Central & peripheral fatigue in male cyclists after 4, 20 & 40 km time-trials

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Short Title: Central & peripheral fatigue after self-paced exercise

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Abstract

Purpose: Few studies have assessed neuromuscular fatigue after self-paced locomotor exercise; moreover, none have assessed the degree of supraspinal fatigue. This study assessed central and peripheral fatigue after self-paced exercise of different durations. **Methods:** Thirteen well-trained male cyclists completed 4 km, 20 km and 40 km simulated time-trials (TTs). Pre- and immediately post-TT (< 2.5 min), twitch responses from the knee-extensors to electrical stimulation of the femoral nerve and transcranial magnetic stimulation of the motor cortex were recorded to assess neuromuscular and corticospinal function. **Results:** Time to complete 4 km, 20 km and 40 km was 6.0±0.2 min, 31.8±1.0 min and 65.8±2.2 min, at average exercise intensities of 96%, 92% and 87% of $\dot{V}O_{2max}$, respectively. Exercise resulted in significant reductions in maximum voluntary contraction, with no difference between TTs (–18%, –15% and –16% for 4, 20 and 40 km respectively). Greater peripheral fatigue was evident after the 4 km (40% reduction in potentiated twitch) compared to the 20 km (31%) and 40 km TTs (29%). In contrast, longer TTs were characterized by more central fatigue, with greater reductions in voluntary activation measured by motor nerve (–11% and –10% for 20 km and 40 km vs. –7% for 4 km) and cortical (–12% and –10% for 20 km and 40 km vs. –6% for 4 km) stimulation. **Conclusions:** These data demonstrate fatigue after self-paced exercise is task-dependent, with a greater degree of peripheral fatigue after shorter, higher intensity (~6 min) TTs and more central fatigue after longer, lower intensity TTs (>30 min).

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Introduction

Paragraph 1. In exercise science fatigue is commonly defined as an exercise-induced impairment in the ability to produce muscular force (17) in the presence of an increased perception of effort (14). Fatigue can be attributed to various processes along the motor pathway that are broadly split in to central and peripheral origins. Peripheral fatigue attributes the decline in force to processes at, or distal to, the neuromuscular junction (17). Central fatigue attributes the decline in force to processes residing within the central nervous system (17), commonly assessed by supramaximally stimulating the peripheral motor nerve during an isometric maximum voluntary contraction (MVC; 28). A subset of central fatigue is supraspinal fatigue, which attributes the decline in force to a sub-optimal output from the motor cortex (48, 49). Transcranial magnetic stimulation (TMS) has been successfully used to demonstrate the presence of supraspinal fatigue across a range of exercise paradigms (18-20, 32, 37, 38). Used in concert, motor nerve and motor cortical stimulation methods can develop a deeper understanding of the processes underpinning fatigue.

Paragraph 2. The extent to which peripheral and central processes contribute to fatigue is dependent on the nature of the exercise task and hence task-dependency remains a central theme in the study of fatigue. During sustained isometric maximal contractions of a single muscle group, peripheral fatigue is dominant, particularly during the early (<60 s) portion of the exercise bout, with central mechanisms increasing in influence as the exercise bout is prolonged (9, 36). During submaximal contractions (sustained or intermittent) at low intensities (<30% MVC) the contribution of central fatigue is higher than observed during higher-intensity submaximal contractions (>30% MVC), where peripheral fatigue predominates and central fatigue is modest or absent (8, 41, 42). Though less data are available, these patterns of central and peripheral fatigue can also be extended to locomotor exercise. Peripheral fatigue develops early during fatiguing locomotor exercise (13) and reductions in voluntary activation are evident when the exercise bout is prolonged (23, 31). While the available literature suggests higher intensity, shorter duration exercise is primarily limited by peripheral fatigue, and central fatigue is exacerbated as the exercise bout lengthens, a direct comparison of the contribution of central and peripheral processes to fatigue after locomotor exercise tasks of different durations is not available.

Paragraph 3. Previous studies investigating fatigue during whole body locomotor exercise have largely employed constant-load exercise protocols; a small number of studies have employed locomotor exercise paradigms that allow self-selected pacing strategies in response to sensations of fatigue and effort (3, 5, 6, 32). A series of recent studies by Amann and colleagues (3, 5, 6) have demonstrated the potential for studying fatigue using self-paced, whole body locomotor exercise modes. The authors proposed that the magnitude of exercise-induced peripheral fatigue is regulated to an individual "critical threshold", as evidenced by a remarkably similar end-exercise peripheral fatigue following self-paced 5 km cycling time-trial exercise in conditions of altered inspired air concentrations (4), pre-fatiguing exercise (3) and impaired afferent feedback (5). This centrally mediated restriction is proposed to be regulated by inhibitory afferent feedback in order to prevent excessive homeostatic disruption (1), perhaps to protect or maintain a muscular reserve capacity (7), and coincides with attainment of an individual "sensory tolerance limit" (17).

Paragraph 4. Amann & Secher (7) were careful to emphasize the critical threshold might be specific to the exercise task, and further work from the same group has demonstrated differences in the magnitude of peripheral fatigue after constant-load single- and double-leg knee extensor exercise modes (34, 35). Some support for a universal critical threshold of muscle fatigue during the same exercise mode has been provided for intermittent submaximal isometric contractions to exhaustion at intensities between 38-55% MVC (11). Interestingly, Burnley *et al*. (11) also observed a lower degree of peripheral fatigue at lower exercise intensities (<31% MVC), suggesting the critical threshold might not be attained in longer duration, lower intensity exercise; though the exercise was capped at 60 min and task failure only occurred in 1 of 9 participants. No study has directly compared the contribution of central and peripheral processes to fatigue after locomotor exercise tasks of different duration, and the existence of a critical threshold for peripheral fatigue after locomotor exercise warrants further investigation. Self-paced exercise offers an interesting test of this question, as the ability to modulate exercise intensity would theoretically permit the athlete to exhaust the available muscular reserve to maximize performance and attain such a threshold of muscle fatigue. In addition, the contribution of central processes to the fatigue observed after self-paced exercise of different durations has yet to be investigated, nor has the contribution of supraspinal fatigue. Accordingly, the aim of the present study was to examine the degree of central and peripheral fatigue induced by self-paced cycling exercise of

different durations. We hypothesized the existence of a consistent critical level of peripheral fatigue between time-trials of different durations, while the degree of central fatigue would increase as the length of the exercise bout is extended.

Methods

Participants

Paragraph 5. Following institutional ethical approval, thirteen well-trained male cyclists (mean ± SD age, 31 ± 8 years; stature, 1.80 ± 0.07 m; body mass, 72.9 ± 9.1 kg; maximum oxygen uptake [$\dot{V}O_{2max}$], 4.26 ± 0.38 L·min^{-1}, Power at $\dot{V}O_{2max}$ [W_{peak}] = 383 ± 29 W) gave written informed consent to take part in the study. All participants were regularly competing in cycling time-trial (TT) events similar in duration to those employed in the study.

Experimental Design

Paragraph 6. Using a repeated measures design, each participant visited the lab on 5 separate occasions to complete a preliminary assessment, a practice time-trial, and three experimental time-trials of 4 km, 20 km and 40 km in length. Trials were separated by a minimum of two and a maximum of seven days, and were conducted at the same time of day (±1 h). The order of experimental trials was randomized and counter-balanced. Prior to each visit, participants were required to refrain from caffeine (for at least 12 h), strenuous exercise (for at least 24 h) and to arrive in a fully rested, hydrated state. Before the first experimental trial participants completed a 48 h food and activity diary and were instructed to replicate their exercise and nutrition as closely as possible for each subsequent trial. Cardiorespiratory, blood lactate and perceptual responses were recorded during each time-trial and measures of central and peripheral fatigue were assessed pre-trial and within 2.5 min post-trial.

Procedures

Preliminary visit

Paragraph 7. Participants attended the laboratory to complete an incremental assessment to measure $\dot{V}O_{2max}$ and W_{peak}. The test started at 200 W and incremented by 5 W every 15 s. Participants cycled to the limit of tolerance and were given strong verbal encouragement in the latter stages. The test was terminated when participants were unable to maintain a cadence within 20 rpm of their self-selected cadence for the test. Maximum oxygen uptake

($L \cdot min^{-1}$) was calculated as the highest 30 s mean value, W_{peak} (W) was recorded as the end test power output.

Practice trial

Paragraph 8. Participants completed a practice trial to habituate to the measurement tools of the study, in particular electrical stimulation of the femoral nerve and magnetic stimulation of the motor cortex. A 4 km time-trial was chosen as the distance for the practice trial as the participant group were regularly competing in trials of distances approximating 20 km and 40 km, but were less practiced in shorter duration time-trials. In addition, previous data from our lab has shown evidence of a learning effect in well-trained cyclists for 4 km (44) but not 20 km (47) simulated time-trials. The reproducibility of time-trial performance across the distances employed is good (CV = 1.6-2.3%; 44, 47). The procedures adopted during the practice trial replicated that of the experimental trials (described below).

Experimental trials

Paragraph 9. Participants completed 4 km, 20 km and 40 km time-trials on separate occasions with instructions to "complete the distance as fast as possible". All exercise was completed on an electromagnetically braked cycle ergometer (Velotron Pro, RacerMate Inc., USA). Participants adjusted the ergometer to mimic their racing position (replicated for each trial) and wore their own cycling shoes and cleats. Visual feedback of distance covered, power output (W) and cadence (rpm) was available to view on a computer screen through the ergometer software (Velotron CS 2008, RacerMate Inc., USA). Participants were able to adjust their power output through variations in cadence and use of an electronic gearing system, and were instructed to remain seated for the duration of the trial. An electric fan was positioned 0.5 m in front of the ergometer for cooling during each trial.

Neuromuscular function

Paragraph 10. Measures of neuromuscular function for the assessment of central and peripheral fatigue were evaluated pre- and post-trial (within <2.5 min of exercise cessation) using transcranial magnetic stimulation (TMS) of the motor cortex and electrical stimulation of the femoral nerve, with evoked responses recorded with surface electromyography (EMG). Pre-time-trial exercise participants completed six isometric maximum voluntary contractions, separated by 60 s rest. The first three contractions ensured adequate potentiation of the knee

extensors. Femoral nerve stimulation was delivered during and 2 s post-MVC to assess voluntary activation and potentiated quadriceps twitch force ($Q_{tw,pot}$), respectively. Subsequently, TMS was delivered during brief (~3-5 s) contractions at 100%, 75% and 50% MVC, separated by ~5 s of rest, for determination of voluntary activation from cortical stimulation (VA_{TMS}). This procedure was repeated 3 times with 15 s rest between each set. Post-time-trial exercise participants completed three MVCs with femoral nerve stimulation, and three sets of contractions at 100%, 75% and 50% MVC with TMS; in line with other investigations that have assessed exercise-induced fatigue of the knee extensors, these measurements were completed within 2.5 min of exercise cessation (18, 35, 38). The rapid nature of this procedure is necessary to capture the magnitude of fatigue induced by the exercise before it dissipates (16), and the duration (2 to 2.5 min) was consistent between trials. Resting MEPs (eight stimuli) were recorded prior to these baseline measures of fatigue, and immediately after the final TMS set post-trial. Further detail on these procedures follows.

Force & EMG recordings

Paragraph 11. Knee-extensor force (N) during voluntary and evoked contractions was measured using a calibrated load cell (MuscleLab force sensor 300, Ergotest technology, Norway) fixed to a custom built chair and connected to a noncompliant strap attached round the participant's right leg superior to the ankle malleoli. The height of the load cell was individually adjusted to ensure a direct line with the applied force. During all measurements participants sat upright with the hips and knees at 90 degrees flexion, and were given specific instruction to maintain seated. Electromyography of the knee extensors and flexors was recorded from the vastus lateralis and lateral head of the biceps femoris, respectively. After the skin was shaved and cleaned, surface electrodes (Ag/AgCl; Kendall H87PG/F, Covidien, Mansfield, MA, USA) were placed 2 cm apart over the belly of each muscle. A reference electrode was placed on the patella. The positions of the electrodes were marked with indelible ink to ensure a consistent placement on repeat trials. The electrodes were used to record the root-mean-square amplitude for maximal voluntary contractions (MVC_{RMS}), the compound muscle action potential (M-wave) from the electrical stimulation of the femoral nerve, and the motor evoked potential (MEP) elicited by TMS. Surface electrode signals were amplified (× 1,000; 1902, Cambridge Electronic Design, Cambridge), band-pass filtered (EMG only; 20-2,000 Hz), digitized (4 kHz, micro 1401, Cambridge Electronic Design) and acquired for off-line analysis (Spike 2 version 7.01, Cambridge Electronic Design).

Femoral nerve stimulation

Paragraph 12. Single electrical stimuli (200 μs duration) were delivered to the right femoral nerve via surface electrodes (CF3200, Nidd Valley Medical Ltd, Harrogate, UK) using a constant-current stimulator (DS7AH, Digitimer Ltd, Welwyn Garden City, UK) at rest and during MVC. The cathode was placed over the nerve high in the femoral triangle; the anode was positioned midway between the greater trochanter and the iliac crest (20). The exact positioning was determined by the response that elicited the maximum quadriceps twitch amplitude (Q_{tw}) and M-wave (M_{max}) at rest. To determine the stimulation intensity, single stimuli were delivered in 20 mA step-wise increments from 100 mA until a plateau in Q_{tw} and M-wave were observed. To ensure a supramaximal stimulus the final intensity was increased by 30% (mean ± SD current = 194 ± 101 mA). The peak-to-peak amplitude and area of the electrically evoked M_{max} was used as a measure of membrane excitability (15). Measures of muscle contractility were derived for each resting twitch; twitch amplitude, maximum rate of force development (MRFD), maximum relaxation rate (MRR), contraction time (CT) and one-half relaxation time ($RT_{0.5}$).

Transcranial magnetic stimulation

Paragraph 13. Using a concave double cone coil (110 mm diameter; maximum output 1.4 T), single pulse magnetic stimuli of 1 ms duration were delivered to the left motor cortex, powered by a monopulse magnetic stimulator (Magstim 200, The Magstim Company Ltd., Whitland, UK). The coil was held and tilted lateral to the vertex (1.5 ± 0.6 cm) to stimulate the left hemisphere (postero-anterior intracranial current flow) over the area relating to Brodmann Area 4, the primary motor cortex. The coil position elicited a large MEP in the vastus lateralis and a concurrent small MEP in the biceps femoris, and was marked on the scalp using indelible ink to ensure consistent placement on repeat trials. Resting motor threshold (rMT) was determined prior to each experimental trial, and was not different between trials (P = 0.49). Starting at sub-threshold intensity (35% of stimulator output), single pulse TMS was delivered over the optimal site of stimulation in 5% increments until the peak-to-peak amplitude of the evoked MEP consistently exceeded 50 μV. Subsequently, the stimulus intensity was reduced in 1% decrements until the MEP response was below 50 μV in more than half of 10 stimuli (33). Resting motor threshold (rMT) for the knee extensors occurred at 49 ± 12% of maximum stimulator output, and subsequently during

experimental trials TMS was delivered at 130% of rMT. This intensity elicited a large MEP in the vastus lateralis (area on average 80% of M_{max} during knee extensor MVC) and a small MEP in the biceps femoris (area on average 6% of the raw quadriceps MEP during MVC).

Cardiorespiratory, Blood [lactate] & Perceptual measures

Paragraph 14. During each trial expired air was analyzed breath-by-breath using an online system (Cortex Metalyser 3b, Biophysik, Germany) and heart rate was measured with short wave telemetry (Polar Electro, Finland). Blood [lactate] was determined from 20 μL samples of fingertip capillary blood immediately analyzed 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. Blood sampling was aligned between trials such that samples occurred at the same distance covered in each, based on sampling blood at 20% of the distance covered in each trial; at 0.8, 1.6, 2.4, 3.2 and 4 km for the 4 km TT, at the same intervals plus 8, 12, 16 and 20 km for the 20 km TT, and then at all of the previously outlined intervals plus 24, 32 and 40 km for the 40 km TT. Ratings of perceived exertion (RPE) were obtained every 20% of trial distance covered using the Borg 6-20 scale. Participants were asked to provide a subjective assessment of RPE taking into account all sensations of physical stress, effort and fatigue (10). After assessment of neuromuscular function and a timed 5 minute standardized cool down participants were asked for a session RPE score that best represented the effort over the entire time-trial.

Data analysis

Paragraph 15. Voluntary activation measured through stimulation of the motor nerve was quantified using the twitch interpolation method (28). Briefly, the amplitude of the superimposed twitch force (SIT) measured during MVC was compared with the amplitude of the potentiated twitch force assessed ~2 s post-MVC at rest. Voluntary activation (%) = $(1 - [SIT/Q_{tw,pot}] \times 100)$. For cortical stimulation, VA_{TMS} was assessed by measurement of the force responses to TMS at 100%, 75% and 50% MVC (see figure, supplemental digital content 1, for an illustration of these methods). Corticospinal excitability increases during voluntary contraction, therefore it is necessary to estimate, rather than directly measure, the amplitude of the resting twitch in response to motor cortex stimulation. The amplitude of the estimated resting twitch (ERT) was calculated as the *y*-intercept of the linear regression between the mean amplitude of the superimposed twitches evoked by TMS at 100%, 75%

and 50% MVC and voluntary force (19, 48, 49); regression analyses confirmed the existence of a linear relationship both pre- and post-exercise (r^2 = 0.96 ± 0.03 and 0.94 ± 0.05 respectively). Voluntary activation (%) was subsequently calculated as (1 – [SIT/ERT] × 100). The reproducibility and validity of this procedure for the knee extensors has been previously established (19, 37). For pre- and post- measures of voluntary activation the median score was used for analysis (17). The peak-to-peak amplitude and area of the evoked M_{max} and MEP responses were quantified offline. The peak-to-peak amplitude was measured as the absolute difference between the maximum and minimum points of the biphasic M-wave or MEP (15). The area was calculated as the integral of the reflected value of the entire M-wave or MEP (15). The area of vastus lateralis MEP was normalized to the M_{max} measured during the MVC to ensure the magnetic stimulus was activating a high proportion of the knee-extensor motor units, and to quantify corticospinal excitability during contraction. Resting corticospinal excitability was quantified as the ratio between the resting MEP and resting M_{max}. The cortical silent period (CSP), was quantified during the MVC as the duration between the point of cortical stimulation until the post-stimulus EMG exceeded ±2 SD of the pre-stimulus EMG for >100 ms (20).

Reproducibility coefficients

Paragraph 16. Typical error (TE) and intra-class correlation coefficients (ICC) between the pre-trial scores were calculated to quantify reproducibility of the outcome measures of interest. Reproducibility was high for MVC (ICC = 0.98, TE = 4.0%), $Q_{tw,pot}$ (ICC = 0.98, TE = 6.6%), motor nerve VA (ICC = 0.96, TE = 3.0%), VA_{TMS} (ICC = 0.98, TE = 1.7%) and moderate for ERT (ICC = 0.91, TE = 10.8%), CSP (ICC = 0.95, TE = 12.8%), M_{max} (ICC = 0.86, TE = 29.1%) and MEP/M_{max} ratio (ICC = 0.74, TE = 12.6%).

Statistical analysis

Paragraph 17. Statistical procedures were planned *a priori*. For all neuromuscular measures, paired samples t-tests were used to assess the expected impact of each time-trial on measures of fatigue (pre- vs. post-trial comparison). The effect of time-trial length on all measures of fatigue and neuromuscular function was assessed using one-way repeated measures ANOVA on the pre- to post-trial change scores, with Tukey's pairwise *post-hoc* comparisons calculated in the event of a significant main effect. The same procedure was used to analyze differences between trials for time-trial performance (power output, W) cardiorespiratory and

blood lactate responses. Where a significant main effect was detected, selected effect sizes for three group comparisons were computed as eta-squared (η^2) and for two group *post-hoc* comparisons as Cohen's *D*. Friedman's ANOVA with *post-hoc* Wilcoxon signed-ranks test were employed for non-parametric data (i.e. RPE). To assess for differences in the pacing strategy, mean power output covered was computed in bins representing 10% of the distance covered for each trial, and expressed relative to the trial mean. This data was then analyzed using 3x10 repeated measures ANOVA, with a focus on the interaction effect to determine whether pacing strategy differed between trials. Previous data have demonstrated association between the degree of peripheral fatigue and capillary blood [lactate] accumulation (38), thus Pearson's product moment correlations were used to determine the relationship between these variables. The assumptions underpinning these statistical procedures were verified as per the guidelines outlined by Newell *et al.* (30) and all data were considered normal. Descriptive data are presented as means ± SD in text, tables and figures unless otherwise indicated. Statistical analysis was conducted using SPSS (IBM SPSS, version 19.0, Chicago, IL.). Statistical significance was assumed at $P < 0.05$.

Results

Paragraph 18. **Exercise Responses.** Mean power output was significantly higher in the 4 km (340 ± 30 W) compared to the 20 km (279 ± 22 W; $D = 0.98$, $P < 0.05$), and the 20 km compared to the 40 km (255 ± 21 W; $D = 0.97$, $P < 0.05$) (Figure 1, panel A). The pacing strategy adopted was not different between trials ($P = 0.57$, Figure 1, panel A). The mean power output during 4, 20 and 40 km corresponded to relative exercise intensities of 89%, 73% and 67% of W_{peak}, and 96%, 92% and 87% of $\dot{V}O_{2max}$ respectively. Mean whole trial values for oxygen uptake ($\dot{V}O_2$), minute ventilation ($\dot{V}_E$), tidal volume (V_T), ventilatory equivalent for oxygen ($\dot{V}_E/\dot{V}O_2$) and respiratory exchange ratio (RER) were higher in the 4 km compared to both the 20 km and 40 km ($P < 0.01$) and RER was higher ($P < 0.01$) in the 20 km compared to 40 km (Table 1). Heart rate was higher in both the 4 km and 20 km in comparison to 40 km ($P < 0.01$, Table 1). Both mean and peak blood lactate were higher in the 4 km (mean = 9.6 ± 1.9 mMol·L^{-1}, peak = 14.5 ± 2.8 mMol·L^{-1}) compared to both 20 km and 40 km ($P < 0.05$), and higher in the 20 km (mean = 7.8 ± 0.9 mMol·L^{-1}, peak = 11.5 ± 1.8 mMol·L^{-1}) compared to the 40 km (mean = 5.1 ± 1.3 mMol·L^{-1}, peak = 8.1 ± 2.2 mMol·L^{-1} ; $P < 0.05$, Figure 2). The evolution of RPE across each trial was similar independent of

distance (Figure 1, panel B), but participants perceived the 4 km to be harder than both the 20 km and 40 km, with differences between both the average RPE and the session RPE (P < 0.05, Table 1).

Pre- and post-exercise responses

Paragraph 19. **Peripheral responses.** Exercise resulted in significant peripheral fatigue in all time-trials ($\Delta Q_{tw,pot}$) along with alterations in muscle contractility (Table 2). Conversely, there were no differences in MVC_{RMS}, or measures of membrane excitability pre- to post-trial (M_{max} amplitude and area, Table 2). The reduction in MVC was not different between trials (102 ± 85 N, 84 ± 62 N and 84 ± 41 N drop for 4 km, 20 km and 40 km, respectively; P = 0.56, η^2 = 0.04, Figure 3, Panel A). The drop in $Q_{tw,pot}$ was different between trials (P = 0.03, η^2 = 0.25). There was evidence of a greater reduction in $Q_{tw,pot}$ after the 4 km trial (61 ± 37 N) compared to both the 20 km trial (46 ± 28 N, P = 0.03, D = 0.46) and the 40 km trial (44 ± 28 N, P = 0.049, D = 0.52) with no difference between 20 km and 40 km (Figure 3, Panel B). Greater decrements in MRFD of the potentiated twitch were observed after the 4 km compared to both 20 and 40 km (P < 0.05), while MRR, CT and $RT_{0.5}$ changed similarly independent of TT length (Table 2). End-trial peak [lactate] was correlated with the reduction in potentiated twitch force for the 4 km trial (r = –0.76, P < 0.01) but not for 20 km (r = –0.37, P = 0.22) or 40 km (r = 0.17, P = 0.66).

Paragraph 20. **Central responses**. Two participants exhibited small responses to TMS ($MEP:M_{max}$ ratio in VL <60%). Low $MEP:M_{max}$ ratios are indicative of an incomplete activation of the available motoneuron pool by the magnetic stimulus, which could invalidate the measurement of voluntary activation (37). These participants were subsequently excluded from analysis of data elicited by TMS (Table 3). Voluntary activation at baseline was similar for both motor nerve and motor cortical stimulation methods (93 ± 6% vs. 93 ± 4%, P > 0.05). Exercise resulted in significant reductions in both motor nerve VA (Table 2) and VA_{TMS} (Table 3). The change in motor nerve VA was different between trials (P = 0.02, η^2 = 0.37). Specifically, the drop in motor nerve VA was less after the 4 km (–7%) compared to both the 20 km (–11%, P = 0.03, D = 0.47) and the 40 km (Δ10%, P = 0.02, D = 0.59, Figure 3, panel C). The reduction in VA_{TMS} was also different between trials (P = 0.02, η^2 = 0.34) and mirrored the pattern observed for motor nerve VA. The decline in VA_{TMS} was less after the 4 km (–6%) compared to both 20 km (–12%, P = 0.01, D = 1.00) and the 40 km (–10%, P

= 0.04, D = 0.55, Figure 3, panel D). Corticospinal excitability during contraction (MEP expressed relative to M_{max} during MVC) was unchanged post-exercise (Table 3). At rest, corticospinal excitability was reduced after the 20 km (P = 0.004) and 40 km (P = 0.04) compared to baseline, but not after the 4 km (Table 3), though analysis of the relative and absolute change revealed no significant effect of time-trial length (P < 0.05). The cortical silent period was unchanged in all trials (Table 3).

Discussion

Paragraph 21. This study assessed the contribution of central and peripheral processes to fatigue after self-paced locomotor exercise of different durations. The main findings demonstrate that the magnitude of peripheral fatigue after high-intensity, short-duration (~6 min) self-paced locomotor exercise is greater than lower-intensity, longer exercise bouts (>30 min), where central fatigue is exacerbated. These data are the first to demonstrate the contributions of central and peripheral processes to the fatigue observed after self-paced locomotor exercise are dependent on the duration and intensity of the exercise task.

Paragraph 22. **Peripheral fatigue after self-paced cycling exercise.** Previous research has demonstrated the existence of an individual critical threshold of peripheral fatigue and associated sensory tolerance limit that is never voluntarily exceeded after high-intensity endurance cycling (3-5). These authors were careful, however, to emphasise this threshold might be task-dependent, and have recently demonstrated differences in the sensory tolerance limit between different modes of exercise requiring small vs. large muscle mass (35). Some support for the concept of a universal critical threshold has however been provided during isolated knee extensor exercise at intensities between 38-55% MVC by Burnley *et al*. (11). Based on this work, we hypothesized that such a threshold might exist across self-paced cycling time-trials of different duration. Self-pacing would theoretically allow participants to modulate power output in response to sensations of fatigue to maximize performance, which would presumably coincide with attainment of the aforementioned critical threshold of peripheral fatigue. However, contrary to our hypothesis, we observed a greater degree of peripheral fatigue after the 4 km (40% reduction in $Q_{tw,pot}$), compared to both the 20 km and 40 km time-trials (31 and 29% reduction, respectively). The reduction in potentiated twitch force after the 4 km TT was similar in magnitude to previous studies that have proposed the

existence of a critical threshold of peripheral fatigue after high-intensity locomotor exercise (~35%: 3, 4, 5), and it is plausible this degree of peripheral fatigue represents such a threshold. However, our data indicate that this threshold is not attained after longer duration, lower intensity self-paced exercise.

Paragraph 23. The most likely explanation for the greater magnitude of peripheral fatigue observed after the 4 km is the higher intensity of this trial. The concept of a critical threshold of peripheral fatigue is currently limited to studies of high-intensity locomotor (2-6) and isolated muscle (11) exercise. During these trials the high exercise intensity elicited responses consistent with non-steady state exercise; including a high and rising blood lactate response (Figure 2), attainment of near-maximal values for oxygen uptake (Table 1) and, in other work, a progressive recruitment of higher threshold motor units (11). The critical intensity (i.e. torque, speed, power) for a given task demarcates the boundary between sustainable, and unsustainable exercise, and exercise above and below this intensity is characterized by distinct physiological responses (11, 22). High-intensity exercise is associated with significant disruption to intramuscular homeostasis (22), and a disproportionate increase in the rate of peripheral fatigue development (11). It is possible that the greater degree of peripheral fatigue observed in the 4 km reflects the distinct physiological responses observed during high-intensity exercise. In contrast, the elevated but stable blood lactate response in the longer TTs indicates the exercise intensity in these trials was sustainable for the majority of the bout (Figure 2). During lower-intensity, longer-duration exercise peripheral fatigue occurs without significant metabolic disturbance (22), and exercise terminates with a substantial motor unit reserve (11). The smaller but significant degree of peripheral fatigue observed after the longer TTs is probably specific to the lower threshold motor units responsible for the exercise task (27), and likely explained by the lower average intensity of the longer trials.

Paragraph 24. **Why was peripheral fatigue different between trials?** The differing degree of peripheral fatigue observed after self-paced locomotor exercise of different durations is therefore likely explained by differences in exercise intensity, and further work is warranted to explicitly test this postulate. However, this proposal does not explain why peripheral fatigue was lower in the longer duration trials where participants were afforded the ability to self-pace. The pacing strategy adopted was also consistent between trials (Figure 1, panel A),

suggesting no influence of this on the observed differences in fatigue. For longer duration TTs, our data suggest factors other than muscle fatigue might play a larger role in limiting performance. Greater demands on temperature regulation, glycogen utilisation, and additional central fatigue occur during sub-maximal exercise (22) which might have limited the attainment of a greater magnitude of peripheral fatigue after the longer time-trials. Alternatively, the longer duration of the trials might have negatively impacted motivation, and the observed difference in peripheral fatigue could reflect a psychological rather than physiological limit to performance. Both mean and peak RPE were higher in the 4 km compared to the 20 and 40 km (Table 1, Figure 1 panel B). In the present study the RPE scale was used as originally defined to measure the total physical and psychic strain of the exercise (10). In this respect the RPE does not distinguish between the perception of effort, defined as the conscious awareness of the central motor command to the active muscles (29) and the perception of exertion or sensation of fatigue that arises due to afferent feedback from the working muscle (43). Consequently, it is unknown whether the higher RPE in the 4 km reflects a higher perception of effort (because of a greater power output) or a higher perception of exertion (because of a stronger afferent signal). Both concepts are limiting factors to self-paced exercise performance (1, 26) but will be balanced against the desire to perform, knowledge of the endpoint and previous experience of similar exertion (24). The shorter duration of the 4 km trial, where the endpoint of exercise is within reach for much of the bout, might permit a higher sensory tolerance limit than could be reached during longer duration trials. In contrast, the substantial degree of both central and peripheral fatigue in the latter stages of the longer time-trials might act collectively to negatively impact motivation, and the effort required to sustain a higher power output might have been perceived as unattainable (25).

Paragraph 25. **Greater central fatigue after longer TTs.** While the degree of peripheral fatigue was lower after longer time-trials, central fatigue (defined as a reduction in the voluntary activation of muscle) was exacerbated. This pattern supports previous research in both single limb and locomotor exercise models that has demonstrated a duration-dependent contribution of central fatigue to reductions in the voluntary force producing capability of skeletal muscle (11, 23, 31). For single-limb exercise, Burnley *et al*. (11) demonstrated that central fatigue decreased as exercise intensities increased above critical torque in the knee extensors. The present study is the first to explicitly compare different durations of locomotor

exercise, but the available literature also suggests a duration-dependent contribution of central processes to fatigue, at least for constant-load exercise. For example, reductions in VA have been observed after 4 hours of cycling (23) and 5 hours of running (31) at 55% of aerobic maximum. For higher intensity constant-load cycling, significant central fatigue has been observed after 30-40 min of repeated 5 minute intervals at 80% of aerobic maximum (38) that only manifests after 80% of the exercise bout is completed (13). For a continuous bout of constant-load cycling to exhaustion at a similar intensity, reductions in VA have been observed after only 8 min of exercise (18). Collectively, the current data and the available literature suggest that central fatigue is exacerbated in a duration-dependent manner, but the intensity of exercise also seems to be of influence. This is further supported by the present data, as central fatigue was similar in the 20 km compared to the 40 km despite the longer duration of the 40 km. Further work that explicitly compares different durations of exercise, both self-paced and constant-load, is warranted to better understand the contribution of central and peripheral processes to the fatigue induced by locomotor exercise.

Paragraph 26. The reduction in voluntary activation measured using TMS followed a similar pattern to that measured using stimulation of the motor nerve, with greater reductions after the 20 km and 40 km compared to the 4 km. This reduction implicates a potential contribution of supraspinal processes to fatigue, or sub-optimal output from motor cortical cells (17). The resting excitability of the corticospinal pathway was also significantly depressed after the 20 and 40 km, with no apparent depression after the 4 km (Table 3). Whether or not this depression could have contributed to the observed central fatigue is not clear, particularly considering corticospinal excitability was unchanged when measured during contraction (Table 3), a finding that has previously been reported after prolonged constant-load locomotor exercise (38). In addition, without a concomitant measure of motoneuron excitability (i.e. via stimulation at the cervicomedullary junction) it is not possible to distinguish between changes in cortical vs. motoneuron excitability. Further work is warranted to better understand the functional consequences of fatigue-induced changes at all levels of the motor pathway.

Paragraph 27. The measurement of fatigue post-exercise in this study was completed within 2.5 min of exercise cessation. Considering significant recovery of muscle function can occur 2 min post-exercise (16) it is likely that the magnitude of central and peripheral fatigue was

under-estimated. This limitation is common to the majority of literature studying fatigue incurred by locomotor exercise modes, and assumes that the fatigue observed after exercise is also present during the bout. This notwithstanding, the time taken to assess fatigue was consistent between trials, significant central and peripheral fatigue was observed after all time-trials and the magnitude of central and peripheral fatigue was influenced by time-trial length. These observations suggest the methods employed were suitable to detect differences in the central and peripheral contributions to fatigue after time-trial exercise of different durations. The time-delay might also have masked changes in processes relating to fatigue that could recover quickly on exercise cessation. For example, membrane excitability in the VL was unchanged post-trial in this study, but has recently been shown to be depressed during, but not post-, a 30 min bout of locomotor exercise (39). In addition, the cortical silent period has consistently been shown to lengthen during sustained isolated muscle exercise, including for knee extensor contractions (21) but recovery on exercise cessation is rapid (12, 45, 46). The lack of difference observed in these variables post-exercise, which have been reported in other similar studies (18, 38) could be due to the time delay between the end of exercise and the assessment of neuromuscular function. Assessing the development of central and peripheral fatigue during exercise is an area warranting further research (40).

Paragraph 28. In conclusion, the contribution of central and peripheral processes to fatigue after self-paced time-trial cycling exercise is task-dependent, with a greater degree of peripheral fatigue evident after shorter, high intensity (~6 min) time-trials, and an increased contribution of central fatigue after longer, lower intensity time-trials (>30 min). These findings suggest an intensity- and duration-dependent influence on the neuromuscular underpinning to fatigue after self-paced exercise, and further research that explicitly compares the central and peripheral contributions to fatigue after locomotor exercise tasks of different demand is warranted.

Author Contributions

All experiments were performed within the Sport Central laboratory facilities at Northumbria University. KT and SG contributed to conception and design of the experiments, data collection, data analysis, data interpretation, manuscript drafting and editorial process. MS contributed to conception and design of the experiments, data collection and manuscript drafting. GH, ASG and LA contributed to conception and design of the experiments, data

interpretation and manuscript drafting. All authors approved the final version of the manuscript.

Disclosures

This project did not receive any funding and has no conflicts of interest to report. The results of the present study do not constitute endorsement by ACSM.

References

1. Amann M. Central and peripheral fatigue: interaction during cycling exercise in humans. *Med Sci Sports Exerc*. 2011;43(11):2039-2045.

2. Amann M, Blain GM, Proctor LT, Sebranek JJ, Pegelow DF, Dempsey JA. Implications of group III and IV muscle afferents for high-intensity endurance exercise performance in humans. *J Physiol*. 2011;589(Pt 21):5299-5309.

3. Amann M, Dempsey JA. Locomotor muscle fatigue modifies central motor drive in healthy humans and imposes a limitation to exercise performance. *J Physiol*. 2008;586(1):161-173.

4. Amann M, Eldridge MW, Lovering AT, Stickland MK, Pegelow DF, Dempsey JA. Arterial oxygenation influences central motor output and exercise performance via effects on peripheral locomotor muscle fatigue in humans. *J Physiol*. 2006;575(Pt 3):937-952.

5. Amann M, Proctor LT, Sebranek JJ, Pegelow DF, Dempsey JA. Opioid-mediated muscle afferents inhibit central motor drive and limit peripheral muscle fatigue development in humans. *J Physiol*. 2009;587(Pt 1):271-283.

6. Amann M, Romer LM, Pegelow DF, Jacques AJ, Hess CJ, Dempsey JA. Effects of arterial oxygen content on peripheral locomotor muscle fatigue. *J Appl Physiol*. 2006;101(1):119-127.

7. Amann M, Secher NH. Point: Afferent feedback from fatigued locomotor muscles is an important determinant of endurance exercise performance. *J Appl Physiol*. 2010;108(2):452-454.

8. Bigland-Ritchie B, Cafarelli E, Vollestad NK. Fatigue of submaximal static contractions. *Acta Physiol Scand Suppl*. 1986;556:137-148.

9. Bigland-Ritchie B, Kukulka CG, Lippold OC, Woods JJ. The absence of neuromuscular transmission failure in sustained maximal voluntary contractions. *J Physiol*. 1982;330:265-278.

10. Borg GA. Psychophysical bases of perceived exertion. *Med Sci Sports Exerc*. 1982;14(5):377-381.

11. Burnley M, Vanhatalo A, Jones AM. Distinct profiles of neuromuscular fatigue during muscle contractions below and above the critical torque in humans. *J Appl Physiol*. 2012;113(2):215-223.

12. Butler JE, Taylor JL, Gandevia SC. Responses of human motoneurons to corticospinal stimulation during maximal voluntary contractions and ischemia. *J Neurosci*. 2003;23(32):10224-10230.

13. Decorte N, Lafaix PA, Millet GY, Wuyam B, Verges S. Central and peripheral fatigue kinetics during exhaustive constant-load cycling. *Scand J Med Sci Sports*. 2012;22(3):381-391.

14. Enoka RM, Stuart DG. Neurobiology of muscle fatigue. *J Appl Physiol*. 1992;72(5):1631-1648.

15. Fowles JR, Green HJ, Tupling R, O'Brien S, Roy BD. Human neuromuscular fatigue is associated with altered Na^+-K^+-ATPase activity following isometric exercise. *J Appl Physiol*. 2002;92(4):1585-1593.

16. Froyd C, Millet GY, Noakes TD. The development of peripheral fatigue and short-term recovery during self-paced high-intensity exercise. *J Physiol*. 2013;591(5):1339-1346.

17. Gandevia SC. Spinal and supraspinal factors in human muscle fatigue. *Physiol Rev*. 2001;81(4):1725-1789.

18. Goodall S, Gonzalez-Alonso J, Ali L, Ross EZ, Romer LM. Supraspinal fatigue after normoxic and hypoxic exercise in humans. *J Physiol*. 2012;590(Pt 11):2767-2782.

19. Goodall S, Romer LM, Ross EZ. Voluntary activation of human knee extensors measured using transcranial magnetic stimulation. *Exp Physiol*. 2009;94(9):995-1004.

20. Goodall S, Ross EZ, Romer LM. Effect of graded hypoxia on supraspinal contributions to fatigue with unilateral knee-extensor contractions. *J Appl Physiol*. 2010;109(6):1842-1851.

21. Hilty L, Lutz K, Maurer K, Rodenkirch T, Spengler CM, Boutellier U, Jancke L, Amann M. Spinal opioid receptor-sensitive muscle afferents contribute to the fatigue-induced increase in intracortical inhibition in healthy humans. *Exp Physiol*. 2011;96(5):505-517.

22. Jones AM, Wilkerson DP, DiMenna F, Fulford J, Poole DC. Muscle metabolic responses to exercise above and below the "critical power" assessed using 31^{P}-MRS. *Am J Physiol*. 2008;294(2):R585-593.

23. Lepers R, Maffiuletti NA, Rochette L, Brugniaux J, Millet GY. Neuromuscular fatigue during a long-duration cycling exercise. *J Appl Physiol*. 2002;92(4):1487-1493.

24. Marcora S. Counterpoint: Afferent feedback from fatigued locomotor muscles is not an important determinant of endurance exercise performance. *J Appl Physiol*. 2010;108(2):454-456; discussion 456-457.

25. Marcora SM. Do we really need a central governor to explain brain regulation of exercise performance? *Eur J Appl Physiol*. 2008;104(5):929-931; author reply 933-925.

26. Marcora SM, Bosio A, de Morree HM. Locomotor muscle fatigue increases cardiorespiratory responses and reduces performance during intense cycling exercise independently from metabolic stress. *Am J Physiol*. 2008;294(3):R874-883.

27. McNeil CJ, Giesebrecht S, Gandevia SC, Taylor JL. Behaviour of the motoneurone pool in a fatiguing submaximal contraction. *J Physiol*. 2011;589(Pt 14):3533-3544.

28. Merton PA. Voluntary strength and fatigue. *J Physiol*. 1954;123(3):553-564.

29. Morree HM, Klein C, Marcora SM. Perception of effort reflects central motor command during movement execution. *Psychophysiology*. 2012;49(9):1242-1253.

30. Newell J, Aitchison T, Grant S. *Statistics for Sports and Exercise Science: A practical approach*. Harlow: Pearson Education; 2010

31. Place N, Lepers R, Deley G, Millet GY. Time course of neuromuscular alterations during a prolonged running exercise. *Med Sci Sports Exerc*. 2004;36(8):1347-1356.

32. Ross EZ, Middleton N, Shave R, George K, Nowicky A. Corticomotor excitability contributes to neuromuscular fatigue following marathon running in man. *Exp Physiol*. 2007;92(2):417-426.

33. Rossini PM, Barker AT, Berardelli A, Caramia MD, Caruso G, Cracco RQ, Dimitrijevic MR, Hallett M, Katayama Y, Lucking CH, et al. Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical

application. Report of an IFCN committee. *Electroencephalogr Clin Neurophysiol*. 1994;91(2):79-92.

34. Rossman MJ, Garten RS, Venturelli M, Amann M, Richardson RS. The role of active muscle mass in determining the magnitude of peripheral fatigue during dynamic exercise. *Am J Physiol*. 2014;306(12):R394-340.

35. Rossman MJ, Venturelli M, McDaniel J, Amann M, Richardson RS. Muscle mass and peripheral fatigue: a potential role for afferent feedback? *Acta Physiol*. 2012;206(4):242-250.

36. Schillings ML, Hoefsloot W, Stegeman DF, Zwarts MJ. Relative contributions of central and peripheral factors to fatigue during a maximal sustained effort. *Eur J Appl Physiol*. 2003;90(5-6):562-568.

37. Sidhu SK, Bentley DJ, Carroll TJ. Cortical voluntary activation of the human knee extensors can be reliably estimated using transcranial magnetic stimulation. *Muscle Nerve*. 2009;39(2):186-196.

38. Sidhu SK, Bentley DJ, Carroll TJ. Locomotor exercise induces long-lasting impairments in the capacity of the human motor cortex to voluntarily activate knee extensor muscles. *J Appl Physiol*. 2009;106(2):556-565.

39. Sidhu SK, Cresswell AG, Carroll TJ. Motor cortex excitability does not increase during sustained cycling exercise to volitional exhaustion. *J Appl Physiol*. 2012;113(3):401-409.

40. Sidhu SK, Cresswell AG, Carroll TJ. Corticospinal responses to sustained locomotor exercises: moving beyond single-joint studies of central fatigue. *Sports Med*. 2013;43(6):437-449.

41. Smith JL, Martin PG, Gandevia SC, Taylor JL. Sustained contraction at very low forces produces prominent supraspinal fatigue in human elbow flexor muscles. *J Appl Physiol*. 2007;103(2):560-568.

42. Sogaard K, Gandevia SC, Todd G, Petersen NT, Taylor JL. The effect of sustained low-intensity contractions on supraspinal fatigue in human elbow flexor muscles. *J Physiol*. 2006;573(Pt 2):511-523.

43. St Clair Gibson A, Baden DA, Lambert MI, Lambert EV, Harley YX, Hampson D, Russell VA, Noakes TD. The conscious perception of the sensation of fatigue. *Sports Med*. 2003;33(3):167-176.

44. Stone MR, Thomas K, Wilkinson M, St Clair Gibson A, Thompson KG. Consistency of perceptual and metabolic responses to a laboratory-based simulated 4,000-m cycling time trial. *Eur J Appl Physiol*. 2011;111(8):1807-1813.

45. Taylor JL, Butler JE, Allen GM, Gandevia SC. Changes in motor cortical excitability during human muscle fatigue. *J Physiol*. 1996;490(2):519-528.

46. Taylor JL, Petersen N, Butler JE, Gandevia SC. Ischaemia after exercise does not reduce responses of human motoneurones to cortical or corticospinal tract stimulation. *J Physiol*. 2000;525 Pt 3:793-801.

47. Thomas K, Stone MR, Thompson KG, St Clair Gibson A, Ansley L. Reproducibility of pacing strategy during simulated 20-km cycling time trials in well-trained cyclists. *Eur J Appl Physiol*. 2012;112(1):223-229.

48. Todd G, Petersen NT, Taylor JL, Gandevia SC. The effect of a contralateral contraction on maximal voluntary activation and central fatigue in elbow flexor muscles. *Exp Brain Res*. 2003;150(3):308-313.

49. Todd G, Taylor JL, Gandevia SC. Measurement of voluntary activation of fresh and fatigued human muscles using transcranial magnetic stimulation. *J Physiol*. 2003;551(Pt 2):661-671.

Table & Figure Legends

Table 1. Performance, cardiorespiratory and perceptual responses to 4, 20 and 40 km cycling time trials. Values are mean ± SD for the whole trial (n = 13).

Table 2. Neuromuscular function and surface EMG responses to electrical stimulation of the motor nerve at rest and during maximum voluntary contraction pre- and post- 4, 20 and 40 km cycling time-trials (Values are mean ± SD, n = 13).

Table 3. Neuromuscular function and surface EMG responses to magnetic stimulation of the motor cortex at rest and during maximum voluntary contraction pre- and post- 4, 20 and 40 km cycling time trials (Values are mean ± SD, n = 11)

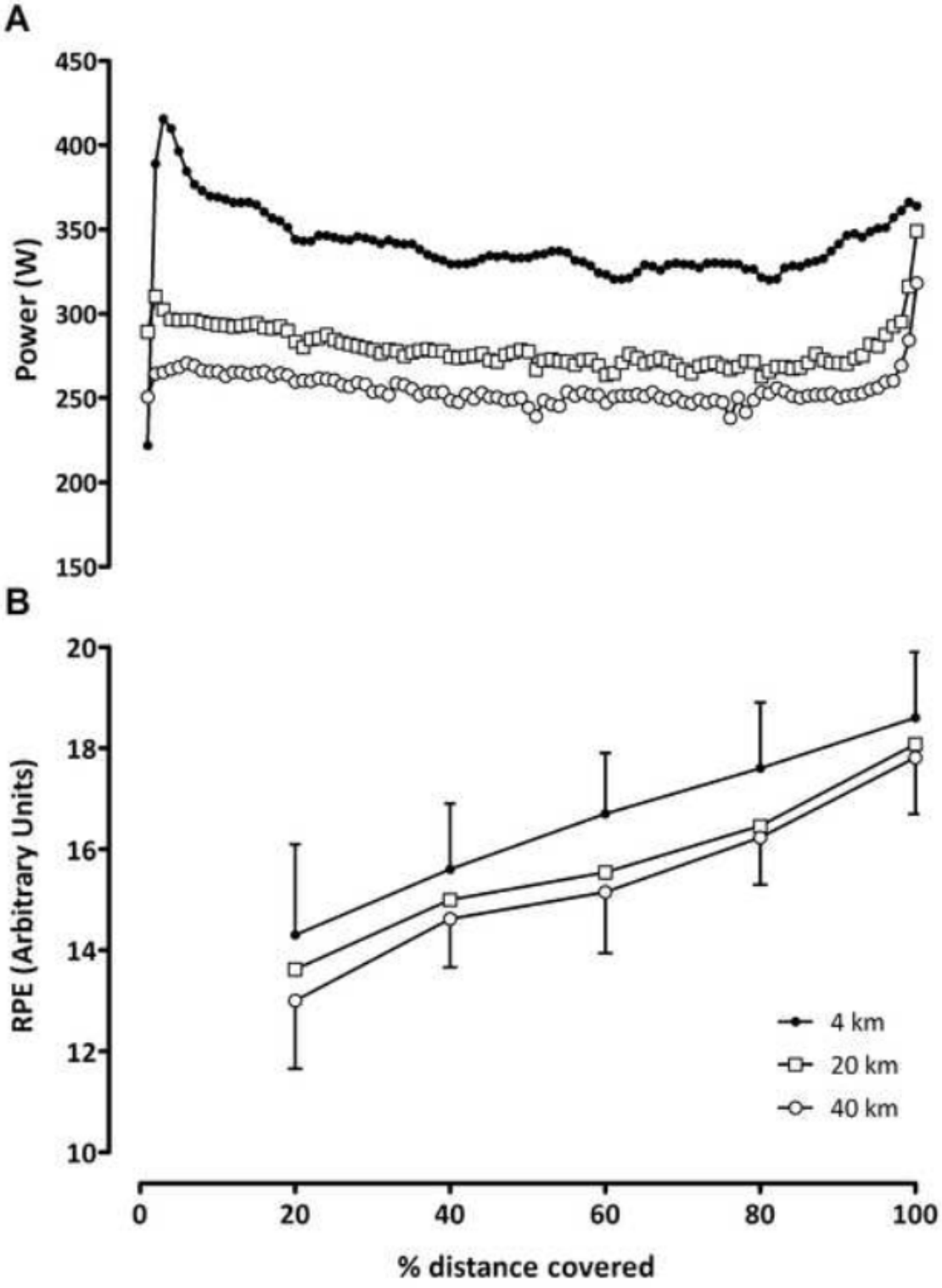
Figure 1. Time-course of power output (A) and rating of perceived exertion (RPE, B) during 4, 20 and 40 km cycling time-trials expressed relative to the distance covered in each trial. Values for power output are 1% means of the total distance covered. Values for RPE are mean ± SD, error bars are omitted for clarity.

Figure 2. Time-course of blood [lactate] ($mMol \cdot L^{-1}$) response to 4, 20 and 40 km cycling time-trials (values are mean ± SD). Capillary blood sampling was aligned between trials such that samples occurred at the same distance covered in each, based on sampling blood at 20% of the distance covered in each trial.

Figure 3. Pre- to post-trial percentage change in maximum voluntary contraction (A), potentiated twitch (B), voluntary activation measured with motor nerve stimulation (C) and voluntary activation measured with cortical stimulation (VA_{TMS}) (D) after 4, 20 and 40 km cycling time-trials. Values are mean + SD. *$P < 0.05$ different from 20 km, †$P < 0.05$ different from 40 km.

Supplemental digital content

Supplemental digital content 1. Raw traces of twitch forces and electromyographic (EMG) responses to transcranial magnetic stimulation (TMS) and peripheral motor nerve stimulation (PNS) from a representative participant. A, superimposed twitch (SIT) forces evoked by TMS during contraction strengths of 50%, 75% and 100% maximum voluntary contraction (MVC, background voluntary force has been offset to allow direct comparison). B, calculation of estimated resting twitch (ERT) force from the linear regression between evoked twitch force and voluntary force, pre- (●) and post- (○) time-trial exercise. C, Motor evoked potentials (MEPs) measured in vastus lateralis (VL) during contractions at 50%, 75% and 100% MVC, and in biceps femoris (BF) during MVC (NB. cortical silent period not shown). D, SIT and potentiated twitch force ($Q_{tw,pot}$) in response to PNS pre- (black line) and post- (grey line) time-trial exercise. E, M-waves measured in VL and BF during MVC.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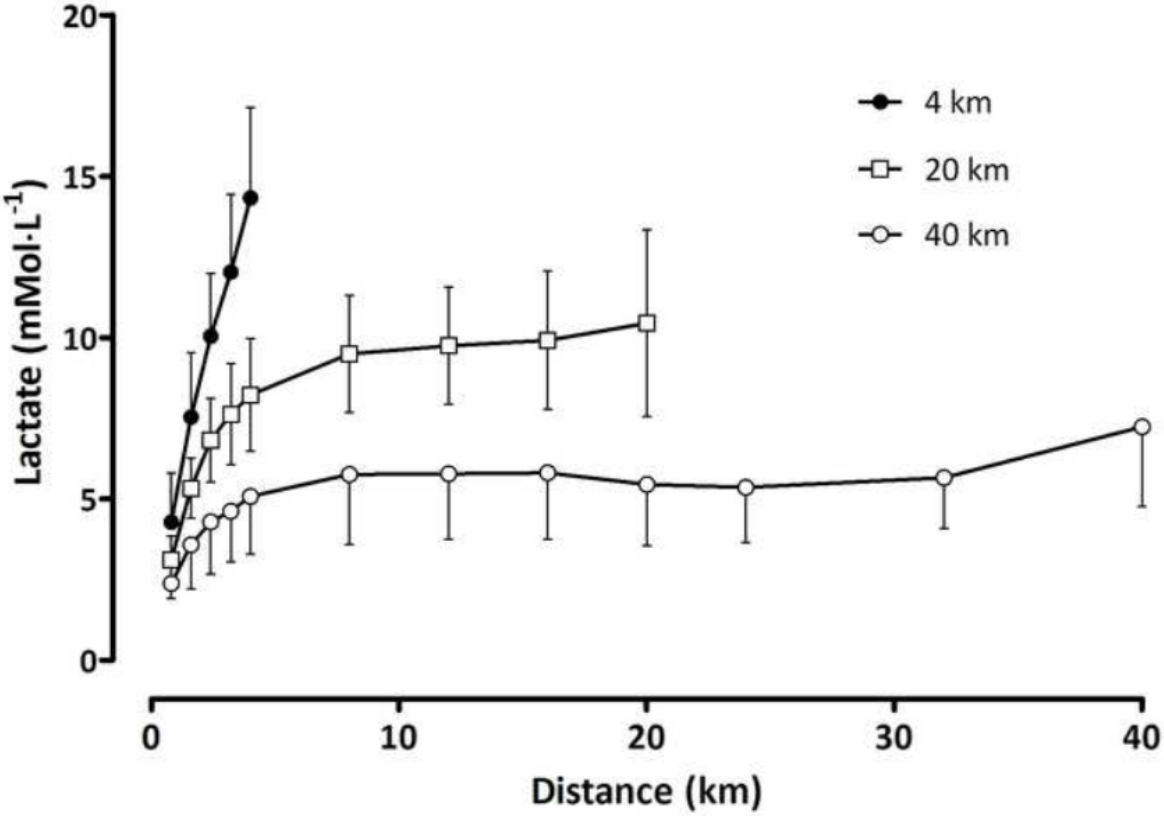
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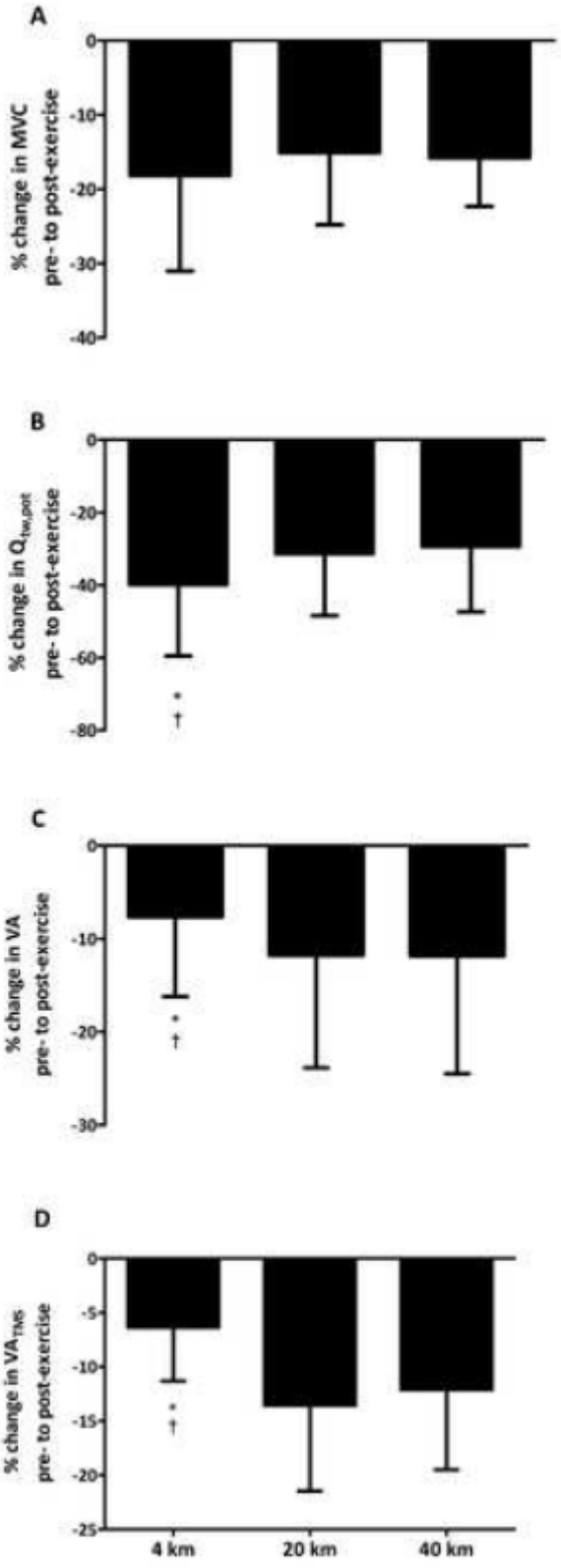
A
% change in MVC
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B
% change in $Q_{tw,pot}$
pre- to post-exercise
0
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-40
-60
-80
*
†
C
% change in VA
pre- to post-exercise
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D
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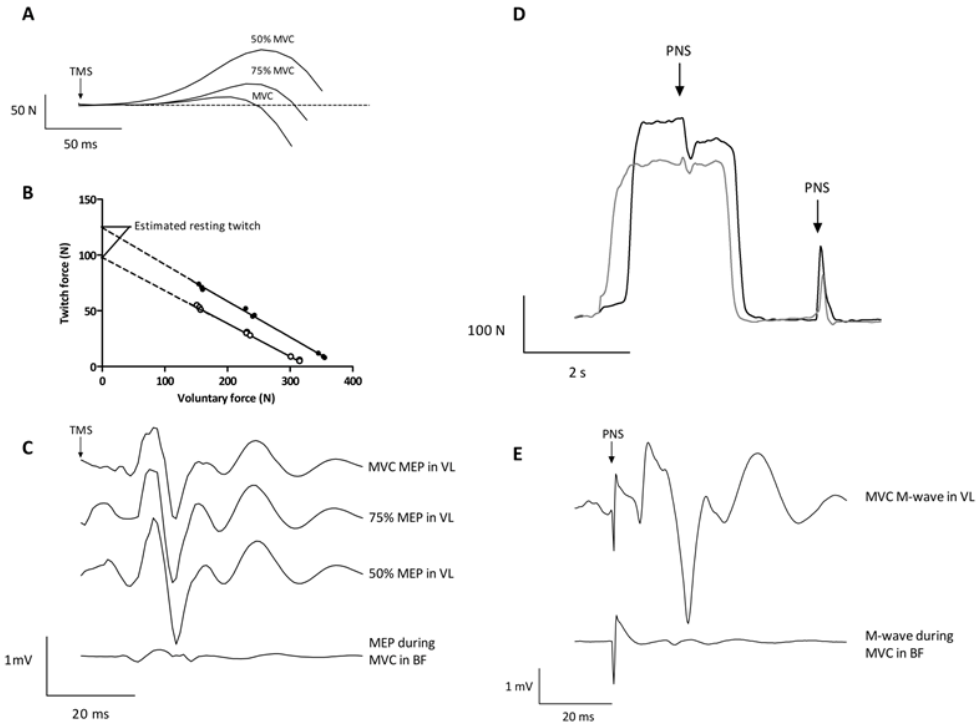

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Table 1. Performance, cardiorespiratory and perceptual responses to 4, 20 and 40 km cycling time trials. Values are mean ±SD for the whole trial (n = 13).

	4 km	20 km	40 km
Exercise time (min)	5.96 ± 0.20[a,b]	31.84 ± 1.04[b]	65.76 ± 2.18
Mean power (W)	340 ± 30[a,b]	279 ± 22[b]	255 ± 21
Cadence (rpm)	100 ± 7[b]	97 ± 3[b]	92 ± 5
$\dot{V}O_2$ ($L \cdot min^{-1}$)	4.10 ± 0.36[a,b]	3.92 ± 0.38[b]	3.70 ± 0.31
$\dot{V}CO_2$ ($L \cdot min^{-1}$)	4.45 ± 0.54[a,b]	3.79 ± 0.36[b]	3.41 ± 0.29
RER	1.08 ± 0.06[a,b]	0.96 ± 0.03[b]	0.92 ± 0.03
$\dot{V}_E$ ($L \cdot min^{-1}$)	152 ± 25[a,b]	130 ± 16[b]	111 ± 16
f_R	55 ± 7[b]	52 ± 7	48 ± 9
V_T (L)	2.78 ± 0.54[a,b]	2.56 ± 0.46[b]	2.34 ± 0.46
$\dot{V}_E/\dot{V}O_2$	36.8 ± 4.1[a,b]	33.2 ± 2.6[b]	30.0 ± 2.9
$\dot{V}_E/\dot{V}CO_2$	34.1 ± 3.6	34.4 ± 2.6	32.4 ± 2.7
Heart rate (bpm)	178 ± 14[b]	177 ± 13[b]	172 ± 14
RPE (mean)	17 ± 1[a,b]	15 ± 1	15 ± 1
RPE (peak)	19 ± 1[a,b]	18 ± 2	18 ± 1
RPE (session)	17 ± 2[a,b]	16 ± 1	16 ± 1

$\dot{V}O_2$; oxygen uptake, $\dot{V}CO_2$, carbon dioxide output; RER; respiratory exchange ratio, $\dot{V}_E$, minute ventilation; f_R; respiratory frequency, V_T; tidal volume, $\dot{V}_E/\dot{V}O_2$, ventilatory equivalent for oxygen; $\dot{V}_E/\dot{V}CO_2$, ventilatory equivalent for carbon dioxide; RPE; rating of perceived exertion. Symbols indicate a statistically significant difference ($P < 0.05$) compared to the following: [a]different from 20 km, [b]different from 40 km.

Table 2. Neuromuscular function and surface EMG responses to electrical stimulation of the motor nerve at rest and during maximum voluntary contraction pre- and post- 4, 20 and 40 km cycling time-trials (Values are mean ± SD, n = 13).

		4 km	20 km	40 km
Global fatigue				
MVC (N)	Pre	548 ± 144	536 ± 143	535 ± 137
	Post	445 ± 137[a]	452 ± 121[a]	451 ± 120[a]
	% change	18 ± 13	15 ± 10	16 ± 7
Peripheral fatigue				
$Q_{tw,pot}$ (N)	Pre	147 ± 34	143 ± 33	145 ± 37
	Post	85 ± 25[a]	97 ± 25[a]	101 ± 28[a]
	% change	40 ± 20[b,c]	31 ± 17	29 ± 18
MRFD ($N \cdot ms^{-1}$)	Pre	4.40 ± 3.32	4.62 ± 3.07	4.72 ± 1.82
	Post	2.22 ± 1.27[a]	3.06 ± 1.71[a]	3.67 ± 1.52[a]
	% change	42 ± 22[b,c]	27 ± 20	22 ± 18
CT (ms)	Pre	86 ± 13	89 ± 15	84 ± 12
	Post	81 ± 11	78 ± 13[a]	76 ± 13[a]
	% change	5 ± 13	10 ± 15	9 ± 10
MRR ($N \cdot ms^{-1}$)	Pre	−1.47 ± 0.85	−1.96 ± 1.13	−1.67 ± 0.72
	Post	−1.15 ± 0.49	−1.60 ± 0.98	−1.59 ± 0.57
	% change	10 ± 37	17 ± 29	1 ± 23
$RT_{0.5}$ (ms)	Pre	87 ± 26	75 ± 25	82 ± 25
	Post	65 ± 22[a]	61 ± 26[a]	60 ± 16[a]
	% change	24 ± 21	18 ± 22	23 ± 22
Central fatigue				
Motor nerve VA (%)	Pre	92 ± 8	92 ± 8	92 ± 6
	Post	85 ± 13[a]	81 ± 15[a]	82 ± 15[a]
	change	7 ± 7[b,c]	10 ± 10	11 ± 10
Surface EMG				
Resting responses				
M_{max} amplitude (mV)	Pre	4.53 ± 2.63	5.21 ± 1.99	5.67 ± 2.98
	Post	4.86 ± 2.36	4.89 ± 1.82	5.17 ± 3.21
M_{max} area ($\mu V \cdot s^{-1}$)	Pre	36.2 ± 18.2	40.9 ± 15.7	48.0 ± 19.6
	Post	42.0 ± 17.9	36.8 ± 11.3	41.5 ± 22.9
During MVC				
MVC_{RMS} (mV)	Pre	0.29 ± 0.13	0.28 ± 0.11	0.34 ± 0.17
	Post	0.27 ± 0.15	0.26 ± 0.14	0.32 ± 0.17
M_{max} amplitude (mV)	Pre	3.96 ± 2.28	4.56 ± 2.10	4.94 ± 2.01
	Post	3.79 ± 2.16	4.24 ± 1.64	4.84 ± 2.37
M_{max} area ($\mu V \cdot s^{-1}$)	Pre	25.5 ± 16.0	26.8 ± 10.5	35.6 ± 15.5
	Post	27.4 ± 16.9	23.8 ± 8.7	30.3 ± 15.4

MVC; maximum voluntary contraction, $Q_{tw,pot}$; potentiated twitch, ERT; estimated resting twitch, CT; contraction time, MRR; maximum rate of relaxation; $RT_{0.5}$; half relaxation time, VA; voluntary activation, M_{max}; maximum M-wave, MVC_{RMS}; root mean square of EMG during maximum voluntary contraction. Symbols indicate a statistically significant ($P < 0.05$) difference compared to the following: [a]different from pre-trial, [b]different from 20 km, [c]different from 40 km.

Table 3. Neuromuscular function and surface EMG responses to magnetic stimulation of the motor cortex at rest and during maximum voluntary contraction pre- and post- 4, 20 and 40 km cycling time trials (Values are mean ± SD, n = 11)

		4 km	20 km	40 km
VA_{TMS} (%)	Pre	94 ± 5	93 ± 7	93 ± 7
	Post	88 ± 8[a]	81 ± 11[a]	83 ± 12[a]
	change	6 ± 5[b,c]	12 ± 6	10 ± 6
ERT (N)	Pre	154 ± 46	137 ± 34	142 ± 46
	Post	92 ± 41[a]	93 ± 39[a]	93 ± 47[a]
	% change	41 ± 20	32 ± 18	36 ± 20
Surface EMG				
Resting responses				
MEP amplitude (mV)	Pre	0.32 ± 0.21	0.32 ± 0.25	0.37 ± 0.32
	Post	0.24 ± 0.17	0.10 ± 0.09	0.09 ± 0.06
MEP/M_{max} (%)	Pre	7.4 ± 5.7	6.2 ± 5.4	6.3 ± 6.8
	Post	6.8 ± 6.1	2.8 ± 3.9[a]	2.1 ± 1.6[a]
During MVC				
CSP (ms)	Pre	165 ± 51	176 ± 57	167 ± 47
	Post	175 ± 50	162 ± 49	173 ± 50
MEP amplitude (mV)	Pre	1.91 ± 0.91	2.11 ± 1.05	2.86 ± 1.68
	Post	2.09 ± 1.02	2.19 ± 0.84	2.75 ± 1.75
MEP area ($\mu V \cdot s^{-1}$)	Pre	17.4 ± 7.4	18.7 ± 6.8	24.4 ± 11.8
	Post	18.9 ± 6.2	17.4 ± 5.5	21.4 ± 11.1
MEP/M_{max} amplitude (%)	Pre	59 ± 16	58 ± 27	55 ± 18
	Post	64 ± 16	55 ± 26	57 ± 15
MEP/M_{max} area (%)	Pre	79 ± 18	76 ± 24	78 ± 27
	Post	84 ± 24	79 ± 32	80 ± 29

VA_{TMS}; voluntary activation measured using transcranial magnetic stimulation, ERT; estimated resting twitch, MEP; motor evoked potential,M_{max}; maximum M-wave, CSP; cortical silent period. Symbols indicate a statistically significant ($P < 0.05$) difference compared to the following: [a]different from pre-trial, [b]different from 20 km, [c]different from 40 km.