Delayed parasympathetic reactivation and sympathetic withdrawal following maximal cardiopulmonary exercise testing (CPET) in hypoxia

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1 **Delayed parasympathetic reactivation and sympathetic withdrawal following maximal**

2 **cardiopulmonary exercise testing (CPET) in hypoxia**

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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

50 **Abbreviations**

51 ANOVA Analysis of variance

52 ANS Autonomic nervous system

53 CPET Cardiopulmonary exercise testing

54 CRR Chronotropic reserve recovery

55 EPOCt Excess of post-exercise oxygen consumption time-constant

56 $EPOC_{MAG}$ Excess of post-exercise oxygen consumption magnitude

57 LF Low-frequency spectral power

58 Ln Natural-logarithm transformation

59 HF High-frequency spectral power

60 HR Heart rate

61 HRR Heart rate recovery

62 HRRt Long-term time constant of heart rate recovery

63 HRV Heart rate variability

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

65 T30 Short-term time constant of heart rate recovery

66 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

118 and Hamlin 2017). For instance, low-intensity hypoxic exercise (e.g. walking) at a simulated

119 altitude of 3000-3500m can be adopted to safely increase the exercise physiological load while

120 reducing the external load in obese patients (Girard et al. 2017).

121 On the other hand, the above-mentioned altitudes are commonly employed by athletes involved in

122 endurance and intermittent sports for performing high-intensity hypoxic exercises (Millet et al.

123 2010; Brocherie et al. 2017). Similarly, high-intensity hypoxic exercises are also performed by

124 athletes for increasing their performance at altitude (Clark et al. 2007).

125 In this regard, hypoxia is well recognized to modify cardiac autonomic modulation at rest (Oliveira

126 et al. 2017) and in response to exercise (Yamamoto et al. 1996; Zupet et al. 2009; Fisher 2015).

127 Alongside, hypoxia acts as a stimulus for an increased sympathetic activity (Hainsworth et al. 2007;

128 Amann and Kayser 2009) and a reduced parasympathetic cardiac control (Perini and Veicsteinas

129 2003; Buchheit et al. 2004; Fisher 2015; Oliveira et al. 2017), that can turn in a slower post-exercise

130 recovery of HR and HRV indices (Al Haddad et al. 2012).

131 For instance, modifications in post-exercise cardiac autonomic modulation, with a delayed

132 parasympathetic reactivation, have been reported in hypoxia (FiO_2=15.4%, 2400m) after sub-

133 maximal exercise intensities (Al Haddad et al. 2012). On the contrary, in the above-mentioned work

134 (Al Haddad et al. 2012), the imposed hypoxic stimulus did not modify parasympathetic recovery

135 after a supra-maximal intensity (20 s sprint "all-out"), probably due to the already maximal

136 homeostatic perturbation induced by a supra-maximal intensity, causing high anaerobic energy

137 contribution and sympathetic activation (Buchheit et al. 2007a; Al Haddad et al. 2012).

138 To date, it is not clear if changes in post-exercise cardiac autonomic modulation can occur in

139 response to exercises performed at more severe hypoxic levels (i.e. FiO_2<15.4%; altitude>2400 m)

140 (Al Haddad et al. 2012). In particular, cardiac autonomic recovery from exercise performed at

141 simulated altitudes of 3000-3500 m, which are relevant for training (Millet et al. 2010; Brocherie et

142 al. 2017; Lizamore and Hamlin 2017) and competition (Clark et al. 2007) purposes, has not yet

143 been investigated.

144 Additionally, according to the above-mentioned scenario, exercises with high cardiorespiratory

145 involvement are widely performed in hypoxia, but post-exercise physiological outcomes have not

146 been specifically studied. It is currently unknown how hypoxia can affect post-exercise cardiac

147 autonomic modulation following a maximal exercise, where cardiovascular and respiratory systems

148 are maximally stressed and pushed to their functional limit (e.g. a maximal cardio-pulmonary

149 exercise test, CPET). This occurrence certainly limits the evaluation of recovery from hypoxic

150 exercise both when used for health assessment or training load quantification purposes (Borresen

151 and Lambert 2008; Ward et al. 2017).

152 Despite the expected lower exercise capacity (i.e. decreased VO_{2max} and peak exercise intensity)

153 (Mollard et al. 2007b), maximal aerobic hypoxic exercise can result in markedly reduced arterial

154 oxygen saturation (Favret and Richalet 2007), comparable cardio-respiratory stress (Ofner et al.

155 2014) and similar level of blood lactate accumulation (this point is still debated (Lundby et al. 2000;

156 van Hall 2007; West 2007). In this case, the homeostatic stress induced by a maximal hypoxic

157 exercise, may produce a more challenging situation for post-exercise cardiac autonomic recovery,

158 further showing amplified post-exercise physiological outcomes indicating increased homeostatic

159 perturbation (Mann et al. 2014).

160 From a practical standpoint, considering the widespread use of hypoxic training, it is also important

161 to establish whether hypoxia influences post-exercise cardiac autonomic recovery in response to a

162 maximal exercise.

163 Therefore, the purpose of this study was to investigate the effects of acute hypoxia on the post-

164 exercise cardiac autonomic modulation following a maximal cardiopulmonary exercise test (CPET).

165 According to previous observations about the influence of the homeostatic perturbation in

166 determining post-exercise outcomes (Buchheit et al. 2007a; Al Haddad et al. 2012), we

167 hypothesized that maximal hypoxic exercise would have been associated to a reduced recovery of

168 fast-phase HRR and HRV indices, reflecting a delayed parasympathetic reactivation, in the

169 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 $\approx$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

220 preceding CPET evaluation. The time-domain HRV index considered was the square root of the

221 sum of successive differences between adjacent normal R-R intervals squared (RMSSD). For

222 frequency-domain HRV indices, low frequency spectral power (LF, 0.04-0.15 Hz), high frequency

223 spectral power (HF, 0.15-0.4 Hz), and total spectral power (TP, 0.04-0.4 Hz) were calculated by

224 Fast Fourier Transform (FFT) (Task Force of the European Society of 1996).

225 Post-exercise heart rate recovery (HRR) indices were calculated with a customized script in Matlab

226 (Matlab, Mathworks Inc., USA). HRR indices were measured from the absolute differences

227 between HR_{peak} and the HR values at 30s, 60 s and 300s of recovery (HRR30, HRR60 and

228 HRR300) in the post-exercise period (averaged over 5s) (Peçanha et al. 2016). HRR was also

229 calculated as the relative decline in HR expressed as a percentage of HR_{peak}

230 (%HRR=HRR/HR_{peak}×100) and as the recovery of the chronotropic reserve (CRR = HRR/(HR_{peak}-

231 HR_{rest})×100) (Molina et al. 2016). T30, the short-time constant of HRR, was calculated as the

232 negative reciprocal of the slope of the regression line of natural-logarithmic transformed HR during

233 the first 30 s of recovery (Buchheit et al. 2007b). HRRt, the long-term time-constant of HRR, was

234 obtained after exponential fitting of the HR during the entire 300s of recovery (Pecanha et al. 2017).

235 This method has been previously suggested to quantify the time-constant of HRR within a time

236 period covering most of the post-exercise HR decay (Pecanha et al. 2017). Additionally, the time-

237 varying vagal-related index, RMSSD, was also calculated for each of the 30-s segments of recovery

238 (Goldberger et al. 2006).

239 The peak power output (PPO), achieved at athlete's exhaustion, was determined according to the

240 equation: PPO (W) = power output last stage completed (W) + [t (s)/stage duration (s) * stage

241 increment (W)], where t is the time of the uncompleted stage (Kuipers, Verstappen, Keizer,

242 Geurten, & Van Kranenburg, 1985). VO_{2peak} and other maximal cardio-respiratory variables were

243 defined as the highest values of a 20-s average (Robergs, Dwyer, & Astorino, 2010). The excess

244 post-exercise oxygen consumption time-constant (EPOCt) was calculated by exponential fitting of 5

245 min VO_2 recovery data (do Nascimento Salvador et al. 2016). Additionally, the excess post-exercise

246   oxygen consumption magnitude (EPOC$_{MAG}$) was determined as the time integral of the 5 min VO$_2$

247   recovery curve values above VO$_2$ baseline (do Nascimento Salvador et al. 2016). Similarly, excess

248   post-exercise Ventilation (ExcessVE) above resting value was also calculated.

249   *Statistical Analysis*

250   Data are presented as means ± standard deviations (SD). Data were tested for normal distribution

251   with Shapiro–Wilk test. If data were not normally distributed, natural logarithm transformation (Ln)

252   was applied to obtain a normal distribution and allow parametric statistical comparisons. Paired *t* -

253   tests were performed to compare cardio-respiratory variables, HR and HRV indices at rest and

254   during sub-maximal exercise period for N and H condition. HRR indices in the post-exercise period

255   were compared using a two-way ANOVA for repeated measures, with "condition" (H and N) and

256   "time" (time points 30s, 60s and 300s) as factors. For time-varying post-exercise HRV indices (Ln-

257   RMSSD), a 2 (condition) × 10 (time) repeated-measures ANOVA was used to examine for main

258   effects and interactions. When statistical significance was identified, a Sidak post hoc test was used

259   to further delineate differences between condition or time (Cunha et al. 2015).

260   The magnitude of the difference between the two conditions was calculated by determining the

261   Cohen d effect size (ES). The difference was considered trivial when ES < 0.2, small when ES 0.2–

262   0.6, moderate when ES 0.6–1.2, and large when ES >1.2 (Hopkins et al. 2009). The relationships

263   between variations from hypoxic and normoxic condition (as Δ%, (Hypoxia-Normoxia)/Normoxia

264   *100) in HR, HRV and cardio-respiratory variables were analyzed using Pearson's correlation.

265   Statistical analysis was completed using a statistical software (SPSS Inc, Chicago, Illinois, USA).

266   The level of statistical significance was set at *p*<0.05.

267   **Results**

268   *Effects of hypoxia at rest*

269   HRV indices and other physiological variables at rest for H and N condition are reported in Table 1.

270   At rest time-domain (Ln-RMSSD) and frequency-domain (Ln-LF, Ln-HF, Ln-TP) HRV indices

271   were not significantly different between H and N (p>0.05). Only an increase in HR (Mean

272 Difference H-N (MD) 4.2 bpm, p=0.025, Effect size (ES) 0.76) was noted for H condition.

273 Respiratory frequency (Rf) and minute ventilation (VE) were not significantly different in H

274 compared with N (p>0.05). SpO_2 was markedly reduced in H (p<0.001).

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

276 *CPET evaluation and post-exercise physiological outcomes*

277 Results from CPET and post-exercise assessment are presented in Table 2. Hypoxia induced a

278 reduction in maximal exercise performance indices. Lower VO_{2peak} and PPO were found in H

279 compared to N (p<0.001). HR_{peak} was significantly reduced in H (MD -6.2 bpm, p<0.001, ES -0.50).

280 Maximal respiratory frequency (Rf) and minute ventilation (VE) were not significantly different in

281 H compared with N (p>0.05). SpO_2 was markedly reduced in H both during and at the end of CPET

282 (p<0.001). Post-exercise physiological outcomes were affected by Hypoxia as well. EPOCt was

283 increased in H (p=0.006), as well as ExcessVE (p=0.031), whereas $EPOC_{MAG}$ and blood lactate

284 accumulation were not different in the two conditions (p>0.05).

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

286 *Effect of hypoxia on post-exercise cardiac autonomic modulation*

287 Indices of post-exercise cardiac autonomic modulation for N and H condition were reported in

288 Table 3. A significant effect of "time" was found in all the HRR and HRV post-exercise recovery

289 indices investigated (p<0.001). The two-way ANOVA for repeated measures showed a significant

290 effect of "condition" (p<0.001) and "time" (p<0.001), with significant "interaction" (p=0.006) on

291 HRR indices expressed in bpm. HRR30, HRR60 and HRR300s were significantly reduced in H

292 (HRR30: MD -6.39 bpm, p=0.005, ES -1.16; HRR60: MD -11.70 bpm, p<0.001, ES -1.23;

293 HRR300: MD -8.78 bpm, p= 0.004, ES -0.84). When expressed as a percentage of peak heart rate

294 (%HRR) a significant effect of "condition" (p=0.005), "time" (p<0.001) and "interaction"

295 (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, $\Delta\%T30$ (r=0.63; p=0.020), $\Delta\%HRR30$ (r=-0.56; p=0.046) and $\Delta\%RMSSD300$ (r=-0.77; p=0.002) were significantly correlated with $\Delta\%HR_{peak}$, whereas no significant relation was observed with $\Delta\%HRR60$ (r=-0.476; p=0.100). In addition, $\Delta\%ExcessVe$ was significantly inversely related to $\Delta\%HRR30$ (r=-0.65; p=0.023) and $\Delta\%RMSSD300$ (r=-0.72; p=0.008), and significantly directly related to $\Delta\%T30$ (r=0.66; p=0.019). $\Delta\%[La]_b$ was directly related to $\Delta\%T30$ (r=0.62; p=0.025) and inversely related to $\Delta\%HRR30$ (r=-0.62; p=0.025) and $\Delta\%RMSSD90$ (r=-0.71; p=0.007) but not to $\Delta\%HRR60$ (r=-0.44, p=0.135). Both $\Delta\%HRR60$ (r=-0.61; p= 0.047) and $\Delta\%RMSSD300$ (r=-0.66; p=0.028) were significantly inversely correlated to $\Delta\%EPOCt$. $\Delta\%EPOC_{MAG}$ was significantly and directly related to $\Delta\%SpO_2$ at peak exercise intensity (r=0.63; p=0.038), $\Delta\%ExcessVe$ (r=0.58; p=0.050), and inversely related to $\Delta\%RMSSD30$ (r=-0.79; p=0.002). Considering slow-phase HRR

321   indices, no significant relation with $\Delta\%HR_{peak}$ was observed for $\Delta\%HRR300$ (r=-0.22; p=0.465)

322   and $\Delta\%HRRt$ (r=0.47, p=0.124). Similarly, $\Delta\%HRR300$ (r=-0.38, p=0.199) and $\Delta\%HRRt$ (r=0.27;

323   p=0.402) were not significantly correlated to $\Delta\%[La]_b$. However, $\Delta\%HRRt$ was significantly

324   inversely related to $\Delta\%SpO_260$ (r=-0.81; p=0.005).

325   **Discussion**

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

327   Despite being extensively investigated under normoxic condition for its implication in evaluating

328   ANS functionality and assessing subject's health (Thayer et al. 2012), clinical (Qiu et al. 2017) and

329   training status (Bellenger et al. 2016), to the best of our knowledge, this the first study examining

330   post-exercise cardiac autonomic modulation, through the recovery of HR and HRV indices, in

331   response to maximal hypoxic exercise. The key finding of this study was that in response to a

332   maximal cardio-pulmonary exercise test (CPET) fast-phase HRR indices (T30, HRR30, HRR60),

333   the recovery of HRV indices (Ln-RMSSD) and slow-phase HRR indices (HRRt) were significantly

334   affected by acute hypoxia (FiO2=13.4%, ≈3500 m). These findings suggest delayed

335   parasympathetic reactivation and sympathetic withdrawal after maximal hypoxic exercise (Pecanha

336   et al. 2017). The delayed cardiac autonomic recovery in hypoxia was associated with a markedly

337   decreased SpO_2, significantly higher EPOCt, similar $EPOC_{MAG}$ and increased ExcessVE, denoting

338   amplified post-exercise physiological responses and increased homeostatic stress induced by

339   hypoxic exercise (Mann et al. 2014).

340   *Effects of hypoxia at rest*

341   Acute hypoxic exposure leads to hemodynamic changes due to increase in sympathetic activation

342   arising from arterial chemoreceptor stimulation (Dinenno 2016) and to decrease in baroreflex

343   (Bourdillon et al. 2017). During rest and sub-maximal exercise, cardiovascular adjustments,

344   including increased HR and cardiac output, and a compensatory vasodilation, occurring despite the

345 sympathoexcitatory effect of hypoxia, operate to face the lower arterial blood oxygen content

346 (Dinenno 2016).

347 Regarding cardiac autonomic modulation, different levels and types of hypoxia (normobaric vs

348 hypobaric hypoxia) appear to induce different HR and HRV responses (Zupet et al. 2009; Giles et

349 al. 2016; Oliveira et al. 2017). Even if variations in HR and HRV indices have been previously

350 documented in healthy men at rest at a simulated altitude of $\approx$2600 m (FiO2=15%) (Iwasaki et al.

351 2006), and at lower altitudes in elite athletes (1200m vs 1800m, real altitude) (Schmitt et al. 2006),a

352 simulated altitude threshold of $\approx$6000m (FiO2=9.8%) has been recently proposed as the minimum

353 required to induce change in resting cardiac autonomic modulation (Giles et al. 2016). In line with

354 this observation, in our study only an increase in resting HR was noted for hypoxic condition

355 (FiO$_2$=13.4%, 3500 m), without any variation in HRV spectral power or time-domain indices of

356 parasympathetic activity at rest (RMSSD). The unchanged resting HRV profile can be partially

357 clarified by the unchanged ventilatory responses (Nobrega et al. 2014; Siebenmann et al. 2015).

358 Indeed, despite a significantly reduced SpO$_2$ (-10.4 %), respiratory variables were not significantly

359 different at rest for the two conditions (Table 1). As participants underwent resting evaluations 30

360 minutes after hypoxic exposure, an attenuated ventilatory response could have occurred (Duffin

361 2007).

362 *Peak exercise and post-exercise physiological outcomes*

363 In line with existing literature (Calbet et al. 2003; Wehrlin and Hallén 2006) in this study a

364 noticeable hypoxic influence on exercise capacity, with marked decreases in maximal oxygen

365 consumption (VO$_{2peak}$) ($\approx$-18%) and peak power out (PPO) ($\approx$-14%), was found (Table 2).

366 Additionally, together with reductions in VO$_{2peak}$ a concurrent reduction in peak heart rate (HR$_{peak}$)

367 at exhaustion was also noted. The decrease ($\approx$-6.2 bpm, $\approx$-3.5%) was in line with previous studies

368 investigating the progressive, and still discussed, reduction in HR$_{peak}$ occurring with increasing

369 levels of hypoxia (Grataloup et al. 2007; Mollard et al. 2007b; Gaston et al. 2016). This occurrence

370 may be likely associated with a decrease in maximal cardiac output, as previously suggested for

371 maximal hypoxic exercise (Calbet et al. 2009).

372 The decrease in VO_{2peak} and HR_{peak} was not accompanied by any variation in maximal respiratory

373 variables (Rf, VE) indicating that CPETs induced comparable maximal respiratory stress at peak

374 exercise intensity (Ofner et al. 2014) (Table 2). Similarly, blood lactate concentration ($[La]_b$

375 indicated similar anaerobic metabolism contribution for the two conditions (Goodwin et al. 2007).

376 However, hypoxic CPET was associated with markedly reduced SpO_2 ($\approx$-16.6%), higher EPOCt

377 ($\approx$24.9%), similar $EPOC_{MAG}$ (4.1 vs 3.9 L, for N and H respectively) and an increased ExcessVE

378 ($\approx$12.1%), when compared to normoxic CPET. Thus, despite the reduced sustained intensity and

379 metabolic requirements of hypoxic exercise at exhaustion, this was accompanied by amplified post-

380 exercise physiological outcomes suggesting an increased homeostatic stress (Mann et al. 2014).

381 Increased chemoreflex stimulation associated with hypoxic exercise can explain the increased

382 ventilatory response observed during hypoxic post-exercise recovery (Somers et al.; Al Haddad et

383 al. 2012). In addition, the reduced post-exercise oxygen availability, in front of a similar exercise-

384 induced metabolites accumulation, as inferred from blood lactate concentration, could have

385 prolonged a sustained metaboreflex activation in the post-exercise period (Peçanha et al. 2016).

386 This could further clarify the increased post-exercise ventilatory responses reported (Peçanha et al.

387 2016). Taken together, these evidences can help to explain the different post-exercise cardiac

388 autonomic modulation observed in hypoxia.

389 *Effect of hypoxia on post-exercise cardiac autonomic modulation*

390 Reductions in post-exercise parasympathetic reactivation have been previously reported in

391 normobaric hypoxia (2400 m, i.e. FiO_2=15.4%) for sub-maximal exercise intensities, but not after

392 supra-maximal intensities (20 s sprint "all-out")(Al Haddad et al. 2012). In this study we tested the

393 hypothesis that a maximal exercise combined with a more severe hypoxic stimulus (FiO_2=13.4%,

394 $\approx$3500 m), would have led to a delayed parasympathetic reactivation. In line with our hypothesis

395 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

The degree of cardiac autonomic recovery impairment was related to the degree of homeostatic stress induced by hypoxic exercise when compared with normoxic exercise (Δ% Hypoxia-Normoxia). At peak exercise indices of cardiac stress (Δ%HR$_{peak}$) and anaerobic metabolism contribution (Δ%[La]$_b$) were significantly related to indices of parasympathetic reactivation (Δ%T30, Δ%HRR30, Δ%RMSSD90). Our results showed that the lower the difference between normoxic and hypoxic HR$_{peak}$, or higher the anaerobic contribution, the more cardiac autonomic recovery was impaired in the immediate post-exercise period. Equally, higher reduction in parasympathetic recovery at 300s (Δ%RMSSD) were reported in subject reaching a higher percentage of normoxic HR$_{peak}$ in hypoxia. Furthermore, parasympathetic reactivation indices were strongly related to measurements reflecting exercise-induced homeostatic stress (Mann, Webster, Lamberts, & Lambert, 2014). For instance, Δ%ExcessVe was associated to Δ%T30, Δ%HRR30 and Δ%RMSSD300. Similarly, higher increases in EPOCt after hypoxic exercise were associated with higher decreases in HRR60 and RMSSD300, denoting delayed parasympathetic recovery. Considering slow-phase HRR indices (Δ%HRR300 and Δ%HRRt), these were neither significant related to indices of cardio-respiratory stress or anaerobic energy contribution. However, an important relation with post-exercise oxygen saturation (Δ%SpO$_2$60) was reported for Δ%HRRt. In this case a higher variation in post-exercise SpO$_2$60 (i.e. decrease) was associated with a higher variation in HRRt (i.e. increase). Overall, these results are in line with existing evidence that higher homeostatic disruptions cause lower post-exercise HR and HRV recovery (Buchheit et al. 2007a; Michael et al. 2017a).

However, a novel finding of this study is that post-exercise cardiac autonomic recovery from a maximal effort (i.e. a maximal exercise intensity) can be further delayed in hypoxia. Accordingly, the findings of this study raise the scientific interest on the cardiac autonomic modulation responses of high-intensity hypoxic exercise.

Limitations

473 Different exercises, characterized by a different muscular involvement, as well as different body

474 positions assumed in the post-exercise period have been shown to induce different response in the

475 recovery of HR and HRV indices (Barak et al. 2011; Cunha et al. 2015). Accordingly, the results

476 obtained in this study may be limited to the specific exercise and the post-exercise recovery

477 modality performed by the participants. Moreover, although the present findings suggested a

478 delayed cardiac autonomic recovery after maximal hypoxic exercise, they were obtained on a

479 homogenous group of moderately aerobically trained healthy men, and therefore further

480 experimental researches are required to confirm this hypothesis on females, due to a possible gender

481 effect, healthy non-active subjects, as well as on different special populations for which hypoxic

482 training sessions may be relevant (Millet et al. 2016).

483 Furthermore, systolic time intervals (STI) investigation, reflecting cardiac sympathetic influences

484 on myocardial contractility (Michael et al. 2017a), could have better elucidated post-exercise

485 cardiac sympathetic modulation responses, also avoiding the confounding factor of resting and

486 maximal HR change in hypoxia. Additionally, a third experimental condition performed at a

487 moderate altitude (e.g. 2000m) would have helped clarifying an eventual progressive decrease of

488 post-exercise cardiac autonomic recovery with increasing levels of hypoxia.

489 *Future perspectives*

490 CPET represents the gold standard laboratory test for cardio-respiratory fitness and exercise

491 capacity evaluation (Albouaini et al. 2007) both in normoxic and hypoxic conditions (Ward et al.

492 2017). In normoxia CPET physiological data (e.g. HR and HRV) are widely adopted for exercise

493 prescription, whereas the evaluation of HRR in the post-exercise period is an important clinical tool

494 for the assessment of ANS functionality (Romero et al. 2017; Qiu et al. 2017). Similarly, when

495 assessed in response to hypoxia, changes in HR and HRV indices are generally believed to reflect

496 ANS responsiveness and body's ability to adapt to this environmental stressor (Oliveira et al. 2017).

497 Nevertheless, information from hypoxic CPET is generally limited to exercising period, with

498 inadequate information from post-exercise period. Accordingly, monitoring cardiac autonomic

499   recovery in response to hypoxic CPET may be useful to evaluate the chronic adaptive changes

500   occurring in cardiac autonomic activity with hypoxic training.

501   Similarly, information regarding cardiac autonomic recovery from hypoxic training sessions is

502   lacking. Accordingly, the implementation of post-exercise cardiac autonomic modulation

503   assessment, together with the investigation of the acute physiological recovery responses, can help

504   in providing effective information relatively the homeostatic stress induced and the body's ability to

505   recover from hypoxic exercise. For instance, based on what we observed, during high-intensity

506   interval training sessions, lower work-to-rest ratios (i.e. increased recovery duration) may be

507   necessary in hypoxia, compared to normoxia, to induce similar post-exercise metabolic and cardiac

508   autonomic modulation responses.

509   **Conclusion**

510   Acute hypoxia (FiO2=13.4%, ≈3500 m) modified post-exercise cardiac autonomic modulation in

511   response to a maximal CPET, causing a reduction in fast-phase HRR, slow-phase HRR and HRV

512   indices. In hypoxia the reduced cardiac autonomic recovery was associated with markedly

513   decreased SpO_2 (≈-16.6%), significantly higher EPOCt (≈24.9%), similar $EPOC_{MAG}$ (4.1 vs 3.9 L,

514   for N and H respectively) and increased ExcessVE (≈12.1%), denoting an amplified post-exercise

515   physiological response and increased homeostatic stress associated with hypoxic exercise.

516   Taken together, these findings suggested both delayed parasympathetic reactivation and

517   sympathetic withdrawal after maximal exercise in hypoxia. Interestingly, as suggested by

518   correlational analysis, the degree of cardiac autonomic recovery impairment seems to be directly

519   related to degree of homeostatic stress induced by hypoxic exercise when compared with normoxic

520   exercise. However, comparable HRV indices and chronotropic reserve restoration indicated that the

521   alterations occurring in cardiac autonomic recovery in hypoxia were restored within 5 minutes after

522   exercise cessation. For the first time, this study showed that post-exercise cardiac autonomic

523   recovery from a maximal effort can be further delayed in hypoxia. These findings have several

524   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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