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Racing an opponent alters pacing, performance and muscle force decline, but not RPE

ORIGINAL INVESTIGATION

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ABSTRACT

Purpose. Performing against a virtual opponent has been shown to invite a change in pacing and improve time trial (TT) performance. This study explored how this performance improvement is established by assessing changes in pacing, neuromuscular function and perceived exertion. Methods. After a peak power output test and a familiarization TT, twelve trained cyclists completed two 4-km TTs in randomized order on a Velotron cycle ergometer. Time trial conditions were riding alone (NO), and riding against a virtual opponent (OP). Knee-extensor performance was quantified before and directly after the TT using maximal voluntary contraction force (MVC), voluntary activation (VA) and potentiated doublet-twitch force (PT). Differences between the experimental conditions were examined using Repeated-measures ANOVAs. Linear regression analyses were conducted to associate changes in pacing to changes in MVC, VA and PT. Results. OP was completed faster than NO (mean power output OP: 289.6±56.1W vs. NO: 272.2±61.6W; p=0.020), mainly due to a faster initial pace. This was accompanied by a greater decline in MVC (MVCpre-vs-post: -17.5±12.4% vs. -11.4±10.9%, P=0.032) and PT (PTpre-vs-post: -23.1±14.0% vs. -16.2±11.4%, P=0.041) after OP compared to NO. No difference between conditions was found for VA (VApre-vs-post: -4.9±6.7% vs. -3.4±5.0%, P=0.274). RPE did not differ between OP and NO. Conclusion. The improved performance when racing against a virtual opponent was associated with a greater decline in voluntary and evoked muscle force compared to riding alone, without a change in perceived exertion, highlighting the importance of human-environment interactions in addition to one’s internal state for pacing regulation and performance.

KEYWORDS: Pacing strategy, Muscle fatigue, Perception, Competition, Cycling
INTRODUCTION

The goal-directed regulation of the exercise intensity over an exercise bout has been defined as pacing, and is widely recognized as an essential determinant for performance.\(^1\) Based on existing theories about pacing, it can be concluded that sensations of fatigue and a willingness to tolerate discomfort (in anticipation of future rewards) are important in this process of action regulation.\(^2\) Concepts such as teleoanticipation\(^3\) and template formation\(^4\) have been pointed out as crucial in the process. In addition, the importance of the interaction of the exerciser and environmental cues has been emphasized recently in the context of pacing.\(^2,5\) Perceptual cues provided by the environment can invite athletes to respond, thereby evoking adaptations of pacing behavior.\(^2,5\) In this sense, an opponent can be perceived as an important environmental cue that represents action possibilities to an athlete in competitive sports.\(^5\)

Indeed, the presence of a virtual opponent has been shown to improve cycling performance\(^6\)–\(^8\), and the pacing behavior of the virtual opponent has been shown to affect the initial pace of cyclists in laboratory-controlled conditions.\(^7\) The performance improvement related to the presence of an opponent appears to remain quite stable, regardless of the level of performance of the opponent.\(^9\) Yet a different level of performance of the opponent appeared to evoke different psychological responses.\(^9\) On top of this, the improvement in performance only seems to occur acutely when the opponent is present, as performance declines back to baseline levels in subsequent time trials riding alone.\(^10\) Possible mechanisms, such as an increased motivation\(^11\) and a change in attentional focus from internal to external aspects,\(^8\) have been suggested in relationship to the performance improvement seen in the presence of a virtual opponent. However, it is yet unclear how this improved performance in the presence of a virtual opponent is established. In this study we explored this by examining performance improvements when riding against a virtual opponent compared to riding alone, and by relating these to neuromuscular adjustments in the knee extensors and perceived exertion. We hypothesized that the presence of a virtual opponent could invite a change in pacing and evoke an improvement in performance, leading to a greater decline in voluntary muscle force after a 4-km time-trial compared to riding alone. In addition, we explored whether a change in pacing and performance would be mainly related to alterations in contractile function or in muscle activation.

METHODS

Participants

Twelve trained male cyclists with at least two years cycling experience at a moderate to high intensity (age: 36.8±10.0 years; body mass: 82.1±13.9 kg; height: 180.1±9.7 cm) participated in this study. Before participating all participants gave written informed consent and completed a health screening questionnaire (Physical Active Readiness Questionnaire\(^12\)). The study was approved by the university’s local ethical committee in accordance with the Declaration of Helsinki.

Experimental procedures

Participants visited the laboratory on four separate occasions. During their first visit, participants performed a maximal incremental test on a Velotron cycle ergometer. In their second to fourth visit participants were asked to perform a self-paced 4-km cycling time-trial (TT) as fast as possible. Prior and after the TTs, maximal voluntary contraction, doublet-twitches at rest and voluntary activation of the quadriceps muscle were determined. The first 4-km TT was always a familiarization TT (FAM). In the final two visits participants completed in a randomized order one of the two different experimental 4-km TT conditions (see Section
Procedures). No verbal coaching or motivation was given to the subjects during any of the TTs. Before each TT condition subjects performed a 5-min warm-up at an intensity of 30% peak power output (PPO).

To minimize circadian variation, TTs were completed at the same time of the day (±2 h) for each participant. Participants were asked to maintain normal activity and sleep pattern throughout the testing period. In addition, participants were asked to refrain from any strenuous exercise and alcohol consumption in the preceding 24-h, and from caffeine and food consumption four and two hours respectively, before the start of the test. Participants were informed that the study was examining the influence of external factors on performance during cycling TTs. To prevent any pre-meditated influence on preparation or pre-exercise state, the specific feedback presented for each trial was only revealed immediately before the start of the TT. All trials were conducted in ambient temperatures between 18-21°C.

Procedures

Maximal incremental test

Participants attended the laboratory to complete a maximal incremental test on the Velotron cycle ergometer (VeloTron Dynafit, Racermate, Seattle, USA) to measure PPO. A 5-min warm-up at 100W was followed by a 3-min rest period before starting the test. The incremental test had an initial workload of 100W and a workload increase of 25W every minute until volitional exhaustion. Subjects were instructed to keep their cadence between 80-100 revolutions per minute (rpm). Participants were given strong verbal encouragement in the latter stages. The highest mean power output achieved during any 60-s period was recorded as the subject’s PPO.

Familiarization and Experimental trials

During the second visit, participants completed a self-paced familiarization 4-km TT. During the third and fourth visit, participants were asked to complete one of the two different experimental, self-paced 4-km TT conditions. The experimental conditions were a TT without virtual opponent (NO), and a TT with virtual opponent (OP). Each 4-km TT started 4 min after completion of the warm-up. Before the trials with a virtual opponent, subjects were told that their virtual opponent would be of similar level of performance in order to make sure a subject would perceive his opponent as competitive. Although participants were unaware of this, the virtual opponent was in fact their own previous performance during FAM. Typically, a modification in pacing strategy towards a less aggressive start occurs after a familiarization trial in TTs of relatively short duration. Therefore, using FAM as basis for the construction of the opponent most likely results in a competitor that uses a different pacing profile compared to our participant in the experimental TT conditions.

Time trials were performed on an advanced cycle ergometer (VeloTron Dynafit, Racermate, Seattle, USA) that has been shown to be a reliable and valid tool to measure cycling performance and pacing behavior. Using the VeloTron 3D software, a straight and flat 4-km TT course with no wind was programmed and projected onto a screen for all trials. During the TTs only feedback regarding the relative distance that still had to be covered was provided. In the opponent conditions, a virtual opponent was projected. Power output, velocity, distance, cadence, and gearing were monitored continuously during each trial (sample frequency = 4 Hz). Rate of perceived exertion (RPE) on a Borg-scale of 6-20 was asked after the warm-up, at 100s, 200s and 300s after starting the TT, and directly after passing the finish line.

Neuromuscular function

Measures of neuromuscular function were evaluated prior and after the trial (within <3 min after finishing TT) using electrical stimulation of the right femoral nerve. Three variables
were obtained to quantify muscle performance; maximal voluntary contraction force (MVC), voluntary activation (VA) and the potentiated doublet-twitch force (PT).

All of these three variables change following exertion. The PT is the highest force of the three repetitions evoked by paired-pulse electrical stimulation administered to the resting muscle, five seconds after the MVC. The VA is determined via the interpolated doublet-twitch technique (ITT) and is estimated by changes in the interpolated doublet-twitch relative to the PT (see equation). The force evoked by the imposed electrical stimulation on top of the MVC is the interpolated doublet-twitch (IT), the force evoked by the electrical stimulation 5s after MVC is PT.

\[
VA(\%) = (1 - \frac{IT}{PT}) \cdot 100
\]

Knee extensor force (N) during voluntary and evoked contractions was measured using a calibrated load cell dynamometer (Kin-Com dynamometer, Chattanooga Group Inc.; Hixon, TN, USA) fixed to a custom-built chair and connected to a noncompliant Velcro strap attached around 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° flexion, and were given specific instruction to remain seated. After the skin was shaved two stimulation pads (Axelgaard ValuTrode 5x9 cm disposable surface electrodes) were placed on the leg and connected to a high voltage stimulator (DS7AH; Digitimer Ltd., Welwyn Garden City, United Kingdom). The cathode pad was placed at the distal side of the middle of the inguinal crease. The anode pad was placed 2-3 cm proximal to the patella, with the knee in a bent position. The sequence of stimulation was controlled by a programmable output system (LabChart 7.0, AD Instruments, United Kingdom). The positions of the electrodes were marked with indelible ink to ensure consistent placement on repeat trials.

Before their TT, participants completed three isometric MVC’s separated by 60s rest. To determine stimulation intensity, paired-pulse stimuli (200 μs duration; 10 ms interval) were delivered in 25 mA stepwise increments from 150 mA and the current that evoked maximal doublet-twitch amplitude at rest was determined. To ensure a supramaximal stimulus, the final intensity was increased by 30% (mean ±SD current: 343±57 mA). Femoral nerve stimulation was delivered during and 5s after MVC to assess VA. Participants completed post-TT exercise another three MVC’s with femoral nerve stimulation. In line with other investigations that have assessed cycling exercise-induced fatigue of the knee extensors, the post-TT measurements were completed within three minutes of exercise cessation. The rapid nature of this procedure is necessary to capture the decline in MVC force, voluntary activation, and potentiated doublet-twitch force induced by the exercise before it dissipates, and the duration was consistent between trials. During all MVC’s participants received verbal encouragement.

Statistical analysis

A two-way repeated-measures ANOVA (condition x time) was used to assess the effect of each time trial on measures of neuromuscular function (comparison of before vs after trial) and to assess the differences between TT conditions. A multiple linear regression analysis (Backward method) was performed to determine the relationship between the change in mean power output per kilometer during OP relative to NO and the absolute VA, and the change in differences in MVC, VA and PT before and after the time-trial in OP relative to NO. Significance was accepted at P<0.05.

To examine 4-km TT performance mean power output, heart rate, cadence, and finish time were calculated. Differences in performance between conditions were assessed using a one-way repeated-measures ANOVA (condition). To assess differences in pacing behavior
between the conditions, average power output, cadence, and split times for each 250m segment were calculated, and differences were tested using a two-way repeated-measures ANOVA (condition x segment). The RPE was evaluated using a two-way repeated-measures ANOVA (condition x asking point). All analyses were performed using SPSS 19.0, and significance was accepted at P<0.05. Data are presented as means ± SD.

RESULTS

Performance analysis

The participants achieved a mean PPO of 351±35 W in the maximal incremental test, and can be classified as trained cyclists based on the guidelines of De Pauw et al. A higher mean power output (OP: 289.6±56.1 W vs. NO: 272.2±61.6 W; F=7.5; p=0.020) and faster finishing times (OP: 382.2±31.9 s vs. NO: 393.6±21.9 s; F=5.1; p=0.046) were reported after OP compared to NO. Completion time of FAM and NO did not differ (p=0.241). In contrast, participants completed their TT faster in OP compared to the FAM/virtual opponent (p=0.003). Mean heart rate over the TTs was higher during OP compared to NO (OP: 164.6±9.0 bpm vs. NO: 158.9±12.4 bpm; F=6.6; p=0.026). No differences in mean cadence were found between OP and NO (OP: 103.9±10.2 rpm vs. NO: 104.7±12.5 rpm; F=0.2; p=0.669).

Pacing analysis

Mean (±SD) power outputs per 250m section are shown in Figure 1. Main effects for condition (F=7.5; p=0.020) and segment (F=5.0; p<0.001), and an interaction effect for condition x segment (F=1.9; p=0.029) were found, indicating differences in pacing profile between conditions. Post hoc analysis revealed a faster initial pace during OP compared to NO, with higher power outputs between 250-500m (p=0.040), 750-1000m (p=0.022), and 1000-1250m (p=0.024). In addition, a faster end spurt (3750-4000m) was noticed in OP compared to NO (p=0.001). Finally, regression analysis showed that the difference in mean power output between OP and NO during the first kilometer could explain 47.9% of the total variance in the relative difference in mean power output between OP and NO over the whole time-trial (R²=0.479, β = 0.692, p=0.013). Participants adopted a slower initial pace in NO (0-250m: p=0.065; 250-500m: p=0.001; 500-750m: p=0.005), but not during OP, in comparison to FAM (and thus the virtual opponent in OP; 0-250m: p=0.187; 250-500m: p=0.148; 500-750m: p=0.216). In addition, participants were faster in OP compared to FAM between 1250-1500m (p=0.032), 2500-2750m (p=0.022), 3250-3500m (p=0.046), and 3750-4000m (p=0.018).

Mean (±SD) heart rates per 250m section are shown in Figure 2. A main effect was found for condition (F=6.6; p=0.026) and segment (F=149.8; p<0.001). An interaction effect was reported for condition x segment (F=1.8; p=0.035). Post hoc tests showed heart rate values were higher in OP compared to NO from 250m until 1750m. A main effect for segment (F=18.756; p<0.001), but no main effect for condition (F=0.2; p=0.669) and no interaction effect for condition x segment (F=0.7; p=0.767) was found for cadence. Mean (± SD) RPE scores per point of asking for each experimental condition are shown in Table 1. A main effects for point of asking (F=29.2; p<0.001), but no main effect for condition (F=4.2; p=0.065), and no interaction effect for condition x point of asking (F=0.7; p=0.560) were found.

Neuromuscular adjustments

Mean (±SD) differences in MVC, PT and VA in the posttest versus the pretest per experimental condition can be found in Table 2. In addition, a typical example of the assessment of neuromuscular function of the knee extensors during and after a MVC using the interpolated doublet-twitch technique is shown in Figure 3. A main effect was found for time (F=23.8;
p<0.001), but not for condition (F=0.3; p=0.596) for the MVC. The main effect for time showed a decrease in MVC force in the posttest compared to the pretest. Furthermore, an interaction effect was reported for condition x time (F=6.1; p=0.032) for the MVC, revealing that the force decline was relatively greater after OP compared to NO.

A main effect for time (F=41.4; p<0.001), but not for condition (F=0.6; p=0.440) was found for the PT, indicating smaller potentiated doublet-twitch force after the TTs compared to before the TTs. An interaction effect for condition x time (F=5.4; p=0.041) showed the decline in potentiated doublet-twitch force was greater after OP compared to NO. A main effect for time (F=11.8; p=0.006), but not for condition (F=0.5; p=0.484) was reported for VA. Moreover, no interaction effect for condition x time (F=1.4; p=0.274) was found for the VA, indicating no difference in voluntary activation was found between NO and OP.

The outcomes of the linear regression analyses used to assess the relationship between the change in power output per kilometer during OP relative to NO, and the change in differences in MVC, VA, and PT before and after the time-trial in OP relative to NO can be found in Table 3. Negative standardized beta coefficients were found between the relative change in power output during the first kilometer in OP compared to NO and both ΔPT (β = -0.50, p=0.036) as well as ΔVA (β = -0.49, p=0.045) after OP compared to NO. These negative beta-values indicate that a relatively faster initial pace in OP is associated with a relatively greater decline in PT and increased reduction in VA after OP compared to NO. The combination of the relative change in PT and VA could explain 60.9% of the total variance in the relative change in power output during the first kilometer in OP compared to NO. The relative change in MVC in OP compared to NO and the absolute voluntary activation did not significantly contribute to the model.

DISCUSSION

Trained cyclists were able to improve their mean power output and finishing time in a self-paced 4-km TT when riding against a virtual opponent. This performance improvement was accompanied by a greater decline in MVC force and PT force, while no difference between TT conditions was found for the voluntary activation. In addition, linear regression analyses showed that the faster initial pace of the participants in OP relative to NO, most likely evoked by their virtual opponent, is associated with a greater relative reduction in doublet-twitch amplitude and voluntary activation after OP relative to NO. Remarkably, participants still perceived a similar level of exertion in both experimental conditions, despite the higher mean power output, the greater decline in MVC force and potentiated doublet-twitch force, and the higher mean heart rate that was found when riding against a virtual opponent.

Previous research has shown before that a virtual opponent could affect pacing behavior and improve performance. In this perspective, the presence of a virtual opponent has been related to a greater external distraction, possibly deterring perceived exertion. However, at the same time a higher level of fatigue has been revealed to alter attentional focus from external to internal factors. Interestingly, if the “competitor” was not visible during the trial, even the prospect of a monetary incentive ($100,-) did not lead to an improvement in 1500m cycling performance. The improvements during a 2-km head to head competition with virtual opponent were shown to be accompanied by a greater anaerobic energy contribution while aerobic contribution remained the same. The present study adds onto this knowledge that the performance improvement in the presence of a virtual opponent is also accompanied by a greater decline in voluntary and evoked muscle force.

Many studies have suggested that muscle fatigue has a crucial impact on the decision-making process regarding exercise regulation and performance. In this respect, afferent feedback generated during high-intensity exercise has been suggested as a potential way to
The present findings indicate the possible effect of afferent feedback on the decision-making process involved in pacing might be counteracted by motivational aspects and/or attentional strategies related to the presence of a virtual opponent. In addition, linear regression analyzes showed that the faster initial pace of the participants in OP relative to NO, most likely evoked by their virtual opponent, was associated to a relative higher reduction in twitch amplitude after OP. This supports the idea that perceptual affordances provided by the environment could invite athletes to respond differently, and might be able to overrule to a certain extent the influence of afferent feedback on the decision-making process involved in pacing. To further our understanding of the complex decision-making process involved in the regulation of the exercise intensity a combination of observational studies (to ensure a high ecological validity; see 31,32) as well as experimental studies (to allow controlled manipulations) will be required.

According to Amann & Dempsey's afferent feedback via group III/IV muscle afferents can also lead to an increased reduction in the voluntary activation of the muscle. However, no difference in the voluntary activation has been found after OP compared to NO. In this respect, it is known that the contribution of the decline in muscle activation to performance fatigability is more apparent in time trials of longer duration, while the contribution of the reduction in contractile function is relatively higher in high-intensity time trials of shorter duration. Interestingly, despite no difference in voluntary activation was found after our experimental conditions, a higher initial pace in OP relative to NO appeared to be associated to a relative higher reduction in voluntary activation after OP compared to NO.

Due to methodological reasons, adjustments in neuromuscular function caused by the TT exercise could only be measured after TT completion but not during the race. This limitation is common in the literature of studying adjustments in neuromuscular function caused by locomotor exercise modes and assumes that the neuromuscular adjustments observed after exercise are also present during the exercise. In addition, we used linear regression analyzes to assess the relationship between the change in mean power output per kilometer during OP relative to NO, and the change in differences in MVC, VA and PT before and after the time-trial in OP relative to NO. The outcomes of the linear regression analyzes indicated that a relatively faster initial pace in OP relative to NO was associated with a relatively larger decline in PT and an increased reduction in VA. The combination of the relative change in PT and VA could explain 60.9% of the total variance in the relative change in mean power output during the first kilometer in OP compared to NO. As a significant recovery of muscle function can occur two minutes after exercise, it is possible that the changes in neuromuscular function caused by the TT exercise were underestimated. Nevertheless, the time taken to assess neuromuscular function was consistent within participants between their trials. Moreover, a significant reduction in all three measured neuromuscular variables was observed after all TTs, while the decline in MVC and PT force was influenced by the TT condition. These observations indicate that the methods used were appropriate to determine differences in the neuromuscular function after the TT exercise in the different experimental conditions. Finally, the reported potentiated doublet-twitch force in this study appeared to be relatively high. This is most likely related to the neuromuscular stimulation of quadriceps, as this effect has been reported earlier for this muscle group.

Practical applications
In the presence of a virtual opponent, cyclists were able to establish an improved performance, maintain a higher mean power output, and able to handle a greater decline in muscle force over a 4-km TT. In this sense, the use of a visual avatar in a simulated competitive situation could be a beneficial, novel tool to use during high-intensity training sessions. In addition, our findings emphasize that external cues are crucial the regulation of the exercise intensity in addition to an athlete’s internal state, and indicate that understanding the interaction between external cues and internal information may be a key for pushing the limits of human performance.

Conclusions
Trained cyclists were able to improve their performance in the presence of a virtual opponent, in line with previous research. The present study has shown that the improved performance during head-to-head competitions compared to individual self-paced cycling time-trials is associated to a greater decline in MVC force and potentiated doublet-twitch force, while still perceiving a similar rate of perceived exertion as when riding alone. Our findings indicate that the regulation of the exercise intensity is not purely based on physiological information related to a decline in muscle force production. An external environmental stimulus appears to be able to evoke the execution of certain actions that were not perceived as possible or necessary when riding alone. To understand the regulation of the exercise intensity, it is crucial to incorporate human-environment interactions in our thinking about how pacing decisions are made in real life competitive situations in sports, and what information is used to inform such decisions.

Acknowledgements
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References


Figure 1. Average power output per 250 m segment for both experimental conditions. In addition, the average power output per 250 segment of the virtual opponent in the experimental condition OP is displayed.

* significant difference between OP and NO (P<0.05)
Figure 2. Average heart rate per 250 m segment for both experimental conditions. *significant difference between OP and NO (P<0.05)
Figure 3. Typical example of the raw data for one of the 5 s MVCs, including the superimposed doublet-twixt during the MVC and the potentiated doublet-twixt 5 s after the MVC. The double arrows indicate the moment of applying the paired-pulse electrical stimuli to the right femoral nerve.
Table 1. Mean ± SD values for the RPE of the participant per experimental condition after completing their warm-up and time trial, and 100 s, 200 s and 300 s after starting their time trial.

<table>
<thead>
<tr>
<th>Warm-up</th>
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<th>TT_{200 sec}</th>
<th>TT_{300 sec}</th>
<th>TT Finish</th>
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<tr>
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<td>13.3 ± 1.5</td>
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<td>OP</td>
<td>9.0 ± 1.8</td>
<td>13.7 ± 2.0</td>
<td>15.7 ± 1.4</td>
<td>17.4 ± 1.7</td>
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Table 2. Mean ± SD values for the neuromuscular function of the knee extensors in terms of maximal voluntary contraction force (MVC), potentiated doublet-twitch force (PT) and voluntary activation (VA) before and after both 4 km time trial conditions.

<table>
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<th></th>
<th>NO</th>
<th>OP</th>
<th></th>
<th>NO</th>
<th>OP</th>
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<td>Post-TT</td>
<td>Decrease%</td>
<td>Pre-TT</td>
<td>Post-TT</td>
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<td>MVC</td>
<td>715±182</td>
<td>633±169</td>
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<td>717±199</td>
<td>592±170</td>
</tr>
<tr>
<td></td>
<td>717±199</td>
<td>592±170</td>
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<td>717±199</td>
<td>592±170</td>
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<tr>
<td>PT</td>
<td>425±70</td>
<td>356±83</td>
<td>16.2±11.4</td>
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<td>331±75</td>
</tr>
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<td></td>
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<td>331±75</td>
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<td>VA</td>
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<td>4.9±6.7</td>
<td>717±199</td>
<td>592±170</td>
</tr>
</tbody>
</table>

A main effect for Trial (pre vs post), B interaction effect for Trial*Condition
Table 3. Multiple linear regression analysis was used to assess the relationship between the change in mean power output per kilometer during OP relative to NO ($\Delta PO$), and the change in MVC, VA, and PT before and after the time-trial in OP relative to NO ($\Delta MVC$, $\Delta VA$, $\Delta PT$ respectively). $R^2$ and Standardized beta coefficients are presented.

<table>
<thead>
<tr>
<th>$\Delta PO$</th>
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<td>$\beta$</td>
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<td>$\Delta VA$</td>
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<td>Sign $\Delta PT$</td>
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<td>-</td>
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<tr>
<td>Sign $\Delta VA$</td>
<td>0.045*</td>
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<td>-</td>
</tr>
</tbody>
</table>

*significant standardized beta coefficient ($P<0.05$)
†$\Delta MVC$ and absolute VA were removed out of the multiple linear regression analysis as they did not contribute significantly to any of the variables
* all variables were removed out of the multiple linear regression analysis