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HEART RATE VARIABILITY ADJUSTMENTS DURING CYCLING EXERCISE AT DIFFERENT INTENSITIES UNTIL EXHAUSTION

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Abstract: Heart rate variability (HRV) is a useful tool to assess the autonomic system modulation during exercise, however to best of our knowledge no one has been verified the responses of HRV during constant workload cycling exercise until exhaustion at two different intensities. **Purpose:** We sought to assess the HRV and physiological changes during cycling exercise until exhaustion at the first lactate threshold (LT_1) and above the second lactate threshold. **Methods:** After a preliminary maximal incremental test to assess the peak power output (W_{PEAK}), subjects (n = 10) performed a constant workload cycling test until exhaustion at two different intensities. In a randomized and counterbalanced order, cycling until exhaustion either at LT_1 or at 25% of the difference between LT_2 and the W_{PEAK} (> LT_2). HRV, blood samples and ratings of perceived exertion were collected throughout both exercise sessions. **Results:** A total parasympathetic withdrawal (SD1 < 3ms) occurs since from exercise beginning at exercise > LT_2 and just at 60% ($p < 0.05$). High Frequency (HF) also showed a main effect of time at LT_1 exercise and time-intensity interaction from 20 to 80% of total exercise ($p < 0.05$). Regarding physiological variables, norepinephrine was increased progressively at > LT_2 ($p < 0.05$) but not at LT_1 ($p > 0.05$). Additionally, respiratory ratio was increased significantly ($p < 0.05$) throughout the exercise at both intensities. **Conclusion:** Our results suggested that HRV and respiratory rate doesn't elicit a steady state behavior neither at LT_1 not > LT_2 and the exercise performed at > LT_2 intensity lead to a parasympathetic withdrawal since from beginning of the exercise while at LT_1 just from 60% of the exercise.

Keywords: Autonomic control, Cycling performance, Catecholamine

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Introduction

Constant workload exercises are traditional and useful exercise mode to investigate physiological responses. It has been suggested that exercises performed at or below the first lactate threshold (LT_1), present a complete steady state in metabolic (i.e. lactate, pH, hormones, etc.) and cardiopulmonary variables[1,2]. In contrast, the traditional understanding further supports that high intensity exercises, which are performed in exercise intensities above the second lactate threshold (LT_2), elicit a progressive increase in metabolic and cardiopulmonary variables, thus representing an exercise intensity in which a physiological steady state cannot be ensured[3,1,4]. However, some studies have challenged the presence of a complete physiological steady state even at LT_1[2,5,4,6]. For example, it was showed that metabolic responses like as epinephrine and norepinephrine, ammonia[6], heart rate (HR)[5] and ratings of perceived exertion[2,5,4] doesn't present a steady state at LT_1. Cardiovascular responses such as autonomic adjustment, surprisingly wasn't yet been studied during cycling exercise test.

Cardiovascular responses are influence by circulating catecholamine's, autonomic nervous system (ANS) activity and respiratory-mechanical rhythms, this last due changes in activation of the sinus node[7]. In this regard, heart rate variability (HRV) assessment is a noninvasive tool to evaluate the autonomic regulation of the cardiovascular system during the exercise, thereby providing important responses regard the sympathovagal balance[8,9]. Currently, is well accepted the use of different responses from HRV during exercise such as time or frequency domain (like a VLF, LF, HF, for example), or geometric and nonlinear methods (like a SD1 and SD2)[10,11,12,13]. Interestingly, several studies have related HRV responses to exercise to physical exercise capacity[14,15], even though just few studies discuss the impact of different exercise intensity and duration on HRV responses[10,16].

The HRV responses during exercise is frequently studied throughout incremental tests[17,18,19] but these responses during constant workload test until exhaustion are poorly understood. For example, Cottin et al.[17] demonstrated that during an incremental exhaustive cycling test, occurs a vagal withdrawal (measured as high frequency (HF) spectral energy) between 60 – 80% of the test. Regard the HRV responses, Cottin et al.[16] showed a prevalence of low-frequency (LF) spectral energy when exercise was performed at moderate exercise (30% below the power at $\dot{V}O_{2MAX}$) while during heavy exercise (all out 10% above the power at $\dot{V}O_{2MAX}$). These results together with others enable the identification of the domains of exercise intensity through indexes derived from HRV.

In this regard, it's might be hypothesized that circulating catecholamine and respiratory

rate would be associate with HRV changes during cycling exercise until exhaustion at different intensities since these variables don't stabilize during this constant workload exercise[5,2]. Surprisingly, no study has been designed to date to investigate HRV response during constant workload cycling exercise performed until exhaustion at different intensities. Therefore, the aim of this study was to verify the HRV adjustments during cycling exercise at first lactate threshold (LT_1) and at 25% of the difference between LT_2 and peak power output (W_{PEAK}).

Our hypothesis is that circulating catecholamines are correlated to HRV responses during cycling exercise and both doesn't show steady state until exhaustion.

Methods

Subjects and Design

Ten healthy men, recreational level of cycling, (28.1 ± 4.5 years, height of 177.7 ± 4.6 cm, weight of 82.0 ± 9.7 kg, and VO_{2PEAK} of 46.8 ± 4.0 $mL \cdot kg^{-1} \cdot min^{-1}$) volunteered to take part in this study. The sample size was calculated by Gpower3.0 (Statistical power of 80%). The benefits and risks of the procedures were fully explained before the participants assign a written consent form. The study procedures were approved by the Ethics Committee of the School of Physical Education and Sport of the University of São Paulo and were conducted according to the Helsinki declaration.

After the familiarization visit, a repeated-measures experimental design, comprising a maximal incremental cycling test during the first experimental visit, followed by two visits where the volunteers performed 15 minutes baseline HRV and constant workload cycling test until exhaustion at the power output correspondent to the LT_1 and 25% between the LT_2 power output and W_{PEAK} (> LT_2) (Figure 1).

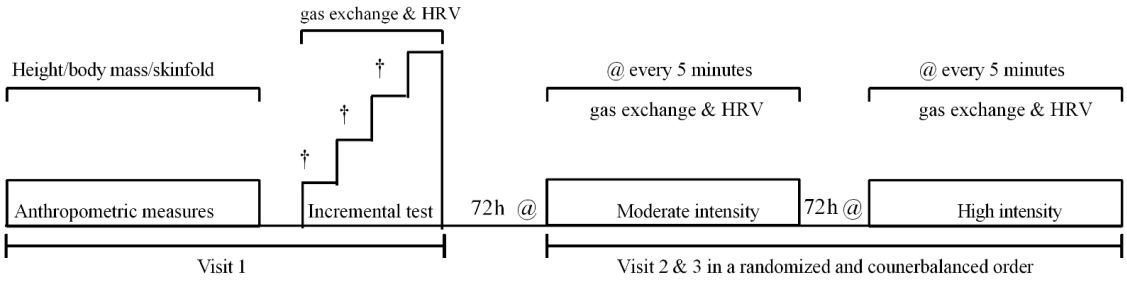

Figure 1. Experimental design. HRV = Heart hate variability.

Both tests were conducted in a randomized and counterbalanced order and interspersed by at least of 72 h to avoid any potential residual fatigue effects from the precedent test. The

maximal incremental cycling test was performed to identify the power output corresponding to LT_1 and LT_2 as well as the peak power output (W_{PEAK}). Constant workload cycling bouts with intensity set at LT_1 power output and at 25 % of the difference between LT_2 power output and W_{PEAK} (>LT_2) were perform until exhaustion with pedal cadence at ~ 70 rpm. Exhaustion was determined when subjects were unable to maintain the target pedal cadence, despite strong verbal encouragement. Cardiopulmonary, plasma catecholamine and HRV responses were obtained throughout all exercise bouts. All tests were carried out in an electrical magnetically-braked cycle ergometer (Standart Lannoy Ergometer, Godart-Statham, Bilthoven, Holland) at the same time of the day, in climatized and humidity-controlled room (23 – 25 °C). Participants were instructed to refrain from intense exercise as well as from the consumption of caffeine, alcoholic beverages or any stimulating substances for at least 24 h before each experimental session.

Procedures and measurements during the maximal incremental cycling test

After a 3 min warm-up cycling at 50 W, participants underwent a maximal incremental cycling test with 20 W increases every 3 min until exhaustion[20]. Verbal encouragement was provided to ensure that maximal effort was reached. Twenty-five microliters (25 µL) of arterialized blood samples were collected from the ear lobe at the end of each stage and immediately analyzed to determine the blood lactate concentration (BLC) (YSI 1500 Sport. Yellow Springs. USA). Gaseous exchange was sampled continuously breath -by-breath through a mask coupled to an open circuit gas analyzer (Quark b2, Cosmed, Rome, Italy), providing measures like as ventilation (VE), oxygen uptake (VO_2) and carbon dioxide (VCO_2). Breath-by-breath VO_2 data was averaged to 10s, so that the $VO2_{PEAK}$ was calculated as de mean of the three highest VO_2 values in the last 60 seconds of the test[21]. The W_{PEAK} was defined as the maximal power output attained at exhaustion. The BLC was plotted as a function of the power output and LT_1 and LT_2 were visually determined by two independent evaluators as the LT_1 and second LT_2 intercept of a linear regression, as reported elsewhere.

Constant Workload Cycling Test

The constant workload cycling tests were performed at LT_1 and > LT_2 and until voluntary exhaustion. During all trials, subjects could drink water *ad libitum*. To avoid any circadian variation, all the tests were performed at the same time day. Before each trial a catheter was inserted in a brachial vein for venous blood samples acquisition. The blood samples (10 ml)

were collected at rest and every 5-min interval between 0 and 30 min of exercise, and every 15-min interval from 30 min of exercise to exhaustion. The blood samples were collected into refrigerated EDTA-treated tubes and centrifuged (1690 g at 3°C) immediately after acquisition. The plasma was stored at -80 ºC for *a posteriori* analysis. Gaseous exchange (Quark b2, Cosmed, Rome, Italy) and HRV (s810 Polar®, Kempele, Finland) were continuously sampled as described for the maximal incremental cycling test, whereas RPE was measured at the blood sampling intervals.

Data Analysis

HRV responses as R-R intervals data set, were obtained at rest (15 min sited in a comfortable chair) and throughout the constant workload cycling bouts. So, HRV parameters of frequency-domain, such as HF and non-linear domain analysis (SD1) were calculate using a free software provided by the Biosignal Analysis and Medical Imaging Group, from the Department of Applied Physics, University of Kuopio, Kuopio, Finland (Kubios v2 Heart Rate Variability software). Initially, data were visually inspected to identify artifacts, premature and ectopic beats (< 3%), which were manually removed and replaced by interpolating the adjacent R-R intervals. Data from both dependent variables (frequency domains (ms^2)) and nonlinear (ms) measures) were expressed as milliseconds (ms). Due to the different exercise durations, data of HRV R-R intervals were decomposed into 5 segments, according to percentages of the total exercise duration (20%, 40%, 60%, 80% and 100%). Furthermore, values of SD1 below 3 ms were assumed as a total parasympathetic withdrawal and an increase in sympathetic activity, as suggested elsewhere[22]. It has been suggested that 3 ms SD1 values may represent the minimum HRV during dynamic exercises, so that the exercise intensity at which SD1 reaches 3 ms would correspond to the anaerobic threshold intensity in incremental test[10,22].

Data of VO_2, VE and respiratory rate (RR) obtained from the 5^{th} min of exercise were averaged to 10 s intervals, and then plotted as a function of the exercise duration. Accordingly, catecholamines obtained from the 5^{th} min of exercise was plotted as a function of the exercise duration. Thereafter, individual curve for each variable was mathematically fitted by a first order linear regression, so that the slope derived from the linear regression was used to indicate the rate of change during each constant cycling bout.

Statistical Analysis

Data were reporting as mean and standard deviation (SD), after checking Gaussian

distribution through Shapiro-Wilk's test. Paired samples *t*-test was used to verify differences between LT_1 and > LT_2 regarding time-to-exhaustion, power output, VO_2, lactate and RPE values. Differences within and between exercise bouts (LT_1 and > LT_2) for SD1, HF, RR and catecholamine's concentrations were assessed by a two-way repeated-measures ANOVA, after data sphericity checking through Mauchly's test. Multiple comparisons were correct by Bonferroni's test always when significant F values were found. All analyses were carried out in a GraphPad Prism software (version 6.01), having the statistical significance set at $p < 0.05$.

Results

Table 1 shows the participants (VO_2 and % VO_{2MAX}) and exercise characterization (power output, time to exhaustion, lactate and RPE) for LT_1 and > LT_2 constant cycling bouts. As expected, exercise outcomes such as power output, time to exhaustion, lactate and RPE were significant higher in the > LT_2 than LT_1 ($p < 0.05$).

Table 1. Characterization of participants and exercise in experimental sessions.

	VO_2 ($l \cdot min^{-1}$)	VO_{2MAX} (%)	Power output (W)	TE (min)	Lactate (mmol)	RPE
LT_1	2.1 ± 0.3*	61.0 ± 12.0*	112.4 ± 22.1*	93.8 ± 18*	1.5 ± 0.7*	13.0 ± 1.9*
> LT_2	2.7 ± 0.3	81.8 ± 9.0	179.8 ± 23.3	22.8± 10.6	3.8 ± 1.0	16.4 ± 0.8

RPE: Ratings of perceived exertion; TE: Time to exhaustion; * $p < 0.05$; > LT_2 are shaded gray

HRV responses to LT_1 and >LT_2 exercise until exhaustion

Figure 2 (panel A) demonstrate SD1 responses during exercise at LT_1 and > LT_2. Regarding LT_1 exercise, SD1 responses at 80 % and 100 % of the time to exhaustion were lower than at 20% and 40% ($p < 0.05$). Accordingly, SD1 at 100 % of the time to exhaustion was lower than at 60% ($p < 0.05$). Additionally, SD1 responses in LT_1 cycling bout was higher than LT_2 exercise at 20, 40 and 60% of the time to exhaustion ($p < 0.05$). Important, >LT_2 exercise elicited SD1 values < 3.0 ms throughout the cycling exercise bout, while LT_1 exercise showed a complete parasympathetic withdrawal (i.e. < 3.0 ms) just after at 60 % of the time-to-exhaustion.

HF responses during LT_1 and > LT_2 were showed on figure 1 (panel B). During 80% of total bout completion, exercise at LT_1 elicited higher HF responses than exercise performed at

> LT_2 intensity ($p < 0.05$). Further just exercise at LT_1 demonstrated time main effect during the exercise bout.

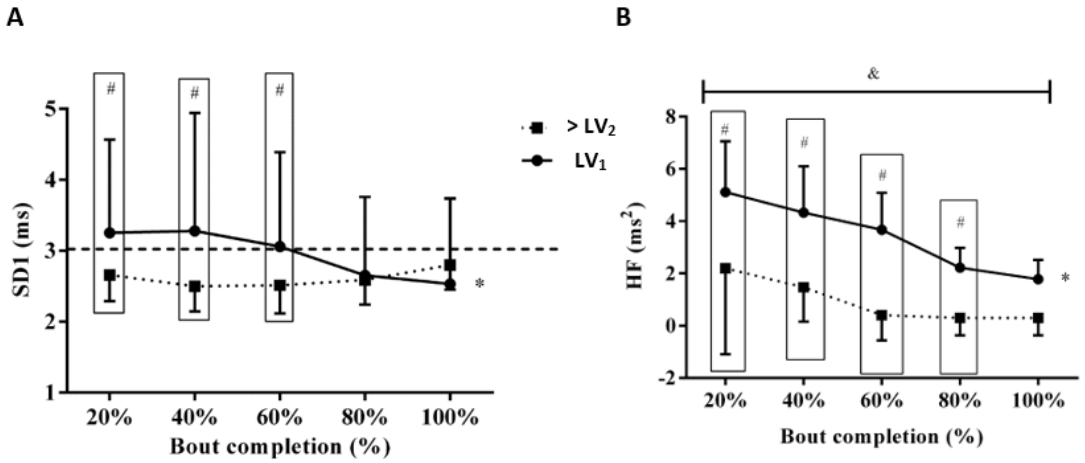

Figure 2. SD1 (panel A) and HF (panel B) responses during exercise at first lactate threshold (LT1) and above the second lactate threshold (> LT_2). * main effect of time ($p < 0.05$), # time-intensity interaction effect ($p < 0.05$) and & main effect of exercise intensity.

Biochemical variables at LT_1 and > LT_2 such as epinephrine, norepinephrine and respiratory rate were demonstrated on figure 3, panels A, B and C, respectively. Epinephrine remained steady throughout the LT_1 and > LT_2 cycling bouts ($p > 0.05$), while norepinephrine increased progressively in >LT_2 ($p < 0.05$), but not in LT_1 cycling bout ($p > 0.05$) and has showed main effect of intensity ($p < 0.05$). Furthermore, respiratory rate increased progressively in both >LT_2 and LT_1 exercises ($p < 0.05$) with main effect of exercise intensity ($p < 0.05$) and interaction effect between both exercise intensities and exercise moment from 40 to 100% ($p < 0.05$).

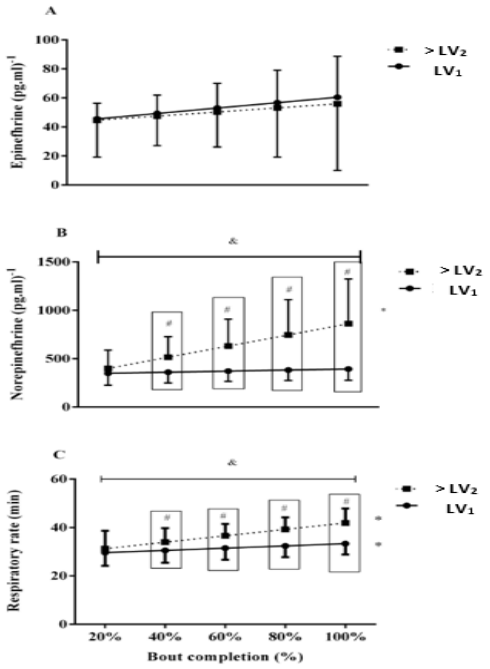

Figure 3. Epinephrine (panel A), norepinephrine (panel B) and respiratory rate (panel C) responses during exercise at first lactate threshold and above the second lactate threshold. * main effect of time ($p < 0.05$), # time-intensity interaction effect ($p < 0.05$) and & main effect of exercise intensity.

Discussion

The main highlights from our study were the absence of steady state on HRV and respiratory rate responses at cycling exercise performed until exhaustion at LT_1 intensity. Exercise performed at LT_1t elicited progressive decrements on SD1, with total parasympathetic tonus withdrawal (marked by the 3ms) after 60 % of the total exercise bout. In contrast, as higher intensity, exercise performed > LT_2 lead to a total parasympathetic tonus withdrawal just from the beginning of the exercise bout (~20%).

Traditional concepts of steady state up to the first lactate threshold (LT_1), in metabolic (i.e. lactate, pH, hormones, etc.) and cardiopulmonary variables[1] has been challenged by studies[3,4,5]. For example, Pires et al.[4] demonstrate a non-steady state in circulating catecholamine, while Lajoie et al.[5] and Hering et al.[2] showed a progressive increase in HR and RPE respectively, during constant exercise at LT_1. Accordingly, results of the present study showed that cycling at LT_1 did not elicit a steady state in HRV and respiratory rate responses. The progressive reduction in SD1 during exercise at LT_1 remains until the complete parasympathetic tonus withdrawal (~3ms). Furthermore, this response has been associated with

a higher perceived stress and feeling of fatigue.

In fact, has been suggested a total withdrawal of parasympathetic tonus during incremental tests around 60 - 80 % VO_{2MAX}[22], but up to date constant workload exercise until exhaustion has not been studied. In the present study, the participants cycled at 61 ± 12 % VO_{2MAX} until exhaustion (LT_1 intensity), and during first half of trial parasympathetic tonus was preserved, with a total parasympathetic tonus withdrawal closest to 60 % of the total exercise bout.

Autonomic responses to exercise are adjusted through central and peripheral nervous mechanisms. For example, the activation of somatomotor centers and *nucleus tractus solitarii* (NTS), provides a feedforward in cardiovascular control[23].

Peripheral and central stimuli to changes in HRV patterns are delivered through different afferent ways, mainly through arterial baroceptor, metabolic , mechano- and chemoreceptors (peripheral and central)[24,25,26,27]. For example, while Amann et al.[24] showed a link between metabolic-sensitive skeletal muscle afferents (group III and IV) and autonomic responses to exercise, others have been attributed a great part of the autonomic regulation to the feedforward influences from central mechanisms such as chemoreflex[26].

In contrast, while in the exercise performed at LT_1 the parasympathetic total withdrawal occurs at ~ 60 % of the total exercise bout, when the exercise was performed > LT_2, it's occurs since from beginning of the bout (~20 %), showing a premature parasympathetic withdrawal at > LT_2 than LT_1 cycling. These results are in accordance with Cottin et al.[12,17], who observed higher decrease in HRV spectral energy when the exercise above the ventilatory threshold. Authors still suggested that is a significant reduction in HRV power spectrum as the exercise intensity was increased [12].

During both exercise bout there was a decrease in HF responses during exercise performed at LT_1 but not at > LT_2 while the respiratory ration increase along the exercise in both intensities. Contrary, Pichon et al.[28] showed that an increase in exercise intensity results in a higher HF peak frequency concomitant to higher respiratory rate. It should be highlighted that in our study both exercises were performed until exhaustion, while Pichon et al.[28] has been used short closed loop exercise. It is possible that the respiratory rate may have affected the HRV responses in LT_1 exercise as previously suggested which some nonneural mechanisms could influence HRV responses[28,29,30]. Previous studies investigating health and heart-denervated has been suggested an intrinsic and non-autonomic mechanism which may establish HR fluctuations due a synchronism with ventilation patterns[28,29]. In our study especially ,

respiratory rate behavior presented a non-steady state which could be influenced by circulating catecholamine leading to changes in HRV responses.

Regarding biochemical responses such as epinephrine and norepinephrine concentrations, was observed an increase just in blood norepinephrine during >LT_2 exercise. Plasma norepinephrine concentration has been considered as an indicator of peripheral sympathetic activity, further norepinephrine is a neurotransmitter present in both central and peripheral nervous system[31]. Thus, norepinephrine concentrations could be one of the factors contributing to the total parasympathetic tonus withdrawal, as noradrenaline is the primary neurotransmitter released by the sympathetic post-ganglionic nerves[31]. However, as norepinephrine did not elicit significant increases in LT_1 cycling bout, results of the present study may suggest that parasympathetic tonus withdrawal in LT_1 exercise was relate to changes in respiratory rate responses, rather than in biochemical changes.

Conclusion

Our study aimed to verify the responses of HRV during constant workload cycling exercise at two different intensities. In conclusion, cycling at LT_1 doesn't elicit a steady state neither in HRV, nor in respiratory rate, so that a total parasympathetic withdrawal was occurred just from the second half of the total exercise duration. In contrast, > LT_2 exercise induced a total parasympathetic tonus withdrawal during whole exercise until exhaustion, from the very beginning of the exercise bout with a non-steady state in no one physiological variables.

Practical application

1) Cycling at LT_1 doesn't elicit a steady and parasympathetic withdrawal occur after some minutes. It is a important physiological information to prescribe long-term cycling training;
2) Cycling at LT_2 induced a total parasympathetic tonus withdrawal during whole exercise until exhaustion. It is a important physiological information to prescribe short-term cycling training;

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