, is termed voluntary activation and provides information on the level of neural drive to the muscle during exercise (Gandevia et al., 1995).

In addition, muscle fatigue can be associated with changes in the excitatory and inhibitory muscle response elicited by TMS during a voluntary contraction and recorded by electromyography (EMG). The excitatory response is a short-latency motor-evoked potential (MEP), which is the sum of several factors such as "excitability" of the underlying motor cortex, the "strength" of the mono- and oligosynaptic corticofugal connections, the "excitability" of the motoneurons and the properties of the muscle fiber action potential (Gandevia, 2001). The inhibitory response is demonstrated by a brief suppression of the ongoing EMG activity, called the silent period (SP), which results from inhibitory intracortical circuits (Gandevia, 2001).

Central and peripheral fatigue have been explored during and after locomotor running or cycling exercises of relatively long duration (Millet et al., 2003), indicating that central fatigue is more likely to be identified after prolonged running than after cycling exercises (Millet & Lepers, 2004). However, the type of fatigue induced by a short cycling bout is understudied. Recently, Decorte et al. (2010) reported that cycling to exhaustion at 80% of maximal power output in intermittent bouts of 6 min leads to a peripheral fatigue that develops early during

constant-load intense cycling, while central fatigue appears to be present toward the end of the exercise. In contrast, Sidhu et al. (2009a) indicated that cycling bouts of 5 min at 80% of maximal power output cause central fatigue, which played a greater role than the peripheral mechanism in the impairment capacity of the knee extensor in sustained MVC. Although these studies provide interesting information about the accumulative effect of several cycling bouts over the muscle fatigue mechanisms, it is still unknown how a single and maximal cycling all-out test affects muscle fatigue.

In order to explore the muscle fatigue effects of a maximal cycling all-out test, we measured the peripheral and central changes of the knee extensor muscle activation after a Wingate test. The Wingate Anaerobic Test, designed by Bar-Or et al. (1977), is relatively easy to administer and has proved to be a very useful tool for testing aspects of muscular work in which the contribution of energy from anaerobic sources may be considered to be relatively high (Bar-Or et al, 1977; Calbet et al., 1997). The Wingate test is a 30-s supramaximal pedaling test in which the resistance is adjusted relative to body weight, such that power output can be computed every 5 s (Bar-Or et al., 1977). An important feature of this test is that the subjects must perform at their maximal effort from the beginning to the end of the test. This test allows us to investigate the impact of this regime exercise over the capacity of the central nervous system to generate volitional force. This may also provide an insight regarding the mechanisms of the regulation of neural drive during exhaustive locomotor exercise.

Thus, the aim of the present study was to investigate how the capacity to activate the knee extensor muscles is affected after the performance of a maximal cycling. To this end, we used TMS and electrical stimulation to explore the peripheral and central mechanisms associated with muscle fatigue. We hypothesized that the Wingate test will result in a decrease in the MVC of the knee and that this impairment will be due to both peripheral and central fatigue.

Material and methods

Subjects

Ten young males participated in this study (age 25 ± 3, height 1.74 ± 0.04, weight 73.87 ± 9.34). The subjects were recruited from the Institute of Physical Education and Sport of Barcelona, Spain. All the subjects were physically active but none of the subjects were undertaking regular sprint-type training. None of them reported neurological impairment and/or contraindications to TMS (Wassermann, 1998). All subjects gave their informed consent after being informed of the possible risks of the study. The experimental procedures conformed to the Declaration of Helsinki and were approved by the local ethics committee.

Experimental protocol

Each subject participated in one familiarization and one experimental session separated by 1 week. The protocol is described schematically in Fig. 1. The familiarization session was used to familiarize the subjects with the Wingate test and voluntary contractions and to determine the individual maximal power output (P_{max}). This was of importance, as the Wingate test demands maximal effort and results in a high blood lactate concentration, so that subjects usually find the following voluntary knee contractions very difficult to perform. The familiarization session included two Wingate tests and five sets of voluntary contraction without the application of electrical or magnetic stimulation.

In the experimental session, subjects were required to perform five sets of voluntary contractions before and after a cycling exercise bout. The experimental session was divided into two blocks, a control and a Wingate block, always performed in that order. In the control block, the cycling exercise consisted of two bouts of 30 s pedaling at a low intensity (25% of P_{max}). In the Wingate block, the cycling exercise consisted of two Wingate tests.

Motor nerve stimulation

A single supramaximal electrical pulse (200 µs) was applied to the femoral nerve via adhesive electrodes (5×5 cm) using a Digitimer DS7AH (Welwyn Garden City, UK). The cathode was placed over the femoral triangle and the anode was positioned midway between the greater trochanter and the iliac crest. Both electrodes were made of carbonized rubber, coated with an electroconductive gel. Before placing the cathode, a small electrode (2×2 cm) was placed on the femoral triangle, in order to localize the best position for the femoral nerve stimulation. The intensity of the electrical pulse was set to 120% of that required to elicit a maximal compound muscle action potential (M_{max}) and maintained constant throughout the protocol.

TMS

A magstim 200^2 TMS (Magstim Company Ltd, Carmarthenshire, Wales, UK) was connected to a larger figure-of-eight-shaped coil (70 mm in external diameter). The optimal coil position to stimulate the knee extensor was explored by moving the coil around the motor cortex. The position and orientation of the coil was determined by localizing the largest motor evoked potential (MEP) in the right rectus femoris, with the lowest motor response in the biceps femoris. The simulator output (75–95%) was set at an intensity that evoked an MEP area in the rectus femoris of $\sim 90\%$ of M_{max} during 50% MVC contractions. Once the optimal intensity was found, it was maintained constant throughout the session for each subject.

Neuromuscular function test

For the isometric knee extension force tests, the subjects were placed in a seated position on an isokinetic Cybex 6000® machine (Lumex Inc., Ronkonkoma, NY, USA) and were securely strapped into the test chair. The chair had a long backrest, providing full back and head support. The hip and knee were at 90° of flexion (0° of knee extension refers to a horizontal leg-thigh).

Force and EMG parameters were measured before and after the cycling exercise bouts. Each set of contractions involved four contractions, following the protocol described by Shidhu et al. (2009a). According to this protocol, TMS was delivered at 100, 50 and 75% of MVC and motor nerve
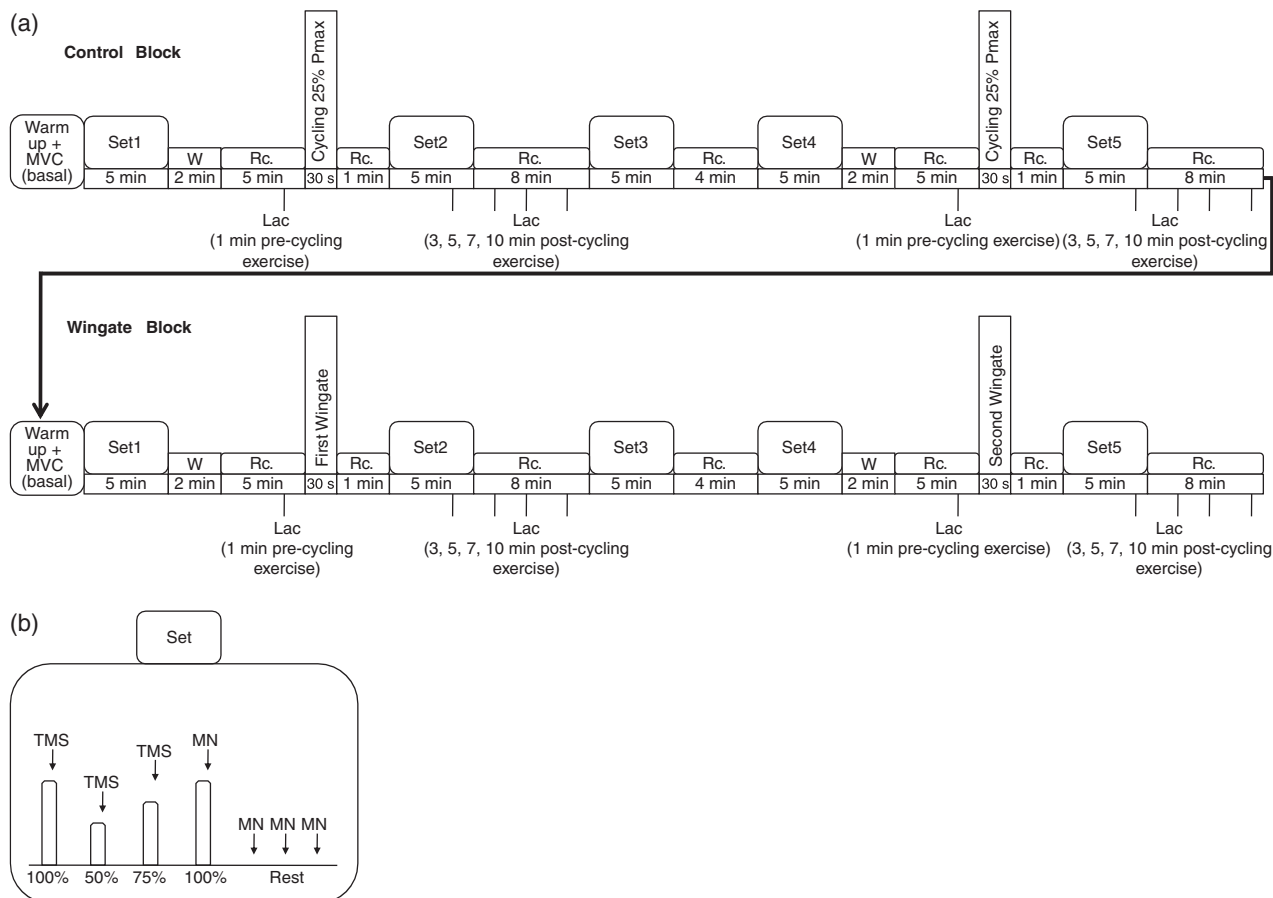
(a)

(b)

Fig. 1. (a) Experimental session. The experimental session consisted of two blocks. The first block (control block) included two cycling bouts performed at 25% of individual P_{max}. The second block (Wingate block) included two Wingate tests. The Wingate block started 8 immediately after the control block. MVC = maximal voluntary contraction, W = cycling warm-up (25% of P_{max}), Rc = recovery period, Lac = lactate samples. (b) Set: Each set consisted of four voluntary contraction during which TMS (100%, 50% and 75% of MVC) and MN (100 % MVC) was applied, followed by three MN stimulations at rest. The interval between each contraction was 6 and 2 s between MN stimulations.

stimulus was delivered at 100% MVC. An additional three motor nerve stimulations were applied at rest with a 2-s interval between them (Fig. 1b). For each contraction, the target force and the force exerted by the subject were displayed on a digital oscilloscope. The subject was required to match the exerted force with the target force and to maintain a steady force for 2–3 s. When the force stabilized at the target level, a single electrical or magnetic pulse was applied. The submaximal target forces were calculated for the MVC performed at the beginning of each block (MVC baseline). According to the recommendations of Gandevia (2001) for a reliable measurement of the MVC, the following methodological points were addressed: (i) all maximal effort was accompanied by instructions and practice, (ii) feedback of performance was given during all the voluntary contractions (visual display), (iii) appropriate standardized verbal encouragement was given by the investigators, (iv) subjects were allowed to reject efforts that they do not regard as "maximal" and (v) as the study involved repeated testing within a session, the gain of real-time visual feedback was varied, so that the subjects were not aware of any decline in their performance.

EMG and force recording

Bipolar silver chloride surface EMG electrodes with an inter-electrode distance of 2 cm were positioned over the muscle belly of the vastus lateralis (VL) and biceps femoris (BF). On the VL, the electrodes were placed 7 cm proximal to the superior border of the patella. On the BF, the electrodes were placed on the long head of the muscle. Before application of the electrodes, the skin surface was shaved and cleansed with alcohol and an electrode conduction gel was applied to the electrode contact face. To reduce movement artifacts, the electrodes were taped firmly in place and a bandage was applied to the thigh to avoid cable movements. The electrodes were kept in place throughout the experimental session. The raw signal was amplified and filtered with a band-pass filter of 30–1 kHz (Digitimer, Welwyn Garden City, UK). Signals were digitized at 2 kHz (CED Power1401, Cambridge Electronic Design, Cambridge, UK) and stored on a laboratory computer for off-line analysis. Voluntary and evoked forces were measured using a linear strain gauge coupled to the chair and the leg with a rigid, noncompliant device.

Cycling exercise

In the control block, the subjects performed two bouts of pedaling at 25% of P_{max}. In the Wingate block, the subjects performed two Wingate tests with a 30-min rest between them. The Wingate test is a 30-s maximal cycle ergometer exercise. The braking resistance was set at 7.5% of the body weight and the subjects were verbally encouraged to maintain as high a pedaling

rate as possible throughout the 30-s duration of the test. The Wingate test was conducted according to the widely accepted recommendations for standardization (Inbar et al., 1996).

Blood sampling

Blood lactate concentration was determined using a Lactat Photometer (Diaglobal DP100 GmbH, Berlin, Germany) from capillary blood samples drawn from the hyperemic ear lobe 1 min before each cycling bout and after the 1st, 3rd, 5th and 10th minute post-cycling bout.

Data analysis

The force evoked by the magnetic and electrical stimulation was identified and measured off-line with the oscilloscope cursors and with the digital software of the force transducer. The amplitude of the evoked twitch was obtained by subtracting the force at the onset of each twitch from the peak force. The amplitude of the resting twitch evoked by TMS was estimated rather than measured directly, as motor cortex excitability increases with activity, according to the method developed by Sidhu et al. (2009b). This method determines the estimated resting twitch using the y-interception of the linear regression between the amplitude of the superimposed twitches evoked by motor cortex stimulation and the voluntary force recorded during 50%, 75% and 100% MVC. Although special attention was paid during the trials that involved an evoked twitch at 100% MVC, it was not possible to apply the magnetic or the electrical stimuli at a correct timing as the subjects were unable to reach a stabilized force. Thus, the electrically evoked twitch at 100% MVC was eliminated from the analysis. For the magnetic pulse, this value was estimated using the linear relationship described in Todd et al. (2003) and adapted to knee extensors by Sidhu et al. (2009b).

Voluntary activation was calculated for the motor cortex stimulation according to the following formula: voluntary activation (%) = {[1 − superimposed twitch/resting twitch]} × 100, where the superimposed twitch is obtained at 100% MVC.

For the Wingate test, the variables measured were the peak power, mean power and pedaling rate. Peak power is the highest mechanical power elicited from the test taken as the average power over any 5-s period. The mean power is the average power maintained throughout the six 5-s segments. Peak and mean power were expressed as W/kg of body mass.

The blood lactate concentration increase was calculated between the sample obtained 1 min before the first Wingate test and the maximum individual value across the samples after the Wingate test (at, 1, 3, 5 and 10 min post-cycling bout).

During contractions in which either electrical or magnetic stimuli were delivered, the amplitudes of M_{max} and MEPs were measured for both VL and BF muscles. The amplitude of each MEP was normalized to the M_{max} evoked during the MVC in each set. The averaged rectified EMG during contractions was measured between 95 and 5 ms before stimulation. The EMG was normalized with the M_{max}. The MEP/EMG and EMG/Force ratios were calculated. The MEP/EMG provides information about the modulation of cortical excitability (Lemon et al., 1995), while EMG/Force determines the level of neural compensation necessary to make up for impaired muscle function (Ross et al., 2010). The SP duration was measured from TMS onset until the reappearance of background EMG.

Statistical analysis

Paired t-tests were used to compare the mechanical recordings between the two Wingate tests. Pearson's correlation coefficients were calculated to determine the relationship between MVC and peak power; mean power and fatigue index; percent increase in blood lactate concentration and percent decrease of MVC immediately after the Wingate tests; percent reduction of resting twitch amplitude and percent decrease of MVC immediately after the Wingate tests; and percent increase in blood lactate concentration and percent reduction of resting twitch amplitude immediately after the Wingate tests. All values are reported as means ± SE.

Two-way ANOVAs of repeated measurements were performed with block (control and Wingate) and set (set1, set2, set3, set4, set5) as factors. The ANOVAs were performed for the following variables: blood lactate, MVC force, resting twitch amplitude and cortical voluntary activation. When a significant interaction was found, separate ANOVAs were performed for each block with set as a main factor. Post-hoc analysis was performed using t-tests with Bonferroni corrections. Statistical significance was set at $P \leq 0.05$.

In order to explore the physiological changes after the two Wingate tests, three-way ANOVAs of repeated measurement were performed with Wingate (1st and 2nd), set (before Wingate and after Wingate) and intensity (75% MVC and 50% MVC) as factors. The ANOVAs were performed for the following variables: MEP amplitudes normalized with the M_{max}, MEP/EMG, EMG/Force ratios and the SP. In this analysis, only 75% and 50% MVC were selected as the corresponding forces remained constant across the experiment. An additional and separate analysis was performed for the MEP amplitudes during the MVCs.

Results
Blood lactate sample

The blood lactate concentration increased significantly ($t = 22.23$, $P \leq 0.0001$) from 1.78 ± 0.31 mM/L before the first Wingate to 12.75 ± 1.68 mM/L after the first Wingate test. One minute before the second Wingate test, the blood lactate concentration remained significantly higher (6.28 ± 1.37 mM/L) in comparison with that measured before the first Wingate test ($t = 10.66$, $P \leq 0.0001$). After the second Wingate test, the blood lactate reached values that were significantly higher (13.83 ± 2.36 mM/L) than the minute before the second Wingate test ($t = 13.01$, $P \leq 0.0001$) and also higher than after the first Wingate test ($t = 2.65$, $P = 0.02$). No significant differences were found in the blood lactate concentration during the control block.

MVC force and Wingate test

No significant difference was found between the MVC baselines (at the beginning of each block). Thus, the ANOVA was performed on the absolute values of force. The results of the MVC are displayed in Fig. 2(a). There was a main effect for block ($F_{1,9} = 47.22$, $P \leq 0.0001$), set ($F_{4,36} = 5.24$, $P = 0.002$) and a significant block × set interaction ($F_{4,36} = 8.84$, $P \leq 0.0001$). Separate ANOVAs were performed for each block that showed a significant main effect for set in the

Wingate block ($F_{4,36} = 10.73$, $P \leq 0.0001$). Post-hoc analysis showed a significant decrease in the MVC force after the first and second Wingate tests in
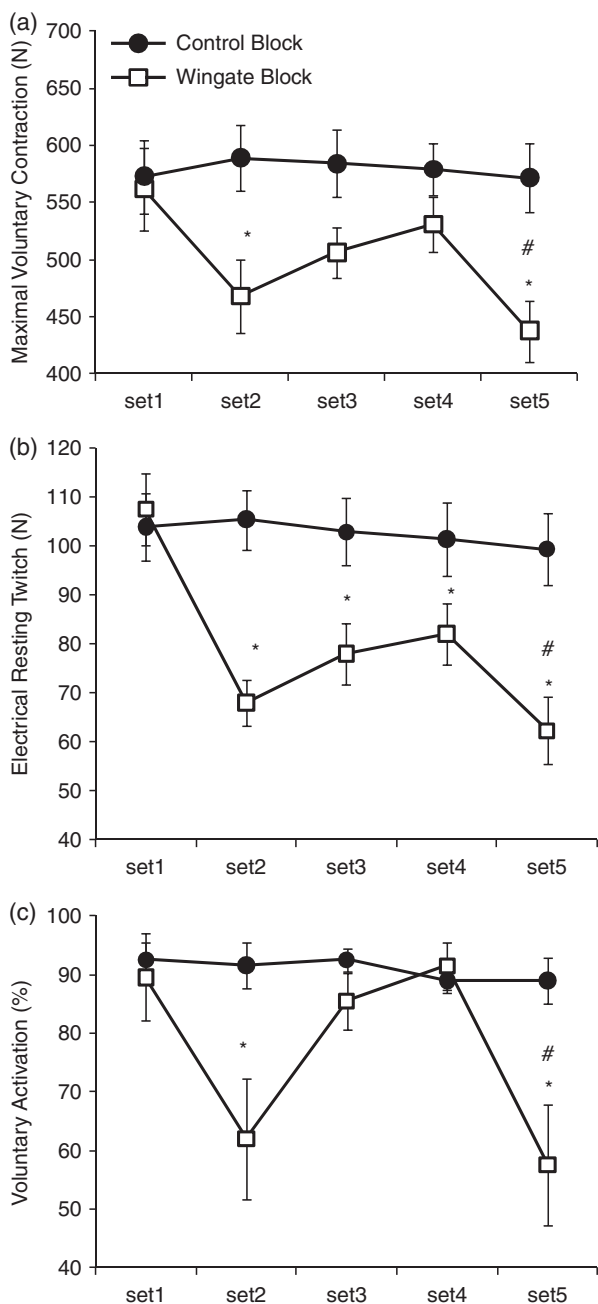

(a)

comparison with the previous sets (set1 vs set2, $t = 4.52$, $P = 0.014$, and set4 vs set5, $t = 4.08$ $P = 0.027$). Maximal voluntary force was reduced to $83.77 \pm 3.2\%$ after the first Wingate and to $82.76 \pm 3.97\%$ after the second test. Interestingly, we did not find significant differences between set1 and set3, showing a recovery of the MVC, 15 min after the first Wingate test. However, as demonstrated in Fig. 2(a), the values remain below the control condition, suggesting that the recovery of the MVC may be due to type II error as a result of the Bonferroni corrections used to perform the post-hoc analysis. The percent reduction in force correlated with the percent increase in blood lactate concentration ($r = 0.6$, $P = 0.04$ and $r = 0.7$, $P = 0.034$ for first and second Wingate tests, respectively).

There were no differences in the peak, mean power and pedaling rate between the two Wingate tests (Table 1). Strong correlations were found between MVC and peak power and between MVC and mean power in both Wingate tests ($r > 0.8$, $P \leq 0.01$ for all correlations).

Resting twitch amplitude

The analysis of the force evoked at rest in response to the supraelectrical stimulus over the femoral nerve showed a main effect for block ($F_{1,9} = 1.45$, $P = 0.004$), set ($F_{4,36} = 3.34$, $P \leq 0.0001$) and block $\times$ set interaction ($F_{4,36} = 4.87$, $P = 0.043$) (see Fig. 2b). No significant effect was found for set in the control block. For the Wingate block, there was a significant main effect for set ($F_{4,36} = 29.53$, $P \leq 0.0001$). Post-hoc analysis showed a significant decrease in the electrically evoked force in the sets performed after the first Wingate test in comparison with the previous set ($t = 8.94$, $P \leq 0.0001$). Potential twitch force was decreased by $36 \pm 9\%$ after the first Wingate test and did not recover in the following sets (set1 vs set2 to set5, $P < 0.01$ for all the pair comparisons). After the second Wingate test, the electrically evoked force decreased significantly in comparison with the previous set (set4 vs set5, $t = 3.56$, $P = 0.006$).

There were no significant correlations between the percent reductions in the resting twitch amplitude and percent reduction in MVC, nor between the percent increase in blood lactate and the percent reduction in the resting twitch amplitude.

Fig. 2. Changes in maximal voluntary contraction (a), electrical resting twitch (c) and voluntary activation (c) during the control and Wingate blocks. Values are means and SE. *Significant differences with set1 and # indicates significant differences with set4.

Table 1. Wingate performances. No significant differences were found between the first and the second Wingate tests

	Peak power (W kg^{-1})	Mean power (W kg^{-1})	Maximal pedal speed (rpm)	Mean pedal speed (rpm)
First Wingate	11.23 ± 0.49	9.04 ± 0.27	154.4 ± 6.85	124.34 ± 3.75
Second Wingate	11.19 ± 0.71	9.19 ± 0.24	153.9 ± 9.93	126.39 ± 3.53

Cortical voluntary activation

The ANOVA showed a main effect for block ($F_{1,9} = 11.64$, $P = 0.027$), set ($F_{4,36} = 7.29$, $P = 0.002$) and a significant block × set interaction ($F_{4,36} = 2.78$, $P = 0.05$). A significant main effect for set was found in the Wingate block ($F_{4,36} = 6.35$, $P = 0.001$) but not in the control block. Post-hoc analysis showed that the voluntary activation decreased significantly after each Wingate test, from $89 \pm 7.43\%$ in set1 to $62 \pm 10.27\%$ in set2 ($t = 2.52$, $P = 0.016$) and from $91.5 \pm 4.03\%$ in set4 to $57.59 \pm 10.31\%$ in set5 ($t = 2.86$, $P = 0.029$). Figure 2(c) demonstrates the recovery of the voluntary activation after set2. No significant differences were found between set1 and set3 or between set1 and set4, indicating a total recovery of the cortical voluntary activation.

Physiological parameters

The MEP amplitude obtained during the MVCs did not change significantly after the Wingate tests. The MEP amplitudes evoked during 75% and 50% of MVC increased significantly in the sets after the Wingate tests in comparison with the previous sets ($F = 12.42$, $P = 0.010$) (Fig. 3a). There was a main effect for the intensity of the MEP/EMG ratio ($F = 12.03$, $P = 0.01$) but no significant main effect for the set, indicating that this ratio was not affected by the Wingate tests (Fig. 3b). The EMG/Force ratio showed a significant increase ($F = 9.18$, $P = 0.023$) after each Wingate test, indicating an increase in neural activation (Fig. 3c). There were no significant differences in the SP between the sets before and after the Wingate tests.

The BF did not change significantly in any parameter related to this muscle, which confirms the correct position of the electrodes during the electrical stimulation and the accurate localization of the extensor muscle hot spot during the magnetic stimulation.

Discussion

The main propose of this study was to explore the effects of the Wingate test on the knee extensors capacity to produce maximal force. Our findings show that 30 s of supramaximal cycling results in a significant decrease of the MVC. This force reduction is associated with peripheral and central fatigue, although the results suggest that central mechanisms may be the main source for this reduction.

MVC force, peripheral and central fatigue

The capacity to generate maximal force in the knee extensors declines to $\sim 82\%$ (16% reduction) after
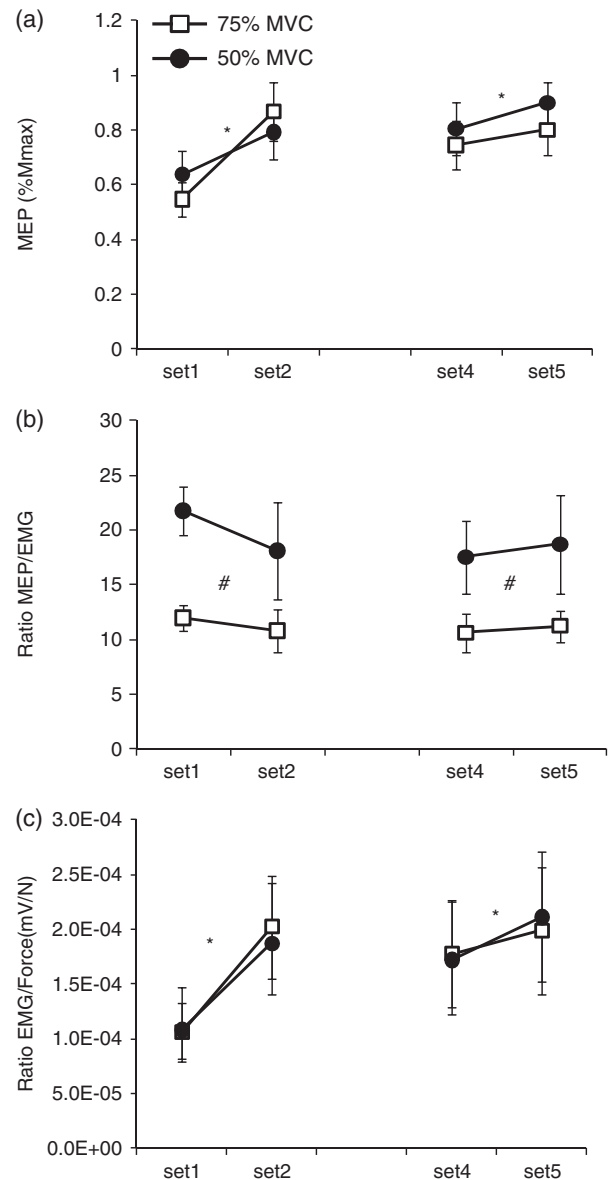

Fig. 3. Changes of MEPs (a), ratio MEP/EMG (b) and ratio EMG/force during submaximal contractions (75% and 50% of MVC) in the Wingate Block before and after the first (set1 vs set2) and second (set4 vs set5) Wingate tests. Values are means and SE. *Significant differences between sets and # indicates significant differences between intensities.

each Wingate test. These reductions in strength are similar to those reported in several studies using longer periods and lower intensities of cycling (for a review, see Millet & Lepers, 2004).

After the Wingate tests, there was a decline in the amplitude of the resting twitches evoked by the motor nerve, indicating the presence of peripheral fatigue. The reduction was around 36% and 41% after the first and the second Wingate test, respectively. These reductions are similar to the values reported in a recent study after 10 min of cycling at a constant load of 83% of the peak power output (Amann & Dempsey, 2008). Interestingly, the blood

lactate concentration in both studies was similar (13 mM/L). In the present study, we did not find a significant correlation between these two parameters, although previous studies have found a strong correlation between the reduction in the amplitude of resting twitches and the blood lactate concentration (Sidhu et al., 2009a). It has been suggested that there is no causal relationship between the lactate concentration and the subsequent peripheral muscle fatigue (Sidhu et al., 2009a) and that the decrease in the resting twitches amplitude may result from impairments in the excitation–contraction coupling (Sidhu et al., 2009a) as a result of metabolite accumulation during the exercise (Duchateau & Hainault 1985; Metzger & Moss, 1990; Westerblad et al., 1993).

Voluntary activation for the knee extensors at the beginning of the experimental session was high (91%), as has been reported previously (Sidhu et al., 2009a, b). The control cycling bouts did not affect the voluntary activation. However, after the Wingate tests, the values declined to approximately 60% (34% *reduction*), indicating an incapacity to drive the motoneurons. Supraspinal fatigue during prolonged running or cycling has been explained using the sertoninergic hypothesis (Newsholme et al., 1992). This hypothesis is based on the fact that there is accumulation of free-tryptophan (the serotonin precursor) during prolonged exercise and that this has a negative effect on the central nervous system. However, this theory is based on major alterations in lypolisis and, thus, it is unlikely that the performance of a brief maximal exercise, such as the Wingate test, is affected by this phenomenon. Moreover, it has been reported that acute and chronic administration of selective serotonin reuptake inhibitors does not affect the Wingate test performance (Parise et al., 2001). Another explanation for the supraspinal fatigue induced by the Wingate test is a possible inhibitory effect of peripheral fatigue on the voluntary activation. In a series of studies (Amann et al., 2006, 2008, 2009), it has been suggested that afferent feedback from fatigued locomotor muscles inhibits central motor drive to the same muscles. However, in our study, the central fatigue recovers fully despite significant peripheral muscle fatigue (sets 3 and 4) during the recovery period. This suggests that the inhibitory afferent feedback related to peripheral locomotor muscle fatigue is not an important determinant of central fatigue.

Approximately 15 min after the first Wingate test, the MVC force was not significantly different in comparison with the MVC before the Wingate test, even though the amplitude of the resting twitches remained small, indicating a persistence of peripheral fatigue. However, the MVC force 15 min after the first Wingate test remains below the control condition, which suggests that the mismatch between peripheral fatigue and MVC may have been overestimated. Nevertheless, in our study, the dynamics of the recovery of the capacity of the motor cortex to drive the motoneurons after the first Wingate test was parallel to the dynamics of the recovery of the MVC.

Wingate tests performance

The subjects were able to achieve the same performance during the second Wingate test as during the first. This recovery in the capacity to perform a short duration of a maximal intensity cycling exercise is in line with a previous study that showed no differences between two Wingate tests performed with a 4-min rest interval (Parise et al., 2001).

An interesting finding is that the peripheral fatigue does not recover even after 35 min before the initiation of the second Wingate test. Thus, it is unlikely that the peripheral fatigue is a limiting factor in the Wingate test as no difference was found between both the tests. In addition, the peripheral fatigue was not significantly different after the second Wingate test compared with that after the first test. Therefore, it is possible that the peripheral fatigue reflects the fatigue of fast-twitch fibers that, once these are fatigued, no more peripheral fatigue can develop (Marcora et al., 2008). Thus, the central fatigue could be the best candidate for the limitation in the Wingate test performance. One alternative explanation for the lack of reduction in the performance during the second Wingate test, even in the presence of peripheral fatigue, may be the effect of changes in muscle temperature. Linnane et al. (2004) recorded a significant high mean power output during a 30-s cycling sprint with an increase in muscle temperature. In addition, it has been reported that dynamic force productions seem to be more dependent on muscle temperature than isometric force productions (Binkhorst et al., 1977; Bergh & Ekblom, 1979). Thus, it is likely that the first Wingate test led to an increase in the muscle temperature that may have compensated the negative effect of peripheral fatigue on maximal power output during the second Wingate test. Nevertheless, in a recent debate (Marcora, 2010), it has been argued that endurance exercise is determined by psychobiological and physiological factors. Therefore, parameters such as the perception of effort may also be an important factor in endurance exercise performance and also in short maximal cycling bouts.

Physiological parameters

The M-wave amplitude did not change after the Wingate tests, which shows that the membrane excitability was not affected for the exhaustive bouts of cycling. Thus, the decrease in the resting twitch

amplitude is mainly due to changes within the quadriceps but not in the electrical transmission from the motor nerve terminals. Therefore, the reduction in response to motor nerve stimulation can result from several factors such as the accumulation of H+ and inorganic phosphate that leads to a reduction in Ca^2 sensitivity and a decrease in the number of strong binding cross bridges (Metzger & Moss, 1990; Allen et al., 2008).

The absence of changes in the MEP/EMG ratio and the SP during brief MVCs after the Wingate tests indicates that this kind of effort does not lead to a change in the corticospinal excitability and inhibition. These results are in line with a previous study (Sidhu et al., 2009a), which showed that neither the MEP amplitude nor the SP were altered during brief MVCs, after 45 min of a cycling exercise. The authors suggested that this kind of exercise does not impair the responsiveness of neurons in the pathway from the motor cortex to the muscle output. Thus, measures of cortical excitability are not necessarily indicative of central fatigue as other fatigue mechanisms can act upstream to the motor cortex output cells (Gandevia, 2001). According to this idea, it has been shown that increasing the cortex excitability (by administrating caffeine) is not associated with enhanced maximal voluntary activation during fatigue or recovery (Kalmar & Cafarelli, 2006). Moreover, the MEPs values can change without significant central fatigue and vice versa (Bigland-Ritchie et al., 1986; Gandevia et al., 1996). Thus, caution should be exercised in the interpretation of data on central fatigue based on MEPs measures during MVCs.

Magnetic stimulation of the motor cortex at 50% and 75% MVCs after the Wingate tests revealed an increase in the superimposed MEP amplitude. We should emphasize that the force achieved during the submaximal isometric contractions was the same absolute value across the experimental session, as it was a percentage of the baseline MVC. Although the interpretation of evoked motor responses during submaximal contractions is controversial, it may be the case that the increased MEP amplitude at submaximal contractions could be a compensatory phenomenon to generate the necessary motor output and to overcome the diminished peripheral force production. The results of the EMG/force ratio support this hypothesis as each Wingate test led to a significant increase in this ratio.

Perspectives

This is a novel study that explores how the capacity to activate the knee extensor muscles is affected after the performance of a Wingate test cycling. To the best of our knowledge, no previous studies have used TMS and motor nerve stimulation in order to explore this issue. In summary, the Wingate test leads to a decrease in MVC associated with peripheral and central fatigue. The peripheral fatigue is likely to be associated with an intramuscular impairment and the central fatigue with mechanisms acting upstream to the motor cortical neurons. The MVC recovered in parallel to the voluntary activation, despite a decrease in the resting twitch response, suggesting that central mechanisms are the main reason for the Wingate test-induced impairment of MVC.

Key words: central fatigue, peripheral fatigue, maximal voluntary contraction, twitch interpolation, transcranial magnetic stimulation, cycling, motor nerve stimulation.

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