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Abstract

Purpose: This study investigated the effects of acute hypoxic exposure on post-exercise cardiac autonomic modulation following maximal cardiopulmonary exercise testing (CPET).

Methods: Thirteen healthy men performed CPET and recovery in normoxia (N) and normobaric hypoxia (H) (FiO_2=13.4%, ≈3500m). Post-exercise cardiac autonomic modulation was assessed during recovery (300s) through the analysis of fast-phase and slow-phase heart rate recovery (HRR) and heart rate variability (HRV) indices.

Results: Both short-term, T30 (Mean Difference (MD) 60.0 s, 95% CI 18.2 to 101.8, p=0.009, ES 1.01) and long-term, HRRt (MD 21.7 s, 95% CI 4.1 to 39.3, p=0.020, ES 0.64), time constants of HRR were higher in H. Fast-phase (30s and 60s) and slow-phase (300s) HRR indices were reduced in H either when expressed in bpm or in percentage of HR_{peak} ($p<0.05$). Chronotropic reserve recovery was lower in H than in N at 30s (MD -3.77 %, 95% CI -7.06 to -0.49, p=0.028, ES -0.80) and at 60s (MD -7.23 %, 95% CI -11.45 to -3.01, p=0.003, ES -0.81), but not at 300s (p=0.436). Concurrently, Ln-RMSSD was reduced in H at 60s and 90s ($p<0.01$) but not at other time points during recovery ($p>0.05$).

Conclusions: Affected fast-phase, slow-phase HRR and HRV indices suggested delayed parasympathetic reactivation and sympathetic withdrawal after maximal exercise in hypoxia. However, a similar cardiac autonomic recovery was re-established within 5 minutes after exercise cessation. These findings have several implications in cardiac autonomic recovery interpretation and in HR assessment in response to high-intensity hypoxic exercise.

Keywords: heart rate recovery; hypoxia; post-exercise recovery, hypoxic exercise; cardiac autonomic activity

Abbreviations

ANOVA Analysis of variance

ANS Autonomic nervous system

CPET Cardiopulmonary exercise testing

CRR Chronotropic reserve recovery

EPOCt Excess of post-exercise oxygen consumption time-constant

$EPOC_{MAG}$ Excess of post-exercise oxygen consumption magnitude

LF Low-frequency spectral power

Ln Natural-logarithm transformation

HF High-frequency spectral power

HR Heart rate

HRR Heart rate recovery

HRRt Long-term time constant of heart rate recovery

HRV Heart rate variability

RMSSD Root mean square of successive differences of R–R intervals

T30 Short-term time constant of heart rate recovery

TP Total spectral power

Introduction

The influence of the autonomic nervous system (ANS) on cardiac activity (i.e. cardiac autonomic modulation) can be non-invasively assessed at rest (Malik 1996), during exercise (Achten and Jeukendrup 2003; Perini and Veicsteinas 2003) and in the transient phases between these two conditions (Pecanha et al. 2017) using heart rate variability (HRV) and heart rate (HR) dynamics analysis (Michael et al. 2017a).

Immediately after exercise, the decrease of HR, defined as heart rate recovery (HRR), and the recovery of HRV indices reflect post-exercise cardiac autonomic modulation (Pecanha et al. 2017; Romero et al. 2017; Michael et al. 2017a). Fast-phase HRR indices (obtained in the first 60 seconds of recovery) mainly reflect parasympathetic reactivation, whereas slow-phase HRR indices (over the first 60s of recovery) represent the combined effects of parasympathetic reactivation and sympathetic withdrawal occurring in the post-exercise period (Pecanha et al. 2017). Together with fast-phase HRR, the analysis of HRV indices over short time-periods (e.g. 30s), such as the root mean square of successive differences of R–R intervals (RMSSD), can be adopted to assess post-exercise parasympathetic reactivation (Goldberger et al. 2006; Buchheit et al. 2007a). These easy-to-obtain indices provide important insight into ANS functionality and reflect subject's health (Thayer et al. 2012), clinical (Qiu et al. 2017) and training status (Bellenger et al. 2016).

Previous studies have investigated post-exercise cardiac autonomic recovery in response to different "stressors", such as different exercise intensities (Terziotti et al. 2001; Cottin et al. 2004; Seiler et al. 2007), exercise durations (Michael et al. 2017b), exercise modalities (e.g. upper vs lower-body muscles involvement (Michael et al. 2018), different whole-body endurance exercises (Cunha et al. 2015)) and modified environmental conditions (e.g. hypoxia) (Al Haddad et al. 2012). In addition the effects of different recovery strategies (e.g. body postures assumed during recovery (Buchheit et al. 2009a) or different water immersion temperatures (Buchheit et al. 2009b; Al Haddad et al. 2010; de Oliveira Ottone et al. 2014) have also been studied.

According to these previous investigations, post-exercise cardiac autonomic modulation is influenced by the degree of the stimulus imposed (Seiler et al. 2007; Michael et al. 2016), with higher homeostatic disruptions (i.e. higher exercise intensities (Buchheit et al. 2007a; Seiler et al. 2007) or durations (Michael et al. 2017b)) causing slower recovery of HR and HRV indices. Additionally, the pre-exercise autonomic state seems also to be of importance (Cunha et al. 2015; Molina et al. 2016). Indeed a higher parasympathetic activity at rest has been associated with a faster recovery of HR and HRV indices in the post-exercise period (Danieli et al. 2014; Cunha et al. 2015). However, the association between resting HRV and post-exercise HRR is still debated due to the conflicting results reported in the literature (Esco et al. 2010).

Moreover, a different influence of previous stimulus characteristics may be observed in the two distinct phases of HRR (i.e. fast and slow-phase), due to the different physiological mechanisms involved in the recovery process (i.e. parasympathetic reactivation in the fast-phase and both parasympathetic reactivation and sympathetic withdrawal in the slow-phase of recovery) (Pecanha et al. 2017).

Nowadays, hypoxic training is commonly employed to induce greater physiological training adaptations in athletic populations (Millet et al. 2010; Brocherie et al. 2017) and has recently emerged as a promising training modality for sedentary and special populations (elderly, obese and hypertensive patients) (Millet et al. 2016; Lizamore and Hamlin 2017). Indeed, whereas positive haematological and non-haematological adaptations, increasing endurance performance, can be obtained with a prolonged hypoxic exposure at altitudes as high as 2000-2500m (>12 h/day), often avoiding the combined stimulus of exercise and hypoxia (live high-train low model, LHTL) (Millet et al. 2010; Chapman et al. 2014), other beneficial training adaptations and/or greater positive exercise-related physiological outcomes can be induced by performing exercise under hypoxic conditions (live low-train high model, LLTH) (Millet et al. 2010, 2016; Brocherie et al. 2017). A recent literature underlined the utilization of hypoxic levels, as high as 3000-3500 m of simulated altitude, for different training interventions (Millet et al. 2010, 2016; Faiss et al. 2013; Lizamore

and Hamlin 2017). For instance, low-intensity hypoxic exercise (e.g. walking) at a simulated altitude of 3000-3500m can be adopted to safely increase the exercise physiological load while reducing the external load in obese patients (Girard et al. 2017).

On the other hand, the above-mentioned altitudes are commonly employed by athletes involved in endurance and intermittent sports for performing high-intensity hypoxic exercises (Millet et al. 2010; Brocherie et al. 2017). Similarly, high-intensity hypoxic exercises are also performed by athletes for increasing their performance at altitude (Clark et al. 2007).

In this regard, hypoxia is well recognized to modify cardiac autonomic modulation at rest (Oliveira et al. 2017) and in response to exercise (Yamamoto et al. 1996; Zupet et al. 2009; Fisher 2015). Alongside, hypoxia acts as a stimulus for an increased sympathetic activity (Hainsworth et al. 2007; Amann and Kayser 2009) and a reduced parasympathetic cardiac control (Perini and Veicsteinas 2003; Buchheit et al. 2004; Fisher 2015; Oliveira et al. 2017), that can turn in a slower post-exercise recovery of HR and HRV indices (Al Haddad et al. 2012).

For instance, modifications in post-exercise cardiac autonomic modulation, with a delayed parasympathetic reactivation, have been reported in hypoxia (FiO_2=15.4%, 2400m) after sub-maximal exercise intensities (Al Haddad et al. 2012). On the contrary, in the above-mentioned work (Al Haddad et al. 2012), the imposed hypoxic stimulus did not modify parasympathetic recovery after a supra-maximal intensity (20 s sprint “all-out”), probably due to the already maximal homeostatic perturbation induced by a supra-maximal intensity, causing high anaerobic energy contribution and sympathetic activation (Buchheit et al. 2007a; Al Haddad et al. 2012).

To date, it is not clear if changes in post-exercise cardiac autonomic modulation can occur in response to exercises performed at more severe hypoxic levels (i.e. FiO_2<15.4%; altitude>2400 m) (Al Haddad et al. 2012). In particular, cardiac autonomic recovery from exercise performed at simulated altitudes of 3000-3500 m, which are relevant for training (Millet et al. 2010; Brocherie et al. 2017; Lizamore and Hamlin 2017) and competition (Clark et al. 2007) purposes, has not yet been investigated.

Additionally, according to the above-mentioned scenario, exercises with high cardiorespiratory involvement are widely performed in hypoxia, but post-exercise physiological outcomes have not been specifically studied. It is currently unknown how hypoxia can affect post-exercise cardiac autonomic modulation following a maximal exercise, where cardiovascular and respiratory systems are maximally stressed and pushed to their functional limit (e.g. a maximal cardio-pulmonary exercise test, CPET). This occurrence certainly limits the evaluation of recovery from hypoxic exercise both when used for health assessment or training load quantification purposes (Borresen and Lambert 2008; Ward et al. 2017).

Despite the expected lower exercise capacity (i.e. decreased VO_{2max} and peak exercise intensity) (Mollard et al. 2007b), maximal aerobic hypoxic exercise can result in markedly reduced arterial oxygen saturation (Favret and Richalet 2007), comparable cardio-respiratory stress (Ofner et al. 2014) and similar level of blood lactate accumulation (this point is still debated (Lundby et al. 2000; van Hall 2007; West 2007). In this case, the homeostatic stress induced by a maximal hypoxic exercise, may produce a more challenging situation for post-exercise cardiac autonomic recovery, further showing amplified post-exercise physiological outcomes indicating increased homeostatic perturbation (Mann et al. 2014).

From a practical standpoint, considering the widespread use of hypoxic training, it is also important to establish whether hypoxia influences post-exercise cardiac autonomic recovery in response to a maximal exercise.

Therefore, the purpose of this study was to investigate the effects of acute hypoxia on the post-exercise cardiac autonomic modulation following a maximal cardiopulmonary exercise test (CPET). According to previous observations about the influence of the homeostatic perturbation in determining post-exercise outcomes (Buchheit et al. 2007a; Al Haddad et al. 2012), we hypothesized that maximal hypoxic exercise would have been associated to a reduced recovery of fast-phase HRR and HRV indices, reflecting a delayed parasympathetic reactivation, in the immediate post-exercise recovery period. Furthermore, we hypothesized that in response to the

maximal cardiovascular, respiratory and metabolic stress induced, the reduced post-exercise oxygen availability would have also led to an impaired recovery of slow-phase HRR indices, also indicating delayed sympathetic withdrawal (Pecanha et al. 2017).

Materials and methods

Participants

Thirteen healthy men (age 34.1 ± 9.7 years, height 175.3 ± 4.6, weight 69.4 ± 6.0 kg) volunteered for this study. All participants were moderate aerobically trained and familiarized with high-intensity exercise. None of them had been at altitude above 2000m for prolonged periods of time (>12 hours) at least 3 months before the study. None of the participants involved had clinical evidence of cardiovascular, metabolic, or musculoskeletal diseases. Before data collection, all participants were properly informed about the experimental protocol and gave their written informed consent for the measures. They were instructed to avoid caffeine, alcohol and high-intensity exercise during the 24-h proceeding each test session. The experimental protocol was approved by the institutional Ethics Committee of the University of Verona (Italy).

Protocol

Each participant visited the laboratory in two occasions at the same time of the day and completed the experimental protocol within 2-week period. Participants randomly performed an evaluation in normoxia (N) and normobaric hypoxia (H). All tests were conducted under controlled laboratory conditions (18°C, 50% relative humidity). The hypoxic environment was created through the manipulation of the FiO_2 by means of an oxygen dilution system based on the Vacuum-Pressure Swing Adsorption principle (B-Cat, Tiel, The Netherlands). For H condition the FiO_2 was set at 13.4% to simulate an altitude of ≈3500m a.s.l.

All the evaluations were performed on a recline cycle ergometer (E1200, Cosmed Srl, Rome, Italy) set at 50° of inclination. Following 30 min of quiet rest on the ergometer participants completed: 6

min of baseline measurements at rest, 10 min of sub-maximal constant load exercise (75W), a maximal cardio-pulmonary exercise test (CPET) and 5 min of post-exercise recovery assessment. CPET started immediately after the sub-maximal exercise with increments of 25W every 1 min until participants' volitional exhaustion. The pedalling cadence during the submaximal exercise and the CPET was kept constant at 90 revolutions/min, using a monitor that provided participants with visual feedback. Throughout rest, exercise and recovery phases, beat-to beat heart rate was continuously recorded using a Polar RS800CX heart rate monitor (Polar, Kempele, Finland). During resting and exercise cardio-respiratory measures were collected continuously with breath-by-breath method using an automated open-circuit gas analysis system (Quark PFT Ergo, Cosmed Srl, Rome, Italy). Careful calibrations of flow sensors and gas analyzers were performed before each measurement according to the manufacturer's instructions. Pulse oxygen saturation (SpO_2) was continuously recorded by fingertip pulse oximetry (Nonin Medical, Minneapolis, MN, USA) at a sampling frequency of 1.0 Hz. To measure blood lactate accumulation a blood sample was collected from the earlobe 3 min after the end of the test (Goodwin et al. 2007; Buchheit et al. 2007b; Al Haddad et al. 2012). The lactate analyser (Biosen C-line, EKF Diagnostics GmbH, Barleben, Germany) was calibrated according to the manufacturer's instructions. The individual rating of perceived exertion (RPE) was assessed at the end of 5-min recovery period using Borg Category Ratio Scale (CR100) (Borg and Borg 2002) .

Data Analysis

The R-R intervals were uploaded using Polar Precision Performance Software (Polar, Kempele, Finland) and then exported as .txt files. Signal artifacts were filtered out by means of a moderate error correction filter with minimum protection zone of 6 bpm (Al Haddad et al. 2012). All the time series of R-R intervals showed low noise (identified errors <5%). HRV analysis was performed using Kubios HRV software (Version 2.1, Biosignal Analysis and Medical Imaging Group, Kuopio, Finland). At rest HRV indices were calculated from the last 5min of the 6-min resting period. Exercise HRV indices were calculated from the last 5-min of the 10-min submaximal exercise

preceding CPET evaluation. The time-domain HRV index considered was the square root of the sum of successive differences between adjacent normal R-R intervals squared (RMSSD). For frequency-domain HRV indices, low frequency spectral power (LF, 0.04-0.15 Hz), high frequency spectral power (HF, 0.15-0.4 Hz), and total spectral power (TP, 0.04-0.4 Hz) were calculated by Fast Fourier Transform (FFT) (Task Force of the European Society of 1996).

Post-exercise heart rate recovery (HRR) indices were calculated with a customized script in Matlab (Matlab, Mathworks Inc., USA). HRR indices were measured from the absolute differences between HR_{peak} and the HR values at 30s, 60 s and 300s of recovery (HRR30, HRR60 and HRR300) in the post-exercise period (averaged over 5s) (Peçanha et al. 2016). HRR was also calculated as the relative decline in HR expressed as a percentage of HR_{peak} (%HRR=HRR/HR_{peak}×100) and as the recovery of the chronotropic reserve (CRR = HRR/(HR_{peak}-HR_{rest})×100) (Molina et al. 2016). T30, the short-time constant of HRR, was calculated as the negative reciprocal of the slope of the regression line of natural-logarithmic transformed HR during the first 30 s of recovery (Buchheit et al. 2007b). HRRt, the long-term time-constant of HRR, was obtained after exponential fitting of the HR during the entire 300s of recovery (Pecanha et al. 2017). This method has been previously suggested to quantify the time-constant of HRR within a time period covering most of the post-exercise HR decay (Pecanha et al. 2017). Additionally, the time-varying vagal-related index, RMSSD, was also calculated for each of the 30-s segments of recovery (Goldberger et al. 2006).

The peak power output (PPO), achieved at athlete's exhaustion, was determined according to the equation: PPO (W) = power output last stage completed (W) + [*t* (s)/stage duration (s) * stage increment (W)], where *t* is the time of the uncompleted stage (Kuipers, Verstappen, Keizer, Geurten, & Van Kranenburg, 1985). VO_{2peak} and other maximal cardio-respiratory variables were defined as the highest values of a 20-s average (Robergs, Dwyer, & Astorino, 2010). The excess post-exercise oxygen consumption time-constant (EPOCt) was calculated by exponential fitting of 5 min VO_2 recovery data (do Nascimento Salvador et al. 2016). Additionally, the excess post-exercise

oxygen consumption magnitude ($EPOC_{MAG}$) was determined as the time integral of the 5 min VO_2 recovery curve values above VO_2 baseline (do Nascimento Salvador et al. 2016). Similarly, excess post-exercise Ventilation (ExcessVE) above resting value was also calculated.

Statistical Analysis

Data are presented as means ± standard deviations (SD). Data were tested for normal distribution with Shapiro–Wilk test. If data were not normally distributed, natural logarithm transformation (Ln) was applied to obtain a normal distribution and allow parametric statistical comparisons. Paired *t* -tests were performed to compare cardio-respiratory variables, HR and HRV indices at rest and during sub-maximal exercise period for N and H condition. HRR indices in the post-exercise period were compared using a two-way ANOVA for repeated measures, with "condition" (H and N) and "time" (time points 30s, 60s and 300s) as factors. For time-varying post-exercise HRV indices (Ln-RMSSD), a 2 (condition) × 10 (time) repeated-measures ANOVA was used to examine for main effects and interactions. When statistical significance was identified, a Sidak post hoc test was used to further delineate differences between condition or time (Cunha et al. 2015).

The magnitude of the difference between the two conditions was calculated by determining the Cohen d effect size (ES). The difference was considered trivial when ES < 0.2, small when ES 0.2–0.6, moderate when ES 0.6–1.2, and large when ES >1.2 (Hopkins et al. 2009). The relationships between variations from hypoxic and normoxic condition (as Δ%, (Hypoxia-Normoxia)/Normoxia *100) in HR, HRV and cardio-respiratory variables were analyzed using Pearson's correlation. Statistical analysis was completed using a statistical software (SPSS Inc, Chicago, Illinois, USA). The level of statistical significance was set at $p<0.05$.

Results

Effects of hypoxia at rest

HRV indices and other physiological variables at rest for H and N condition are reported in Table 1. At rest time-domain (Ln-RMSSD) and frequency-domain (Ln-LF, Ln-HF, Ln-TP) HRV indices were not significantly different between H and N ($p>0.05$). Only an increase in HR (Mean

Difference H-N (MD) 4.2 bpm, p=0.025, Effect size (ES) 0.76) was noted for H condition. Respiratory frequency (Rf) and minute ventilation (VE) were not significantly different in H compared with N (p>0.05). SpO_2 was markedly reduced in H (p<0.001).

**********Table 1 about here**********

CPET evaluation and post-exercise physiological outcomes

Results from CPET and post-exercise assessment are presented in Table 2. Hypoxia induced a reduction in maximal exercise performance indices. Lower VO_{2peak} and PPO were found in H compared to N (p<0.001). HR_{peak} was significantly reduced in H (MD -6.2 bpm, p<0.001, ES -0.50). Maximal respiratory frequency (Rf) and minute ventilation (VE) were not significantly different in H compared with N (p>0.05). SpO_2 was markedly reduced in H both during and at the end of CPET (p<0.001). Post-exercise physiological outcomes were affected by Hypoxia as well. EPOCt was increased in H (p=0.006), as well as ExcessVE (p=0.031), whereas $EPOC_{MAG}$ and blood lactate accumulation were not different in the two conditions (p>0.05).

**********Table 2 about here**********

Effect of hypoxia on post-exercise cardiac autonomic modulation

Indices of post-exercise cardiac autonomic modulation for N and H condition were reported in Table 3. A significant effect of “time” was found in all the HRR and HRV post-exercise recovery indices investigated (p<0.001). The two-way ANOVA for repeated measures showed a significant effect of “condition” (p<0.001) and “time” (p<0.001), with significant “interaction” (p=0.006) on HRR indices expressed in bpm. HRR30, HRR60 and HRR300s were significantly reduced in H (HRR30: MD -6.39 bpm, p=0.005, ES -1.16; HRR60: MD -11.70 bpm, p<0.001, ES -1.23; HRR300: MD -8.78 bpm, p= 0.004, ES -0.84). When expressed as a percentage of peak heart rate (%HRR) a significant effect of “condition” (p=0.005), “time” (p<0.001) and “interaction” (p=0.021) was also noted. %HRR was significantly reduced in H compared with N at 30s (MD -

3.22 %, p= 0.012, ES-0.97), 60s (MD -6.06 %, p<0.001, ES -1.00) and 300s (MD -3.38 %, p=0.045, ES -0.53) of the post-exercise recovery period. A significant effect of "condition" (p= 0.021), "time" (p<0.001) and "interaction" (p=0.021) was reported in HRR indices, when expressed as percentage of the chronotropic reserve (CRR). CRR was reduced in H compared with N at 30s (MD -3.77 %, p=0.028, ES -0.80) and at 60s (MD -7.23 %, p=0.003, ES -0.81), but not at 300s (p=0.436). Both short-term time constant, T30, and long-term time constant of HRR, HRRt, were significantly higher in H, indicating a slower decay of HR and a reduced HRR recovery. Concurrently, a non-significant effect of "condition" (p=0.183), with significant effects of "time" (p=0.010) and "interaction" (p=0.009), was reported on Ln-RMSSD. This index was significantly reduced in H at 60s (p=0.007) and at 90s (p=0.010) but not at other time points during the recovery (p>0.05).

**********Table 3 about here**********

Correlational analysis

For complete correlational analysis results please refer to electronic supplementary material 1 available online from the journal (ESM-1). Considering indices of parasympathetic reactivation, Δ%T30 (r=0.63; p=0.020), Δ%HRR30 (r=-0.56; p=0.046) and Δ%RMSSD300 (r=-0.77; p=0.002) were significantly correlated with $\Delta\%HR_{peak}$, whereas no significant relation was observed with Δ%HRR60 (r=-0.476; p=0.100). In addition, Δ%ExcessVe was significantly inversely related to Δ%HRR30 (r=-0.65; p=0.023) and Δ%RMSSD300 (r=-0.72; p=0.008), and significantly directly related to Δ%T30 (r=0.66; p=0.019). $\Delta\%[La]_b$ was directly related to Δ%T30 (r=0.62; p=0.025) and inversely related to Δ%HRR30 (r=-0.62; p=0.025) and Δ%RMSSD90 (r=-0.71; p=0.007) but not to Δ%HRR60 (r=-0.44, p=0.135). Both Δ%HRR60 (r=-0.61; p= 0.047) and Δ%RMSSD300 (r=-0.66; p=0.028) were significantly inversely correlated to Δ%EPOCt. $\Delta\%EPOC_{MAG}$ was significantly and directly related to $\Delta\%SpO_2$ at peak exercise intensity (r=0.63; p=0.038), Δ%ExcessVe (r=0.58; p=0.050), and inversely related to Δ%RMSSD30 (r=-0.79; p=0.002). Considering slow-phase HRR

indices, no significant relation with $\Delta\%HR_{peak}$ was observed for Δ%HRR300 (r=-0.22; p=0.465) and Δ%HRRt (r=0.47, p=0.124). Similarly, Δ%HRR300 (r=-0.38, p=0.199) and Δ%HRRt (r=0.27; p=0.402) were not significantly correlated to $\Delta\%[La]_{b.}$ However, Δ%HRRt was significantly inversely related to $\Delta\%SpO_2$60 (r=-0.81; p=0.005).

Discussion

**********Fig 1 about here**********

Despite being extensively investigated under normoxic condition for its implication in evaluating ANS functionality and assessing subject's health (Thayer et al. 2012), clinical (Qiu et al. 2017) and training status (Bellenger et al. 2016), to the best of our knowledge, this the first study examining post-exercise cardiac autonomic modulation, through the recovery of HR and HRV indices, in response to maximal hypoxic exercise. The key finding of this study was that in response to a maximal cardio-pulmonary exercise test (CPET) fast-phase HRR indices (T30, HRR30, HRR60), the recovery of HRV indices (Ln-RMSSD) and slow-phase HRR indices (HRRt) were significantly affected by acute hypoxia (FiO2=13.4%, ≈3500 m). These findings suggest delayed parasympathetic reactivation and sympathetic withdrawal after maximal hypoxic exercise (Pecanha et al. 2017). The delayed cardiac autonomic recovery in hypoxia was associated with a markedly decreased SpO_2, significantly higher EPOCt, similar $EPOC_{MAG}$ and increased ExcessVE, denoting amplified post-exercise physiological responses and increased homeostatic stress induced by hypoxic exercise (Mann et al. 2014).

Effects of hypoxia at rest

Acute hypoxic exposure leads to hemodynamic changes due to increase in sympathetic activation arising from arterial chemoreceptor stimulation (Dinenno 2016) and to decrease in baroreflex (Bourdillon et al. 2017). During rest and sub-maximal exercise, cardiovascular adjustments, including increased HR and cardiac output, and a compensatory vasodilation, occurring despite the

sympathoexcitatory effect of hypoxia, operate to face the lower arterial blood oxygen content (Dinenno 2016).

Regarding cardiac autonomic modulation, different levels and types of hypoxia (normobaric vs hypobaric hypoxia) appear to induce different HR and HRV responses (Zupet et al. 2009; Giles et al. 2016; Oliveira et al. 2017). Even if variations in HR and HRV indices have been previously documented in healthy men at rest at a simulated altitude of ≈2600 m (FiO2=15%) (Iwasaki et al. 2006), and at lower altitudes in elite athletes (1200m vs 1800m, real altitude) (Schmitt et al. 2006),a simulated altitude threshold of ≈6000m (FiO2=9.8%) has been recently proposed as the minimum required to induce change in resting cardiac autonomic modulation (Giles et al. 2016). In line with this observation, in our study only an increase in resting HR was noted for hypoxic condition (FiO_2=13.4%, 3500 m), without any variation in HRV spectral power or time-domain indices of parasympathetic activity at rest (RMSSD). The unchanged resting HRV profile can be partially clarified by the unchanged ventilatory responses (Nobrega et al. 2014; Siebenmann et al. 2015). Indeed, despite a significantly reduced SpO_2 (-10.4 %), respiratory variables were not significantly different at rest for the two conditions (Table 1). As participants underwent resting evaluations 30 minutes after hypoxic exposure, an attenuated ventilatory response could have occurred (Duffin 2007).

Peak exercise and post-exercise physiological outcomes

In line with existing literature (Calbet et al. 2003; Wehrlin and Hallén 2006) in this study a noticeable hypoxic influence on exercise capacity, with marked decreases in maximal oxygen consumption (VO_{2peak}) (≈-18%) and peak power out (PPO) (≈-14%), was found (Table 2). Additionally, together with reductions in VO_{2peak} a concurrent reduction in peak heart rate (HR_{peak}) at exhaustion was also noted. The decrease (≈-6.2 bpm, ≈-3.5%) was in line with previous studies investigating the progressive, and still discussed, reduction in HR_{peak} occurring with increasing levels of hypoxia (Grataloup et al. 2007; Mollard et al. 2007b; Gaston et al. 2016). This occurrence

may be likely associated with a decrease in maximal cardiac output, as previously suggested for maximal hypoxic exercise (Calbet et al. 2009).

The decrease in VO_{2peak} and HR_{peak} was not accompanied by any variation in maximal respiratory variables (Rf, VE) indicating that CPETs induced comparable maximal respiratory stress at peak exercise intensity (Ofner et al. 2014) (Table 2). Similarly, blood lactate concentration ($[La]_b$ indicated similar anaerobic metabolism contribution for the two conditions (Goodwin et al. 2007).

However, hypoxic CPET was associated with markedly reduced SpO_2 (≈-16.6%), higher EPOCt (≈24.9%), similar $EPOC_{MAG}$ (4.1 vs 3.9 L, for N and H respectively) and an increased ExcessVE (≈12.1%), when compared to normoxic CPET. Thus, despite the reduced sustained intensity and metabolic requirements of hypoxic exercise at exhaustion, this was accompanied by amplified post-exercise physiological outcomes suggesting an increased homeostatic stress (Mann et al. 2014). Increased chemoreflex stimulation associated with hypoxic exercise can explain the increased ventilatory response observed during hypoxic post-exercise recovery (Somers et al.; Al Haddad et al. 2012). In addition, the reduced post-exercise oxygen availability, in front of a similar exercise-induced metabolites accumulation, as inferred from blood lactate concentration, could have prolonged a sustained metaboreflex activation in the post-exercise period (Peçanha et al. 2016). This could further clarify the increased post-exercise ventilatory responses reported (Peçanha et al. 2016). Taken together, these evidences can help to explain the different post-exercise cardiac autonomic modulation observed in hypoxia.

Effect of hypoxia on post-exercise cardiac autonomic modulation

Reductions in post-exercise parasympathetic reactivation have been previously reported in normobaric hypoxia (2400 m, i.e. FiO_2=15.4%) for sub-maximal exercise intensities, but not after supra-maximal intensities (20 s sprint “all-out”)(Al Haddad et al. 2012). In this study we tested the hypothesis that a maximal exercise combined with a more severe hypoxic stimulus (FiO_2=13.4%, ≈3500 m), would have led to a delayed parasympathetic reactivation. In line with our hypothesis fast-phase HRR indices (i.e. the heart rate recovery within the first 30 or 60 s) were significantly

396 reduced under hypoxic condition (Fig 1.A, 1.B, and 1.C). HRR was reduced either when expressed
397 in bpm (Fig 1.A) or in percentage of HR_{peak} (Fig 1.B). Furthermore, beside the two aforementioned
398 widespread methods, HRR can be expressed as the recovery occurring in chronotropic reserve
399 (CRR) (Molina et al. 2016). This method may help HRR interpretation in hypoxic environments
400 where chronotropic reserve is reduced (Mollard et al. 2007b). Also CRR was reduced in hypoxia
401 (Fig 1.C). Together, these findings on HRR suggest a delayed parasympathetic reactivity after
402 normobaric hypoxic exercise.
403 Interestingly, comparing our results with those of Al Haddad et al. (2012), obtained in subjects with
404 similar fitness level (VO_{2max}), in line with existing evidence, parasympathetic reactivation assessed
405 through HRR60 was faster after maximal normoxic CPET (45±11bpm) than after supra-maximal
406 normoxic exercise (36±7 bpm). However, in this study we found that HRR60 after maximal CPET
407 at 3500m was similar to that observed after supra-maximal exercise at 2400m (34±8 vs 37±10
408 bpm). Despite the two different exercise modalities and the two different altitudes (2400m vs
409 3500m, i.e. moderate altitude vs high altitude), this occurrence may suggest a progressive decrease
410 in post-exercise parasympathetic recovery with increasing altitude, that needs to be further
411 investigated.
412 The delayed parasympathetic reactivation (Imai et al. 1994; Pecanha et al. 2017) in hypoxia was
413 further underlined by the increase (+35%) occurring in T30. When assessed in response to different
414 bouts of aerobic exercise, T30 is strongly dependent on previous exercise intensity, with higher
415 intensities causing higher increase in this index (Michael et al. 2016). Moreover, the highest values
416 of T30 (i.e. reduced recovery) have been documented after supra-maximal exercises (Buchheit et al.
417 2007a). According to this scenario, the same effects on T30 can be observed when a maximal
418 exercise is performed at sufficiently severe hypoxic levels.
419 Alongside the observed increase in HR, exercise is known to reduce HRV indices (e.g. RMSSD),
420 that tend to return to pre-exercise level at exercise stimulus cessation (Pecanha et al. 2017; Michael
421 et al. 2017a), or may remain depressed (up to 48h) when intensity exceeds the first ventilatory

threshold (Seiler et al. 2007). When assessed in the immediate post-exercise period the recovery of RMSSD can characterize the level of parasympathetic reactivation (Goldberger et al. 2006). In line with our findings on fast phase HRR indices, Ln-RMSSD was significantly reduced at 60s and 90s of recovery for hypoxic condition (Fig 1.D), demonstrating depressed HRV and a delayed recovery of parasympathetic cardiac control.

HRR300 and the long-term time-constant (HRRt), covering both the fast and slow phase of HRR, are considered markers of both parasympathetic reactivation and sympathetic withdrawal (Peçanha et al. 2016; Pecanha et al. 2017). In the study we hypothesized that the standardized maximal respiratory, cardiovascular and metabolic stress produced by a CPET combined with hypoxic post-exercise recovery would have led to a delayed sympathetic withdrawal. In line with our hypothesis, despite the larger effect size (moderate-large) observed in fast-phase HRR indices, also slow-phase HRR indices (HRRt) were reduced in hypoxia (Table 3). Indeed, HRRt was significantly increased by ≈30.4% after hypoxic exercise, suggesting a more sustained sympathetic activity during recovery for hypoxic condition (Peçanha et al. 2016).

In this case, when expressed as bpm or as $\%HR_{peak}$, HRR300 showed impaired recovery in hypoxia. Nevertheless, it should be noted that when adequately normalized for the changes already observable in HR at rest and at maximal exercise intensity (i.e. change in chronotropic reserve) (Molina et al. 2016), slow-phase HRR index indicated similar chronotropic reserve restoration within 5 min of recovery (CRR300 69.5±10.8 vs 68.0±7.7 %, in N and H, respectively) (Fig 1.C). These results, together with the comparable parasympathetic reactivation level observed (RMSSD, Fig1.D) suggested that, after an initial impairment, a similar cardiac autonomic recovery is re-established within 5 minutes post-exercise. However, different methods of evaluating post-exercise cardiac autonomic recovery can produce different results and observations in response to hypoxic exercise, and caution in therefore required in the interpretation of HRR in hypoxia due to the modification occurring in chronotropic reserve (Mollard et al. 2007a).

Correlational analysis

448 The degree of cardiac autonomic recovery impairment was related to the degree of homeostatic
449 stress induced by hypoxic exercise when compared with normoxic exercise (Δ% Hypoxia-
450 Normoxia). At peak exercise indices of cardiac stress ($\Delta\%HR_{peak}$) and anaerobic metabolism
451 contribution ($\Delta\%[La]_b$) were significantly related to indices of parasympathetic reactivation
452 (Δ%T30,Δ%HRR30, Δ%RMSSD90). Our results showed that the lower the difference between
453 normoxic and hypoxic HR_{peak}, or higher the anaerobic contribution, the more cardiac autonomic
454 recovery was impaired in the immediate post-exercise period. Equally, higher reduction in
455 parasympathetic recovery at 300s (Δ%RMSSD) were reported in subject reaching a higher
456 percentage of normoxic HR_{peak} in hypoxia. Furthermore, parasympathetic reactivation indices were
457 strongly related to measurements reflecting exercise-induced homeostatic stress (Mann, Webster,
458 Lamberts, & Lambert, 2014). For instance, Δ%ExcessVe was associated to Δ%T30, Δ%HRR30 and
459 Δ%RMSSD300. Similarly, higher increases in EPOCt after hypoxic exercise were associated with
460 higher decreases in HRR60 and RMSSD300, denoting delayed parasympathetic recovery.
461 Considering slow-phase HRR indices (Δ%HRR300 and Δ%HRRt), these were neither significant
462 related to indices of cardio-respiratory stress or anaerobic energy contribution. However, an
463 important relation with post-exercise oxygen saturation ($\Delta\%SpO_260$) was reported for Δ%HRRt. In
464 this case a higher variation in post-exercise SpO_260 (i.e. decrease) was associated with a higher
465 variation in HRRt (i.e. increase). Overall, these results are in line with existing evidence that higher
466 homeostatic disruptions cause lower post-exercise HR and HRV recovery (Buchheit et al. 2007a;
467 Michael et al. 2017a).
468 However, a novel finding of this study is that post-exercise cardiac autonomic recovery from a
469 maximal effort (i.e. a maximal exercise intensity) can be further delayed in hypoxia. Accordingly,
470 the findings of this study raise the scientific interest on the cardiac autonomic modulation responses
471 of high-intensity hypoxic exercise.
472 *Limitations*

Different exercises, characterized by a different muscular involvement, as well as different body positions assumed in the post-exercise period have been shown to induce different response in the recovery of HR and HRV indices (Barak et al. 2011; Cunha et al. 2015). Accordingly, the results obtained in this study may be limited to the specific exercise and the post-exercise recovery modality performed by the participants. Moreover, although the present findings suggested a delayed cardiac autonomic recovery after maximal hypoxic exercise, they were obtained on a homogenous group of moderately aerobically trained healthy men, and therefore further experimental researches are required to confirm this hypothesis on females, due to a possible gender effect, healthy non-active subjects, as well as on different special populations for which hypoxic training sessions may be relevant (Millet et al. 2016).

Furthermore, systolic time intervals (STI) investigation, reflecting cardiac sympathetic influences on myocardial contractility (Michael et al. 2017a), could have better elucidated post-exercise cardiac sympathetic modulation responses, also avoiding the confounding factor of resting and maximal HR change in hypoxia. Additionally, a third experimental condition performed at a moderate altitude (e.g. 2000m) would have helped clarifying an eventual progressive decrease of post-exercise cardiac autonomic recovery with increasing levels of hypoxia.

Future perspectives

CPET represents the gold standard laboratory test for cardio-respiratory fitness and exercise capacity evaluation (Albouaini et al. 2007) both in normoxic and hypoxic conditions (Ward et al. 2017). In normoxia CPET physiological data (e.g. HR and HRV) are widely adopted for exercise prescription, whereas the evaluation of HRR in the post-exercise period is an important clinical tool for the assessment of ANS functionality (Romero et al. 2017; Qiu et al. 2017). Similarly, when assessed in response to hypoxia, changes in HR and HRV indices are generally believed to reflect ANS responsiveness and body's ability to adapt to this environmental stressor (Oliveira et al. 2017). Nevertheless, information from hypoxic CPET is generally limited to exercising period, with inadequate information from post-exercise period. Accordingly, monitoring cardiac autonomic

recovery in response to hypoxic CPET may be useful to evaluate the chronic adaptive changes occurring in cardiac autonomic activity with hypoxic training.

Similarly, information regarding cardiac autonomic recovery from hypoxic training sessions is lacking. Accordingly, the implementation of post-exercise cardiac autonomic modulation assessment, together with the investigation of the acute physiological recovery responses, can help in providing effective information relatively the homeostatic stress induced and the body's ability to recover from hypoxic exercise. For instance, based on what we observed, during high-intensity interval training sessions, lower work-to-rest ratios (i.e. increased recovery duration) may be necessary in hypoxia, compared to normoxia, to induce similar post-exercise metabolic and cardiac autonomic modulation responses.

Conclusion

Acute hypoxia (FiO2=13.4%, ≈3500 m) modified post-exercise cardiac autonomic modulation in response to a maximal CPET, causing a reduction in fast-phase HRR, slow-phase HRR and HRV indices. In hypoxia the reduced cardiac autonomic recovery was associated with markedly decreased SpO_2 (≈-16.6%), significantly higher EPOCt (≈24.9%), similar $EPOC_{MAG}$ (4.1 vs 3.9 L, for N and H respectively) and increased ExcessVE (≈12.1%), denoting an amplified post-exercise physiological response and increased homeostatic stress associated with hypoxic exercise.

Taken together, these findings suggested both delayed parasympathetic reactivation and sympathetic withdrawal after maximal exercise in hypoxia. Interestingly, as suggested by correlational analysis, the degree of cardiac autonomic recovery impairment seems to be directly related to degree of homeostatic stress induced by hypoxic exercise when compared with normoxic exercise. However, comparable HRV indices and chronotropic reserve restoration indicated that the alterations occurring in cardiac autonomic recovery in hypoxia were restored within 5 minutes after exercise cessation. For the first time, this study showed that post-exercise cardiac autonomic recovery from a maximal effort can be further delayed in hypoxia. These findings have several implications in cardiac autonomic recovery interpretation and in HR assessment in response to

high-intensity hypoxic exercise and raise the scientific interest on cardiac autonomic modulation responses of hypoxic training.

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Author Contribution Statement

AF, AS, SS, LB, LM and BP participated in study conception and design. AF, AS and SS participated in data acquisition. AF, FSt, GB and AZ participated in data analysis. AF and LM were responsible for data interpretation. AF, AS, SS, GB, AZ, LM and BP contributed to the draft of the paper. AF, AS, SS, GB, AZ, FSc, LM and BP critically reviewed the manuscript. All authors approved the final version of the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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