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Original investigation

Are the oxygen uptake and heart rate off-kinetics influenced by the intensity of prior exercise?

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ABSTRACT

The aim of this study was to investigate the effect of prior exercise on the heart rate (HR) and oxygen uptake (VO_2) off-kinetics after a subsequent high-intensity running exercise. Thirteen male futsal players (age 22.8 ± 6.1 years) performed a series of high-intensity bouts without prior exercise (control), preceded by a prior same intensity continuous exercise (CE_{+CE}) and a prior sprint exercise (SE_{+CE}). The magnitude of excess post-exercise oxygen consumption ($EPOC_m$ – 4.25 ± 0.19 vs. 3.69 ± 0.20 L min^{-1} in CE_{+CE} and 3.62 ± 0.18 L min^{-1} in control; $p < 0.05$) and the parasympathetic reactivation (HRR_{60s} – 33 ± 3 vs. 37 ± 3 bpm in CE_{+CE} and 42 ± 3 bpm in control; $p < 0.05$) in the SE_{+CE} were higher and slower, compared with another two conditions. The EPOCτ (time to attain 63% of total response; 53 ± 2 s) and the heart rate time-course (HRτ – 86 ± 5 s) were significantly longer after the SE_{+CE} condition than control transition (48 ± 2 s and 69 ± 5 s, respectively; $p < 0.05$). The SE_{+CE} induce greater stress on the metabolic function, respiratory system and autonomic nervous system regulation during post-exercise recovery than CE, highlighting that the inclusion of sprint-based exercises can be an effective strategy to increase the total energy expenditure following an exercise session.

1. Introduction

The dynamic recovery of resting homeostasis upon cessation of exercise is widely investigated in exercise physiology, with oxygen uptake (VO_2) and heart rate (HR) responses as the two most commonly used variables to investigate the post-exercise recovery process (Poole and Jones, 2012). The magnitude of excess post-exercise oxygen consumption ($EPOC_m$) seems to be mediated by the extent of phosphocreatine (PCr) breakdown, replenishment of O_2 stores in blood and muscle, metabolites removal (e.g., lactate), increased body temperature and circulating catecholamines (Bahr and Sejersted 1991; Børsheim and Bahr 2003; Townsend et al., 2013). On the other hand, the post-exercise autonomic nervous system activity, measured indirectly through HR recovery, is modulated by complex interactions of factors related to sympathetic withdrawal and parasympathetic reactivation (Imai et al., 1994; Buchheit et al., 2007).

Some previous studies have shown that the VO_2 recovery pattern after a square-wave transition is strikingly influenced by exercise intensity, supporting the notion of a curvilinear relationship between the $EPOC_m$ and exercise intensity (Børsheim and Bahr, 2003; Jones and Carter, 2000; Mann et al., 2014; Ozyener et al., 2001). Regarding the HR recovery kinetics, Mann et al. (2014) showed that the time constant of HR off-kinetics (HRτ) was greater when the exercise intensity was increased from 60% to 80% of

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maximal VO_2 (VO_{2max}). However, to our knowledge, the effect of prior exercise on the VO_2 and HR recovery curves after subsequent exercise remains to be investigated, mainly comparing the prior exercise performed at different intensities (e.g., submaximal vs. supramaximal) and patterns (e.g., continuous vs. intermittent).

Repeated sprints training may be considered as a time-efficient strategy for inducing important muscle metabolic adaptations (Bailey et al., 2009). The sprints exercise (SE) could be adopted as a training model to improve some physiological functions and performance. According to previous studies, sprints-based training might promote improvement in the ability to repeat high intensity exercise, glycolytic and oxidative enzymes activity, muscle buffering capacity and increases in aerobic fitness in humans (Bailey et al., 2009; Gibala et al., 2006). Moreover, intermittent repeated or single sprints have been used as a viable alternative to the traditional submaximal warm-up, aiming to accelerate the "overall" VO_2 on-kinetics response (i.e., mean response time—MRT) (Burnley et al., 2002; do Nascimento et al., 2015; Lanzi et al., 2012; Wilkerson et al., 2004). Several studies have reported that prior exercise performed above the gas exchange threshold (GET) normally accelerates the MRT, either increasing the amplitude of the VO_2 primary component and/or decreasing the amplitude of the VO_2 slow component, without changes to the time constant (i.e., τ) of both (Burnley et al., 2002, 2006; Jones et al., 2003; Bailey et al., 2009; Lanzi et al., 2012).

Sprints exercise are characterized by a greater anaerobic energy contribution (i.e., PCr hydrolysis and glycolysis) and higher levels of muscle power output than submaximal continuous running exercise (Buchheit et al., 2007). Furthermore, high-intensity runs with changes of direction may elicit greater HR, blood lactate concentration ([La]) and rating of perceived exertion (RPE) values compared with straight-line high-intensity efforts (Dellal et al., 2010). Despite these differences in the metabolic and neuromuscular requirements during running bouts of different activity patterns (continuous vs. intermittent or straight-line vs. shuttle run), it has been shown that both prior repeated sprints and straight-line submaximal continuous exercise had a similar effect on VO_2 on-kinetics parameters (do Nascimento et al., 2015). However, to date, the literature is still sparse regarding the physiological stress imposed by a bout of square-wave exercise preceded by SE on the post-exercise autonomic cardiac activity and respiratory responses.

The balance between training stimulus and recovery after different combinations of exercise is considered as a key component to induce performance gains (Stanley et al., 2013). Understanding the impact of prior exercise intensity and type on post-exercise autonomic cardiac activity can be an interesting approach to detect the recovery profile according to different types of tasks typically incorporated into exercise programs. In addition, if the difference on the $EPOC_m$ caused by the prior exercise intensity and type (i.e., SE_{+CE} vs. CE_{+CE}) is physiologically significant, then this may have implications for the design of exercise protocols which aim at weight loss or maintenance. The resulting information will be of great interest not only for the assessment of the exercise load and for the monitoring fatigue but also for individualized training prescription and establishing adequate recovery periods according to different types of tasks (Cunha et al., 2016; Del Rosso et al., 2016; Stanley et al., 2013).

Thus, the aim of the present study was to investigate how prior exercise of different intensities and patterns (i.e., SE_{+CE} vs. CE_{+CE}) affects post-exercise autonomic control and VO_2 off-kinetics parameters following a high-intensity exercise bout in trained subjects. Since the VO_2 and HR off-kinetics are stimulus dependent, our hypothesis was that high-intensity running preceded by a prior SE would induce a higher $EPOC_m$ and would elicit slower metabolic and cardiac responses than when preceded by a CE.

2. Materials and methods

2.1. Participants

Thirteen male amateur futsal players (age 22.8 ± 6.1 years; body mass 76.0 ± 10.2 kg; height 178.7 ± 6.6 cm; VO_2max 58.1 ± 4.5 mL kg^{-1} min^{-1}) volunteered to participate in the present study. At the time of study, the participants had at least four years experience in futsal training. Participants performed three to five regular training sessions per week (6.7 ± 1.9 h) and participated in games on weekends (74.6 ± 18.5 min). After being fully informed of the risks and stresses associated with the study, the participants gave their written informed consent to participate. The experimental protocol was approved by the local Ethics Committee of the University and was performed according to the Declaration of Helsinki.

2.2. Experimental design

The participants were required to report to the laboratory on three separate occasions over a two-week period. On the first visit, they performed a maximal incremental running test for the determination of the GET and VO_{2max} in order to determine the exercise intensities used during the main experimental protocol. Following this preliminary test session, the subsequent visits were used to evaluate the HR and VO_2 off-kinetics. During the second visit, the participants completed a CE without prior exercise (control) at 50% Δ (running speed corresponding to 50% of the difference between the VO_2 at GET and VO_{2max}), followed by another CE performed at the same intensity (with prior exercise—CE_{+CE}), each lasting 6 min and separated by 6 min of recovery. After 60 min of rest (Burnley et al., 2006), the participants performed a prior SE, followed 6 min later by a 6-min CE at 50% Δ (SE_{+CE}) and 6 min of recovery. The third visit was identical to the second. Therefore, the 6-min square-wave transient was performed twice in each condition (see Fig. 1). The SE was performed on an outdoor futsal court and composed by six sprints of 40 m, with 60 s of passive recovery between each sprint. Each sprint was designed with three changes of direction of 180°, one every 10 m. Each participant was verbally encouraged to complete all sprints as fast as possible. All tests were separated by at least 48 h and were performed at the same time of day in controlled environmental laboratory conditions (19–22 °C; 50–60% RH) to minimize the effects of diurnal biological variation on the results (Carter et al., 2002). The participants were instructed to avoid any intake of alcohol and strenuous exercise in the 24 h preceding each test session and to arrive at the laboratory in a rested and fully hydrated state, at least 2 h postprandial.

2.3. Measurement of GET and VO_{2max}

The participants completed a ramp incremental test on the motorized treadmill (Millenium Super Atl 10.200, Inbramed, Brazil) to volitional exhaustion in order to determine the GET and VO_{2max}. The test started with the participants walking at 6.0 km h^{-1} and 1% gradient (Jones and Doust, 1996) followed by an increase of 0.5 km h^{-1} every 30 s thereafter until exhaustion. Capillary blood samples (25 μL) were obtained from the ear lobe of each participant at the beginning and at the end of the test and the [La] was measured using an electrochemical analyzer (YSL 2700 STAT, Yellow Springs, Ohio, USA). The analyzer was calibrated in accordance with the manufacturer's recommended procedures. Each participant was verbally encouraged to perform a maximal effort. The VO_2 and HR responses were recorded continuously throughout the test and subsequently averaged over 15-s intervals (Quark PFTergo, Cosmed, Rome, Italy). The VO_{2max} was considered as the highest 15-s average obtained during the test, or, in the presence of a plateau

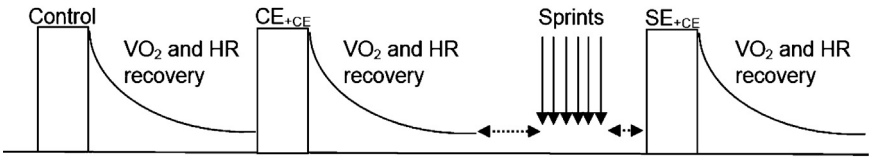

Fig. 1. Experimental design of study. CE_{+CE} = two bouts of continuous exercise. SE_{+CE} = continuous exercise preceded by sprint exercise. VO_2 = oxygen uptake. HR = heart rate. See more details in text.

in the VO_2 response, it was considered as the average of the final minute of exercise (Day et al., 2003). The attainment of VO_{2max} was defined using the criteria proposed by Bassett and Howley (2000). The peak values of velocity (PV) and HR (HR_{peak}) were considered as the highest velocity and HR achieved by the participants during the test, respectively. The GET was determined using the V-slope method (Beaver et al., 1986), constituting the first disproportionate increase in CO_2 production (VCO_2) relative to the increase in VO_2. An increase in the ventilatory equivalent for VO_2 (VE/VO_2) with no increase in the ventilatory equivalent for VCO_2 (VE/VCO_2) was subsequently verified upon visual inspection. The $[La]_{peak}$ was considered as [La] obtained at the end of the ramp incremental test.

2.4. VO_2 off-kinetics

During the main experimental sessions, the participants completed a series of 6-min square-wave transitions from rest to exercise (and from exercise to recovery) at 50% Δ. The running speed at 50% Δ was calculated by interpolation of the VO_2-speed linear regression during the incremental test. This excluded the first few minutes of walking, as well as the time following the attainment of any plateau in VO_2. All 6-min transitions were preceded by 3-min of rest with the resting VO_2 measured breath-by-breath for the identification of VO_2 "baseline". In the last 5 s of exercise, participants were given a countdown to dismount the treadmill and begin the 6-min recovery phase. The participants supported their body mass with their hands on the handrails of the treadmill and stepped to the side of the treadmill belt. The treadmill belt was stopped immediately and the participant stepped back onto the stationary treadmill belt and there remained stood upright without moving or speaking. The transition from exercise to recovery was typically achieved within 5–6 s. During the tests, capillary blood samples were collected from the ear lobe in the 30 s before and after the exercise.

2.5. Data analysis

Respiratory and pulmonary gas exchange variables were measured during all protocols with an automated breath-by-breath system (Quark PFTergo, Cosmed, Rome, Italy), calibrated before each test according to the manufacturer's instructions. The HR was continuously recorded throughout the tests by a HR monitor incorporated into the gas analyzer. In each recovery phase the breath-by-breath VO_2 data were initially examined to exclude outliers caused by sighs, swallowing, coughs, and so on. As suggested by Lamarra et al. (1987), occasional breath values were omitted from the analysis, using an exclusion criterion of greater than three standard deviations from the local mean. Therefore, for each recovery phase, the breath-by-breath VO_2 data were then time-aligned to the end of exercise (i.e., last 60 s), linearly interpolated to 1-s intervals and ensemble-averaged to yield a single profile to give a single response for each participant. The single profile data were reduced to 5-s stationary averages for improved parameter estimation (Whipp and Rossiter, 2005). The first 20 s of data after the onset of recovery (i.e., the phase I response) were not included in the analysis (Ozyener et al., 2001). Non-linear regression techniques were used to fit the data after the onset of recovery with an exponential function. An iterative process ensured that the sum of squared error was minimized. The mathematical model consisted of a mono-exponential function (Barstow and Molé, 1991). Based on previous literature (Ozyener et al., 2001 Mann et al., 2014), the model was constrained to the VO_{2end} to aid in identification of the key parameters according to equation 1:

$$VO2(t) = VO2end - Ax\left[1 - e^{-\left(\frac{t-TD}{\tau}\right)}\right] \quad (1)$$

Where: $VO_2(t)$ represents the value of VO_2 at a given time (t); VO_{2end} is the average value over the last 60-s of exercise; A is the asymptotic amplitude for the exponential term describing changes in VO_2 from exercise to its asymptote; τ is the time constant; and the TD is the time delay. Moreover, to provide a description of the HR kinetics during recovery, the HR kinetics was modelled via a similar mono-exponential function to VO_2, without a time delay from the end of exercise (i.e., the last 16 s) as described by Buchheit et al. (2007) and Marwood et al. (2011) according to Eq. (2):

$$HR(t) = HRend - Ax\left[1 - e^{-\left(\frac{t}{\tau}\right)}\right] \quad (2)$$

Where: HR(t) represents the value of HR at a given time (t); HR_{end} is the average value over the last 16 s exercise; A is the asymptotic amplitude for the exponential term describing changes in HR from exercise to its asymptote; and the τ is the time constant. The HR initial recovery index (HRR_{60s}) was defined as the absolute difference between the HR_{end} and the HR recorded 60 s after exercise (mean of 5 s) (Buchheit et al., 2007; Mann et al., 2014). The 5-s average in this part of recovery was chosen because of the rapid decrease in HR values (Buchheit et al., 2007). The HRR_{60s} index is the simplest and most used measure attributed primarily to parasympathetic reactivation (Buchheit et al., 2007; Mann et al., 2014). The $EPOC_m$ was determined as the time integral of the VO_2 recovery curve values above VO_2 baseline.

The mono-exponential modeling provided an adequate characterization of the off-transient VO_2 kinetics in the present study. The adjusted R^2 was not different when compared to a bi-exponential model, except for SE_{+CE} (0.96 ± 0.03 vs. 0.96 ± 0.02; $p > 0.05$ for control exercise; 0.95 ± 0.04 vs. 0.96 ± 0.02; $p > 0.05$ for CE_{+CE}; 0.94 ± 0.02 vs. 0.96 ± 0.02; $p < 0.05$ for SE_{+CE}), but the F of ANOVA was significantly higher than the bi-exponential model, except for SE_{+CE} (3136.5 ± 1423.9 vs. 2047.9 ± 928.3; $p < 0.01$ for control exercise; 2686.8 ± 1013.3 vs. 1858.1 ± 737.8; $p < 0.01$ for CE_{+CE}; 2813.3 ± 914.1 vs. 2399.6 ± 1480.1; $p > 0.05$ for SE_{+CE}). Thus, the F test was used to decide which model led to a significant reduction in the sum of squared residuals.

2.6. Statistical analyses

Descriptive statistics are expressed as mean ± SD unless stated otherwise. Assumptions of sphericity were assessed using the Mauchly test, and any violations were corrected using the Greenhouse-Geisser correction factor. Main effects for all variables were determined using one-way repeated-measures analysis of variance (ANOVA). When significant effects were observed, a Bon-

Table 1
Physiological responses during incremental test.

Variables	Mean + SD	95%CI	
		Lower bound	Upper bound
VO_{2max} ($L\,min^{-1}$)	4.4 ± 0.6	4.0	4.7
PV ($km\,h^{-1}$)	17.9 ± 1.3	17.2	18.7
HR_{peak} (bpm)	193 ± 10	187	199
GET ($L\,min^{-1}$)	3.0 ± 0.4	2.8	3.3
GET (%VO_{2max})	70.4 ± 7.5	65.8	74.9
GET ($km\,h^{-1}$)	11.0 ± 0.8	10.5	11.5
GET (%PV)	61.4 ± 3.2	59.5	63.3
50% Δ ($L\,min^{-1}$)	4.0 ± 0.7	3.6	4.4
50% Δ ($km\,h^{-1}$)	13.8 ± 1.0	13.2	14.4
$[La]_{peak}$ ($mmol\,L^{-1}$)	9.3 ± 2.2	8.0	10.6

VO_{2max} = maximal oxygen uptake. PV = peak velocity. HR_{peak} = peak heart rate. GET = gas exchange threshold. 50% Δ = 50% of difference between the VO_2 at GET and VO_{2max}. $[La]_{peak}$ = peak blood lactate concentration.

ferroni post hoc test was used for comparisons. The Shapiro-Wilk test was used to verify the normality of residuals and to ensure a Gaussian distribution of the data (n < 50). When any measure (i.e., VO_{2end} and HRτ) did not demonstrate normality of residuals, the data were log transformed (or Box-cox for VO_{2end}). For these measurements, a repeated-measures ANOVA and Bonferroni post hoc test were performed on the log-transformed values. Linear regression techniques and Pearson product moment correlations were used to assess the relationships between any variables. Effect size (ES) was calculated according to Cohen (1988). The following criteria for effect sizes were used: trivial (<0.2), small (0.2–0.5), moderate (0.5–0.8) or large (>0.8). The statistical analyzes of the data were executed with the Statistical Package for Social Sciences Windows® (SPSS Inc. version 17.0; Chicago, IL, USA), and the mathematical analyzes were performed using Origin 8.0 software. The level of significance was set at $p \leq 0.05$.

3. Results

3.1. Incremental test

Table 1 shows physiological responses during incremental test. The baseline values of [La] and HR were $1.2 \pm 0.4\,mmol\,L^{-1}$ and 85 ± 14 bpm, respectively.

3.2. Exercise responses during square-wave bouts

Physiological responses during all experimental conditions are shown in Table 2. Velocity during exercise at 50% Δ was $13.8 \pm 1.0\,km\,h^{-1}$ (77.1 ± % PV). The VO_2 at the end of exercise ($VO_{2exercise}$) averaged ~91% VO_{2max} in all conditions (i.e., control, CE_{+CE} and SE_{+CE}), with no difference (p > 0.05) found among them. On the other hand, there was a significant difference (p < 0.05) and a large ES for the [La] between the SE_{+CE} and the other two conditions. However, there was no difference between the control and CE_{+CE} (p > 0.05). The HR at the end of exercise ($HR_{exercise}$) showed higher values (p < 0.05) and a large ES in SE_{+CE} compared to both CE_{+CE} and control conditions. Moreover, the $HR_{exercise}$ at CE_{+CE} was higher than control (p < 0.05).

Table 2
Oxygen uptake and heart rate recovery kinetics on square-wave bouts with prior high-intensity exercise and prior sprint exercise.

	Inferential statistics			Effect size		
	Control	CE_{+CE}	SE_{+CE}	Control vs. CE_{+CE}	Control vs. SE_{+CE}	CE_{+CE} vs. SE_{+CE}
Exercise						
$VO_{2exercise}$ ($L\,min^{-1}$)	3.98 ± 0.18 (95%CI: 3.59–4.37)	3.99 ± 0.18 (95%CI: 3.59–4.37)	3.97 ± 0.17 (95%CI: 3.59–4.37)	0.1 Trivial	0.1 Trivial	0.1 Trivial
%VO_{2max}	91.4 ± 1.4 (95%CI: 88.4–94.4)	91.6 ± 1.4 (95%CI: 88.7–94.5)	91.2 ± 0.9 (95%CI: 89.2–93.2)	0.1 Trivial	0.1 Trivial	0.1 Trivial
[La] ($mmol\,L^{-1}$)	6.7 ± 0.6 (95%CI: 5.4–8.0)	7.5 ± 0.8 (95%CI: 5.8–9.3)	11.3 ± 0.8*,# (95%CI: 9.5–13.2)	0.8 Moderate	2.1 Large	2.2 Large
$HR_{exercise}$ (bpm)	177 ± 3 (95%CI: 171–184)	186 ± 3* (95%CI: 179– 192)	189 ± 3*,# (95%CI: 182–196)	3.3 Large	3.6 Large	2.0 Large
%HR_{peak}	91.7 ± 1.1 (95%CI: 89.3–94.0)	95.9 ± 1.1* (95%CI: 93.4–98.4)	97.7 ± 1.0*,# (95%CI: 95.5–99.9)	3.5 Large	3.6 Large	1.9 Large
Recovery						
$EPOC_m$ ($L\,min^{-1}$)	3.62 ± 0.18 (95%CI: 3.23–4.00)	3. 69 ± 0.20 (95%CI: 3.27–4.12)	4.25 ± 0.19*,# (95%CI: 3.83–4.66)	0.2 Small	0.8 Moderate	1.0 Large
A_VO_2 ($L\,min^{-1}$)	3.26 ± 0.16 (95%CI: 2.92–3.61)	3.26 ± 0.16 (95%CI: 2.91–3.61)	3.14 ± 0.15*,# (95%CI: 2.82–3.46)	0.0 Trivial	0.9 Large	0.8 Moderate
EPOCτ (s)	48.2 ± 1.8 (95%CI: 44.3–52.1)	50.4 ± 1.5 (95%CI: 47.1–53.7)	53.1 ± 2.3* (95%CI: 48.0–58.2)	0.6 Moderate	0.8 Moderate	0.5 Moderate
HRR_{60s} (bpm)	42 ± 3 (95%CI: 35–48)	37 ± 3* (95%CI: 31–43)	33 ± 3*,# (95%CI: 26–39)	1.2 Large	1.5 Large	0.9 Large
A_HR (bpm)	78 ± 3 (95%CI: 72–84)	79 ± 3 (95%CI: 72–86)	75 ± 3# (95%CI: 68–81)	0.3 Small	0.7 Moderate	1.0 Large
HRτ (s)	68.8 ± 4.9 (95%CI: 58.1–79.5)	80.5 ± 6.4* (95%CI: 66.3–94.7)	85.6 ± 5.2* (95%CI: 74.2–97.0)	1.4 Large	0.9 Large	0.2 Small
Recovery curve plateaus						
VO_{2end} ($L\,min^{-1}$)	0.72 ± 0.04 (95%CI: 0.63–0.81)	0.73 ± 0.04 (95%CI: 0.65–0.81)	0.83 ± 0.04*,# (95%CI: 0.75–0.91)	0.3 Small	1.0 Large	1.1 Large
HR_{end} (bpm)	99 ± 4 (95%CI: 91–107)	106 ± 4* (95%CI: 99–114)	114 ± 3*,# (95%CI: 107–122)	2.2 Large	2.6 Large	1.8 Large

Values are means ± standard error. Control = without prior exercise. CE_{+CE} = two bouts of continuous exercise. SE_{+CE} = continuous exercise preceded by sprint exercise. $VO_{2exercise}$ = oxygen uptake during the last minute exercise. [La] = blood lactate concentration. $HR_{exercise}$ = heart rate attained during exercise. VO_{2max} = maximal oxygen uptake. HR_{peak} = peak heart rate. $EPOC_m$ = magnitude of excess post-exercise oxygen consumption. A_VO_2 and EPOCτ = represent the amplitude and time constant of the oxygen uptake recovery curve. HRR_{60s} = 1-min heart rate recovery. A_HR and HRτ are the amplitude and time constant of the heart rate recovery curve. VO_{2end} and HR_{end} = VO_2 and HR during recovery plateau.

* Significantly different of control p < 0.05.

\# Significantly different in relation the CE p < 0.05.

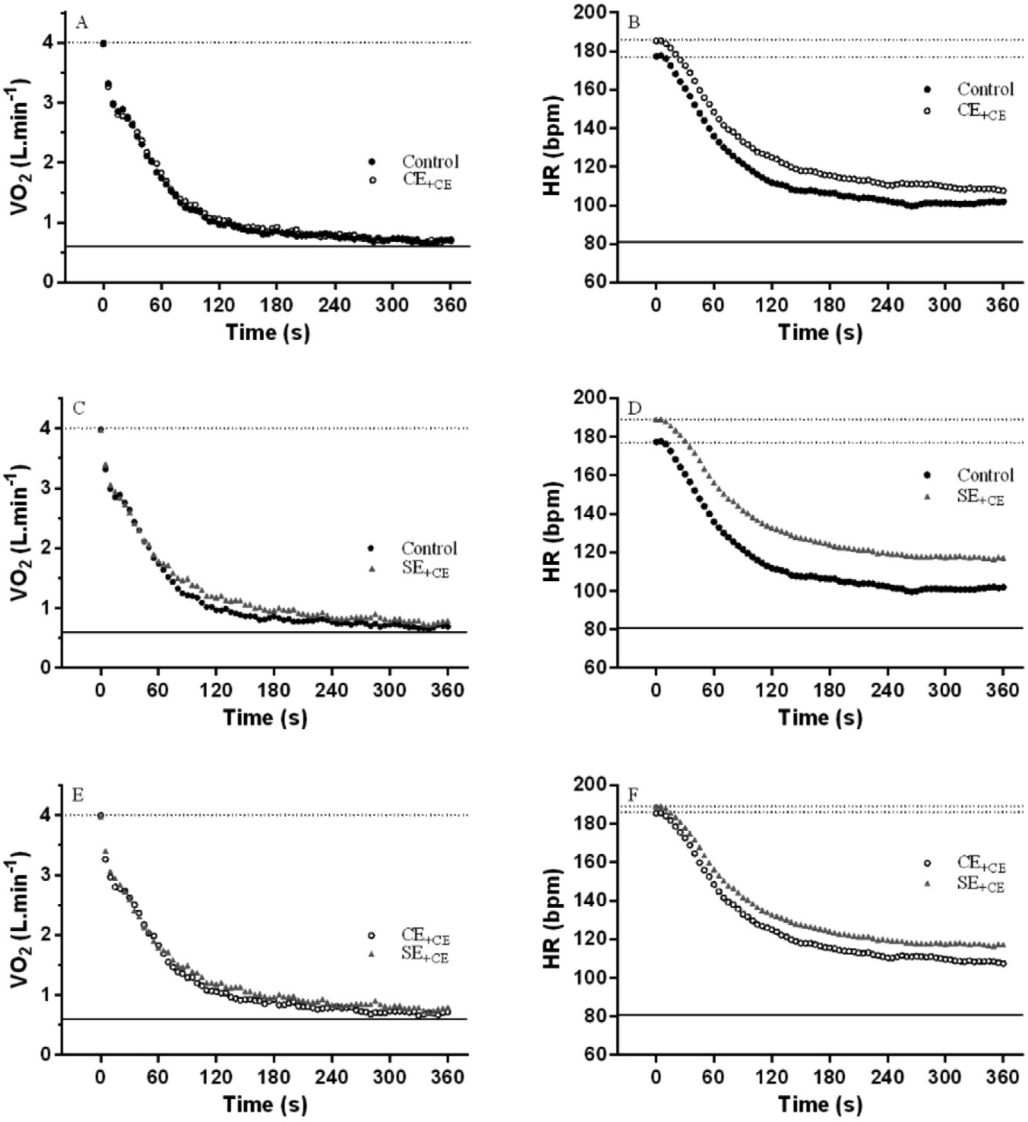

Fig. 2. CE_{+CE} = two bouts of continuous exercise. SE_{+CE} = continuous exercise preceded by sprint exercise. Mean group values of oxygen uptake (VO_2) and heart rate (HR) recovery curve responses in all square-wave exercise transitions. Closed symbols represent the control recovery condition in all panels. Open symbols represent the recovery after the CE_{+CE}. The gray triangles are the recovery condition after SE_{+CE}. The dotted lines represent the VO_2 and HR values attained during every exercise condition. The continued line showed the VO_2 and HR values (baseline) before the exercise.

3.3. VO_2 and HR off-kinetics

The VO_2 and HR recovery kinetics parameters are presented in Table 2 and illustrated in Figs. 2 and 3. The $EPOC_m$ after the SE_{+CE} was higher than the other two conditions (moderate to large ES; $p < 0.05$) and the EPOCτ after the SE_{+CE} was slower than control (moderate ES; $p < 0.05$). However, the EPOCτ was not different between the SE_{+CE} and CE_{+CE} ($p > 0.05$). The VO_{2end} after the SE_{+CE} was higher than the plateau values observed after both CE_{+CE} and control conditions (large ES; $p < 0.05$). In addition, the VO_{2end} after the SE_{+CE} was significantly greater than the $VO_{2baseline}$, (0.83 ± 0.13 vs. $0.55 \pm 0.09\ L min^{-1}$; $p < 0.01$). However, no difference ($p > 0.05$) was found between the VO_{2end} and $VO_{2baseline}$ in the other two conditions. Interestingly, there was a significant correlation ($r = 0.68$; $R^2 = 0.46$; $p = 0.01$) between the $EPOC_m$ and [La] in the SE_{+CE} (Fig. 4). On the other hand, the HRτ was slower after the SE_{+CE} and CE_{+CE} compared to control (large ES; $p < 0.05$), but with no significant difference found between the CE_{+CE} and SE_{+CE} (small ES; $p > 0.05$).

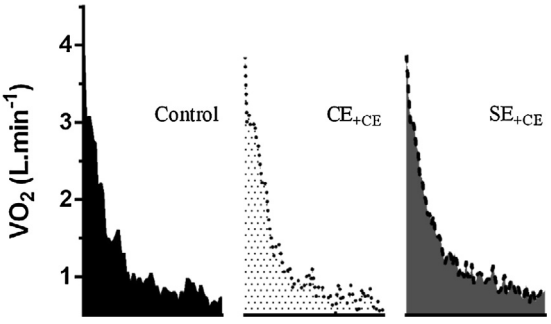

Fig. 3. CE_{+CE} = two bouts of continuous exercise. SE_{+CE} = continuous exercise preceded by sprint exercise. The magnitude of excess post-oxygen consumption ($EPOC_m$) for a representative subject. Notice that the $EPOC_m$ after the SE_{+CE} condition was higher than CE_{+CE} and control conditions.

The HRR_{60s} and HR_{end} were significantly different among the three conditions (large ES; $p < 0.05$).

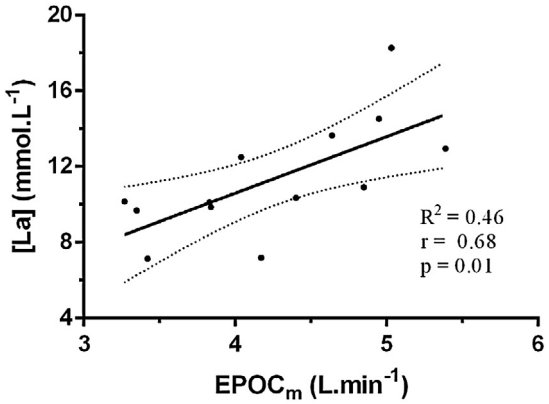

Fig. 4. The relation between the magnitude of excess post-oxygen consumption ($EPOC_m$) and the blood lactate concentration ([La]) after the continuous exercise preceded by sprint exercise (SE_{+CE}). Notice that the [La] could explain ~46% of the $EPOC_m$ variation after the SE_{+CE}.

4. Discussion

The aim of this study was to analyze the effects of prior exercise performed at different intensities and patterns (i.e., CE vs. SE) on the HR and VO_2 kinetics recovery curves after a high-intensity running exercise performed at the same relative exercise intensity (50% Δ). The main findings of the present study were: (1) $EPOC_m$ and HRR_{60s} in SE_{+CE} were higher and slower compared to CE_{+CE} and the control conditions, respectively, and (2) the EPOCτ and the HRτ were significantly longer after the SE_{+CE} than the control transition (Table 2). Collectively, these results are in accordance with our hypothesis that the square-wave condition with prior SE, which requires a greater muscle power engagement and anaerobic contribution than CE, would induce a greater stress on metabolic function and the autonomic nervous system regulation during post-exercise recovery.

In the current study, running bouts with different activity patterns (continuous vs. intermittent) varying in intensity (submaximal vs. supramaximal) were chosen to precede a square-wave exercise performed at 50% Δ. Regarding the VO_2 response, no difference was found for $VO_{2exercise}$ among the conditions. However, $HR_{exercise}$ and [La] were consistently higher after SE_{+CE} in comparison to control and CE_{+CE}. Furthermore, $HR_{exercise}$ was significantly different between the CE_{+CE} and the control trial, whereas there was no [La] difference between the trials. These findings suggest that the greater anaerobic contribution in the SE_{+CE} can lead to a different VO_2 and HR recovery curve responses towards resting homeostasis.

Previous studies reported that $EPOC_m$ is exercise intensity-dependent (see review Børsheim and Bahr, 2003). For instance, Townsend et al. (2013) showed that the $EPOC_m$ after 3 × 30 s all-out efforts interspersed by 4 min of rest was greater than following a bout of moderate-intensity running (30 min at 60% HR reserve). In addition, it has been shown that walking with muscular blood flow restriction (BFR) may result in a greater $EPOC_m$ compared to that seen in the non-BFR condition (Mendonca et al., 2015). However, these previous studies have compared maximal/supramaximal intensities vs. submaximal intensities relatively lower (60%HR reserve or 40–50% HR_{max}) than that the submaximal intensity used in the present study (> 90% HR_{max}). Contrary to this perspective, the novelty of this study was to investigate whether the inclusion of SE to traditional high-intensity aerobic exercises would be able to have a significant impact on the $EPOC_m$. Our data demonstrated that a 6-min exercise performed at 50% Δ has a greater $EPOC_m$ (~14%) when it is preceded by a SE (i.e., 6 × 40 m; interspersed with 60 s) than a CE performed with the same duration and intensity (i.e., 6 min at 50% Δ). It suggests, that the addition of a small volume of sprint-based exercise (~60 s) to high intensity aerobic protocols, can be effectively incorporated in exercise programs applied in the clinical and team-sports setting aimed at producing a greater $EPOC_m$. In addition, our findings highlight that a combination between intermittent and continuous exercises rather than a continuous exercise can be recommended where the primary outcome of the exercise prescription is to promote greater increases in $EPOC_m$ (Cunha et al., 2016).

The $EPOC_m$ has been considered a more sensitive measure to changes in exercise intensity than EPOCτ (Mann et al., 2014). Our findings are in accordance with this, since the $EPOC_m$ was significantly higher in SE_{+CE} than in the other two conditions (i.e., CE_{+CE} and control), whereas the EPOCτ appeared to be unchanged when comparing both SE_{+CE} and CE_{+CE}. Among the main physiological mechanisms underlying the $EPOC_m$, it has been suggested that the metabolites derived from anaerobic metabolism are the principal determinants (Børsheim and Bahr, 2003). In the current study, the [La] after the SE_{+CE} was positively correlated with the $EPOC_m$ (r = 0.68; p = 0.01; Fig. 4), highlighting that anaerobic glycolysis contribution could explain around 46% of the inter-individual variation in the $EPOC_m$. This finding is in agreement with that reported by Aguiar et al. (2015), which found a significant correlation of the $EPOC_m$ with the lactate accumulation during exercise (r = 0.74) and the quantity of lactate removed from 0 to 10 min post exercise (r = 0.61) after a 1-min all-out test. Conversely, we did not find any relationship between the $EPOC_m$ and [La] in the CE_{+CE} and control conditions, thus supporting the notion that [La] appears to have a higher contribution to $EPOC_m$ mainly when supramaximal exercise is performed (i.e., when higher [La] is elicited). Obviously it should be considered that there are other primary candidates for mediating the effect of exercise intensity on $EPOC_m$, such as the metabolic cost of glycogen resynthesis, normalization of blood pH, circulating catecholamine levels, replenishment of O_2 stores in blood and muscle, increased ventilation and body temperature (Børsheim and Bahr, 2003; LaForgia et al., 2006; Mann et al., 2014; Aguiar et al., 2015). It is noteworthy that in the higher exercise intensities (e.g., supramaximal efforts) greater changes in these aforementioned variables can occur. However, the evaluation of these physiological mechanisms is beyond the scope of this study.

Previous studies have reported that the greatest $EPOC_m$ identified at higher exercise intensities can also be related to either a longer EPOCτ or a plateau of the VO_{2end} augmented during post-exercise recovery (Bahr and Sejersted, 1991; Børsheim and Bahr, 2003; Mann et al., 2014). Our results are in agreement with these findings, demonstrating that VO_{2end} remains elevated above the baseline values only after the SE_{+CE}. Of interest, the VO_{2end} was also largely correlated with [La] after the SE_{+CE} (r = 0.68; p = 0.01). Accordingly, Bahr and Sejersted (1991) and Mann et al. (2014) highlight that the higher plateau in VO_{2end} may be explained by factors such as increased metabolite levels and higher levels of catecholamines following high compared to low exercise intensities. Furthermore, the slower VO_2 off-kinetics towards resting homeostasis after the SE_{+CE} suggest that the residual effects of this exercise on VO_2 and $EPOC_m$ tends to remain for a longer period of time than only 6 min as considered in the present study. Finally, it is noteworthy that an additional bout of exercise at the same intensity (i.e., CE_{+CE}) did not influence the VO_2 off-response, suggesting that it seems to be more influenced by intensity than duration of exercise.

In addition to $EPOC_m$, the cardiac autonomic recovery after exercise has important physiological and clinical significance. HR recovery has been used as an important parameter for individualize the training prescription, for monitoring short-term exercise-induced changes in cardiac performance and as an indicator of internal workload and fitness level (Stanley et al., 2013). In the present study, the HRR_{60s} was significantly lower after the SE_{+CE}

when compared to the control and CE_{+CE} conditions, whereas the HRτ values after the CE_{+CE} and SE_{+CE} were longer than the control condition. Our findings are supported by Buchheit et al. (2007), who also found that HRR_{60s} and HRτ were highly impaired after repeated running sprints compared to moderate-intensity CE of equal net energy expenditure. These data suggest that either a slower parasympathetic reactivation or sympathetic withdraw are expected during short-term autonomic regulation of HR after repeated sprints in comparison to submaximal CE (Buchheit et al., 2007). However, as HRR_{60s}, in contrast to HRτ, was influenced by the type of prior exercise in the present study, it reasonable to suggest from our data that the slower HR kinetics recovery after the SE_{+CE} than CE_{+CE} may be caused by a slower parasympathetic reactivation. In accordance with our findings, Heffernan et al. (2006) have reported slower HR kinetics recovery following acute resistance (10-repetition maximum test) vs. endurance (30-min cycling at 65% VO_{2max}) exercise. These authors showed that the greater tachycardia after the resistance workout, which also requires higher levels of muscular power output in comparison to endurance exercise, appeared to be primarily determined by a reduced cardiac vagal tone rather than a sympathetic hyperactivity during the recovery period. Collectively, the available studies in the literature (Spencer et al., 2005; Buchheit et al., 2007) have consistently shown that the anaerobic contribution and other parameters associated with a high level of fast-twitch fiber recruitment are among the main factors in determining the level of parasympathetic reactivation after supramaximal exercise (e.g., repeated running sprints).

It is important to highlight that we intentionally recruited amateur futsal players for the present study because we expected that they would cope better with this type of exercise (i.e., intermittent sprints). Therefore, the present results should be viewed with caution when inferences are made as to what might occur in other subjects. However, we are aware that sprint-based training has been widely conducted with healthy/physically active individuals (Bailey et al., 2009; Gibala et al., 2006). Thus, our finding shows that a slower short-term parasympathetic reactivation after the SE_{+CE} condition may be of particular importance to clinicians wishing to prescribe intermittent training to sedentary individuals and clinical populations, given that a reduced cardiac vagal tone during post-exercise recovery may denote a poor cardioprotective background to cardiovascular events (e.g. ischemic heart disease and sudden cardiac death) (Billman, 2002). Whether individuals with low-activity levels show a similar impairment of parasympathetic reactivation following SE needs to be confirmed. Longitudinal studies are required to examine the effect of long-term repeated sprint training on autonomic control of the heart, parasympathetic reactivation and cardiovascular risk prognostics.

Although the current investigation has a number of strengths, it is not without its limitations. Unfortunately, our experimental design does not allow us to separate the effects of shuttle vs straight line runs from the effects of higher vs lower intensity exercise on the HR and VO_2 kinetics recovery parameters. Further studies investigating the effects of the shuttle vs straight line runs in different exercise intensities on HR and VO_2 recovery patterns could provide helpful insights regarding the recovery profile according to the type of task.

4.1. Conclusion

In summary, the present study has shown that early $EPOC_m$ and parasympathetic reactivation after the SE_{+CE} trial were higher and slower than CE_{+CE} and control conditions, respectively, resulting in a higher plateau of the VO_2 and HR at the end of the recovery period. Furthermore, it was demonstrated that EPOCτ and HRτ, in contrast to $EPOC_m$ and HRR_{60s}, were similar between the SE_{+CE} and CE_{+CE} conditions, suggesting that these two parameters are less sensitive to changes in exercise intensity. From a practical perspective, we demonstrated that the combination of SE and CE can be an effective approach to increase the total energy expenditure following an exercise session. Finally, the HRR_{60s} can be a useful marker for establishing a suitable cardiovascular recovery period and to guide the individualized training prescription.

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