Metabolic and performance-related consequences of exercising at and slightly above MLSS

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Running head:

MLSS and the limit of exercise tolerance

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Abstract

Exercising at the maximal lactate steady state (MLSS) results in increased but stable metabolic responses. We tested the hypothesis that even a slight increase above MLSS (10 W), by altering the metabolic steady-state, would reduce exercise performance capacity. Eleven trained men in our study performed: one ramp-incremental tests; two to four 30-min constant-load cycling exercise trials to determine the PO at MLSS ($MLSS_p$), and ten watts above MLSS ($MLSS_{p+10}$), which were immediately followed by a time-to-exhaustion test; and a time-to-exhaustion test with no-prior exercise. Pulmonary O_2 uptake ($\dot{V}O_2$) and blood lactate concentration ($[La^-]_b$) as well as local muscle O_2 extraction ([HHb]) and muscle activity (EMG) of the vastus lateralis (VL) and rectus femoris (RF) muscles were measured during the testing sessions. When exercising at $MLSS_{p+10}$, although $\dot{V}O_2$ was stable, there was an increase in ventilatory responses and EMG activity, along with a non-stable $[La^-]_b$ response ($P<0.05$). The [HHb] of VL muscle achieved its apex at $MLSS_p$ with no additional increase above this intensity, whereas the [HHb] of RF progressively increased during $MLSS_{p+10}$ and achieved its apex during the time-to-exhaustion trials. Time-to-exhaustion performance was decreased after exercising at $MLSS_p$ (37.3±16.4 %) compared to the no-prior exercise condition, and further decreased after exercising at $MLSS_{p+10}$ (64.6±6.3 %) ($P<0.05$). In summary, exercising for 30 min slightly above MLSS led to significant alterations of metabolic responses which disproportionately compromised subsequent exercise performance. Furthermore, the [HHb] signal of VL seemed to achieve a "ceiling" at the intensity of exercise associated with MLSS.

Key words

Maximal lactate steady state, exercise tolerance, O_2 extraction, fatigue

Introduction

The maximal lactate steady state (MLSS) represents the highest intensity of exercise at which lactate production and utilization/removal are in equilibrium, so that blood lactate concentration ($[La^-]_b$) remains stable [1]. As a progressive acceleration of the glycolytic rate leading to $[La^-]_b$ accumulation occurs when constant-load exercise is performed above this intensity, MLSS, by definition, experimentally demarcates the upper boundary at which the aerobic metabolism solely accounts for the overall energy expenditure and metabolic responses are elevated but stabilized [1]. For these reasons MLSS is often defined as the physiological landmark separating the heavy from the severe exercise intensity domain.

The acute responses of exercising in these two different domains have been described quite extensively [2–5], with the critical power (CP) concept used in these studies as the paradigm to separate the heavy from the severe-intensity domain, which, similarly to MLSS, is supposed to identify the "critical intensity" of exercise (i.e., the highest) sustained exclusively by oxidative sources of energy [6–9]. However, as the estimation of this intensity through the CP model can be subject to errors, it could be possible that in some circumstances the highest and true metabolic steady state might be under- or over-predicted. For instance, CP presents a typical error that is usually reported to be within a 5% range [6] which might arise from biases associated with the protocols and models employed [10,11]. For example, Pringle et al. [12], and more recently Mattioni Maturana et al. [13], observed that the power output (PO) at CP was on average ~20 W higher than that at MLSS. Considering these potential differences, it might be possible that the metabolic disturbances associated with exercising in the severe-intensity domain have been only described in the literature at intensities greatly exceeding MLSS. Thus, it is unknown if and to what extent a very small increase in PO above MLSS (but within the reported typical error assigned to CP) would alter physiological responses and consequent maximal exercise performance.

In relation to the physiological responses associated with the upper limit of sustainable exercise (i.e., heavy-to-severe boundary), a growing amount of evidence has shown that, during ramp-incremental

cycling exercise to exhaustion, following a rather linear increase from the onset of exercise, the NIRS-derived deoxygenated haemoglobin [HHb] signal of the vastus lateralis (VL) muscle displays a breakpoint ($[HHb]_{BP}$) that occurs at ~75-85% of $\dot{V}O_{2peak}$ [14–20]. This leads to a plateau-like response in the [HHb] signal, with controlling mechanisms for this "apparent" upper limit still being a subject of debate [16,20]. The $[HHb]_{BP}$ has been linked to a metabolic rate similar to MLSS and CP [8], and is also seen (albeit with a different profile) in the rectus femoris (RF) muscle [19]. These distinctive responses in the [HHb] profiles are thought to reflect the changes in the dynamic balance between local muscle blood flow ($\dot{Q}_m$) and $\dot{V}O_2$ ($\dot{V}O_{2m}$) ($\dot{Q}_m$-to-$\dot{V}O_{2m}$ ratio) that results from alterations within the intra and extracellular environments as well as from variations in muscle fibres recruitment patterns that occur during incremental exercise [19,21–23]. However, during constant-load exercise when the intensity is sustained at or slightly above the PO associated with MLSS, the behaviour of the [HHb] response is unknown. Given the physiological alterations observed during exercise in the severe-intensity domain, it might be possible that local changes in the $\dot{Q}_m$-to-$\dot{V}O_{2m}$ ratio may affect local O_2 extraction. Thus, characterization of the behaviour of the [HHb] signal during prolonged constant-load exercise at, and slightly above the intensity associated with MLSS is warranted.

Therefore, the aim of this study was to *i*) evaluate the metabolic and performance-related consequences of exercising at an intensity slightly exceeding MLSS, and *ii*) characterize the behaviour of the local muscle oxygen extraction ([HHb]) signal in the VL and RF muscles during constant-load exercise. We tested the hypothesis that prolonged exercise sustained only 10 W (~3-5%) above MLSS would result in a significant increase in physiological variables linked to metabolic activity (i.e., $[La^-]_b$ and $\dot{V}O_2$) which would lead to a greater decrease in time-to-exhaustion performance compared to exercise sustained at the PO associated with MLSS. Furthermore, given the similar $\dot{V}O_2$ associated with the plateau of the [HHb] signal of the VL muscle (during ramp-incremental exercise) and MLSS, it was hypothesized that during constant-PO exercise the [HHb] signal of the VL muscle would achieve its peak values during constant-load exercise at the intensity

corresponding to MLSS, with additional increments above this intensity only possible in the RF muscle.

Material and Methods

Participants

Eleven recreationally-active men (31±10 yrs; 78.4±10.5 kg), who indicated during a preliminary interview that they routinely performed endurance training at least three times per week, voluntarily participated in the study. Participants were non-smokers, non-obese and not undergoing any medical treatment that could affect their cardiovascular responses to exercise. Participants were made aware of all testing procedures as well as the risks and benefits of participating in the study, and they all signed an informed consent. All procedures were approved by the Conjoint Health Research Ethics Board of the University of Calgary, in compliance with the Declaration of Helsinki.

Procedures

All participants completed the following testing procedures on an electromagnetically braked cycle ergometer (Velotron; RacerMate, Seattle, WA) within a 2-week period: *i*) One ramp-incremental tests to exhaustion *ii*) two to four, 30-min constant-PO rides for determination of the PO associated with MLSS ($MLSS_p$) and 10 W above MLSS ($MLSS_{p+10}$) immediately followed by time-to-exhaustion trials (i.e., TTE_{MLSSp} and $TTE_{MLSSp+10}$), and *iii*) a baseline time-to-exhaustion trial (i.e., not preceded by exercise) (TTE_b). All tests were carried in an environmentally controlled room (temperature: 19-20° C; humidity 50-60%) at a similar time of the day (the time could vary by ± 30 min). Prior to each exercise testing session, all participants were instructed to avoid consumption of food and caffeinated beverages for at least 2 and 8 hours, respectively, and to abstain from vigorous physical activity for 24 hours. A minimum of 48 hours and a maximum of 72 hours of rest was allowed between each visit. During each session participants were blinded to the PO and the elapsed time, but received visual feedback on their pedal cadence.

Ramp-incremental test. A ramp-incremental exercise test to the limit of tolerance was performed to determine $\dot{V}O_{2peak}$, the gas exchange threshold (GET), the respiratory compensation point (RCP), maximal heart rate (HR_{max}), peak PO (PO_{peak}), and the near-infrared spectroscopy (NIRS)-derived HHb-breakpoint ($[HHb]_{BP}$) of the VL muscle. After a 4-min period of cycling at 50 W, the PO was increased in a ramp-like manner by 30 $W \cdot min^{-1}$ (1 W every 2 s). Participants were instructed to cycle at their preferred cadence in a range between 75 and 95 rpm. The ramp-incremental test was stopped when participants failed to maintain the targeted cadence by 10 rpm for more than ten consecutive seconds despite strong verbal encouragement, or when volitional exhaustion ensued.

MLSS determination trials. On successive appointments, participants performed constant-PO tests of 30 min of duration. $MLSS_p$ corresponded to the highest PO that resulted in a difference in $[La^-]_b$ of less than 1 $mmol \cdot L^{-1}$ between the 10^{th} and 30^{th} min of exercise [24]. Each trial was initiated with a 4-min baseline cycling at 80 W after which the PO was instantaneously increased to a predetermined value. For all the constant-PO trials participants were asked to cycle at the preferred cadence established during the previous ramp-incremental test. To determine the resistance of the first constant-PO trial, participants were asked to self-select a load that could correspond to MLSS [25], from a set of proposed loads derived from a mathematical model for MLSS estimation recently developed in our laboratory [26]. Briefly, this mathematical model, by using the respiratory compensation point (RCP) (expressed in $W \cdot Kg^{-1}$), $\dot{V}O_{2peak}$ (expressed in $ml \cdot Kg^{-1} \cdot min^{-1}$), and body weight (Kg) as predictive variables, is capable to estimate with a high degree of accuracy the PO associated with MLSS. This model allowed us to notably reduce the number of laboratory visits necessary to determine $MLSS_p$. Regardless, the constant-PO rides were repeated as long as the criteria for $MLSS_p$ and $MLSS_{p+10}$ identification were satisfied. In this regard, depending on whether the $[La^-]_b$ response from the first 30-min constant-PO test was greater, or less (or equal) than 1 $mmol \cdot L^{-1}$, the PO for the subsequent constant-PO ride was either decreased or increased by 10 W, respectively. Five subjects exercised at $MLSS_{p+10}$ during the first determination trial, whereas the remaining six at $MLSS_p$, with this leading to a homogeneous distribution of the rides at and above $MLSS_p$. $[La^-]_b$ was assessed

during baseline (within the first 3-min) and every 5 min after the PO was increased. Furthermore, at the 10^{th} and 30^{th} min $[Lac]_b$ was taken in duplicates and the average of the two measures was used for subsequent analysis.

Time-to-exhaustion trials. At the end of each 30-min constant-PO test and after a three-minute recovery period while cycling at 50 W, participants performed a time-to-exhaustion trial with the resistance set at 80% of PO_{peak} previously recorded at the end of the ramp-incremental exercise. Participants were asked to maintain the same cadence as during the trials for MLSS determination. The test was continued until volitional exhaustion. A time-to-exhaustion trial preceded by only 4-min of baseline cycling (80 W) was also performed on a separate occasion at the end of the experimental protocol. The percentage of PO_{peak} during the time-to-exhaustion trials was selected to elicit exhaustion within approximately 5-6 min in the condition not preceded by the 30 min ride. Immediately after each time-to-exhaustion trial, a sample of capillary blood was taken for $[La^-]_b$ assessment.

Data collection

A metabolic cart (Quark CPET, Cosmed, Rome, Italy) was used to measure breath-by-breath gas exchanges. The turbine flowmeter was calibrated using a syringe of known volume (3 L). Gas analyzers were calibrated using a gas mixture of known concentration (16% O_2; 5% CO_2; balance N_2), as per the manufacturer recommendations. Heart rate (HR) was recorded using radiotelemetry (SP0180 Polar Transmitter; Polar Electro, Inc., Kempele, Finland) and $[La^-]_b$ was measured with a portable lactate analyzer (Lactate Scout, SensLab GmbH, Leipzig, Germany). Briefly, after wiping the finger with an alcohol swab and performing a pin-prick, a 2 µL capillary sample of whole blood was collected and immediately analyzed for determination of $[La^-]_b$.

A two-channel NIRS system (Oxiplex TS^{TM}, ISS, Champaign, IL) was used in our study to monitor local [HHb] and total-haemoglobin (*tot*[Hb]) signals. Each NIRS probe was composed of eight laser diodes operating at two wavelengths (λ = 690 and 828 nm, four at each wavelengths), which were

pulsed in rapid succession, and a photomultiplier tube. The lightweight plastic NIRS probes (connected to laser diodes and a photomultiplier tube by optical fibres) consisted of two parallel rows of light-emitting fibres and one detector fibre bundle; the source–detector separations for this probe were 2.0, 2.5, 3.0 and 3.5 cm for both wavelengths. The two NIRS probes were placed on the belly of the VL muscle (midpoint between the greater trochanter of the femur and the knee joint) and of the distal portion of the RF muscle of the right leg. The areas of placement were carefully prepared by shaving hair and gently wiping the skin. Double-sided tape as well as an elastic bandage were used to secure in place the probes. An optically dense, black vinyl sheet was used to cover the probes to avoid the intrusion of external light. The apparatus was calibrated on each testing day after a warm-up of at least 30 min, as per the manufacturer recommendations. Data were stored online at an output frequency of 2 Hz, and reduced to 1-s bins for all subsequent analyses within the present study. The areas of probes' placement in VL and RF were marked and recorded to ensure consistency for the following visits.

A multi-channel surface electromyography system (Delsys Inc, Boston, MA) was used for monitoring muscle activity (i.e., EMG) at a sampling rate of 1000 Hz. Two bipolar surface electrodes (41 × 20 × 5 mm) (DE-2.1, Delsys Inc. Boston, MA) were placed on the belly of the VL and RF muscles in close proximity (longitudinally) of the NIRS probes after the skin area was carefully prepared. Briefly, excessive hair was shaved and the area of placement was gently abraded and cleaned with alcohol swab to reduce skin impedance. Electrodes were secured in place by bi-adhesive and surgical tape, and were connected to an EMG amplifier which was connected to the acquisition apparatus (Power Lab, ADInstruments, Bella Vista, Australia) linked to a computer software (LabChart 8, ADInstruments, Bella Vista, Australia). Probe placement was recorded to ensure consistency between visits.

Data and statistical analyses

Ventilatory and gas exchange data. Two experimenters independently inspected the ramp-incremental test performed by each participant to identify GET and RCP from the gas exchange and

ventilatory variables that were plotted against $\dot{V}O_2$. In the circumstance of a disagreement of more than 100 ml·min^{-1}, the experimenters re-evaluated together the profiles to form a consensus. GET was determined as the point at which $\dot{V}CO_2$ began to increase out of proportion in relation to $\dot{V}O_2$, coincidental with a systematic rise in the $\dot{V}_E$-to-$\dot{V}O_2$ relation and end-tidal PO_2, whereas the ventilatory equivalent of $\dot{V}CO_2$ ($\dot{V}_E/\dot{V}CO_2$) and end-tidal PCO_2 were stable [27]. RCP was identified as the point at which end-tidal PCO_2 began to fall after a period of isocapnic buffering. This point was confirmed by examining the $\dot{V}_E/\dot{V}CO_2$ and $\dot{V}_E/\dot{V}O_2$ relationships as well as by identifying the second breakpoint in the $\dot{V}_E$-to-$\dot{V}O_2$ relation [28]. The highest $\dot{V}O_2$ value computed from a 30-s rolling average during the ramp-incremental test was considered as $\dot{V}O_{2peak}$. The same strategy was employed to determine $\dot{V}O_{2peak}$ during each time-to-exhaustion trial. PO_{peak} was the highest PO value recorded at the end of the ramp-incremental test for each participant. The $\dot{V}O_2$, $\dot{V}_E$, frequency of breathing (fB), and heart rate (HR), at $MLSS_p$ and $MLSS_{p+10}$ at the 10th and 30th minutes were calculated as the average of two minutes data surrounding the 10th minute (9th – 11th min) and the last two minutes, respectively, of the 30-min constant-PO exercise. Baseline $\dot{V}O_2$ and the average of the last 10 min of the $\dot{V}O_2$ response during the 30-min constant-PO exercise was used to compute the functional gain of the $\dot{V}O_2$ during each MLSS trial determination.

The $\dot{V}O_2$ mean response time of the ramp-incremental exercise test was determined as previously suggested [29]. Briefly, $\dot{V}O_2$ vs time was plotted and time-aligned such that time "zero" corresponded to the onset of the ramp portion of the test. A preliminary linear fit of the $\dot{V}O_2$ data during both the baseline and the ramp-incremental portion of the test was used to identify and remove data points lying ± 3 SD from the local mean. Afterwards, processed $\dot{V}O_2$ data were linearly interpolated to 1 s intervals and the best fit of a double-linear function applied from the last 2 min of the baseline to the previously established GET using non-linear least squares regression procedures (Origin, Origin Lab, Northampton, USA). The slope of the first segment of the double-linear was fixed at "0" and the breakpoint reflected the mean response time. The $\dot{V}O_2$ data were subsequently left-shifted by the

individual-specific mean response time and the $\dot{V}O_2$ as well as the PO corresponding to RCP were identified.

Adipose tissue thickness correction of [HHb] and tot[Hb] signals. Both the [HHb] and *tot*[Hb] signals were analysed after accounting for the adipose tissue thickness of each of the muscles investigated. Briefly, a Harpenden skin caliper (Baty Int., West Sussex, UK) was used to measure the adipose tissue thickness (mm) in the areas of NIRS probe placements. The same investigator took measurements in duplicate and the average of the two was used. In case of a discrepancy of more than 0.4 mm, a third measurement was taken and the average of the closest two was used. Subsequently, a linear regression analysis of the relationship between the adipose tissue thickness and resting *tot*[Hb] was calculated and the measured [HHb] and *tot*[Hb] data were corrected to a common adipose tissue thickness of 0 mm.

[HHb] during ramp incremental test. The [HHb] data recorded during the ramp-incremental test on the VL muscle were plotted against time and modeled with the following piece-wise "double-linear" fit, as previously described [14]:

$f = \text{if } (x < BP, g(x), h(x))$

$g(x) = i_1 + (s_1 \cdot x)$

$i_2 = i_1 + (s_1 \cdot BP)$

$h(x) = i_2 + (s_2 \cdot (x - BP))$

fit f to y,

where f is the double-linear function, x is time and y is [HHb], BP is the time coordinate corresponding to the interception of the two regression lines (i.e., $[HHb]_{BP}$), i_1 and i_2 are the intercepts of the first and second linear function, respectively, and s_1 and s_2 are the slopes. Model parameter estimates for each participant were determined by linear least-square regression analysis. A preliminary fit was used to identify and delete aberrant data that were ± 3 SD from the local mean.

The double linear fit was used from the onset of the systematic increase in the [HHb] signal until the last data point corresponding to the end of the test. A linear interpolation was then used to retrieve the $\dot{V}O_2$ (left shifted to account for the mean response time) and PO values corresponding to the $[HHb]_{BP}$. Finally, the slope of change in the [HHb] signal in the RF muscle was determined from linear regression in two distinctive portions of the response: below and above the visually determined point at which the [HHb] would systematically change its slope [30]. The slopes of increase/decrease in the signals and the values of $\dot{V}O_2$ and PO associated with the $[HHb]_{BP}$ for each individual from both ramp-incremental tests were averaged together.

Surface electromyography. The EMG data recorded during the 30-min constant-PO exercise and subsequent time-to-exhaustion trials was amplified, band-pass filtered (5 – 500 Hz), rectified, and computed as 1-s root mean square (RMS) amplitude. Subsequently the processed EMG data were normalized to the averaged last two minutes of the baseline cycling at 80 W. This normalization strategy was chosen as it is representative of the actual dynamic muscular patterns during cycling.

[HHb], tot[Hb], and EMG data during 30-min constant-PO and time-to-exhaustion trials. The [HHb], *tot*[Hb], and EMG data were plotted against time and the following bin-averaging strategy was employed at the time points of interest for successive statistical analysis: *bsln1*: last two minutes of the 4-min baseline cycling at 80 W; *10^{th} min*: two minutes surrounding the 10^{th} minute mark; *30^{th} min*: last two minutes of the 30-min constant-PO exercise; *bsln2*: last two minutes of the 3-min recovery period while cycling at 50 W; *TTE*: last 30 seconds of the time-to-exhaustion trial.

Rating of Perceived Effort. A 0-10 rating of perceived effort (RPE) scale was used to monitor perceptual responses to exercise. The scale was displayed to the participants during baseline and every five minutes during the rides for MLSS determination and immediately at the end of every time-to-exhaustion tests.

Statistics. Data are presented as means ± SD. All statistics were performed using SPSS version 23 (SPSS, IBM, Chicago, IL). A repeated-measures ANOVA was used to compare *i)* the $\dot{V}O_2$ values

corresponding to $MLSS_p$, RCP, and $[HHb]_{BP}$; *ii)* the time duration of each time-to-exhaustion trial performed *iii)* the peak ventilatory, gas exchange, $[La^-]_b$, and RPE values recorded at end of the ramp-incremental test and at the end of each time-to-exhaustion trial; *iv)* changes in $\dot{V}O_2$, $\dot{V}_E$, HR, $[La^-]_b$, [HHb], *tot*[Hb], and EMG variables from min 10 to min 30 during the 30-min constant-PO trials and time-to-exhaustion trials; *v)* $\dot{V}O_2$, $\dot{V}_E$, fB, HR, $[La^-]_b$, RER, and RPE responses between $MLSS_p$ and $MLSS_{p+10}$. Where appropriate, a Bonferroni post hoc was applied. Statistical significance was accepted at $\alpha < 0.05$.

Results

The average PO_{peak} recorded at the end of the ramp-incremental cycling test was 396±55 W. The average 80% of PO_{peak} used for all the time-to-exhaustion trials was 317±43 W. Peak physiological responses measured at the end of ramp-incremental, TTE_{MLSSp}, $TTE_{MLSSp+10}$ and TTE_b tests are shown in Table 1. The $\dot{V}O_{2peak}$ recorded during $TTE_{MLSSp+10}$ was approximately 7% lower than the $\dot{V}O_{2peak}$ values recorded during the other TTE and ramp-incremental tests (P<0.05). A lower peak ventilatory response (~10%) was also found at the end of the $TTE_{MLSSp+10}$ compared to the other exercise conditions (P<0.05). The absolute durations of TTE_b, TTE_{MLSSp}, and $TTE_{MLSSp+10}$ were 349±78 s, 220±75 s, and 125±40 s, respectively. The percent decreases in TTE_{MLSSp} and $TTE_{MLSSp+10}$ tests compared to TTE_b were 37.3±16.4 % and 64.6±6.3 %, respectively (P<0.05) (Figure 1).

The $\dot{V}O_2$ corresponding to RCP (3.29±0.48 $L \cdot min^{-1}$ or 79.2±4.0% of $\dot{V}O_{2max}$), $MLSS_p$ (3.31±0.53 $L \cdot min^{-1}$ or 79.5±5.3% of $\dot{V}O_{max}$) and $[HHb]_{BP}$ of the VL muscle (3.44±0.5 $L \cdot min^{-1}$ or 84.5±8.3% of $\dot{V}O_{2peak}$) were not different (P>0.05). The PO at RCP, $MLSS_p$, and $[HHb]_{BP}$ were 270±48 W (68±5% of PO_{peak}), 231±49 W (58±5% of PO_{peak}), and 292±46 (74±6% of PO_{peak}), respectively, and were different from each other (P<0.05). Baseline and peak values for the [HHb] signal from the ramp-incremental test were 28.1±11.2 and 43.6±20.1 for the VL muscle, and 18.2±6.3 and 29.7±11.2 for the RF muscle, respectively. The slopes of the linear regression in the [HHb] signal before and after the $[HHb]_{BP}$ of the VL muscle during the ramp-incremental test were 0.032±0.017 and 0.010±0.013,

respectively (P<0.05). In the RF muscle these slopes in the [HHb] signal, calculated below and above ~80% of the $\dot{V}O_{2peak}$, were 0.012±0.011 and 0.043±0.038, respectively (P<0.05).

Figure 2 shows $\dot{V}O_2$, V_E, fB, HR, $[La^-]_b$, and RPE responses during the 30-min constant-PO trials at $MLSS_p$ and $MLSS_{p+10}$. The $\dot{V}O_2$ response stabilized during both $MLSS_p$ (min 10, 3.27±0.58 L·min^{-1}; min 30, 3.30±0.51 L·min^{-1}; P>0.05) and $MLSS_{p+10}$ trials (min 10, 3.41±0.56 L·min^{-1}; min 30, 3.46±0.52 L·min^{-1}) (P>0.05), despite a more pronounced upward drift during this latter condition. The $\dot{V}O_2$ functional gain was lower during $MLSS_p$ (10.1±1.2 ml·W^{-1}) than during $MLSS_{p+10}$ (10.6±1.2 ml·W^{-1}) (P<0.05). $\dot{V}_E$ increased from min 10 (102±18 L·min^{-1}) to min 30 (108±20 L·min^{-1}; +5.8%) during $MLSS_p$ (P<0.05) and it increased to a greater extent during $MLSS_{p+10}$ (min 10, 115±22 L·min^{-1}; min 30, 131±25 L·min^{-1}; +13.8%; P<0.05). fB during $MLSS_p$ was 39.1±7.7 breath·min^{-1} at min 10 and 42.5±8.1 breath·min^{-1} at min 30; during $MLSS_{p+10}$ fB was 42.7±8.0 breath·min^{-1} at min 10 and 50.8±11.9 breath·min^{-1} at min 30. The magnitude of change of fB was greater during $MLSS_{p+10}$ (+18.9±7.5%) than during $MLSS_p$ (+8.7±3.2%). HR rose significantly from min 10 to min 30 in both conditions [min 10, 156±9 bpm; min 30, 162±9 bpm (+3.8%) during $MLSS_p$ (P<0.05); min 10, 162±9 bpm; min 30, 169±9 bpm (4.3%) during $MLSS_{p+10}$ (P<0.05)], and it was higher during $MLSS_{p+10}$ compared to $MLSS_p$ (P<0.05). $[La^-]_b$ values were higher during $MLSS_{p+10}$ compared to $MLSS_p$. During $MLSS_p$, $[La^-]_b$ was 5.1±1.8 mMol·L^{-1} at min 10 and 5.6±1.7 mMol·L^{-1} at min 30 (Δ=0.5±0.3 mMol·L^{-1}). During $MLSS_{p+10}$, $[La^-]_b$ raised from 6.2±1.9 mMol·L^{-1} at min 10 to 7.9±2.3 mMol·L^{-1} at min 30 (Δ=1.7±1.1 mMol·L^{-1}) (P<0.05). The delta change in $[La^-]_b$ from min 10 to min 30 was greater during $MLSS_{p+10}$ compared to $MLSS_p$ (P<0.05). RER values during $MLSS_{p+10}$ were greater than during $MLSS_p$ (0.97±0.05 vs. 0.95±0.04 respectively, P<0.05). RPE values were consistently higher throughout the exercise at $MLSS_{p+10}$ compared to $MLSS_p$ (P<0.05).

Table 2 and Table 3 summarize the values of [HHb] and *tot*[Hb], respectively, for each time point of interest during the constant-PO tests in the VL and RF muscles. Figure 3 displays the group mean profile for [HHb], *tot*[Hb], and EMG (RMS) for VL and RF muscles during $MLSS_p$, $MLSS_{p+10}$, and the subsequent time-to-exhaustion trials. The [HHb] signal in the VL muscle was stable during

$MLSS_p$ as well as during $MLSS_{p+10}$ and no further increase in the [HHb] signal was observed during the time-to-exhaustion trials performed immediately after each condition (P>0.05). The [HHb] signal was stable in the RF muscle throughout the trials performed at $MLSS_p$ but progressively increased during the trial at $MLSS_{p+10}$ so that the values were greater at 30 min compared to 10 min (P<0.05). Furthermore, during the TTE performed following the $MLSS_{+10}$ ride, the [HHb] signal in the RF muscle rose above the levels achieved during the previous 30-min constant-PO trials (P<0.05). The *tot*[Hb] values remained unchanged in the VL muscle at min 10 and min 30 during both $MLSS_p$ and $MLSS_{p+10}$ trials as well as during the TTE trials that followed each of those tests (P>0.05). On the other hand, the *tot*[Hb] values progressively increased in the RF muscle in both the $MLSS_p$ and $MLSS_{p+10}$ trials from min 10 to min 30 (P<0.05), with a further significant increase observed during TTE_{MLSSp} and $TTE_{MLSSp+10}$ (P<0.05). The baseline RMS values for VL and RF during $MLSS_p$ were 0.032±0.016 and 0.090±0.086, respectively. The baseline RMS values for VL and RF during $MLSS_{p+10}$ were 0.031±0.021 and 0.089±0.083, respectively. The EMG signal of the VL muscle increased progressively from min 10 to min 30 during $MLSS_p$ (from 85±73% to 132±159%) and $MLSS_{p+10}$ (from 180±126% to 224±187%) (P<0.05). The increases in the EMG signal of VL during the TTE_{MLSSp} and $TTE_{MLSSp+10}$ from baseline were 276±159% and 282±240%, respectively (P<0.05). In the RF muscle, the EMG signal was stable during $MLSS_p$ (min 10, 111±99%; min 30, 98±153%) but progressively rose during $MLSS_{p+10}$ (min 10, 76±111%; min 30, 108±237%, (P<0.05)). During the TTE_{MLSSp} and $TTE_{MLSSp+10}$, the EMG signal of RF increased to 337±355% and 253±321% from baseline, respectively (P<0.05).

Discussion

The novel findings of the study were that: *1)* the greater increases in physiological responses while exercising only 10 W (~100 $ml \cdot kg^{-1} \cdot min^{-1}$) above MLSS remarkably reduced time-to-exhaustion performance by approximately 50%; *2)* participants achieved lower $\dot{V}O_{2peak}$ values during the $TTE_{MLSSp+10}$ compared to those recorded during the TTE_{MLSSp}, TTE_b, and ramp-incremental test; *3)* the [HHb] signal in the VL muscle achieved its peak amplitude at the intensity of exercise associated

with MLSS, with no further increases during $MLSS_{p+10}$ or any of the subsequent time-to-exhaustion trials; *4*) the behaviour of the [HHb] signal in the RF muscle markedly differed from that of the VL muscle, as it rose progressively when exercising at $MLSS_{p+10}$ and increased further during the following time-to-exhaustion trial.

Physiological implications of exercising at a PO associated with and slightly above MLSS.

A stable $\dot{V}O_2$ response was observed during $MLSS_p$ and, contrary to our hypothesis, also during $MLSS_{p+10}$. However, when exercising at $MLSS_{p+10}$ a progressive accumulation of blood lactate and a greater ventilatory response as well as greater rating of perceived effort occurred. An accumulation of lactate in the blood that cannot be stabilized is indicative of an accelerated glycolytic flux, as well as an inability for lactate clearance to match lactate production [31]. Concomitantly with the accumulation of lactate, a greater ventilatory response when exercising at $MLSS_{p+10}$ was also observed. Multiple factors might have contributed to this exacerbated response, such as augmented metabolic acidosis, body temperature, respiratory muscle fatigue, and perception of effort [2,32–34]. The greater increase in $\dot{V}_E$, along with the accumulation of lactate, seems to be a defining characteristic of exercising slightly above MLSS (compared to "at" MLSS).

The stability in the $\dot{V}O_2$ response while exercising at $MLSS_{p+10}$ is in contrast with a previous finding that showed a progressive rise in $\dot{V}O_2$ throughout the constant-PO trial above MLSS [12]. The reason for these contrasting results may lie on the different delta PO adopted. Indeed, while the present study employed a 10 W difference between the exercise trials for MLSS determination, the other investigation used on average a much larger increase (19 W). Increments greater than 10 W may induce non-stable $\dot{V}O_2$ responses when exercising above MLSS and may not provide sufficient sensitivity for identifying the highest PO associated with a stable lactate response (i.e., MLSS). In this perspective, these findings highlight that, despite the metabolic perturbations (e.g., lactate accumulation, greater increase in $\dot{V}_E$) and greater functional gain (~0.5 $ml \cdot W^{-1}$), $\dot{V}O_2$ can still appear to stabilize if the increment in metabolic demand above MLSS is relatively small. This emphasizes the fact that a stable $\dot{V}O_2$ response is not necessarily reflective of the overall metabolic steady state

during exercise at this intensity. This suggestion is supported by a previous study that demonstrated that a 30-min constant-PO exercise performed at the PO associated with MLSS altered pH and blood ammonia concentration, in spite of stable $\dot{V}O_2$, $\dot{V}_E$, and $[La^-]_b$ responses [35]. As suggested by these authors, MLSS may represent the highest intensity of exercise at which, despite some ongoing physiological changes, compensatory mechanisms may still be able to preserve overall metabolic steady-state. This also implies that if a careful "validation" of the individual critical intensity during dynamic exercise (i.e., cycling, running) is required, this should be performed by monitoring $[La^-]_b$ in response to small changes in sustained PO.

The progressive loss of metabolic steady-state and increased fatigue accumulation is also evidenced by the increased EMG activity of VL during $MLSS_p$ and $MLSS_{p+10}$. On the other hand, the EMG activity of the RF was stable during $MLSS_p$ but progressively increased during $MLSS_{p+10}$. Further increases were then observed in both muscles during the time-to-exhaustion trials. The increased central motor drive (firing rate and/or motor unit recruitment) observed during the 30-min constant-PO exercises may reflect the necessity to compensate for the progressive failure of peripheral contractile capacity as motor units fatigue [36]. In the case of the RF, it may also signify a greater need to support the action of the prime mover muscle (i.e., VL). The RF is a bi-articular muscle and a relatively greater activation of this muscle would be particularly needed to enhance hip flexion during the recovery phase of the pedal cycle and the generation of forward force during the early phase of the knee extension [37].

Performance implications of exercising at a PO associated with and slightly above MLSS.

The consequences of exercising at and slightly above MLSS on exercise performance capacity are highlighted by the reduction in the time-to-exhaustion tests. According to the traditional view, any exercise carried at an intensity equivalent or lower than that supposedly eliciting the highest but stable metabolic responses (i.e., MLSS and/or CP) can be sustained indefinitely without fatigue accumulation [38]. However, the present study demonstrates that exercising for 30 minutes at MLSS, with elevated but stable metabolic responses (i.e., $[La^-]_b$ and $\dot{V}O_2$), results in a significant reduction

in subsequent exercise performance. This is in agreement with a recent study showing that exercising for 2 h in the heavy-intensity domain (at an intensity even lower than the one used in the present study) reduced subsequent work capacity carried in the severe-intensity domain [39].

In addition to this, the present study found a disproportionate decrement in time-to-exhaustion performance (~50%) after exercising at $MLSS_{p+10}$ which was accompanied by a decrease in $\dot{V}O_{2peak}$ values (~7%) observed during the $TTE_{MLSSp+10}$. This reduction in performance highlights the "non-linear" nature of the decrease in maximal exercise capacity when sustaining work in the severe-intensity domain which may be caused by a significant greater decrease of glycogen stores and/or a disproportionate build-up of metabolites. Regarding the lower $\dot{V}O_{2peak}$ values recorded, although a decreased maximal capacity to deliver and/or utilize O_2 cannot be excluded, it can be possible that in some subjects (n=4) the premature exhaustion, that occurred within 1.5 min, could have limited the full expression of the $\dot{V}O_2$ kinetics, thus truncating the $\dot{V}O_2$ response before the achievement of peak values. Furthermore, the contribution of the higher perception of effort after exercising at $MLSS_{p+10}$ must be also considered when explaining this greater reduction of performance. Indeed, RPE values were consistently higher during exercise at $MLSS_{p+10}$ compared to $MLSS_p$. Thus, a higher perception of effort at the onset of the $TTE_{MLSSp+10}$ may have contributed to a more rapid achievement of maximal RPE and an earlier termination of the exercise (i.e., task disengagement). This effort-based decision to disengage from exhaustive exercise prematurely is postulated to occur when individuals, due to a "higher-than-normal" perception of effort, are no longer willing to exert effort [32,40].

After exercising in the severe-intensity domain at a PO substantially above MLSS a reduction in performance is to be expected. The findings from the present study are notable as they demonstrate that even a very small increase in PO (+10 W, ~3-5%) above MLSS, reflected in a ~100 $ml \cdot min^{-1}$ increase in $\dot{V}O_2$, results in a progressive rise in $[La^-]_b$ and disproportionately impairs subsequent exercise performance. This is an important observation, as most methods utilized to estimate the PO associated with MLSS, and similar thresholds or critical intensities (supposedly eliciting stable $[La^-]_b$ and $\dot{V}O_2$ responses) generally have an error in their estimate that is greater than 10 W [12,13]. Where

an accurate determination of the heavy-to-severe intensity boundary of exercise is required (e.g., for exercise prescription), the PO value estimated from any model should be carefully confirmed against validated protocols and physiological criteria to minimise unintended responses.

Muscle deoxygenation during constant-PO and time-to-exhaustion trials in the VL and RF muscles.

The exercise design adopted in the current study allowed, for the first time, for the characterization of the [HHb] profiles at exercise intensities surrounding MLSS. In accordance to our hypothesis, these findings experimentally demonstrated what previous investigations describing the [HHb] signal during ramp-incremental test had speculated [8,18]. That is, the achievement of peak values in the [HHb] signal of the VL muscle at a metabolic rate corresponding to MLSS, that is independent from the exercise protocol adopted (i.e., ramp-incremental versus constant-PO exercise), and the existence of a potential for further increase in this signal in the RF muscle beyond this intensity of exercise.

Overall, these data corroborate previous evidence highlighting the diverse relative contribution of each of the muscles (or muscle portions) engaged in the exercise task to the whole-body arteriovenous O_2 difference [16,19,21,30] which arises from the heterogeneity in motor unit recruitment patterns [19,21], fibre-type expression [23], and the resulting vascular dynamic controls [22,23] that characterize the active muscles. What is interesting is that the greater muscle activity in the VL muscle (i.e., greater EMG signal) during $MLSS_{p+10}$ and subsequent time-to-exhaustion trials was not accompanied by an increase in the [HHb] signal (in contrast to the RF muscle). It could be expected that the additional recruitment of muscle fibres would be accompanied by a greater fractional O_2 extraction, reflected in a rise in the [HHb] signal. However, this was clearly not the case in the VL muscle. These differences between the VL and RF [HHb] signals resemble those observed in this and previous studies[19,21] when exercising above the RCP during ramp-incremental test.

The physiological mechanisms underlying these different patterns of behaviour of the VL and RF muscles in regulating the delivery and utilization of O_2 are a current topic of debate [15,16,20,21,41–43]. What is important in relation to the findings of the present study is that these behaviour patterns might

be reflective of the metabolic changes occurring when transitioning from the heavy to the severe exercise intensity domain. In this perspective, these may be representative not only of divergent local capacities to regulate the $\dot{Q}_m$-to-$\dot{V}O_{2m}$ ratio, but they may also be indicative of the fact that some muscle areas (i.e., VL vs RF) may contribute more than others to the rise in metabolites linked to the progressive acceleration of the glycolytic flux and fatigue accumulation when exercising in the severe-intensity domain.

Limitations

The present study used the PO corresponding to MLSS to separate the heavy from the severe-intensity domain. Although MLSS experimentally determines the highest PO associated with elevated but "stable" metabolic responses (e.g., stable $[La^{-1}]_b$), it is important to acknowledge that this "method" also present a measurement error. For example, in the current study the measurement error could have been as high as 9 W (given the 10 W delta we used), leading to an underestimation of the PO corresponding with MLSS. Considering this, it is possible that in some circumstances the increase in PO above the "true" MLSS (during the $MLSS_{p+10}$ condition) could have been even less than 10 W. However, this observation strengthens the interpretation of the findings of the present study.

It is important to acknowledge that TTE_b was always performed as the last testing session. Although we cannot exclude that this choice exacerbated the differences in time-to-exhaustion performance (e.g., due to a possible greater motivation of the participants), however, considering that *i*) the rides at MLSSp and $MLSS_{p+10}$ were evenly distributed with marked differences recorded, *ii*) the $\dot{V}O_{2peak}$ values were similar between TTE_{MLSSp} and TTE_b, and *iii*) also the RPE values recorded at the end of the time-to-exhaustion trials were similar across the tested conditions, we believe that this factor marginally contributed to the observed differences.

Another aspect to consider is the interpretation of the EMG data. Although we attempted to reduce the variability of the EMG signal by normalizing it against baseline cycling at 80 W [19,44], it is important to acknowledge that this variability remained quite large. Furthermore, we cannot ascertain

whether an increase in the EMG signal is truly reflective of differences in muscle fibres recruitment (e.g., greater number of muscle fibres recruited reflected in a greater force production). Therefore, the EMG findings should be interpreted with some caution.

Perspective

The novelty of the present study is that we used a very small increase in PO (10 W) above the MLSS to evaluate how such small increments in PO affect metabolic responses and consequent performance capacity. We found that exercising for 30 min slightly above the PO at MLSS disproportionally reduces subsequent exercise performance. The findings from this study have important implications for exercise prescription and they suggest that the adoption of stringent methods is required when establishing the heavy-to-severe exercise intensity boundary with accuracy. Furthermore, the different dynamics in the behavior of the [HHb] signal confirms that the regulation of the delivery and utilization of O_2 is different across the quadriceps muscles, and may reveal that metabolic perturbations of exercising in the severe-intensity domain may be greater in some muscles compared to others. However, further investigations will be needed to clarify the specific influence of these heterogeneities when exercising above MLSS.

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Figure 1. Time-to-exhaustion performance recorded at baseline (TTE_b), after exercising at $MLSS_p$ (TTE_{MLSSp}), and $MLSS_{p+10}$ ($TTE_{MLSSp+10}$).

***** different than TTE_b; **§** different than TTE_{MLSSp}

Figure 2. Group mean data (with SD bars) displaying $\dot{V}O_2$ (*A*) ventilation ($\dot{V}_E$) (*B*), frequency of breathing (fB) (*C*), heart rate (*D*), blood lactate concentration ($[La^-]_b$) (*E*), and Rating of Perceived Exertion (RPE) (*F*) during 30-min constant-PO trials at $MLSS_p$ (open circles) and $MLSS_{p+10}$ (grey circles). Refer to results section for statistical significances.

Figure 3. Group mean (with SD bars) data displaying NIRS-derived local deoxygenated haemoglobin ([HHb]) (*panels A and B*) and total haemoglobin (*tot*[Hb]) (*panels C and D*) signals as well as EMG (*panels E and F*) signal during 30-min constant-PO trials at $MLSS_p$ (left panels) and $MLSS_{p+10}$ (right panels) for VL (open circles) and RF (grey circles). Refer to Table 2, Table 3, and results section for statistical significances.