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

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26 **Abstract**

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

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

33 *Results:* Both short-term, T30 (Mean Difference (MD) 60.0 s, 95% CI 18.2 to 101.8, $p=0.009$, ES
34 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
35 HRR were higher in H. Fast-phase (30s and 60s) and slow-phase (300s) HRR indices were reduced
36 in H either when expressed in bpm or in percentage of HR_{peak} ($p<0.05$). Chronotropic reserve
37 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)
38 and at 60s (MD -7.23 %, 95% CI -11.45 to -3.01, $p=0.003$, ES -0.81), but not at 300s ($p=0.436$).
39 Concurrently, Ln-RMSSD was reduced in H at 60s and 90s ($p<0.01$) but not at other time points
40 during recovery ($p>0.05$).

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

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47

48 **Keywords:** heart rate recovery; hypoxia; post-exercise recovery, hypoxic exercise; cardiac
49 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 EPOC_t 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 HRR_t 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

67 **Introduction**

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

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

84 Previous studies have investigated post-exercise cardiac autonomic recovery in response to different
85 “stressors”, such as different exercise intensities (Terziotti et al. 2001; Cottin et al. 2004; Seiler et
86 al. 2007), exercise durations (Michael et al. 2017b), exercise modalities (e.g. upper vs lower-body
87 muscles involvement (Michael et al. 2018), different whole-body endurance exercises (Cunha et al.
88 2015)) and modified environmental conditions (e.g. hypoxia) (Al Haddad et al. 2012). In addition
89 the effects of different recovery strategies (e.g. body postures assumed during recovery (Buchheit et
90 al. 2009a) or different water immersion temperatures (Buchheit et al. 2009b; Al Haddad et al. 2010;
91 de Oliveira Ottone et al. 2014) have also been studied.

92 According to these previous investigations, post-exercise cardiac autonomic modulation is
93 influenced by the degree of the stimulus imposed (Seiler et al. 2007; Michael et al. 2016), with
94 higher homeostatic disruptions (i.e. higher exercise intensities (Buchheit et al. 2007a; Seiler et al.
95 2007) or durations (Michael et al. 2017b)) causing slower recovery of HR and HRV indices.
96 Additionally, the pre-exercise autonomic state seems also to be of importance (Cunha et al. 2015;
97 Molina et al. 2016). Indeed a higher parasympathetic activity at rest has been associated with a
98 faster recovery of HR and HRV indices in the post-exercise period (Danieli et al. 2014; Cunha et al.
99 2015). However, the association between resting HRV and post-exercise HRR is still debated due to
100 the conflicting results reported in the literature (Esco et al. 2010).

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

106 Nowadays, hypoxic training is commonly employed to induce greater physiological training
107 adaptations in athletic populations (Millet et al. 2010; Brocherie et al. 2017) and has recently
108 emerged as a promising training modality for sedentary and special populations (elderly, obese and
109 hypertensive patients) (Millet et al. 2016; Lizamore and Hamlin 2017). Indeed, whereas positive
110 haematological and non-haematological adaptations, increasing endurance performance, can be
111 obtained with a prolonged hypoxic exposure at altitudes as high as 2000-2500m (>12 h/day), often
112 avoiding the combined stimulus of exercise and hypoxia (live high-train low model, LHTL) (Millet
113 et al. 2010; Chapman et al. 2014), other beneficial training adaptations and/or greater positive
114 exercise-related physiological outcomes can be induced by performing exercise under hypoxic
115 conditions (live low-train high model, LLTH) (Millet et al. 2010, 2016; Brocherie et al. 2017). A
116 recent literature underlined the utilization of hypoxic levels, as high as 3000-3500 m of simulated
117 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

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

173 **Materials and methods**

174 *Participants*

175 Thirteen healthy men (age 34.1 ± 9.7 years, height 175.3 ± 4.6 , weight 69.4 ± 6.0 kg) volunteered
176 for this study. All participants were moderate aerobically trained and familiarized with high-
177 intensity exercise. None of them had been at altitude above 2000m for prolonged periods of time
178 (>12 hours) at least 3 months before the study. None of the participants involved had clinical
179 evidence of cardiovascular, metabolic, or musculoskeletal diseases. Before data collection, all
180 participants were properly informed about the experimental protocol and gave their written
181 informed consent for the measures. They were instructed to avoid caffeine, alcohol and high-
182 intensity exercise during the 24-h proceeding each test session. The experimental protocol was
183 approved by the institutional Ethics Committee of the University of Verona (Italy).

184 *Protocol*

185 Each participant visited the laboratory in two occasions at the same time of the day and completed
186 the experimental protocol within 2-week period. Participants randomly performed an evaluation in
187 normoxia (N) and normobaric hypoxia (H). All tests were conducted under controlled laboratory
188 conditions (18°C , 50% relative humidity). The hypoxic environment was created through the
189 manipulation of the FiO_2 by means of an oxygen dilution system based on the Vacuum-Pressure
190 Swing Adsorption principle (B-Cat, Tiel, The Netherlands). For H condition the FiO_2 was set at
191 13.4% to simulate an altitude of $\approx 3500\text{m}$ a.s.l.

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

194 min of baseline measurements at rest, 10 min of sub-maximal constant load exercise (75W), a
195 maximal cardio-pulmonary exercise test (CPET) and 5 min of post-exercise recovery assessment.
196 CPET started immediately after the sub-maximal exercise with increments of 25W every 1 min
197 until participants' volitional exhaustion. The pedalling cadence during the submaximal exercise and
198 the CPET was kept constant at 90 revolutions/min, using a monitor that provided participants with
199 visual feedback. Throughout rest, exercise and recovery phases, beat-to beat heart rate was
200 continuously recorded using a Polar RS800CX heart rate monitor (Polar, Kempele, Finland).
201 During resting and exercise cardio-respiratory measures were collected continuously with breath-
202 by-breath method using an automated open-circuit gas analysis system (Quark PFT Ergo, Cosmed
203 Srl, Rome, Italy). Careful calibrations of flow sensors and gas analyzers were performed before
204 each measurement according to the manufacturer's instructions. Pulse oxygen saturation (SpO₂)
205 was continuously recorded by fingertip pulse oximetry (Nonin Medical, Minneapolis, MN, USA) at
206 a sampling frequency of 1.0 Hz. To measure blood lactate accumulation a blood sample was
207 collected from the earlobe 3 min after the end of the test (Goodwin et al. 2007; Buchheit et al.
208 2007b; Al Haddad et al. 2012). The lactate analyser (Biosen C-line, EKF Diagnostics GmbH,
209 Barleben, Germany) was calibrated according to the manufacturer's instructions. The individual
210 rating of perceived exertion (RPE) was assessed at the end of 5-min recovery period using Borg
211 Category Ratio Scale (CR100) (Borg and Borg 2002) .

212 *Data Analysis*

213 The R-R intervals were uploaded using Polar Precision Performance Software (Polar, Kempele,
214 Finland) and then exported as .txt files. Signal artifacts were filtered out by means of a moderate
215 error correction filter with minimum protection zone of 6 bpm (Al Haddad et al. 2012). All the time
216 series of R-R intervals showed low noise (identified errors <5%). HRV analysis was performed
217 using Kubios HRV software (Version 2.1, Biosignal Analysis and Medical Imaging Group, Kuopio,
218 Finland). At rest HRV indices were calculated from the last 5min of the 6-min resting period.
219 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 ($\%HRR = HRR / HR_{peak} \times 100$) and as the recovery of the chronotropic reserve ($CRR = HRR / (HR_{peak} -$
231 $HR_{rest}) \times 100$) (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). HRR_t, 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: $PPO (W) = \text{power output last stage completed (W)} + [t (s) / \text{stage duration (s)} * \text{stage}$
241 $\text{increment (W)}]$, 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 (EPOC_t) 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 \pm 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) \times 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 $\Delta\%$, (Hypoxia-Normoxia)/Normoxia
264 $\times 100$) 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 ($p>0.05$). SpO₂ 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 ($p>0.05$). SpO₂ 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 -

296 3.22 %, $p=0.012$, ES-0.97), 60s (MD -6.06 %, $p<0.001$, ES -1.00) and 300s (MD -3.38 %, $p=0.045$,
297 ES -0.53) of the post-exercise recovery period. A significant effect of “condition” ($p=0.021$),
298 “time” ($p<0.001$) and “interaction” ($p=0.021$) was reported in HRR indices, when expressed as
299 percentage of the chronotropic reserve (CRR). CRR was reduced in H compared with N at 30s (MD
300 -3.77 %, $p=0.028$, ES -0.80) and at 60s (MD -7.23 %, $p=0.003$, ES -0.81), but not at 300s
301 ($p=0.436$). Both short-term time constant, T30, and long-term time constant of HRR, HRR_t, were
302 significantly higher in H, indicating a slower decay of HR and a reduced HRR recovery.
303 Concurrently, a non-significant effect of “condition” ($p=0.183$), with significant effects of “time”
304 ($p=0.010$) and “interaction” ($p=0.009$), was reported on Ln-RMSSD. This index was significantly
305 reduced in H at 60s ($p=0.007$) and at 90s ($p=0.010$) but not at other time points during the recovery
306 ($p>0.05$).

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

308 *Correlational analysis*

309 For complete correlational analysis results please refer to electronic supplementary material 1
310 available online from the journal (ESM-1). Considering indices of parasympathetic reactivation,
311 $\Delta\%T30$ ($r=0.63$; $p=0.020$), $\Delta\%HRR30$ ($r=-0.56$; $p=0.046$) and $\Delta\%RMSSD300$ ($r=-0.77$; $p=0.002$)
312 were significantly correlated with $\Delta\%HR_{peak}$, whereas no significant relation was observed with
313 $\Delta\%HRR60$ ($r=-0.476$; $p=0.100$). In addition, $\Delta\%ExcessVe$ was significantly inversely related to
314 $\Delta\%HRR30$ ($r=-0.65$; $p=0.023$) and $\Delta\%RMSSD300$ ($r=-0.72$; $p=0.008$), and significantly directly
315 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
316 inversely related to $\Delta\%HRR30$ ($r=-0.62$; $p=0.025$) and $\Delta\%RMSSD90$ ($r=-0.71$; $p=0.007$) but not to
317 $\Delta\%HRR60$ ($r=-0.44$, $p=0.135$). Both $\Delta\%HRR60$ ($r=-0.61$; $p=0.047$) and $\Delta\%RMSSD300$ ($r=-0.66$;
318 $p=0.028$) were significantly inversely correlated to $\Delta\%EPOC_t$. $\Delta\%EPOC_{MAG}$ was significantly and
319 directly related to $\Delta\%SpO_2$ at peak exercise intensity ($r=0.63$; $p=0.038$), $\Delta\%ExcessVe$ ($r=0.58$;
320 $p=0.050$), 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 $\Delta\%HRR_{300}$ ($r=-0.22$; $p=0.465$)
322 and $\Delta\%HRR_t$ ($r=0.47$, $p=0.124$). Similarly, $\Delta\%HRR_{300}$ ($r=-0.38$, $p=0.199$) and $\Delta\%HRR_t$ ($r=0.27$;
323 $p=0.402$) were not significantly correlated to $\Delta\%[La]_b$. However, $\Delta\%HRR_t$ was significantly
324 inversely related to $\Delta\%SpO_2_{60}$ ($r=-0.81$; $p=0.005$).

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 ($FiO_2=13.4\%$, ≈ 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 (Dinanno 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 ≈ 2600 m ($FiO_2=15\%$) (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 ≈ 6000 m ($FiO_2=9.8\%$) 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 (≈ -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 ≈ 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 ± 11 bpm) 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

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

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

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

447 *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 ($\Delta\%$ 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 ($\Delta\%T30, \Delta\%HRR30, \Delta\%RMSSD90$). 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 ($\Delta\%RMSSD$) 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, $\Delta\%ExcessVe$ was associated to $\Delta\%T30, \Delta\%HRR30$ and
459 $\Delta\%RMSSD300$. 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 ($\Delta\%HRR300$ and $\Delta\%HRRt$), 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 $\Delta\%HRRt$. 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*

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 ($F_{iO_2}=13.4\%$, ≈ 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 ($\approx -16.6\%$), significantly higher $EPOC_t$ ($\approx 24.9\%$), similar $EPOC_{MAG}$ (4.1 vs 3.9 L,
514 for N and H respectively) and increased $ExcessVE$ ($\approx 12.1\%$), 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

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

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532 **Author Contribution Statement**

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

538 **Conflict of interest**

539 The authors declare that they have no conflict of interest.

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