Prior exercise impairs subsequent performance in an intensity- and duration-dependent manner

Authors:

Madison M. Fullerton[1], Louis Passfield[1,2], Martin J. MacInnis[1], Danilo Iannetta[1], Juan M. Murias[1]

[1]*Faculty of Kinesiology, University of Calgary, Calgary, AB, Canada*

[2]*School of Sport and Exercise Sciences, University of Kent, Canterbury, United Kingdom*

Corresponding author:

Dr. Juan M Murias

Associate Professor,

Faculty of Kinesiology, University of Calgary

KNB 434, 2500 University Drive NW, Calgary, Alberta, Canada, T2N 1N4

+1 (403) 220-7955

jmmurias@ucalgary.ca

Abstract

Prior constant-load exercise performed for 30-min at or above maximal lactate steady state ($MLSS_p$) significantly impairs subsequent time-to-task failure (TTF) compared with TTF performed without prior exercise. We tested the hypothesis that TTF would decrease in relation to the intensity and the duration of prior exercise compared to a baseline TTF trial. Eleven individuals (6 men, 5 women, 28 ± 8 yrs) completed the following tests on a cycle ergometer (randomly assigned after $MLSS_p$ was determined): *i*) a ramp-incremental test, *ii*) a baseline TTF trial performed at 80% of peak power (TTF_b), *iii*) five 30-min constant-PO rides at 5% below lactate threshold ($LT_{-5\%}$), halfway between LT and $MLSS_p$ ($Delta_{50}$), 5% below $MLSS_p$ ($MLSS_{-5\%}$), $MLSS_p$, and 5% above $MLSS_p$ ($MLSS_{+5\%}$), and *iv*) 15- and 45-min rides at $MLSS_p$ ($MLSS_{15}$ and $MLSS_{45}$, respectively). Each condition was immediately followed by a TTF trial at 80% of peak power. Compared to TTF_b (330 ± 52s), there was 8.0 ± 24.1, 23.6 ± 20.2, 41.0 ± 14.8, 52.2 ± 18.9, and 75.4 ± 7.4% reduction in TTF following $LT_{-5\%}$, $Delta_{50}$, $MLSS_{-5\%}$, $MLSS_p$, and $MLSS_{+5\%}$, respectively. Following $MLSS_{15}$ and $MLSS_{45}$ there were 29.0 ± 20.1 and 69.4 ± 19.6% reductions in TTF, respectively (P <0.05). It is concluded that TTF is reduced following prior exercise of varying duration at $MLSS_p$ and at submaximal intensities below MLSS.

Novelty

- Prior constant-PO exercise, performed at intensities below $MLSS_p$, reduces subsequent TTF performance.
- Subsequent TTF performance is reduced in a linear fashion following an increase in the duration of constant-PO exercise at $MLSS_{p.}$

Key Words: Performance, exercise intensity domains, maximal lactate steady state, time to task failure, exercise tolerance, fatigue

Introduction

The lactate threshold (LT) and the critical intensity of exercise (commonly estimated by measures of maximal lactate steady state (MLSS) or critical power (CP) (Jamnick et al. 2020; Black et al. 2017), partition the exercise intensity spectrum into three domains of intensity: moderate (<LT), heavy (>LT but <MLSS/CP), and severe (>MLSS/CP) (Whipp 1994). The physiological responses to exercise performed in each of these domains are highly predictable (Black et al. 2017), such that this intensity domain "schema" represents a useful tool to prescribe and "normalize" the exercise intensity (i.e., metabolic stimulus) amongst individuals (Iannetta et al. 2020; Jamnick et al. 2020). From this perspective, the metabolic and neuromuscular perturbations associated with constant-PO exercise performed within each intensity domain have been well characterized (Black et al. 2017; Keir et al. 2016; Cannon et al. 2011). Briefly, within the moderate-intensity domain, exercise is sustainable with a steady-state $\dot{V}O_2$ response, a small contribution of anaerobic metabolism limited to the short kinetic phase of $\dot{V}O_2$, and no major changes in the concentration of exercise-induced metabolites (i.e., lactate) in muscle and blood compared to baseline (Cannon et al. 2011; Black et al. 2017). Within the heavy-intensity domain the attainment of $\dot{V}O_2$ steady-state can be delayed by as much as 15-min and is associated with an overall acceleration of the glycolytic rate, which leads to increased levels of exercise-induced metabolites in muscle and blood compared to baseline (Black et al. 2017). Exercise within the severe-intensity domain is characterised by a $\dot{V}O_2$ that projects to its maximum and marked changes in exercise-induced metabolites that accumulate in muscle and blood (Black et al. 2017; Cannon et al. 2011; Iannetta, Inglis, et al. 2018; Burnley, Vanhatalo, and Jones 2012; Schäfer, Hayes, and Dekerle 2019). Therefore, while neuromuscular fatigue is modest, or absent, in the moderate-intensity domain, it becomes increasingly apparent with exercise in the heavy- and severe-intensity domain (Black et al. 2017; Cannon et al. 2011). Despite these observations, it is poorly understood how exercise performed at specific domain-derived intensities affects cycling performance within the severe-intensity domain (as measured by a time-to-task failure (TTF) trial).

We have previously demonstrated that 30-min of cycling at the power output (PO) corresponding to MLSS and at 10 W above MLSS reduced subsequent TTF performance by ~40% and ~65%, respectively, in relation to a control TTF trial with no prior exercise (Iannetta, Inglis, et al. 2018). Additionally, Clark et al. (2018) have shown that two hours of cycling within the heavy-intensity domain reduced the PO associated with predicted CP (~8%) and the capacity to perform work above CP by ~20%. Collectively, these observations suggest that exercise performed below MLSS/CP can impair subsequent performance, and that small increases in sustained intensity exacerbate this impairment. In other words, the magnitude of the impairment on subsequent performance is different across the intensity domains. From these observations, it could be hypothesized a progressive impairment in the capacity to perform exercise as the intensity (across domains) or duration (for a given workload) of exercise increases. To our knowledge, no study has evaluated the changes in TTF performance following systematic and progressive changes in domain-derived intensities and durations in the same group of individuals. Investigating the changes in TTF is important as it could clarify the effect that intensity and duration of prior exercise have on subsequent TTF performance and be informative for coaches and practitioners when prescribing exercise and predicting residual exercise capacity within the severe-intensity domain.

In addition to the physiological responses discussed above, perceptual responses may also be related to subsequent exercise capacity within the severe-intensity domain (Pageaux and Lepers 2016; Noakes 2004). The rate of perceived exertion (RPE) increases with exercise intensity across the different domains and is postulated to reflect the integration of afferent feedback from exercising and non-exercising muscles as well as corollary discharge rate (Pires et al. 2011; Pageaux and Lepers 2016). Consequently, RPE may be used to determine the proximity of an individual to their sensory tolerance limit (i.e., TTF) (Pageaux and Lepers 2016). For example, Pires et al. (2011) demonstrated that the slope of the increase in RPE during constant-PO exercise accurately predicted TTF. As the sensory tolerance limit is heavily influenced by the metabolic status of the muscle (Pageaux and Lepers 2016; Hureau, Romer, and Amann 2018), it is of interest to also understand the relationship between $[La^-]_b$ and any reduction in TTF. As RPE can increase despite $[La^-]_b$ remaining stable during constant-load exercise (Iannetta, Inglis, et al. 2018), it is possible that RPE but not $[La^-]_b$ is associated with subsequent changes in TTF.

The aim of the present study was to: (*i*) assess whether a reduction in TTF performance occurs following 30-min constant-PO exercise performed at normalized, domain-derived intensities (same duration, different intensity), particularly at sub-MLSS intensities, (*ii*) assess whether a reduction in TTF performance occurs following prior constant-PO exercise performed for different durations at $MLSS_p$ (same intensity, different duration); and (*iii*) determine whether physiological measures and RPE immediately after a bout of prior exercise were associated with a subsequent reduction in TTF. We hypothesized that there would be a reduction in TTF performance as the intensity of exercise for a fixed duration increased. We also hypothesised that increasing the duration of prior exercise at a fixed intensity would lead to a progressive reduction in TTF performance; and (*iii*) by then exploring differences between intensity and duration of prior exercise, we also hypothesized that RPE, but not $[La^-]_b$, would be predictive of the reductions in TTF.

Methods

Participants

Eleven recreationally active and well-trained men (n=6) and women (n=5) (28 ± 8 yrs, 67.9 ± 9.0 kg), who took part in endurance-based exercise at least three times per week voluntarily participated in this study. Participants were non-smokers, non-obese (BMI ≤ 30 kg/m^2) and were not undergoing any medical treatment. Prior to testing, each participant completed the CSEP Physical Activity Readiness Questionnaire (PAR-Q+) and provided written informed consent. All procedures were approved by the Conjoint Health and Research Ethics Board of the University of Calgary (REB18-1890).

Experimental Design

All participants completed all testing procedures on an electromagnetically braked cycle ergometer (Velotron; RacerMate, Seattle, WA), in an environmentally controlled room (temperature: 19-20° C; humidity: 50-60%), at a similar time of the day (±30 min). The visits to the laboratory included, *i*) one ramp-incremental test to task failure, *ii*) five 30-min constant-PO rides at 5% below LT ($LT_{-5\%}$), halfway between LT and $MLSS_p$ ($Delta_{50}$), 5% below $MLSS_p$ ($MLSS_{-5\%}$), $MLSS_p$, and 5% above $MLSS_p$ ($MLSS_{+5\%}$). Each of these rides was preceded by a 4-min 50 W baseline period and immediately followed by a TTF trial (i.e., $TTF_{LT-5\%}$, $TTF_{Delta50}$, $TTF_{MLSS-5\%}$, TTF_{MLSSp}, and $TTF_{MLSS+5\%}$, respectively), *iii*) 15- and 45-min constant-PO rides at $MLSS_p$ immediately followed by a TTF trial (TTF_{MLSS15} and TTF_{MLSS45}, respectively), and *iv*) a baseline TTF trial (TTF_b), serving as "control" condition, only preceded by a 4-min 50 W baseline. After the ramp-incremental test and the determination of $MLSS_p$, the remaining visits were performed in a randomized order. All tests were performed over the course of five consecutive weeks with a minimum of 48 hours between each visit (see below for order of visits). For each testing session, participants were able to view their cycling cadence but were blinded to the condition they were performing, the PO and elapsed time. Moreover, participants were not provided with any feedback related to their performance before concluding the study. Prior to each visit, participants were instructed to avoid the consumption of food and caffeinated beverages for at least 2 and 8 hours, respectively, and to abstain from vigorous physical activity for 24 hours. Participants were also asked to maintain a similar diet throughout the course of the study, self-recording what they ate the day before and day of each laboratory visit.

Ramp-incremental test. On the first visit to the laboratory, participants completed a ramp-incremental test to task failure. The session began with a 4-min baseline at 20 W, followed by a moderate step-transition (6 min at 100 W) to facilitate the accurate computation of the $\dot{V}O_2$ mean response time (MRT) of the ramp-incremental test (Iannetta, Murias, and Keir 2019). Immediately following the moderate step-transition, participants cycled for 4 min at 50 W leading to the incremental portion of the test (25 $W \cdot min^{-1}$ for women (1 W every 2.4 s) and 30 $W \cdot min^{-1}$ (1 W every 2 s) for men). The test was stopped once participants reached volitional exhaustion or when the cycling cadence dropped below 70 rpm for longer than ten consecutive seconds, despite strong verbal encouragement.

Constant-PO trials. The second laboratory visit consisted of cycling for 30 min at each participant's predicted $MLSS_p$. $MLSS_p$ prediction was based on a mathematical model developed in our laboratory (see Iannetta et al. (Iannetta, Fontana, et al. 2018)). $MLSS_p$ was established as the highest PO where $[La^-]_b$ between the 10th and 30th minute differed by no more than 1 $mmol \cdot L^{-1}$ (Beneke 2003). When the $[La^-]_b$ between the 10th and 30th minute differed by greater or less than (or equal to) 1 $mmol \cdot L^{-1}$, the PO was decreased or increased by 5%, respectively, for the subsequent visit. Upon the determination of $MLSS_p$ and the completion of the corresponding confirmation ride (i.e., $MLSS_{+5\%}$ and/or $MLSS_{-5\%}$), the remaining conditions were performed in a randomized order and consisted of cycling either at a constant-PO for 30 min at $LT_{-5\%}$, $Delta_{50}$, $MLSS_{-5\%}$, for 15 or 45 min at $MLSS_p$, or TTF_b. For all conditions, the constant-PO rides were initiated after a 4-min baseline at 50 W; during these rides participants were instructed to cycle consistently at their preferred cadence determined during the ramp-incremental test.

Time-to-exhaustion trials. Each constant-PO condition was followed by a TTF trial in the severe-intensity domain. 10 s before the end of the constant-PO ride, participants were informed that the TTF trial was about to begin. The PO for this TTF trial corresponded to 80% of the peak power (PO_{peak}) from the ramp-incremental test. The intensity of 80% PPO for the TTF trials was selected to ensure severe-intensity domain exercise (Iannetta et al. 2019, 2020). The TTF_b condition was proceeded by a 4-min baseline at 50 W only and conducted randomly after determination of $MLSS_p$. All TTF trials were stopped when participants reached volitional exhaustion, or their cadence dropped below 70 rpm for 10 s.

Data collection

Breath-by-breath gasexchange and ventilatory variables were measured continuously during each testing session with a metabolic cart (Quark CPET, Cosmed, Rome, Italy). Calibration of the system was performed according to the manufacturer's instructions with a 3-L syringe and a standard gas mixture (16% O_2, 5% CO_2, and balance N_2). For measures of $[La^-]_b$, a 20 µL finger-prick blood sample was collected into a capillary tube and immediately analysed (EKF Biosen C-Line Analyzer, EKF Diagnostics, Barleben, Germany). For each condition, $[La^-]_b$ was assessed during the 4-min baseline phase, every 5 min of each constant-PO ride, and immediately after each TTF trial. At the 10^{th} and 30^{th} min of the constant-PO rides, three blood samples were taken in rapid succession, and the two closest measures were averaged for subsequent analyses. The 0-10

Borg scale (Borg 1982) was used to measure RPE at the same time points as the $[La^-]_b$ measurements.

Data and Statistical Analyses

Ventilatory and gas exchange variables. For each ramp-incremental test, the raw ventilatory and gas exchange variables were plotted against $\dot{V}O_2$ to estimate LT (Beaver, Wasserman, and Whipp 1986). LT corresponded to the point at which carbon dioxide output ($\dot{V}CO_2$) began to increase out of proportion in relation to $\dot{V}O_2$, coincidentally with a systematic rise in the ventilation ($\dot{V}_E$)/$\dot{V}O_2$ ventilatory equivalent and end-tidal PO_2, whilst the $\dot{V}_E/\dot{V}CO_2$ ventilatory equivalent and end-tidal PCO_2 were stable (Beaver, Wasserman, and Whipp 1986). To accurately identify the PO associated with the estimated LT, the ramp-$\dot{V}O_2$ was corrected by the MRT to account for the muscle-lung transit time delay and the kinetics of $\dot{V}O_2$ at ramp-onset (Iannetta, Murias, and Keir 2019).

After the raw ventilatory and gas-exchange variables were edited on the basis of $\dot{V}O_2$ (Lamarra et al. 1987), peak $\dot{V}O_2$ ($\dot{V}O_{2peak}$) was computed from the highest 30-s rolling average during the ramp-incremental test. The same strategy was employed to determine $\dot{V}O_{2peak}$, as well as the peak $\dot{V}_E$, respiratory exchange rate (RER), breathing frequency (f_R), and heart rate response during each TTF trial. PO_{peak} corresponded to the highest PO value at the end of the ramp-incremental test. To compute the average $\dot{V}O_2$, $\dot{V}_E$, f_R, and RER response during each constant-PO ride, the last 5 min of each condition were averaged.

Total Work. The total work for each constant-PO condition, including each TTF trial, was calculated as follows.

$$\text{Total Work (kJ)} = \text{time (s)} \times \text{power output (W)} / 1000$$

Statistical Analyses

Sample size was calculated based on means and SD for TTF (s) following constant-PO exercise at $MLSS_p$ and $MLSS_{+10W}$ from previously published data (Iannetta, Inglis, et al. 2018). Considering a type I error rate of 5% (2-tailed) and a power of 80%, the minimum sample size required to identify significant differences between conditions was 10 participants. Data are presented as means ± SD, and all statistical analyses were performed using SPSS version 25 (SPSS, IBM, Chicago, IL). For the five 30-min constant-PO conditions, a repeated-measures ANOVA was used to compare: *i*) the absolute duration of each TTF trial, *ii*) the reduction in each TTF trial, expressed as a percentage of TTF_b; *iii*) $\dot{V}O_2$, $\dot{V}_E$, $[La^-]_b$, and RPE responses at the 30^{th} minute of each constant-PO condition; *iv*) the average $\dot{V}O_2$, $\dot{V}_E$, f_R, RER responses during each constant-PO condition; and *v*) the total work performed during each constant-PO condition and subsequent TTF trial. The same comparisons were used to evaluate responses for the three durations of cycling at $MLSS_p$, and for the comparison of $\dot{V}O_2$ and $\dot{V}_E$ at iso-time in these rides (i.e., 15^{th} minute of $MLSS_{15}$, $MLSS_p$, and $MLSS_{45}$). Prior to performing the repeated-measures ANOVAs, all assumptions were met (independence between samples, normality of distribution, and sphericity). For all repeated-measures ANOVA, when a main effect was found a Bonferroni post hoc was performed and statistical significance was accepted at $\alpha < 0.05$.

For each individual, the Pearson's correlation coefficient was used to assess the relationship between the percent reduction in TTF performance and, *i)* RPE, *ii)* $[La^-]_b$, and *iii)* $\dot{V}_E$, measured at the end of each constant-PO condition, *iv)* the constant-PO duration at $MLSS_p$, and *v*) the total work performed during each constant-PO duration at $MLSS_p$. The Pearson's correlation coefficient was also used to evaluate the association between RPE and $\dot{V}_E$ at the end of each constant-PO condition.

Results

The average PO_{peak} at the end of the ramp-incremental cycling test was 336 ± 59 W and the derived 80% of PO_{peak} used for the TTF trials was 269 ± 47 W. Table 1 presents the average for each constant-PO condition, as well as the corresponding responses in $\dot{V}O_2$, RER, $\dot{V}_E$, and f_R.

Profiles of $\dot{V}O_2$ as well as of $\dot{V}_E$, $[La^-]_b$, and RPE during each constant-PO condition are displayed in Figure 1. During each 30-min trial, the $\dot{V}O_2$ and $\dot{V}_E$ responses at the 30th minute of each exercise intensity were all different from each other (Figure 1, A and C, respectively). No difference was found in the $\dot{V}O_2$ at the end of each $MLSS_p$ condition of different duration (F (2,20) = 1.63, P = 0.221) (Figure 1, B). The $\dot{V}_E$ at the end of $MLSS_{15}$ was lower than both $MLSS_p$ and $MLSS_{45}$ rides (P = 0.001, and P = 0.001, respectively), with no difference found between $MLSS_p$ and $MLSS_{45}$ (P > 0.05) (Figure 1, D). Moreover, no difference was found at iso-time for $\dot{V}O_2$ (F (2,20) = 0.18, P = 0.982) or $\dot{V}_E$ (F (2,20) = 0.17, P = 0.843) (i.e., assessed at the 15th minute of $MLSS_{15}$, $MLSS_p$, and $MLSS_{45}$). As the intensity of each constant-PO ride increased up to $MLSS_p$, progressively higher but stable $[La^-]_b$ responses were observed (F (4,40) = 87.54, P = 0.000) (Figure 1, E). The Bonferroni post-hoc revealed that all pairwise differences among means were significant (P < 0.05). A disproportionate increase in $[La^-]_b$ occurred during $MLSS_{+5\%}$ compared to all other conditions (P < 0.05). $[La^-]_b$ values recorded at the end of $MLSS_{15}$, $MLSS_p$, and $MLSS_{45}$ were different from one another (F (2,20) = 7.31, P = 0.004) (Figure 1, F). The Bonferroni post-hoc revealed that $[La^-]_b$ at the end of $MLSS_p$ was greater than the $[La^-]_b$ at the end of $MLSS_{15}$ (P = 0.004). The RPE responses throughout each constant-PO condition are shown in Figure 1 (G and H).

Peak ventilatory and gas exchange, heart rate, $[La^-]_b$, and perceptual responses during the ramp-incremental and each TTF trial are shown in Table 2. The percent reduction in TTF with respect to TTF_b is shown in Figure 2 (A-D). The mean reduction in TTF performance after each 30-min constant-PO ride, plotted against each 30-min condition expressed as a percentage of $MLSS_p$, decreased curvilinearly (Figure 2, C). Conversely, a linear decrease was observed in response to different exercise durations at $MLSS_p$ (Figure 2, D). The total amount of work performed during each constant-PO condition and TTF trial are shown in Figure 4 (A, B), as well as the reduction in TTF in relation to the total amount of work completed during each constant-PO condition (C, D). An analysis of variance showed that the effect of total work (30-min constant-PO condition + TTF) was significant (F (1,10) = 35.68, P = 0.000). A Bonferroni post-hoc revealed that the total work during $LT_{-5\%}$ + $TTF_{LT-5\%}$ was less than all other 30-min conditions (P < 0.001) (Table 1, Figure 4, A). The analysis of variance indicated that the effect of total work, completed for each duration condition at $MLSS_p$ when including each corresponding TTF trial, were significant (F (2,20) = 438.13, P = 0.000). The Bonferroni post-hoc revealed that all pairwise differences among means were significant (P = 0.000) (Table 1, Figure 4, B).

The relationship between the percent reduction in the different TTF trials and $[La^-]_b$ and RPE recorded immediately prior to each TTF trial is presented in Figure 3. The percent reduction in TTF, for each individual, with increasing intensity of the constant-PO rides was strongly correlated to the progressive increase in $[La^-]_b$ (r= -0.93 ± 0.04) and RPE (r= -0.93 ± 0.07) (Figure 3, A and C). However, for the $MLSS_{15}$, $MLSS_p$, and $MLSS_{45}$ conditions (Figure 3, B and D), the reduction in TTF performance was correlated with RPE (r = -0.79 ± 0.41) but not $[La^-]_b$ (r = -0.18 ± 0.59). The percent reduction in TTF performance with increasing intensity and duration of the constant-PO rides, for each individual, was strongly correlated to the progressive increase in $\dot{V}_E$ (r = -0.94 ± 0.06 and -0.79 ± 0.22, respectively). The relationship between $\dot{V}_E$ and RPE, immediately prior to each TTF trial, was strongly correlated following an increase in intensity of the constant-PO rides ($r = 0.96 \pm 0.03$), whereas a moderate correlation was found following an increase in the duration at $MLSS_p$ ($r = 0.68 \pm 0.27$).

Discussion

The current investigation shows that prior exercise performed at a range of specific, domain-derived intensities (i.e., $LT_{-5\%}$, $Delta_{50}$ (midway between LT and $MLSS_p$), $MLSS_{-5\%}$, $MLSS_p$, and $MLSS_{+5\%}$) and a range of durations (i.e., 15, 30, and 45 min at $MLSS_p$) progressively impairs subsequent cycling performance within the severe-intensity domain, and that this impairment is predictable by the RPE levels measured prior to TTF trial.

Reduction in cycling performance following exercise at different intensities and durations.

Following 30 min of cycling at a constant-PO, there was a progressive impairment in TTF performance with increasing exercise intensity ($LT_{-5\%}$:~ 8% , $Delta_{50}$: ~ 24%, $MLSS_{-5\%}$: ~ 41%, and $MLSS_p$: ~ 52%, $MLSS_{+5\%}$: ~75 %) (Figure 2, A and C). When these distinct reductions in TTF performance were plotted against each constant-PO expressed as a percentage of $MLSS_p$, a curvilinear downward pattern seemed to emerge (Figure 2, C). Regardless, all prior intensities of exercise investigated herein significantly impaired subsequent TTF performance in the severe-intensity domain, in relation to a TTF trial without prior exercise. Interestingly, this observation is true even following 30 min of exercise in the moderate-intensity domain, within which depletion of substrates and accumulation of metabolites during such exercise is likely minimal (Black et al. 2017). Moreover, manipulating the duration of the exercise also had marked effects on TTF performance (Figure 2, B and D). Indeed, as the duration of exercise at $MLSS_p$ was increased (from 15, to 30, and to 45 min), the TTF performance was reduced in a seemingly linear pattern (from ~ 29%, to ~ 52%, and to ~ 69 %) (Figure 2, D). This finding demonstrates that changes in intensity and duration pose different challenges that contribute differently in the reduction of subsequent TTF performance.

During submaximal exercise (in the moderate- and heavy-intensity domains), the amplitude of the changes in intramuscular substrates and metabolites from baseline become progressively greater with increasing metabolic rates (Jones, Wilkerson, and Fulford 2008; Rossiter et al. 2002). However, while in the moderate- and heavy-intensity domains the metabolic milieu remains relatively stable, exercise within the severe-intensity domain results in an inexorable fall in substrate availability (i.e., [PCr]) and accumulation of metabolites (i.e., [Pi], [ADP], and $[H^+]$) (Black et al. 2017; Jones, Wilkerson, and Fulford 2008; Vanhatalo et al. 2016; Rossiter et al. 2002; Fitts 2008), such that, independently of the external workload (i.e., PO), exercise performed within the severe-intensity domain typically culminates with the attainment of a consistent intramuscular metabolic milieu (Jones and Vanhatalo 2017). Therefore, the progressive reduction in TTF performance might be explained by the fact that various TTF trials were initiated from progressively higher levels of metabolic perturbation leading to the attainment of this “critical” metabolic milieu being reached earlier (Jones and Vanhatalo 2017). Interestingly, the combined mechanical work expressed during the 30-min constant-PO rides and subsequent TTF trials were not different across conditions, except for $LT_{-5\%}$ (Figure 4, A), suggesting an inextricable link between the level of metabolic perturbations in the heavy-intensity domain and exercise capacity within the severe-intensity domain.

From inspecting the group mean data, it seems that the pattern of reduction in TTF was curvilinear (Figure 2, C), suggesting that slight increases of prior intensity can lead to disproportionate reductions of exercise capacity within the severe-intensity domain. Additional studies may be required to resolve the exact pattern of reduction in TTF following prior exercise performed at different exercise intensities. However, it is notable that our observed response is consistent with the exponential nature of the power-duration curve, as increases in PO in the severe-intensity domain have disproportionate effects on tolerable durations of exercise compared to those in the other domains (i.e., moderate and heavy) (Poole et al. 2016). Regardless, Passfield and Doust (2000) demonstrated that cycling efficiency declines during sustained exercise within the moderate-intensity domain and that this decline is highly correlated with subsequent severe-intensity performance. Similarly, Cannon et al. (2011) and more recently Keir et al. (2016) have reported a close association between the progression of the $\dot{V}O_2$ slow component and the time course of peripheral fatigue, demonstrating a link between the development of metabolic inefficiency and the capacity of the muscle to sustain the required levels of force. These studies could suggest that, as these metabolic inefficiencies become progressively greater with increasing metabolic rates, they could contribute to the disproportional reduction in capacity in the severe-intensity domain.

Collectively, the findings of the present study indicate that the effects of intensity and duration should be considered separately when comparing or prescribing varying exercise bouts or evaluating their impact on subsequent performance. Specifically, it appears that an increase in intensity of prior exercise (even if prior exercise is matched for total work) has a greater detrimental consequence than an increase in duration (at least within the time durations tested herein) (Figure 4). As previously discussed, this reflects the greater physiological stress of an increase in intensity, and it may offer partial support to the observation that varying the intensity and duration of exercise training can alter the magnitude of adaptation in $\dot{V}O_{2max}$ (Seiler et al. 2013).

Lactate and perceptual responses: relationship with time-to-exhaustion performance.

An interesting finding of the present study is the relationship between $[La^-]_b$ and RPE, assessed immediately prior to each TTF trial, and the reduction in exercise capacity within the severe-intensity domain. As the intensity of the constant-PO trials increased, the percent reduction in TTF performance was also strongly correlated with both the progressive increase in $[La^-]_b$ and RPE (Figure 3, A and C). The progressive increase in $[La^-]_b$ at the end of each exercise intensity condition (from $LT_{-5\%}$ to $MLSS_{+5\%}$) is indicative of an increasing glycolytic flux leading to some level of metabolites accumulation (Stainsby 1986) (Figure 1, E).

However, although there were strong associations between both $[La^-]_b$ and RPE and TTF performance for the 30-min exercise bouts (performed at different intensities), the decline in TTF performance following progressively longer rides (at $MLSS_p$) provide a different perspective. At the end of each duration condition performed at $MLSS_p$, the reduction in TTF performance was associated with RPE (Figure 3, D) but was not with $[La^-]_b$ (Figure 3, B). This lack of relationship would indicate that a given $[La^-]_b$ is not necessarily predictive of a reduction in subsequent TTF performance (Figure 3, B), and may suggest that other factors in addition to the ones related to the metabolic status of exercising muscles may be responsible for the reduction in TTF performance.

Several studies have shown that RPE during constant-PO exercise increases linearly with the duration to task-failure (Horstman et al. 1979; Baldwin et al. 2003; Eston et al. 2007; Pires et al. 2011; Pageaux and Lepers 2016). The present study shows that RPE at the end of each constant-PO ride (regardless of intensity or duration) negatively correlates with reductions in TTF performance. Therefore, these findings may support the sensory tolerance limit model of fatigue, as it has been suggested that RPE is reflective of the integration of a diverse range of peripheral and central feedback and feedforward responses to exercise (Pageaux and Lepers 2016; Gibson et al. 2003; Hureau, Romer, and Amann 2018). As RPE responses increase with increasing exercise intensities and durations, it is not surprising that levels of RPE strongly predict reductions in TTF performance. In addition to this, $\dot{V}_E$, a response argued to be affected by central and peripheral responses (Hureau, Romer, and Amann 2018; Amann et al. 2010; Fukuba et al. 2007; Bruce and White 2012), has been shown to influence RPE, especially as the intensity of exercise increases (Robertson 1982). Consequently, several studies have established a strong relationship between RPE and $\dot{V}_E$ during exercise (Robertson 1982; Noble et al. 1973; Edwards et al. 1972; Nicoló et al. 2014). Consistent with these findings, we found a strong association between RPE and $\dot{V}_E$ at the end of each constant-PO condition, regardless of its intensity and duration. Moreover, $\dot{V}_E$ at the end of each constant-PO condition was strongly correlated with the reduction in subsequent TTF performance ($r = -0.94 \pm 0.06$ and -0.79 ± 0.22 for intensities and durations correlations, respectively). Taken together, these data may indicate a role for both peripheral (i.e., metabolic state of the muscle) and central mechanisms (i.e., corollary discharge) affecting subsequent exercise capacity within the severe-intensity domain.

Practical implications: total mechanical work

The practical implications of this study arise from the observation that TTF is increasingly compromised by 30 min of prior exercise, even when the intensity of the preceding rides may not be necessarily considered highly demanding. Interestingly, the decrease in TTF though related to the duration, is unrelated to the total work done in the prior exercise bout (Figure 4, A). The latter observation is particularly important as many studies use total work done as the basis for comparing different exercise bouts or programs. Indeed, the concept of training load, increasingly used to prescribe, monitor and evaluate athletes' training, also normalises for total work done or its equivalent. The findings of the present study indicate these practices might not be correct (Nicolò, Passfield, and Sacchetti 2016) and that the confound may lie in the need to consider the influence of exercise duration separately from intensity (Kesisoglou, Nicolò, and Passfield 2020; Seiler et al. 2013). Another notable finding from the present study is the strong relationship between RPE and subsequent TTF that was present across a range of exercise intensities and durations (Figure 3, C and D). In contrast, the same strength of relationship was not apparent for [La^-], implying that following prior exercise, TTF is more strongly influenced by perceived exertion than the metabolic milieu.

Conclusion

This study characterized the effects of prior exercise performed at different intensities and durations on subsequent TTF performance. Notably, when a bout of exercise was performed at sub-MLSS intensities, subsequent performance was reduced. We found a strong correlation between the reduction in performance following constant-PO exercise across intensity domains and a range of durations of preceding exercise. The current study also highlighted that perceptual responses, such as RPE, relate to the exercise capacity within the severe-intensity domain.

Acknowledgments

We express our gratitude to the participants in this study. Dr. Murias research is supported by Natural Science and Engineering Research Council of Canada (RGPIN-2016-03698) and the Heart & Stroke Foundation of Canada (1047725).

Competing interests

The authors declare that they have no competing interests.

References

Amann, Markus, Gregory M. Blain, Lester T. Proctor, Joshua J. Sebranek, David F. Pegelow, and Jerome A. Dempsey. 2010. “Group III and IV Muscle Afferents Contribute to Ventilatory and Cardiovascular Response to Rhythmic Exercise in Humans.” *Journal of Applied Physiology* 109 (4): 966–76. https://doi.org/10.1152/japplphysiol.00462.2010.

Baldwin, Jacinta, Rodney J. Snow, Martin J. Gibala, Andrew Garnham, Krista Howarth, and Mark A. Febbraio. 2003. “Glycogen Availability Does Not Affect the TCA Cycle or TAN Pools during Prolonged, Fatiguing Exercise.” *Journal of Applied Physiology* 94 (6): 2181–87. https://doi.org/10.1152/japplphysiol.00866.2002.

Beaver, William L., Karlman Wasserman, and Brian J. Whipp. 1986. “A New Method for Detecting Anaerobic Threshold by Gas Exchange.” *Journal of Applied Physiology* 121 (6): 2020–27. https://doi.org/10.1152/jappl.1986.60.6.2020.

Beneke, Ralph. 2003. “Methodological Aspects of Maximal Lactate Steady State-Implications for Performance Testing.” *European Journal of Applied Physiology* 89 (1): 95–99. https://doi.org/10.1007/s00421-002-0783-1.

Black, Matthew I., Andrew M. Jones, Jamie R. Blackwell, Stephen J. Bailey, Lee J. Wylie, Sinead T. J. McDonagh, Christopher Thompson, et al. 2017. “Muscle Metabolic and Neuromuscular Determinants of Fatigue during Cycling in Different Exercise Intensity Domains.” *Journal of Applied Physiology* 122 (3): 446–59. https://doi.org/10.1152/japplphysiol.00942.2016.

Borg, Gunnar A.V. 1982. “Psychophysical Bases of Perceived Exertion.” *Medicine & Science in Sports & Exercise*. https://doi.org/-.

Bruce, Richard M., and Michael J. White. 2012. “Muscle Afferent Activation Causes Ventilatory and Cardiovascular Responses during Concurrent Hypercapnia in Humans.” *Experimental Physiology* 97 (2): 208–18. https://doi.org/10.1113/expphysiol.2011.061606.

Burnley, Mark, Anni Vanhatalo, and Andrew M. Jones. 2012. “Distinct Profiles of Neuromuscular Fatigue during Muscle Contractions below and above the Critical Torque in Humans.” *Journal of Applied Physiology* 113 (2): 215–23. https://doi.org/10.1152/japplphysiol.00022.2012.

Cannon, Daniel T., Ailish C. White, Melina F. Andriano, Fred W. Kolkhorst, and Harry B. Rossiter. 2011. “Skeletal Muscle Fatigue Precedes the Slow Component of Oxygen Uptake Kinetics during Exercise in Humans.” *Journal of Physiology* 589 (3): 727–39. https://doi.org/10.1113/jphysiol.2010.197723.

Edwards, R. H.T., A. Melcher, C. M. Hesser, O. Wigertz, and L. -G Ekelund. 1972. “Physiological Correlates of Perceived Exertion in Continuous and Intermittent Exercise with the Same Average Power Output.” *European Journal of Clinical Investigation* 2 (2): 108–14. https://doi.org/10.1111/j.1365-2362.1972.tb00578.x.

Eston, Roger, James Faulkner, Alan St Clair Gibson, Tim Noakes, and Gaynor Parfitt. 2007. “The Effect of Antecedent Fatiguing Activity on the Relationship between Perceived Exertion and Physiological Activity during a Constant Load Exercise Task.” *Psychophysiology* 44 (5): 779–86. https://doi.org/10.1111/j.1469-8986.2007.00558.x.

Fitts, Robert H. 2008. “The Cross-Bridge Cycle and Skeletal Muscle Fatigue.” *Journal of Applied Physiology* 104 (2): 551–58. https://doi.org/10.1152/japplphysiol.01200.2007.

Fukuba, Yoshiyuki, Asami Kitano, Naoyuki Hayashi, Takayoshi Yoshida, Hatsumi Ueoka, Masako Yamaoka Endo, and Akira Miura. 2007. “Effects of Femoral Vascular Occlusion on Ventilatory Responses during Recovery from Exercise in Human.” *Respiratory Physiology and Neurobiology* 155 (1): 29–34. https://doi.org/10.1016/j.resp.2006.02.017.

Gibson, Alan St Clair, Denise A Baden, Mike I Lambert, E Vicki Lambert, X R Harley, Dave Hampson, Vivienne A Russell, and Tim D Noakes. 2003. “The Conscious Perception of the Sensation of Fatigue.” *Sports Medicine* 33 (3): 167–76. http://link.springer.com/article/10.2165/00007256-200333030-00001.

Horstman, D. H., W. P. Morgan, A. Cymerman, and J. Stokes. 1979. “Perception of Effort during Constant Work to Self-Imposed Exhaustion.” *Perceptual and Motor Skills* 48 (3): 1111–26. https://doi.org/10.2466/pms.1979.48.3c.1111.

Hureau, Thomas J., Lee M. Romer, and Markus Amann. 2018. “The ‘Sensory Tolerance Limit’: A Hypothetical Construct Determining Exercise Performance?” *European Journal of Sport Science* 18 (1): 13–24. https://doi.org/10.1080/17461391.2016.1252428.

Iannetta, Danilo, Rafael De Almeida Azevedo, Daniel A. Keir, and Juan M. Murias. 2019. “Establishing the VO2 versus Constant-Work-Rate Relationship from Rampincremental Exercise: Simple Strategies for an Unsolved Problem.” *Journal of Applied Physiology* 127 (6): 1519–27. https://doi.org/10.1152/japplphysiol.00508.2019.

Iannetta, Danilo, Federico Y. Fontana, Felipe Mattioni Maturana, Erin Calaine Inglis, Silvia Pogliaghi, Daniel A. Keir, and Juan M. Murias. 2018. “An Equation to Predict the Maximal Lactate Steady State from Ramp-Incremental Exercise Test Data in Cycling.” *Journal of Science and Medicine in Sport* 21 (12): 1274–80. https://doi.org/10.1016/j.jsams.2018.05.004.

Iannetta, Danilo, Erin Calaine Inglis, Christopher Fullerton, Louis Passfield, and Juan M. Murias. 2018. “Metabolic and Performance-Related Consequences of Exercising at and Slightly above MLSS.” *Scandinavian Journal of Medicine and Science in Sports* 28 (12): 2481–93. https://doi.org/10.1111/sms.13280.

Iannetta, Danilo, Erin Calaine Inglis, Anmol T. Mattu, Federico Y. Fontana, Silvia Pogliaghi, Daniel A. Keir, and Juan M. Murias. 2020. “A Critical Evaluation of Current Methods for Exercise Prescription in Women and Men.” *Medicine and Science in Sports and Exercise* 52 (2): 466–73. https://doi.org/10.1249/MSS.0000000000002147.

Iannetta, Danilo, Juan M. Murias, and Daniel A. Keir. 2019. “A Simple Method to Quantify the V-O 2 Mean Response Time of Ramp-Incremental Exercise.” *Medicine and Science in Sports and Exercise* 51 (5): 1080–86. https://doi.org/10.1249/MSS.0000000000001880.

Jamnick, Nicholas A., Robert W. Pettitt, Cesare Granata, David B. Pyne, and David J. Bishop. 2020. “An Examination and Critique of Current Methods to Determine Exercise Intensity.” *Sports Medicine* 50 (10): 1729–56. https://doi.org/10.1007/s40279-020-01322-8.

Jones, Andrew M., and Anni Vanhatalo. 2017. “The ‘Critical Power’ Concept: Applications to Sports Performance with a Focus on Intermittent High-Intensity Exercise.” *Sports Medicine* 47 (s1): 65–78. https://doi.org/10.1007/s40279-017-0688-0.

Jones, Andrew M., Daryl P. Wilkerson, and Jonathan Fulford. 2008. “Muscle [Phosphocreatine]Dynamics Following the Onset of Exercise in Humans: The Influence of Baseline Work-Rate.” *Journal of Physiology* 586 (3): 889–98. https://doi.org/10.1113/jphysiol.2007.142026.

Keir, Daniel A., David B. Copithorne, Michael D. Hodgson, Silvia Pogliaghi, Charles L. Rice, and John M. Kowalchuk. 2016. “The Slow Component of Pulmonary O2 Uptake Accompanies Peripheral Muscle Fatigue during High-Intensity Exercise.” *Journal of Applied Physiology* 121 (2): 493–502. https://doi.org/10.1152/japplphysiol.00249.2016.

Kesisoglou, Antonis, Andrea Nicolò, and Louis Passfield. 2020. “Cycling Performance and Training Load: Effects of Intensity and Duration.” *International Journal of Sports Physiology and Performance* 1 (aop): 1–9. https://doi.org/10.1123/ijspp.2020-0072.

Lamarra, N., B. J. Whipp, S. A. Ward, and K. Wasserman. 1987. “Effect of Interbreath Fluctuations on Characterizing Exercise Gas Exchange Kinetics.” *Journal of Applied Physiology* 62 (5): 2003–12. https://doi.org/10.1152/jappl.1987.62.5.2003.

Nicoló, Andrea, Ilenia Bazzucchi, Jonida Haxhi, Francesco Felici, and Massimo Sacchetti. 2014. “Comparing Continuous and Intermittent Exercise: An ‘Isoeffort’ and ‘Isotime’ Approach.” *PLoS ONE* 9 (4). https://doi.org/10.1371/journal.pone.0094990.

Nicolò, Andrea, Louis Passfield, and Massimo Sacchetti. 2016. “Investigating the Effect of Exercise Duration on Functional and Biochemical Perturbations in the Human Heart: Total Work or ‘isoeffort’ Matching?” *Journal of Physiology* 594 (11): 3157–58. https://doi.org/10.1113/JP272421.

Noakes, Timothy D. 2004. “Linear Relationship between the Perception of Effort and the Duration of Constant Load Exercise That Remains [Letter to the Editor].” *Journal of Applied Physiology* 96: 1571–73. https://doi.org/10.1080/13518040701205365.

Noble, Bruce J., Kenneth F. Metz, Kent B. Pandolf, and Enzo Cafarelli. 1973. “Perceptual Responses to Exercise: A Multiple Regression Study.” *Medicine and Science in Sports*. https://doi.org/10.1249/00005768-197300520-00020.

Pageaux, Benjamin, and Romuald Lepers. 2016. “Fatigue Induced by Physical and Mental Exertion Increases Perception of Effort and Impairs Subsequent Endurance Performance.” *Frontiers in Physiology* 7 (NOV). https://doi.org/10.3389/fphys.2016.00587.

Pires, Flávio O., Timothy D. Noakes, Adriano E. Lima-Silva, R. Bertuzzi, Carlos Ugrinowitsch, Fabio S. Lira, and Maria Augusta P.D.M. Kiss. 2011. “Cardiopulmonary, Blood Metabolite and Rating of Perceived Exertion Responses to Constant Exercises Performed at Different Intensities until Exhaustion.” *British Journal of Sports Medicine* 45 (14): 1119–25. https://doi.org/10.1136/bjsm.2010.079087.

Poole, David C, Mark Burnley, Anni Vanhatalo, Harry B Rossiter, M Andrew, Exercise Sciences, and Health Sciences. 2016. “Critical Power: An Important Fatigue Threshold in Exercise Physiology.” *Medicine and Science in Sports and Exercise* 48 (11): 2320–34. https://doi.org/10.1249/MSS.0000000000000939.Critical.

Robertson, R. J. 1982. “Central Signals of Perceived Exertion during Dynamic Exercise.” *Medicine and Science in Sports and Exercise* 14 (5): 390–96. https://doi.org/10.1249/00005768-198205000-00014.

Rossiter, H. B., S. A. Ward, J. M. Kowalchuk, F. A. Howe, J. R. Griffiths, and B. J. Whipp. 2002. “Dynamic Asymmetry of Phosphocreatine Concentration and O2 Uptake between the On- and off-Transients of Moderate- and High-Intensity Exercise in Humans.” *Journal of Physiology* 541 (3): 991–1002. https://doi.org/10.1113/jphysiol.2001.012910.

Schäfer, Lisa U., Mark Hayes, and Jeanne Dekerle. 2019. “The Magnitude of Neuromuscular Fatigue Is Not Intensity Dependent When Cycling above Critical Power but Relates to Aerobic and Anaerobic Capacities.” *Experimental Physiology* 104 (2): 209–19.

https://doi.org/10.1113/EP087273.

Seiler, S, K Joranson, B. V Olesen, and K. J Hetledid. 2013. “Adaptations to Aerobic Interval Training: Interactive Effects of Exercise Intensity and Total Work Duration.” *Scandinavian Journal of Medicine and Science in Sports* 23: 74–83.

Stainsby, W.N. 1986. “Biochemical and Physiological Bases for Lactate Production.” *Medicine & Science in Sports & Exercise* 18 (3): 341–43.

Vanhatalo, Anni, Matthew I. Black, Fred J. DiMenna, Jamie R. Blackwell, Jakob Friis Schmidt, Christopher Thompson, Lee J. Wylie, et al. 2016. “The Mechanistic Bases of the Power–Time Relationship: Muscle Metabolic Responses and Relationships to Muscle Fibre Type.” *Journal of Physiology* 594 (15): 4407–23. https://doi.org/10.1113/JP271879.

Whipp, Brian J. 1994. “Domains of Aerobic Function and Their Limiting Parameters.” In *The Physiology and Pathophysiology of Exercise Tolerance*, 83–89. https://doi.org/10.1038/sc.1966.33.

Table 1. The average PO, ventilatory and gas exchange variables during the last 5-min of each constant-PO condition and the total work performed during each constant-PO condition and subsequent TTF trial is presented.

Variable	LT$_{-5\%}$	Delta$_{50}$	MLSS$_{-5\%}$	MLSS$_{p}$	MLSS$_{+5\%}$
PO (W)	142 ± 25	$174 \pm 31^{*, \ddagger, \S, \text{¢}}$	$188 \pm 38^{*, \S, \text{¢}}$	$198 \pm 39^{*, \text{¢}}$	$208 \pm 41^{*}$
$\dot{V}O_2$ (L·min^{-1})	2.29 ± 0.33	$2.62 \pm 0.32^{*, \ddagger, \S, \text{¢}}$	$2.76 \pm 0.40^{*, \S, \text{¢}}$	$2.88 \pm 0.44^{*, \text{¢}}$	$3.04 \pm 0.56^{*}$
RER	0.91 ± 0.03	0.91 ± 0.03	0.94 ± 0.06	0.94 ± 0.04	0.93 ± 0.04
$\dot{V}_E$ (L·min^{-1})	63 ± 7	$78 \pm 9^{*, \ddagger, \S, \text{¢}}$	$87 \pm 11^{*, \S, \text{¢}}$	$96 \pm 13^{*, \text{¢}}$	$115 \pm 20^{*}$
f_R (breath·min^{-1})	32.3 ± 5.4	$38.7 \pm 6.8^{*, \text{¢}}$	$41.4 \pm 9.3^{*, \text{¢}}$	$44.2 \pm 10.1^{*, \text{¢},}$	$52.9 \pm 8.5^{*}$
Total Work (kJ)	337 ± 67	$382 \pm 78^{*}$	$391 \pm 79^{*}$	$399 \pm 85^{*}$	$396 \pm 76^{*}$

Variable	MLSS$_{15}$	MLSS$_{p}$	MLSS$_{45}$
PO (W)	198 ± 39	198 ± 39	198 ± 39
$\dot{V}O_2$ (L·min^{-1})	2.84 ± 0.41	2.88 ± 0.44	2.90 ± 0.47
RER	0.94 ± 0.05	0.94 ± 0.04	0.93 ± 0.05
$\dot{V}_E$ (L·min^{-1})	88 ± 10	$96 \pm 13^{\pounds}$	$103 \pm 23^{\pounds}$
f_R (breath·min^{-1})	39.8 ± 8.2	$44.2 \pm 10.1^{\pounds}$	$49.9 \pm 12.1^{\pounds}$
Total Work (kJ)	242 ± 59	$399 \pm 85^{\pounds, \infty}$	$561 \pm 106^{\pounds}$

* Different from LT$_{-5\%}$

‡ Different from MLSS$_{-5\%}$

§ Different from $MLSS_p$
¢ Different from $MLSS_{+5\%}$
£ Different from $MLSS_{15}$
∞ Different from $MLSS_{45}$
$P < 0.05$

Table 2. Peak ventilatory and gas exchange, heart rate, blood lactate, and perceptual responses during the ramp-incremental test and each TTF trial.

Variable	Ramp-incremental	TTF_b	$TTF_{LT-5\%}$	$TTF_{Delta50}$	$TTF_{MLSS-5\%}$	TTF_{MLSSp}	$TTF_{MLSS+5\%}$
TTF duration (s)	-	330 ± 52	300 ± 74 $^{†, ‡, §, ¢}$	251 ± 70 $^{€, §, ¢}$	194 ± 55 $^{€, ¢}$	155 ± 62 $^{€, ¢}$	80 ± 26 $^{€}$
$\dot{V}O_{2peak}$ ($L·min^{-1}$)	3.39 ± 0.16	3.48 ± 0.18	3.45 ± 0.21	3.37 ± 0.16	3.36 ± 0.15	3.28 ± 0.16	3.22 ± 0.18
$\dot{V}_E$ ($L·min^{-1}$)	160 ± 29	155 ± 28	151 ± 31	151 ± 32	147 ± 33	147 ± 31	141 ± 27
HR (bpm)	181 ± 7	174 ± 6	177 ± 5	178 ± 8	180 ± 5	178 ± 11	179 ± 5
$[La^-]_b$	11.2 ± 2.0	10.6 ± 2.5	10.2 ± 3.1	9.3 ± 2.9	9.1 ± 1.9	9.5 ± 2.6	9.4 ± 3.0
RPE	8.9 ± 1.1	9.2 ± 1.1	9.3 ± 0.8	9.6 ± 0.9	9.5 ± 0.9	9.5 ± 0.7	9.7 ± 0.5

Variable	Ramp-incremental	TTF_b	TTF_{MLSS15}	TTF_{MLSSp}	TTF_{MLSS45}
TTF duration (s)	-	330 ± 52	234 ± 69 $^{€, §, ∞}$	155 ± 62 $^{€, ∞}$	101 ± 66 $^{€}$
$\dot{V}O_{2peak}$ ($L·min^{-1}$)	3.39 ± 0.16	3.48 ± 0.18	3.46 ± 0.17	3.28 ± 0.16	3.15 ± 0.15
$\dot{V}_E$ ($L·min^{-1}$)	160 ± 29	155 ± 28	154 ± 31	147 ± 31	131 ± 25
HR (bpm)	181 ± 7	174 ± 6	175 ± 7	178 ± 11	178 ± 6
$[La^-]_b$	11.2 ± 2.0	10.6 ± 2.5	9.3 ± 2.9	9.5 ± 2.6	7.1 ± 2.5
RPE	8.9 ± 1.1	9.2 ± 1.1	9.6 ± 0.7	9.5 ± 0.7	9.5 ± 0.9

Note: Each variable was computed as the highest 30-second rolling average during the ramp-incremental and each TTF trial.
TTF duration (s) results:

€ Different from TTF_b

† Different from $TTF_{Delta50}$

‡ Different from $TTF_{MLSS-5\%}$

§ Different from TTF_{MLSSp}

¢ Different from $TTF_{MLSS+5\%}$

∞ Different from TTF_{MLSS45}

$P < 0.05$

Figure 1. Group mean data (with SD bars) (n=11) displaying $\dot{V}O_2$ (A, B), $\dot{V}_E$ (C, D), $[La^-]_b$ (E, F), RPE (G, H) during 30-min constant-PO rides at $MLSS_{+5\%}$ (light grey circles), $MLSS_p$ (grey circles), $MLSS_{-5\%}$ (white circles), $Delta_{50}$ (dark grey diamonds), $LT_{-5\%}$ (white diamonds), and during $MLSS_{15}$ (light grey circles) and $MLSS_{45}$ (dark grey circles). For each variable, the average of the last 2 min of each condition were compared.

* Different from $TTF_{LT-5\%}$

‡ Different from $TTF_{MLSS-5\%}$

§ Different from TTF_{MLSSp}

¢ Different from $TTF_{MLSS+5\%}$

£ Different from TTF_{MLSS15}

∞ Different from TTF_{MLSS45}

($P < 0.05$)

Figure 2. Group mean data (with SD bars) (n=11) displaying the individual responses in TTF performance expressed as a percentage of TTF_b (A, B), and the reduction in TTF performance and each 30-min intensity expressed as a percentage of $MLSS_p$ (C) and each exercise duration at $MLSS_p$ (D). Correlations are based on the individual data and the black line represents the line of best fit.

* Different from $TTF_{LT\text{-}5\%}$

‡ Different from $TTF_{MLSS\text{-}5\%}$

§ Different from TTF_{MLSSp}

$^{¢}$ Different from $TTF_{MLSS+5\%}$

$^{£}$ Different from TTF_{MLSS15}

$^{\infty}$ Different from TTF_{MLSS45}

($P < 0.05$).

Figure 3. Group mean data (with SD bars) (n=11) displaying the relationship between the reduction in TTF performance expressed as a percentage of TTF_b, and $[La^-]_b$ (A, B) and RPE (C, D) evaluated immediately prior to the commencement of each TTF trial. Correlations are based on the individual data and the black line represents the line of best fit.

Figure 4. Group mean data (with SD bars) (n=11) displaying the total amount of work performed during each constant-PO and TTF trial (A, B), and the reduction in TTF in relation to the total work during each constant-PO condition (C, D). Correlations are based on the individual data and the black line represents the line of best fit.

* Different from $TTF_{LT\text{-}5\%}$

$^{£}$ Different from TTF_{MLSS15}

$^{\infty}$ Different from TTF_{MLSS45}

($P < 0.05$).