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DOCTORAL THESIS

"Acute and post-exercise physiological responses to different hypoxic exercises"

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Abstract

A better understanding of the acute and post-exercise physiological responses to hypoxic exercise (i.e. exercise combined with hypoxic stress) can provide knowledge to improve human tolerance to high altitude environments, also providing helpful information for the adoption of safe exercise protocols in individuals engaged in hypoxic exercise training. This doctoral thesis investigated the acute exercise and post-exercise physiological responses evoked by hypoxic exercises of various intensities and nature, providing new insights into the understanding of the complex interaction between exercise and hypoxic stimuli in inducing the aforementioned responses.

Study 1 investigates the effects of hypoxia (FiO_2=13.4%, ≈3500 m) on the acute exercise and post-exercise cardiorespiratory and cardiac autonomic responses to a maximal cardiopulmonary exercise test. The study shows that cardiac autonomic recovery is delayed in response to a maximal cardiopulmonary exercise test in hypoxia and that the degree of cardiac autonomic recovery impairment is directly related to the increase of exercise-induced physiological stress associated with hypoxic exercise.

Study 2 aims at examining the effects of hypoxic exercise (FiO_2=14.2%, ≈3000 m) performed at the same absolute intensity (i.e. 80% of the power output at the first ventilatory threshold) or same relative intensity (i.e. heart rate matched exercise) of normoxic exercise on the exercise cardiac autonomic and cardiorespiratory responses. The study shows that moderate heart rate matched hypoxic exercise triggers similar cardiac autonomic and physiological responses to normoxic exercise with a reduced mechanical load; whilst the same absolute intensity exercise in hypoxia is associated with increased exercise-induced physiological stress and delayed cardiac autonomic recovery.

Study 3 aims at examining the effects of hypoxic exercise (FiO_2=14.2%, ≈3000 m) performed at the same absolute (i.e. work rate matched exercise) or same relative (i.e. heart rate matched exercise) normoxic exercise intensity on the post-exercise cardiac autonomic and cardiovascular responses. The study shows that moderate heart rate matched hypoxic exercise (≈75% HRmax) does not affect cardiac baroreflex sensitivity and does not blunt cardiac autonomic recovery during post-exercise

recovery, but does not induce significant post-exercise hypotension. Conversely, work rate matched hypoxic exercise, resulting in greater physiological stress, delays cardiac autonomic recovery, temporarily decreases cardiac baroreflex sensitivity and evokes prolonged post-exercise hypotension.

Study 4 aims at investigating the effects of a simulated mountain hike in a cold and hypoxic environment (-25°C, FiO2=11%, ≈5000 m) on cardiac autonomic activity, and the influence of two different strategies (i.e. different work-rest durations, long vs short) on the physiological and perceptual responses associated with the simulated ascent. The study shows that reduced work-rest durations are associated with improved perceptual responses and less perturbation of cardiac autonomic balance compared to longer work-rest durations in response to a simulated hike at high altitude.

Investigating the acute exercise and post-exercise physiological responses evoked by hypoxic exercises of various intensities and nature, this doctoral thesis and the data presented herein want to expand our understanding of hypoxic exercise and stimulate new research on this topic.

List of publications

- Fornasiero, A., Savoldelli, A., Skafidas, S., Stella, F., Bortolan, L., Boccia, G., Zignoli, A. , Schena, F., Mourot L. & Pellegrini, B. (2018). Delayed parasympathetic reactivation and sympathetic withdrawal following maximal cardiopulmonary exercise testing (CPET) in hypoxia. *European journal of applied physiology*, 118(10), 2189-2201. https://doi.org/10.1007/s00421-018-3945-5
- Fornasiero, A., Skafidas, S., Stella, F., Zignoli, A., Savoldelli, A., Rakobowchuk, M., Pellegrini, B., Schena, F. & Mourot, L. (2019). Cardiac autonomic and physiological responses to moderate-intensity exercise in hypoxia. *International journal of sports medicine*, 40(14), 886-896. DOI: 10.1055/a-1015-0647
- Fornasiero, A., Savoldelli, A., Stella, F., Callovini, A., Bortolan, L., Zignoli, A., Low, D., Mourot, L., Schena, F. & Pellegrini, B. (2020). Shortening Work-Rest Durations Reduces Physiological and Perceptual Load During Uphill Walking in Simulated Cold High-Altitude Conditions. *High Altitude Medicine & Biology.* 21 (3) 249-257. https://doi.org/10.1089/ham.2019.0136
- Fornasiero, A., Zignoli, A., Rakobowchuk M., Stella, F., Skafidas, S, Savoldelli, A, Pellegrini, B., Schena, F., Mourot, L. (2021) Post-exercise cardiac autonomic and cardiovascular responses to heart rate matched and work rate matched hypoxic exercise; *European journal of applied physiology,* 1-16. https://doi.org/10.1007/s00421-021-04678-5

Abbreviations

[La] blood lactate accumulation

ANOVA Analysis of variance

ANS Autonomic nervous system

CaO_2 arterial oxygen content

cBRS Cardiac Baroreflex Sensitivity

CO Cardiac output

CPET Cardiopulmonary exercise testing

CRR Chronotropic reserve recovery

DBP diastolic arterial pressure

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

EPOCt Excess of post-exercise oxygen consumption time-constant

ExcessVE Excess of post-exercise ventilation

LF Low-frequency spectral power

Ln Natural-logarithm transformation

HF High-frequency spectral power

HR Heart rate

HRR Heart rate recovery

HRRt Long-term time constant of heart rate recovery

HRV Heart rate variability

MAP: mean arterial pressure

PEH post-exercise hypotension

PiO_2 inspired pressure of oxygen

PO power output

Rf Respiratory frequency

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

RPE Rating of perceived exertion

SaO_2 arterial oxygen saturation

SAP systolic arterial pressure

SDNN standard deviation of normal to normal R-R intervals

SpO_2 pulse oxygen saturation

SS squat-stand

SV Stroke volume

T30 Short-term time constant of heart rate recovery

TP Total spectral power

TPR total peripheral resistance

VCO_2 carbon dioxide production

VE minute ventilation

VO_2 oxygen consumption

WR work rate

Background

Hypoxia is a condition in which the tissues of the body receive reduced oxygen supply (i.e. reduced oxygen availability) and naturally occurs at altitude (Burtscher et al. 2018). With increasing ascent to higher altitudes, a progressive decline in barometric pressure and inspired pressure of oxygen (PiO_2) leads to a drop in arterial partial pressure of oxygen (PaO_2) and arterial oxygen saturation (SaO_2) (i.e. progressive increase in the degree of hypoxic stress)(Favret and Richalet 2007; Mazzeo 2008) (Fig. 1). This occurrence has profound effects on human physiology, causing significant disruption in resting homeostasis and evoking numerous short-term and long-term physiological responses needed to help the body better cope with hypoxic stress (Favret and Richalet 2007; Mazzeo 2008; Burtscher et al. 2018).

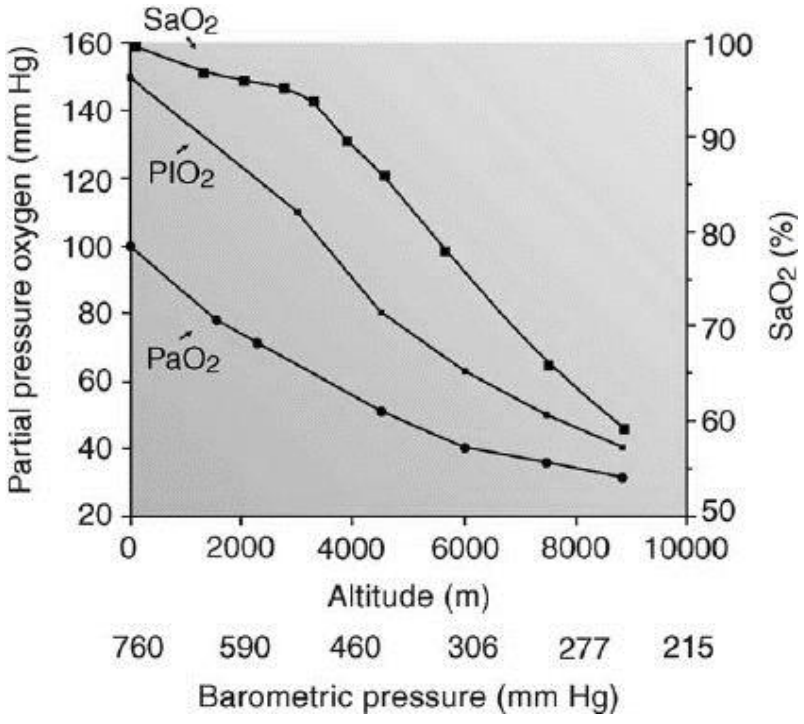

Figure 1. From Gallagher and Hackett (2004). Relationship of altitude and barometric pressure to inspired pressure of oxygen (PiO_2), arterial partial pressure of oxygen (PaO_2) and arterial oxygen saturation (SaO_2).

The physiological changes that take place to adapt to hypoxic environments are known as acclimatization and have been thoroughly investigated in the literature (Favret and Richalet 2007; Shah et al. 2015; Burtscher et al. 2018). This process, which include both haematological (e.g. increase in blood oxygen-carrying capacity) and non-haematological adaptions that increase human tolerance to hypoxic stress (Favret and Richalet 2007; Fulco et al. 2013; Burtscher et al. 2018), has also been shown to be potentially relevant for improving exercise performance in individuals (Mazzeo 2008; Millet et al. 2010; Shah et al. 2015). This has led to the implementation of various protocols of altitude/hypoxic training within the training programs of endurance athletes (Millet et al. 2010).

Traditional modalities of altitude/hypoxic training include the live high-train high (LHTH) and live high-train low (LHTL) approaches (Millet et al. 2010). In the LHTH approach people, mostly athletes, live and train for several weeks at moderate altitude (1,800–2,500 m above sea level), whilst in the LHTL approach they live at altitude but train nearer to sea level, so as not to decrease their normal absolute exercise intensities as occurs with LHTH approach (Millet et al. 2010). The pros and cons of LHTH and LHTL approaches have been explained in detail by Millet and colleagues (Millet et al. 2010), with the ultimate goal being the administration of a hypoxic dose/training stimuli that are adequate to induce a significant improvement in athletes' sea level performance (Millet et al. 2010).

Nowadays, exposures to hypoxic stimuli, commonly experienced at altitude, can be equally recreated in artificial environments, either using hypobaric (i.e. decreasing barometric pressure and consequently the inspired pressure of oxygen, PiO_2) or normobaric approaches (i.e. reducing the fraction of inspired oxygen, FiO_2) and hypoxic exposures/hypoxic training interventions have gained popularity (Millet et al. 2016; Girard et al. 2020).

Hypoxic stimuli of various degree and nature, also associated with various exercise stimuli/manoeuvres, are today implemented in a wide range of contexts for different health and sports performance purposes, within the so-called "living low-training high" (LLTH) approach (Girard et

al. 2020). In a recent perspective article Girard et al. (Girard et al. 2020) provided us with an updated panorama of the several LLTH hypoxic methods currently available, which include the use of systemic and local hypoxic stimuli, with both passive (i.e. rest) and active (i.e. exercise) hypoxic exposure modalities (Fig.2).

As regards the active modalities, the use of an additional hypoxic stress has now been proposed for most of the different types of exercise, from aerobic to resistance exercise, and implemented into most of the specific training modalities (e.g. continuous aerobic exercise, repeated sprint training, sprint interval training) (Millet and Brocherie 2020; Girard et al. 2020). The addition of hypoxic stress to other forms of exercise has also been proposed (e.g. pilates) (Jung et al. 2020)

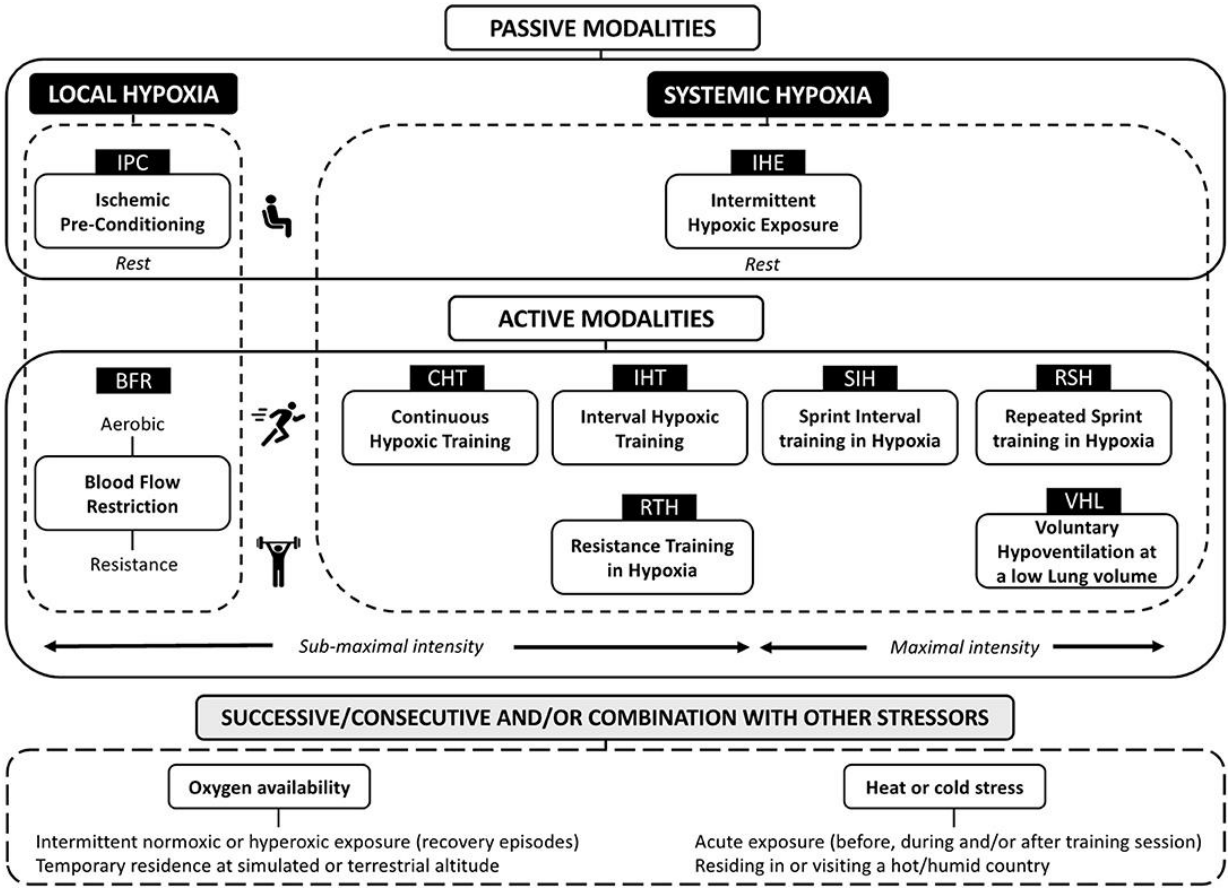

Figure 2. From Girard et al. (2020). Currently available "living low-training high" (LLTH) hypoxic methods.

Interestingly, hypoxic exercise (i.e. exercise combined with hypoxic stress within the LLTH approach), which has been more traditionally adopted to induce advantageous physiological training adaptations in athletic populations (Brocherie et al. 2017; Girard et al. 2020), has also more recently emerged as a suitable training modality for a wide range of individuals, including sedentary and special populations (e.g. elderly, obese and hypertensive patients) (Millet et al. 2016; Lizamore and Hamlin 2017; Girard et al. 2020). Numerous studies have highlighted hypoxic exercise as a new promising nonpharmacological therapeutic intervention, able to promote several positive health-related outcomes (Millet et al. 2016; Millet and Girard 2017; Lizamore and Hamlin 2017; Brocherie and Millet 2020).

By reducing the mechanical load needed for adequate cardiovascular stimulation, hypoxic exercise represents a suitable option for obese and elderly individuals to help meet exercise recommendations (Haufe et al. 2008; Girard et al. 2017; Pramsohler et al. 2017; Hobbins et al. 2017). For instance, there is good evidence that simulated altitudes of 3000-3500m, commonly employed by athletes involved in endurance and intermittent sports for performing high-intensity hypoxic exercises (within the LLTH approach) (Brocherie et al. 2017; Girard et al. 2020), are adequate for obese (Girard et al. 2017) and elderly patients (Pramsohler et al. 2017) to perform moderate-intensity hypoxic exercise (e.g. walking).

Moreover, hypoxic exercise can improve weight loss and cardio-metabolic health in overweight and obese patients (Netzer et al. 2008; Hobbins et al. 2017; Ramos-Campo et al. 2019), further representing a promising approach for insulin resistance and type 2 diabetes prevention and treatment (Mackenzie et al. 2012; De Groote et al. 2018; Mai et al. 2019). Potential applications of hypoxic exercise in patients with various cardiovascular diseases have also been highlighted and are likely to be further investigated (Wee and Climstein 2015; Millet et al. 2016). In addition, hypoxic exercise has also been associated to improved vascular health and autonomic balance (Montero and Lundby 2016; Lizamore and Hamlin 2017).

The present scenario suggests that the number of exercise training interventions including hypoxic exercise is likely to increase in the near future (Millet et al. 2016; Brocherie and Millet 2020).

Acute physiological responses to hypoxic exercise

The interest in hypoxic exercise arises from its potential to promote greater physiological and health-related adaptions compared to normoxic exercise (Millet et al. 2016; Girard et al. 2020) in the long-term (i.e. chronic effects), stemming from the markedly different acute physiological responses (i.e. acute effects). Indeed, according to the degree of hypoxic and exercise stimuli, acute hypoxic exercise can result in altered physiological and perceptual responses and in increased exercise-induced perturbation of homeostasis (Mazzeo 2008), that must be taken into account when prescribing/practising exercise under hypoxic environments. Moreover, both external (e.g. exercise load) and internal (e.g. heart rate) markers, commonly used for setting exercise intensity (Garber et al. 2011), are affected during acute hypoxic exercise, making exercise prescription more challenging. Above all, an acute impairment of aerobic exercise capacity in hypoxia is observed, which is progressively increased with increasing altitude levels (i.e. severity of hypoxia) (Fulco et al. 1998; Wehrlin and Hallén 2006) (Fig. 3).

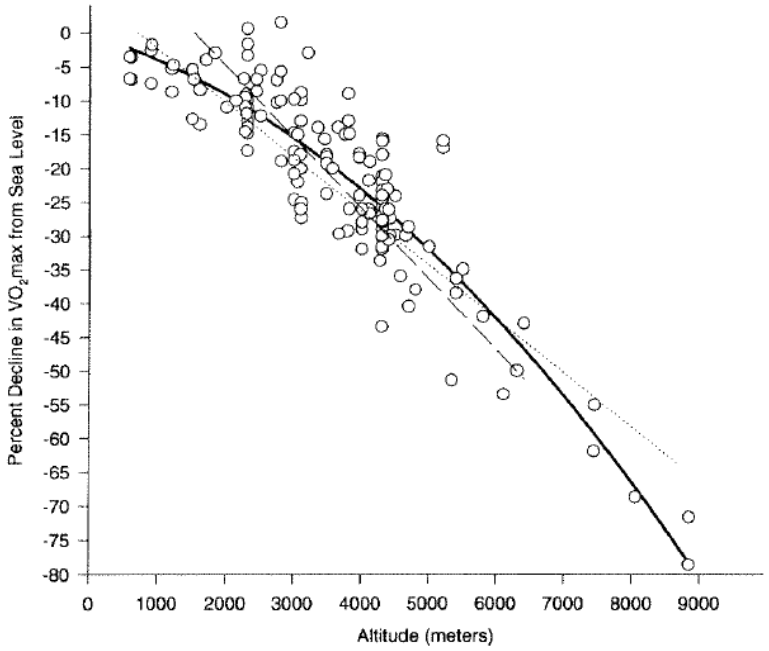

Figure 3. From Fulco et al. (1998). Decrease in maximal oxygen consumption with increasing altitude

During hypoxic exercise, hypoxia-induced arterial chemoreceptor stimulation promotes greater sympathetic activation and withdrawal of parasympathetic activity (Hainsworth et al. 2007; Amann and Kayser 2009; Fisher 2015; Siebenmann et al. 2018), which lead to increased cardiovascular and ventilatory responses (Calbet et al. 2009; Sheel et al. 2010; Fisher 2015; Winkler et al. 2017) to cope with the lower blood oxygen content (Bartsch and Gibbs 2007; Fisher 2015). Compensatory vasodilation of vascular beds, facilitating blood and oxygen delivery to the working muscles, also occurs (Joyner and Casey 2014; Dinenno 2016).

The greater sympathetic activation and parasympathetic withdrawal (Amann and Kayser 2009; Nobrega et al. 2014; Siebenmann et al. 2018) result in a further increase of heart rate (HR), stroke volume (SV), cardiac output (CO), and blood pressure (BP) (Calbet et al. 2009; Fisher 2015; Winkler et al. 2017), and in greater respiratory involvement (e.g. increase in minute ventilation, VE) (Sheel et al. 2010) compared to the same absolute exercise intensity in normoxia. Similarly, blood lactate levels ([La]) are increased in hypoxia at the same absolute submaximal exercise load due to the greater relative exercise intensity and the increased anaerobic energy contribution (Mazzeo 2008).

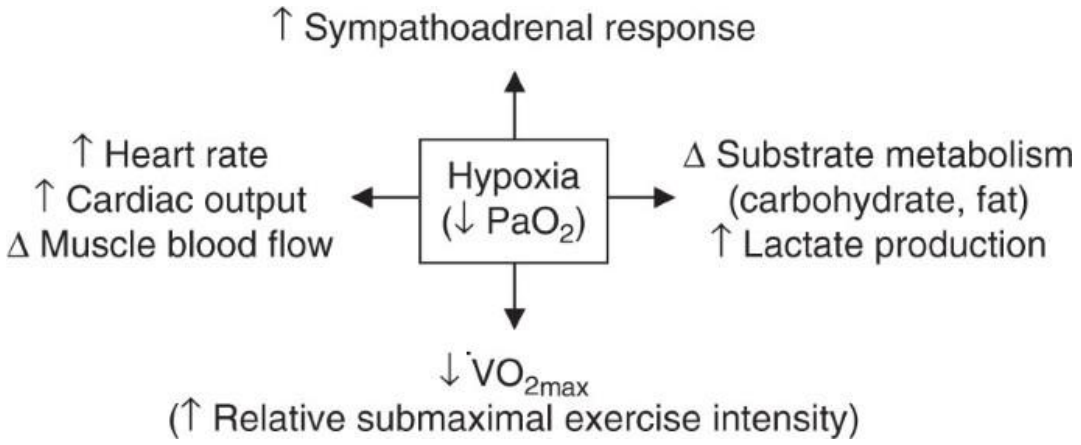

Figure 4. From Mazzeo 2008. Some of the altered physiological responses during acute hypoxic exercise

As a result, any same absolute exercise intensity represents a greater physiological challenge during hypoxic exercise (Mazzeo 2008) (Fig. 4).

An exacerbated increase of exercise-induced physiological stress of hypoxic exercise, can result in greater Autonomic Nervous System (ANS) disturbance (Hainsworth et al. 2007; Amann and Kayser 2009; Fisher 2015; Siebenmann et al. 2018), delayed recovery of autonomic balance (i.e. progressive restoration of normal resting balance between parasympathetic and sympathetic activity) and affected post-exercise cardiovascular responses (Romero et al. 2017; Michael et al. 2017a), which may be particularly relevant for patients involved in hypoxic exercise protocols and people sojourning at high altitude. For instance, it has been suggested that acute post-exercise blood pressure reduction (i.e. post-exercise hypotension, PEH) (Halliwill et al. 2013) may be intensified after hypoxic exercise (Horiuchi et al. 2016, 2018; Saito et al. 2019), and autonomic blood pressure control (e.g. cardiac baroreflex sensitivity, cBRS) affected, increasing the risk of orthostatic intolerance and hypotension (Halliwill et al. 2014). Moreover, an inadequate autonomic recovery, and the increased exercise-induced physiological stress associated with hypoxic exercise, may facilitate the development of

maladaptive responses to exercise training (Kiviniemi et al. 2007) and to high altitude environments (Sutherland et al. 2017; Boos et al. 2018).

In this regard, the investigation of cardiac autonomic activity via Heart Rate Variability (HRV) analysis (Malik 1996), at rest and during post-exercise recovery periods, has been suggested as a valid tool to examine the disturbance induced in ANS balance by an acute bout of exercise (Seiler et al. 2007; Kiviniemi et al. 2007; Michael et al. 2017a). Similarly, HRV can provide useful information about ANS status in response to acute hypoxic stimuli and in response to hypoxic exercise (Yamamoto et al. 1996; Zupet et al. 2009; Fisher 2015), where sympathetic and parasympathetic activities levels may be affected (Fisher 2015).

Particularly, post-exercise recovery of HR (HRR) and of vagal-related HRV indices can inform about recovery of parasympathetic activity (e.g. post-exercise parasympathetic reactivation) and are the most employed indices to investigate post-exercise cardiac autonomic recovery (Pecanha et al. 2017; Michael et al. 2017a). A clear relationship has been shown between exercise-induced perturbation of homeostasis and post-exercise cardiac autonomic recovery (Goldberger et al. 2006; Michael et al. 2017a). Higher exercise intensities (Terziotti et al. 2001; Buchheit et al. 2007a; Seiler et al. 2007) and durations (Castrillón et al. 2017; Michael et al. 2017b), as well as the presence of environmental conditions influencing ANS activity, such as hypoxia (Al Haddad et al. 2012; Koelwyn et al. 2013), have been shown to increase exercise-induced perturbation of homeostasis and lead to a delayed recovery of HR and vagal-related HRV indices (Michael et al. 2017a). Information regarding the acute exercise and post-exercise cardiac autonomic responses induced by various protocols of exercise in hypoxia seems to be of paramount importance to understand the impact of hypoxic exercise on ANS balance and recovery status. However, despite being extensively studied during normoxic exercise, information regarding exercise and post-exercise cardiac autonomic recovery responses to hypoxic exercise, as well as their relationship with other physiological variables related to exercise-induced stress (Mann et al. 2014), remains limited. In light of these observations, a further

effort seems to be required to better characterize exercise and post-exercise physiological responses to different protocols of hypoxic exercise.

General aim and overview of the thesis

The general aim of the present doctoral thesis was to contribute to a better understanding of the acute and post-exercise physiological responses to hypoxic exercise, so as to provide knowledge to improve human tolerance to high altitude environments, also providing helpful information for the adoption of safe exercise protocols in individuals engaged in hypoxic exercise training.

With this aim, in this paper-based doctoral thesis I present and discuss some new data regarding the acute and post-exercise physiological responses evoked by different protocols of hypoxic exercise. The data included and discussed in this thesis were obtained from 3 distinct data collections, where a number of healthy subjects completed different protocols of hypoxic exercise in an environmental chamber, which recreated the different hypoxic environments (i.e. normobaric hypoxia), according to the specific experimental conditions described in the following sections of the thesis.

In the thesis, special focus is given to the monitoring of exercise and recovery cardiac autonomic modulation responses (i.e. HR and HRV), and their relationship with other exercise physiological markers, as they can provide meaningful information about the impact of hypoxia and hypoxic exercise on whole body homeostasis and recovery status (Mann et al. 2014; Pecanha et al. 2017).

More specifically, *Study 1*, entitled "Cardiac autonomic and physiological responses to maximal cardiopulmonary exercise testing (CPET) in hypoxia" aims at investigating the effects of hypoxia (FiO_2=13.4%, ≈3500 m) on the acute exercise and post-exercise cardiorespiratory and cardiac autonomic responses to a maximal cardiopulmonary exercise test (CPET).

Study 2, entitled "Cardiac autonomic and physiological responses to moderate-intensity exercise in hypoxia", aims at examining the effects of hypoxic exercise (FiO_2=14.2%, ≈3000 m) performed at the same absolute intensity (i.e. 80% of the power output at the first ventilatory threshold) or same

relative intensity (i.e. heart rate matched exercise) of normoxic exercise on the exercise cardiac autonomic and cardiorespiratory responses.

Study 3, entitled "Post-exercise cardiac autonomic and cardiovascular responses to heart rate matched and work rate matched hypoxic exercise", aims at examining the effects of hypoxic exercise (FiO_2=14.2%, ≈3000 m) performed at the same absolute (i.e. 80% of the power output at the first ventilatory threshold) or same relative (i.e. heart rate matched exercise) normoxic exercise intensity on the post-exercise cardiac autonomic and cardiovascular responses.

Study 4, entitled " Shortening work-rest durations reduces physiological and perceptual load during uphill walking in simulated cold high-altitude conditions", aims at investigating the effects of a simulated mountain hike in a cold and hypoxic environment (-25°C, FiO2=11%, ≈5000 m) on cardiac autonomic activity, and the influence of two different strategies (i.e. different work-rest durations, long vs short) on the physiological and perceptual responses associated with the simulated ascent.

Investigating the acute exercise and post-exercise physiological responses evoked by hypoxic exercises of various intensities and nature, this doctoral thesis and the data presented herein want to expand our understanding of hypoxic exercise and stimulate new research on this topic.

1. Study 1

Cardiac autonomic and physiological responses to maximal cardiopulmonary exercise testing (CPET) in hypoxia

Based on the article

Fornasiero, A., Savoldelli, A., Skafidas, S., Stella, F., Bortolan, L., Boccia, G., Zignoli, A. , Schena, F., Mourot L. & Pellegrini, B. (2018). Delayed parasympathetic reactivation and sympathetic withdrawal following maximal cardiopulmonary exercise testing (CPET) in hypoxia. *European journal of applied physiology*, 118(10), 2189-2201. https://doi.org/10.1007/s00421-018-3945-5

Abstract

This study investigated the effects of acute hypoxic exposure on post-exercise cardiac autonomic modulation following maximal cardiopulmonary exercise testing (CPET). Thirteen healthy men performed CPET 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. 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 (MD -0.42, 95% -0.71 to -0.14, p= 0.007, ES -0.87) but not at 300s (p=0.578). Affected fast-phase, slow-phase HRR and HRV indices suggested delayed parasympathetic reactivation and reduced 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.

1.1 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 (Cottin et al. 2004; Seiler et al. 2007), exercise durations (Michael et al. 2017b) and modified environmental conditions (e.g. hypoxia) (Al Haddad et al. 2012). 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 is also of importance (Cunha et al. 2015; Molina et al. 2016), indeed a higher parasympathetic activity at rest is associated with a faster recovery of HR and

HRV indices in the post-exercise period (Danieli et al. 2014; Cunha et al. 2015). 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 (Pecanha et al. 2017).

Hypoxic training is commonly employed to induce greater physiological training adaptations in athletic populations (Brocherie et al. 2017) and has recently emerged as a promising training modality for sedentary and pathological populations (Millet et al. 2016; Lizamore and Hamlin 2017). In this regard, hypoxia is well recognized to modify cardiac autonomic modulation at rest (Oliveira et al. 2017) and in response to exercise (Yamamoto et al. 1996; Zupet et al. 2009; Fisher 2015).

Alongside, hypoxia acts as a stimulus for an increased sympathetic activity (Hainsworth et al. 2007; Amann and Kayser 2009) and a reduced parasympathetic cardiac control (Perini and Veicsteinas 2003; Buchheit et al. 2004; Fisher 2015; Oliveira et al. 2017), that can turn in a slower post-exercise recovery of HR and HRV indices (Al Haddad et al. 2012). For instance, modifications in post-exercise cardiac autonomic modulation, with a delayed parasympathetic reactivation, have been reported in hypoxia (FiO_2=15.4%, 2400m) after sub-maximal exercise intensities (Al Haddad et al. 2012). On the contrary, in the above-mentioned work (Al Haddad et al. 2012), the imposed hypoxic stimulus did not modify parasympathetic recovery after a supra-maximal intensity (20 s sprint “all-out”), probably due to the already maximal homeostatic perturbation induced by a supra-maximal intensity, causing high anaerobic energy contribution and sympathetic activation (Buchheit et al. 2007a; Al Haddad et al. 2012).

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

Additionally, exercises with high cardiorespiratory involvement are widely performed in hypoxia, but post-exercise physiological outcomes have not been specifically studied. In particular, brief

periods of supra-maximal exercise are not known to induce maximal cardiovascular and respiratory stress (Buchheit and Laursen 2013). Thus, it is currently unknown how hypoxia can affect post-exercise cardiac autonomic modulation following a maximal exercise, where cardiovascular and respiratory systems are maximally stressed and pushed to their functional limit (e.g. a maximal cardio-pulmonary exercise test, CPET) (Albouaini et al. 2007). This occurrence certainly limits the evaluation of recovery from hypoxic exercise both when used for health assessment or training load quantification purposes (Ward et al. 2017).

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

Therefore, the purpose of this study was to investigate the effects of acute hypoxia on the post-exercise cardiac autonomic modulation following a maximal cardiopulmonary exercise test (CPET). According to previous observations about the influence of the homeostatic perturbation in determining post-exercise outcomes (Buchheit et al. 2007a; Al Haddad et al. 2012), we hypothesized that maximal hypoxic exercise would have been associated to a reduced recovery of fast-phase HRR and HRV indices, reflecting a delayed parasympathetic reactivation, in the immediate post-exercise recovery period. Furthermore, we hypothesized that in response to the maximal cardiovascular, respiratory and metabolic stress induced, the reduced post-exercise oxygen availability would have also led to an impaired recovery of slow-phase HRR indices, also indicating delayed sympathetic withdrawal (Pecanha et al. 2017).

1.2 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 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 Local Ethics Committee.

Protocol

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

All the evaluations were performed on a recline cycle ergometer (E1200, Cosmed Srl, Rome, Italy) set at 50° of inclination. Following 30 min of quiet rest on the ergometer participants completed: 6 min of baseline measurements at rest, 10 min of sub-maximal constant load exercise (75W), a maximal cardio-pulmonary exercise test (CPET) and 5 min of post-exercise recovery assessment. CPET started immediately after the sub-maximal exercise with increments of 25W every 1 min until participants' volitional exhaustion. The pedalling cadence during the submaximal exercise and the CPET was kept constant at 90 revolutions/min, using a monitor that provided participants with visual feedback. Throughout rest, exercise and recovery phases, beat-to beat heart rate was continuously recorded using a Polar RS800CX heart rate monitor (Polar, Kempele, Finland). During resting and exercise cardio-respiratory measures were collected continuously with breath-by-breath method

using an automated open-circuit gas analysis system (Quark PFT Ergo, Cosmed Srl, Rome, Italy). Careful calibrations of flow sensors and gas analyzers were performed before each measurement according to the manufacturer's instructions. Pulse oxygen saturation (SpO_2) was continuously recorded by fingertip pulse oximetry (Nonin Medical, Minneapolis, MN, USA) at a sampling frequency of 1.0 Hz. To measure maximal lactate accumulation a blood sample was collected from the earlobe 3 min after the end of the test (Goodwin et al. 2007). The lactate analyser (Biosen C-line, EKF Diagnostics GmbH, Barleben, Germany) was calibrated according to the manufacturer's instructions. The individual rating of perceived exertion (RPE) was assessed at the end of 5-min recovery period using Borg Category Ratio Scale (CR100) (Borg and Borg 2002) .

Data Analysis

The R-R intervals were uploaded using Polar Precision Performance Software (Polar, Kempele, Finland) and then exported as .txt files. Signal artifacts were filtered out by means of a moderate error correction filter with minimum protection zone of 6 bpm (Al Haddad et al. 2012). All the time series of R-R intervals showed low noise (identified errors <5%). HRV analysis was performed using Kubios HRV software (Version 2.1, Biosignal Analysis and Medical Imaging Group, Kuopio, Finland). At rest HRV indices were calculated from the last 5min of the 6-min resting period. Exercise HRV indices were calculated from the last 5-min of the 10-min submaximal exercise preceding CPET evaluation. The time-domain HRV index considered was the square root of the sum of successive differences between adjacent normal R-R intervals squared (RMSSD). For frequency-domain HRV indices, low frequency spectral power (LF, 0.04-0.15 Hz), high frequency spectral power (HF, 0.15-0.4 Hz), and total spectral power (TP, 0.04-0.4 Hz) were calculated by Fast Fourier Transform (FFT) (Task Force of the European Society of Cardiology 1996).

Post-exercise heart rate recovery (HRR) indices were calculated with a customized script in Matlab (Matlab, Mathworks Inc., USA). HRR indices were measured from the absolute differences between HR_{peak} and the HR values at 30s, 60 s and 300s of recovery (HRR30, HRR60 and HRR300) in the post-exercise period (averaged over 5s) (Peçanha et al. 2016). HRR was also calculated as the relative

decline in HR expressed as a percentage of HR_{peak} (%HRR=HRR/HR_{peak}×100) and as the recovery of the chronotropic reserve (CRR = HRR/(HR_{peak}-HR_{rest})×100) (Molina et al. 2016). T30, the short-time constant of HRR, was calculated as the negative reciprocal of the slope of the regression line of natural-logarithmic transformed HR during the first 30 s of recovery (Buchheit et al. 2007b). HRRt, the long-term time-constant of HRR, was obtained after exponential fitting of the HR during the entire 300s of recovery (Pecanha et al. 2017). Additionally, the time-varying vagal-related index, RMSSD, were also calculated for each of the 30-s segments of recovery (Goldberger et al. 2006). The time points used for statistical analysis were 30 s, 60 s and 300s in the post-exercise recovery period (RMSSD30, RMSSD60, RMSSD300).

The peak power output (PPO), achieved at athlete's exhaustion, was determined according to the equation: PPO (W) = power output last stage completed (W) + [*t* (s)/step duration (s) * step increment (W)], where *t* is the time of the uncompleted stage. VO_{2peak} and other maximal cardio-respiratory variables were defined as the highest values of a 20-s average. The excess post-exercise oxygen consumption time-constant (EPOCt) was calculated by exponential fitting of 5 min VO_2 recovery data (do Nascimento Salvador et al. 2016). Additionally, the excess post-exercise oxygen consumption magnitude ($EPOC_{MAG}$) was determined as the time integral of the 5 min VO_2 recovery curve values above VO_2 baseline (do Nascimento Salvador et al. 2016). Similarly, excess post-exercise Ventilation (Excess VE) above resting value were also calculated.

Statistical Analysis

Data are presented as means ± standard deviations (SD). Data were tested for normal distribution with Shapiro–Wilk test. If data were not normally distributed, natural logarithm transformation (Ln) was applied to obtain a normal distribution and allow parametric statistical comparisons. Paired *t* -tests were performed to compare cardio-respiratory variables, HR and HRV indices at rest and during sub-maximal exercise period for N and H condition. HRR and HRV indices in the post-exercise period were compared using a two-way ANOVA for repeated measures, with "condition" (H and N) and "time" (time points 30s, 60s and 300s) as factors. When a significant interaction was noted post

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

1.3 Results

Effects of hypoxia at rest

HRV indices and other physiological variables at rest for H and N condition are reported in Table 1.1 At rest time-domain (Ln-RMSSD) and frequency-domain (Ln-LF, Ln-HF, Ln-TP) HRV indices were not significantly different between H and N ($p>0.05$). Only an increase in HR (Mean Difference H-N (MD) 4.2 bpm, $p=0.025$, Effect size (ES) 0.76) was noted for H condition. Respiratory frequency (Rf) and minute ventilation (VE) were not significantly different in H compared with N ($p>0.05$). SpO_2 was markedly reduced in H ($p<0.001$).

Table 1.1 HRV and cardiorespiratory indices at rest in Normoxia and Hypoxia

		NORMOXIA			**HYPOXIA**				95% CI for the Difference				
		Mean	±	Sd	Mean	±	Sd	Mean Difference (H-N)	Lower Bound	Upper Bound	p	Effect size	Descriptor
HR	bpm	53.0	±	5.8	57.2	±	5.2	4.2	0.6	7.8	**0.025**	0.76	moderate
Ln-RMSSD	ms	4.06	±	0.55	3.89	±	0.40	-0.17	-0.37	0.03	0.094	-0.35	small
Ln-LF	ms^2	7.20	±	0.90	7.11	±	0.79	-0.09	-0.66	0.48	0.743	-0.10	trivial
Ln-HF	ms^2	6.80	±	1.29	6.55	±	0.94	-0.25	-0.64	0.15	0.199	-0.22	small
Ln-TP	ms^2	8.50	±	0.80	8.29	±	0.74	-0.21	-0.71	0.29	0.378	-0.27	small
SpO_2	%	96.6	±	1.6	86.2	±	4.1	-10.4	-12.8	-8.0	**<0.001**	-3.35	large
Ve	L/min	9.8	±	2.6	10.2	±	1.7	0.4	-1.2	2.0	0.628	0.17	trivial
Rf	$b*min^{-1}$	13.9	±	3.4	15.2	±	2.9	1.3	-0.3	2.9	0.105	0.41	small

Values are Mean ± SD. HR: Heart rate; VE: Ventilation; Rf: Respiratory frequency; RMSSD: Root mean square of successive differences of R–R intervals; Ln: Natural-logarithm transformation; LF Low-frequency spectral power; HF: High-frequency spectral power; TP: Total spectral power; SpO_2: oxygen saturation.

CPET evaluation and post-exercise physiological outcomes

Results from CPET and post-exercise assessment are presented in Table 1.2. Hypoxia induced a reduction in maximal exercise performance indices. Lower VO_{2peak} and PPO were found in H compared to N ($p<0.001$). HR_{peak} was significantly reduced in H (MD -6.2 bpm, $p<0.001$, ES -0.50). Maximal respiratory frequency (Rf) and minute ventilation (VE) were not significantly different in H compared with N ($p>0.05$). SpO_2 was markedly reduced in H both during and at the end of CPET ($p<0.001$). Post-exercise physiological outcomes were affected by Hypoxia as well. EPOCt was increased in H ($p=0.006$), as well as ExcessVE ($p=0.031$), whereas $EPOC_{MAG}$ and blood lactate accumulation were not different in the two conditions ($p>0.05$).

Table 1.2 Cardiopulmonary exercise testing and post-exercise outcomes													
		NORMOXIA			**HYPOXIA**				95% CI for the Difference				
		Mean	±	Sd	Mean	±	Sd	Mean Difference (H-N)	Lower Bound	Upper Bound	p	Effect size	Descriptor
Peak exercise intensity													
HR_{peak}	bpm	177.5	±	12.6	171.3	±	12.2	-6.2	-9.3	-3.2	**<0.001**	-0.50	small
VO_{2peak}	L/min	3.80	±	0.50	3.08	±	0.28	-0.72	-0.92	-0.52	**<0.001**	-1.78	large
PPO	W	309.2	±	42.2	265.9	±	27.4	-43.3	-54.7	-32.0	**<0.001**	-1.22	large
VE	L/min	147.9	±	20.7	151.9	±	23.1	4.0	-4.0	12.0	0.295	0.18	trivial
Rf	$b*min^{-1}$	59.6	±	10.3	60.2	±	9.3	0.6	-3.9	5.2	0.776	0.06	trivial
SpO_2	%	94.0	±	3.1	77.4	±	5.0	-16.6	-20.1	-13.1	**<0.001**	-3.99	large
$[La]_b$	mmol/L	10.6	±	2.6	11.6	±	2.2	0.9	-1.0	2.9	0.302	0.39	small
RPE		98.1	±	10.7	98.8	±	6.2	0.8	-4.3	5.8	0.746	0.09	trivial
Post-exercise													
HR30	bpm	152.4	±	14.9	152.5	±	13.6	0.2	-6.1	6.4	0.954	0.01	trivial
HR60	bpm	132.1	±	20.1	137.6	±	15.4	5.5	-1.4	12.3	0.107	0.31	small
HR300	bpm	91.9	±	17.8	94.2	±	13.9	2.2	-4.4	8.8	0.477	0.14	trivial
VO_230	L/min	2.59	±	0.31	2.40	±	0.22	-0.20	-0.31	-0.08	**0.003**	-0.74	moderate
VO_260	L/min	1.42	±	0.23	1.48	±	0.18	0.06	-0.02	0.14	0.126	0.30	small
VO_2300	L/min	0.57	±	0.11	0.60	±	0.09	0.03	-0.04	0.09	0.364	0.26	small
VE30	L/min	100.6	±	22.3	114.2	±	19.0	13.5	1.3	25.8	**0.034**	0.65	moderate
VE60	L/min	75.1	±	17.5	78.1	±	14.0	3.0	-3.1	9.1	0.305	0.19	trivial
VE300	L/min	27.2	±	7.2	32.5	±	7.9	5.2	1.9	8.6	**0.006**	0.69	moderate
SpO_230	%	94.9	±	2.1	76.6	±	4.9	-18.3	-21.6	-15.0	**<0.001**	-4.80	large
SpO_260	%	96.1	±	2.0	79.0	±	5.6	-17.1	-20.9	-13.3	**<0.001**	-4.06	large
SpO_2300	%	94.9	±	2.5	84.7	±	2.7	-10.2	-11.5	-8.9	**<0.001**	-3.97	large
EPOCt	s	40.6	±	4.3	50.8	±	11.5	10.2	3.7	16.8	**0.006**	1.18	moderate
$EPOC_{MAG}$	L	4.1	±	0.9	3.9	±	0.6	-0.3	-0.7	0.13	0.163	-0.38	small

Excess VE	L	214	±	48	237	±	53	23	3	44	**0.031**	0.46	small

Values are Mean ± SD. HR: Heart rate; VO_{2max} Peak oxygen uptake; PPO: Peak Power Output; VE: Ventilation; Rf: Respiratory frequency; SpO_2: oxygen saturation; [La]b: maximal blood lactate accumulation; RPE: Rate of Perceived Exertion; EPOCt: Excess of post-exercise oxygen consumption time-constant; $EPOC_{MAG}$: Excess of post-exercise oxygen consumption magnitude; Excess VE: excess of post-exercise Ventilation

Effect of hypoxia on post-exercise cardiac autonomic modulation

Indices of post-exercise cardiac autonomic modulation for N and H condition were reported in Table 1.3. A significant effect of "time" was found in all the HRR and HRV post-exercise recovery indices investigated ($p<0.001$). The two-way ANOVA for repeated measures showed a significant effect of "condition" ($p<0.001$) and "time" ($p<0.001$), with significant "interaction" ($p=0.006$) on HRR indices expressed in bpm. HRR30, HRR60 and HRR300s were significantly reduced in H (HRR30: MD -6.39 bpm, $p=0.005$, ES -1.16; HRR60: MD -11.70 bpm, $p<0.001$, ES -1.23; HRR300: MD -8.78 bpm, $p= 0.004$, ES -0.84). When expressed as a percentage of peak heart rate (%HRR) a significant effect of "condition" ($p=0.005$), "time" ($p<0.001$) and "interaction" ($p=0.021$) was also noted. %HRR was significantly reduced in H compared with N at 30s (MD -3.22 %, $p= 0.012$, ES-0.97), 60s (MD -6.06 %, $p<0.001$, ES -1.00) and 300s (MD -3.38 %, $p=0.045$, ES -0.53) of the post-exercise recovery period. A significant effect of "condition" ($p= 0.021$), "time" ($p<0.001$) and "interaction" ($p=0.021$) was reported in HRR indices, when expressed as percentage of the chronotropic reserve (CRR). CRR was reduced in H compared with N at 30s (MD -3.77 %, $p=0.028$, ES -0.80) and at 60s (MD -7.23 %, $p=0.003$, ES -0.81), but not at 300s ($p=0.436$). Both short-term time constant, T30, and long-term time constant of HRR, HRRt, were significantly higher in H, indicating a slower decay of HR and a reduced HRR recovery. Concurrently, a significant effect of "condition" ($p=0.029$) and "time" ($p<0.001$) with "interaction" ($p=0.036$), was reported on Ln-RMSSD. This index was significantly reduced in H at 60s ($p=0.007$) but not at 30s and at 300s during the recovery ($p>0.05$).

Table 1.3 HRR and HRV indices reflecting post-exercise cardiac autonomic modulation													
		NORMOXIA			**HYPOXIA**				95% CI for the Difference				
		Mean	±	Sd	Mean	±	Sd	Mean Difference (H-N)	Lower Bound	Upper Bound	p	Effect size	Descriptor
T30	s	201.6	±	48.9	261.6	±	67.9	60.0	18.2	101.8	**0.009**	1.01	moderate
HRRt	s	85.2	±	30.8	107.0	±	37.0	21.7	4.1	39.3	**0.020**	0.64	moderate
HRR30	bpm	25.1	±	6.6	18.7	±	4.2	-6.4	-10.4	-2.4	**0.005**	-1.16	moderate
HRR60	bpm	45.4	±	11.0	33.7	±	7.8	-11.7	-16.5	-6.9	**<0.001**	-1.23	large
HRR300	bpm	85.9	±	12.8	77.1	±	7.2	-8.8	-14.3	-3.3	**0.004**	-0.84	moderate
%HRR30	%	14.2	±	3.8	11.0	±	2.7	-3.2	-5.6	-0.8	**0.012**	-0.97	moderate
%HRR60	%	25.9	±	7.0	19.8	±	4.9	-6.1	-9.1	-3.0	**<0.001**	-1.00	moderate
%HRR300	%	48.6	±	7.5	45.2	±	5.0	-3.4	-6.7	-0.1	**0.045**	-0.53	small
CRR30	%	20.3	±	5.4	16.6	±	3.9	-3.8	-7.1	-0.5	**0.028**	-0.80	moderate
CRR60	%	37.0	±	10.3	29.8	±	7.2	-7.2	-11.5	-3.0	**0.003**	-0.81	moderate
CRR300	%	69.5	±	10.8	68.0	±	7.7	-1.4	-5.3	2.5	0.436	-0.15	trivial
Ln-RMSSD30	ms	0.82	±	0.39	0.75	±	0.20	-0.07	-0.24	0.09	0.356	-0.23	small
Ln-RMSSD60	ms	1.25	±	0.61	0.82	±	0.31	-0.42	-0.71	-0.14	**0.007**	-0.87	moderate
Ln-RMSSD300	ms	1.76	±	0.66	1.69	±	0.62	-0.07	-0.36	0.21	0.578	-0.12	trivial

Values are Mean ± SD. HRR: Heart rate recovery, HRV: Heart rate variability; T30: Short-term time constant of heart rate recovery; HRRt: Long-term time constant of heart rate recovery; Ln: Natural-logarithm transformation; %HRR: percentage of HRR; CRR: Chronotropic reserve recovery; RMSSD Root mean square of successive differences of R–R intervals.

Correlational analysis

Complete correlational analysis results were reported in Table 4. Considering indices of parasympathetic reactivation, Δ%T30 (r=0.63; p=0.020), Δ%HRR30 (r=-0.56; p=0.046) and Δ%RMSSD300 (r=-0.77; p=0.002) were significantly correlated with $\Delta\%HR_{peak}$, whereas no significant relation was observed with Δ%HRR60 (r=-0.476; p=0.100). In addition, Δ%ExcessVe was significantly inversely related to Δ%HRR30 (r=-0.65; p=0.023) and Δ%RMSSD300 (r=-0.72; p=0.008), and significantly directly related to Δ%T30 (r=0.66; p=0.019). $\Delta\%[La]_b$ was directly related to Δ%T30 (r=0.62; p=0.025) and inversely related to Δ%HRR30 (r=-0.62; p=0.025), but not to Δ%HRR60 (r=-0.44, p=0.135). Both Δ%HRR60 (r=-0.61; p= 0.047) and Δ%RMSSD300 (r=-0.66; p=0.028) were significantly inversely correlated to Δ%EPOCt. $\Delta\%EPOC_{MAG}$ was significantly and directly related to $\Delta\%SpO_2$ at peak exercise intensity (r=0.63; p=0.038), Δ%ExcessVe (r=0.58; p=0.050), and inversely related to Δ%RMSSD30 (r=-0.79; p=0.002). Considering slow-phase HRR indices, no significant relation with $\Delta\%HR_{peak}$ was observed for Δ%HRR300 (r=-0.22; p=0.465) and Δ%HRRt (r=0.47, p=0.124). Similarly, Δ%HRR300 (r=-0.38, p=0.199) and Δ%HRRt (r=0.27; p=0.402) were not significantly correlated to $\Delta\%[La]_b$. However, Δ%HRRt was significantly inversely related to $\Delta\%SpO_260$ (r=-0.81; p=0.005).

Table 1.4 Correlations between variations (Δ% Hypoxia-Normoxia) in HR, HRV, cardio-respiratory and metabolic variables

		HR and HRV recovery							**Peak exercise**					**Post-exercise**						**Rest**			
	Δ%	T30	HRR60	RMSSD30	RMSSD60	RMSSD300	HRRt	HRR300	HR_{peak}	VO_{2peak}	SpO_2	VE	$[La]_b$	EPOCt	$EPOC_{MAG}$	Excess VE	$SpO_2$30	$SpO_2$60	$SpO_2$300	HR	RMSSD	HF	SpO_2
HR and HRV recovery	T30																						
	HRR60	**-0.75**																					
	RMSSD30	0.01	-0.13																				
	RMSSD60	-0.25	0.30	0.41																			
	RMSSD300	**-0.71**	0.49	0.24	0.08																		
	HRRt	**0.58**	**-0.83**	0.33	-0.30	-0.36																	
	HRR300	**-0.60**	**0.66**	-0.45	-0.16	0.46	-0.47																
Peak exercise	HR_{peak}	**0.63**	-0.48	-0.35	-0.23	**-0.77**	0.47	-0.22															
	VO_{2peak}	0.25	0.08	-0.47	-0.42	-0.34	-0.30	0.04	0.22														
	SpO_2	0.23	-0.15	-0.30	-0.08	-0.11	-0.43	-0.10	0.10	0.27													
	VE max	0.02	0.29	-0.41	-0.30	-0.27	-0.53	0.12	0.13	**0.78**	0.44												
	$[La]_b$	**0.62**	-0.44	-0.19	-0.22	-0.44	0.27	-0.38	0.27	0.46	0.48	0.09											
Post-exercise	EPOCt	0.50	**-0.61**	-0.11	-0.31	**-0.66**	0.52	-0.39	0.52	-0.08	0.17	-0.04	0.30										
	$EPOC_{MAG}$	0.28	-0.17	**-0.79**	-0.46	-0.13	-0.38	0.13	0.25	**0.67**	**0.63**	0.51	0.46	-0.08									
	Excess VE	**0.66**	-0.50	-0.47	-0.35	**-0.72**	0.10	-0.39	**0.64**	0.56	0.47	0.56	0.30	0.33	**0.58**								
	$SpO_2$30	0.17	-0.16	-0.28	0.12	0.05	-0.51	-0.05	-0.16	0.37	**0.78**	0.15	0.58	-0.21	**0.77**	0.24							
	$SpO_2$60	-0.26	0.44	-0.25	0.05	0.47	**-0.81**	0.32	-0.50	0.47	0.46	0.42	0.21	-0.63	0.62	-0.13	**0.71**						
	$SpO_2$300	-0.26	0.25	0.52	0.44	0.30	-0.02	-0.03	-0.01	-0.19	-0.53	-0.05	**-0.61**	-0.47	-0.30	-0.30	-0.34	0.01					
Rest	HR	0.44	-0.46	0.17	-0.20	**-0.58**	**0.71**	-0.53	0.38	-0.08	-0.38	-0.25	0.23	**0.73**	-0.42	0.12	-0.52	**-0.78**	-0.23				
	RMSSD	-0.39	0.32	0.00	0.11	0.45	-0.31	0.51	-0.08	0.16	-0.03	0.17	-0.36	**-0.75**	0.27	-0.08	0.19	0.46	0.60	**-0.77**			
	HF	-0.28	0.32	0.21	0.35	0.45	-0.28	0.34	-0.21	0.03	-0.10	-0.02	-0.26	**-0.87**	0.08	-0.20	0.23	0.47	**0.60**	**-0.75**	**0.89**		
	SpO_2	0.18	-0.34	**-0.72**	-0.37	-0.37	0.26	0.14	**0.60**	-0.04	0.15	-0.06	0.01	0.35	0.40	**0.70**	-0.02	-0.38	-0.40	0.10	-0.07	-0.22	

HR: Heart rate; HRR: Heart rate recovery, T30: Short-term time constant of heart rate recovery; HRRt: Long-term time constant of heart rate recovery; RMSSD: Root mean square of successive differences of R–R intervals; VE: Ventilation; LF Low-frequency spectral power; HF: High-frequency spectral power; TP: Total spectral power; SpO2: oxygen saturation; [La]b: maximal blood lactate accumulation; EPOCt: Excess of post-exercise oxygen consumption time-constant; $EPOC_{MAG}$: Excess of post-exercise oxygen consumption magnitude; Excess VE: excess of post-exercise Ventilation.

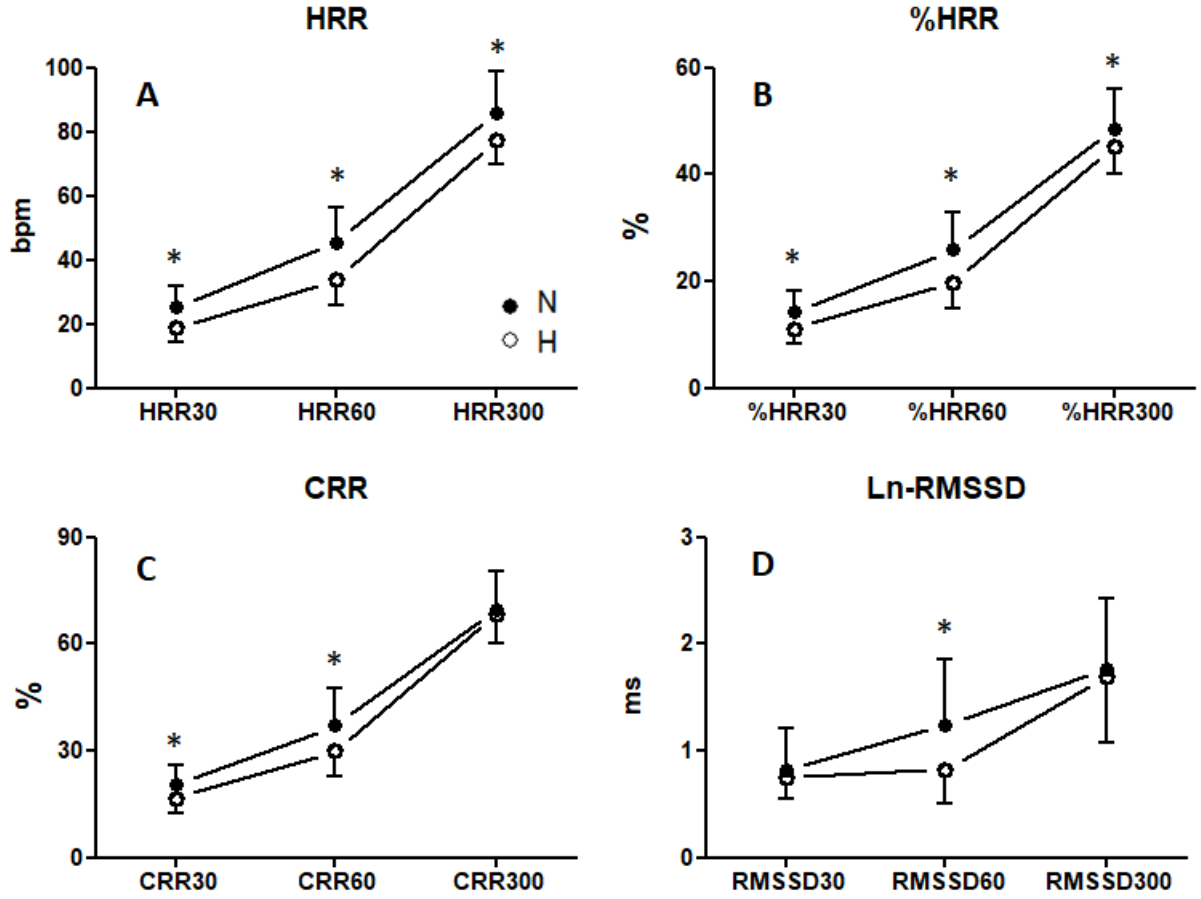

Figure 1.1 Post-exercise cardiac autonomic modulation in response to normoxic and hypoxic cardiopulmonary exercise testing (CPET). Filled circles (●) and open circles (○) represent Normoxic (N) and Hypoxic (H) condition, respectively, at different time points during recovery (30, 60, 300 s). Fig 1A: Heart Rate Recovery expressed in bpm (HRR); Fig 1.B: Heart Rate Recovery expressed as percentage (%) of HR_{peak} (%HRR); Fig 1.C: Chronotropic Reserve Recovery (CRR); Fig 1.D: recovery of natural-logarithm transformation of the root mean square of successive differences of R–R intervals (Ln-RMSSD).

1.4 Discussion

Despite being extensively investigated under normoxic condition for its implication in evaluating ANS functionality and assessing subject's health (Thayer et al. 2012), clinical (Qiu et al. 2017) and training status (Bellenger et al. 2016), to the best of our knowledge, this the first study examining post-exercise cardiac autonomic modulation, through the recovery of HR and HRV indices, in response to maximal hypoxic exercise. The key finding of this study was that in response to a maximal

cardio-pulmonary exercise test (CPET) fast-phase HRR indices (T30, HRR30, HRR60), the recovery of HRV indices (Ln-RMSSD) and slow-phase HRR indices (HRRt) were significantly affected by acute hypoxia (FiO2=13.4%, ≈3500 m). These findings suggest delayed parasympathetic reactivation and reduced sympathetic withdrawal after maximal hypoxic exercise (Pecanha et al. 2017). The reduced cardiac autonomic recovery in hypoxia was associated with a markedly decreased SpO_2, significantly higher EPOCt, similar $EPOC_{MAG}$ and increased ExcessVE, denoting amplified post-exercise physiological responses and increased homeostatic stress induced by hypoxic exercise (Mann et al. 2014).

Effects of hypoxia at rest

Different levels of hypoxia appear to induce different HR and HRV responses (Zupet et al. 2009; Giles et al. 2016; Oliveira et al. 2017). Even if variations in HR and HRV indices have been previously documented at rest at a simulate altitude of ≈2600 m (FiO2=15%) (Iwasaki et al. 2006), an altitude threshold of ≈6000m (FiO2=9.8%) has been recently proposed as the minimum required to induce change in resting cardiac autonomic modulation (Giles et al. 2016). In line with this observation, in our study only an increase in resting HR was noted for hypoxic condition (FiO_2=13.4%, 3500 m), without any variation in HRV spectral power or time-domain indices of parasympathetic activity at rest (RMSSD). The unchanged resting HRV profile can be partially clarified by the unchanged ventilatory responses (Nobrega et al. 2014; Siebenmann et al. 2015). Indeed, despite a significantly reduced SpO_2 at rest (-10.4 %), respiratory variables were not significantly different at rest for the two conditions (Table 1.1).

Peak exercise and post-exercise physiological outcomes

In line with existing literature (Calbet et al. 2003; Wehrlin and Hallén 2006) in this study a noticeable hypoxic influence on exercise capacity, with marked decreases in maximal oxygen consumption (VO_{2peak}) (≈-18%) and peak power out (PPO) (≈-14%), was found (Table 1.2). Additionally, together with reductions in VO_{2peak} a concurrent reduction in peak heart rate (HR_{peak}) at exhaustion was also

noted. The decrease (≈-6.2 bpm, ≈-3.5%) was in line with previous studies investigating the progressive, and still discussed, reduction in HR_{peak} occurring with increasing levels of hypoxia (Grataloup et al. 2007; Mollard et al. 2007b; Gaston et al. 2016).

The decrease in VO_{2peak} and HR_{peak} was not accompanied by any variation in maximal respiratory variables (Rf, VE) indicating that CPETs induced comparable maximal respiratory stress at peak exercise intensity (Ofner et al. 2014) (Table 1.2). Similarly, blood lactate concentration ($[La]_b$ indicated similar anaerobic metabolism contribution for the two conditions (Goodwin et al. 2007). However, hypoxic CPET was associated with markedly reduced SpO_2 (≈-16.6%) higher EPOCt (≈24.9%), similar $EPOC_{MAG}$ (4.1 vs 3.9 L, for N and H respectively) and an increased ExcessVE (≈12.1%), when compared to normoxic CPET. Thus, despite the reduced sustained intensity and metabolic requirements of hypoxic exercise at exhaustion, this was accompanied by amplified post-exercise physiological outcomes suggesting an increased homeostatic stress (Mann et al. 2014). This evidence can help to explain the different post-exercise cardiac autonomic modulation observed in hypoxia.

Effect of hypoxia on post-exercise cardiac autonomic modulation

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

In line with our hypothesis fast-phase HRR indices (i.e. the heart rate recovery within the first 30 or 60 s) were significantly reduced under hypoxic condition (Fig 1.1.A, 1.1.B, and 1.1.C). HRR was reduced either when expressed in bpm (Fig 1.1.A) or in percentage of HR_{peak} (Fig 1.1.B). Furthermore, beside the two aforementioned widespread methods, HRR can be expressed as the recovery occurring in chronotropic reserve (CRR) (Molina et al. 2016). This method may help HRR interpretation in hypoxic environments where chronotropic reserve is reduced (Mollard et al. 2007b).

Also CRR was reduced in hypoxia (Fig 1.1.C). Together, these findings on HRR suggest a delayed parasympathetic reactivity after normobaric hypoxic exercise.

Interestingly, comparing our results with those of Al Haddad & colleagues (Al Haddad et al. 2012), obtained in subjects with similar fitness level (VO_{2max}), in line with existing evidence, parasympathetic reactivation assessed through HRR60 was faster after maximal normoxic CPET (45±11bpm) than after supra-maximal normoxic exercise (36±7 bpm). However, in this study we found that HRR60 after maximal CPET at 3500m was similar to that observed after supra-maximal exercise at 2400m (34±8 vs 37±10 bpm). Despite the two different exercise modalities and the two different altitudes (2400m vs 3500m, i.e. moderate altitude vs high altitude), this occurrence may suggest a progressive decrease in post-exercise parasympathetic recovery with increasing altitude, that needs to be further investigated.

The delayed parasympathetic reactivation (Imai et al. 1994; Pecanha et al. 2017) in hypoxia was further underlined by the increase (+35%) occurring in T30. When assessed in response to different bouts of aerobic exercise, T30 is strongly dependent on previous exercise intensity, with higher intensities causing higher increase in this index (Michael et al. 2016). Moreover, the highest values of T30 (i.e. reduced recovery) have been documented after supra-maximal exercises (Buchheit et al. 2007a). According to this scenario, the same effects on T30 can be observed when a maximal exercise is performed at sufficiently severe hypoxic levels.

Alongside the observed increase in HR, exercise is known to reduce HRV indices (e.g. RMSSD), that tend to return to pre-exercise level at exercise stimulus cessation (Pecanha et al. 2017; Michael 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 of recovery for hypoxic condition (Fig 1.1.D), demonstrating depressed HRV and a delayed recovery of parasympathetic cardiac control.

Accordingly, our results indicated that alterations in parasympathetic reactivation can occur in response to maximal exercise performed at a simulated altitude of ≈3500m.

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 1.3). 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 1.1.C). These results, together with the comparable parasympathetic reactivation level observed at 300s (Ln-RMSSD) (Table 1.3) 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 ($\Delta\%HR_{peak}$) and anaerobic metabolism contribution ($\Delta\%[La]_b$)

were significantly related to indices of parasympathetic reactivation (Δ%T30 and Δ%HRR30). 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 (first 30s). 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 reduced 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 ($\Delta\%SpO_2 60$) 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).

Limitations

Different exercises, characterized by a different muscular involvement, as well as different body positions assumed in the post-exercise period have been shown to induce different response in the recovery of HR and HRV indices (Barak et al. 2011; Cunha et al. 2015). Accordingly, the results obtained in this study may be limited to the specific exercise and the post-exercise recovery modality performed by the participants.

Future perspectives

CPET represents the gold standard laboratory test for cardio-respiratory fitness and exercise capacity evaluation (Albouaini et al. 2007) both in normoxic and hypoxic conditions (Ward et al. 2017). In normoxia CPET physiological data (e.g. HR and HRV) are widely adopted for exercise prescription,

whereas the evaluation of HRR in the post-exercise period is an important clinical tool for the assessment of ANS functionality (Romero et al. 2017; Qiu et al. 2017). Similarly, when assessed in response to hypoxia, changes in HR and HRV indices are generally believed to reflect ANS responsiveness and body's ability to adapt to this environmental stressor (Oliveira et al. 2017). Nevertheless, information from hypoxic CPET is generally limited to exercising period, with inadequate information from post-exercise period. Accordingly, the implementation of post-exercise cardiac autonomic modulation assessment, together with the investigation of the acute physiological recovery responses, can help in provide effective information relatively the homeostatic stress induced and the body's ability to recover from hypoxic exercise.

1.5 Conclusions

Acute hypoxia (FiO2=13.4%, ≈3500 m) modified post-exercise cardiac autonomic modulation in response to a maximal CPET, causing a reduction in fast-phase HRR, slow-phase HRR and HRV indices. These findings suggested both delayed parasympathetic reactivation and reduced sympathetic withdrawal after maximal exercise in hypoxia.

However, comparable HRV indices and chronotropic reserve restoration indicated that the alterations occurring in cardiac autonomic recovery in hypoxia were restored within 5 minutes after exercise cessation. In hypoxia the reduced cardiac autonomic recovery was associated with markedly decreased SpO_2 (≈-16.6%), significantly higher EPOCt (≈24.9%), similar $EPOC_{MAG}$ (4.1 vs 3.9 L, for N and H respectively) and increased ExcessVE (≈12.1%), denoting an amplified post-exercise physiological response and increased homeostatic stress associated with hypoxic exercise. Interestingly, as suggested by correlational analysis, the degree of cardiac autonomic recovery impairment seems to be directly related to degree of homeostatic stress induced by hypoxic exercise when compared with normoxic exercise. These findings have several implications in cardiac autonomic recovery interpretation and in HR assessment in response to high-intensity hypoxic exercise.

2. Study 2

Cardiac autonomic and physiological responses to moderate-intensity exercise in hypoxia

Based on the article

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Abstract

We investigated cardiac autonomic and physiological responses to different hypoxic training protocols. Thirteen men performed three exercise sessions (5x5-min; 1-min passive recovery): normoxic exercise at 80% of the power output (PO) at the first ventilatory threshold (N), hypoxic exercise (FiO_2 =14.2%) with the same PO as N (HPO) and hypoxic exercise at the same heart rate (HR) as N (HHR). PO was lower in HHR (21.1±9.3%) compared to N and HPO. Mean HR was higher in HPO (154±11 bpm, $p<0.01$) than N and HHR (139±10 vs 138±9 bpm; $p=0.80$). SpO_2 was reduced ($p<0.01$) to a similar extent ($p>0.05$) in HPO and HHR compared to N. HR recovery (HRR) and HR variability indices were similar in N and HHR ($p>0.05$) but reduced in HPO ($p<0.05$), mirroring a delayed parasympathetic reactivation. Blood lactate and ventilation were similar in N and HHR ($p>0.05$) and increased in HPO ($p<0.001$). During recovery oxygen consumption and ventilation were similar in N and HHR ($p>0.05$) and increased in HPO ($p<0.01$). Moderate HR-matched hypoxic exercise triggers similar cardiac autonomic and physiological responses to normoxic exercise with a reduced mechanical load. On the contrary, the same absolute intensity exercise in hypoxia is associated with increased exercise-induced metabolic stress and delayed cardiac autonomic recovery.

2.1 Introduction

Regular exercise training has an important impact on autonomic nervous system (ANS) function (Joyner and Green 2009), improving cardiac parasympathetic regulation, reducing sympathetic tone, increasing baroreflex sensitivity and heart rate variability (HRV), further providing protective effects against cardiovascular and metabolic diseases (Joyner and Green 2009; Fiuza-Luces et al. 2018) and reducing all-cause mortality (Blair et al. 1989; Hupin et al. 2017).

Hypoxic exercise (i.e. exercise under reduced oxygen availability) has been shown to represent a valid alternative to normoxic exercise to provide an adequate training stimulus, promoting beneficial physiological training adaptations as well as positive health-related outcomes (Millet et al. 2016; Montero and Lundby 2016; Hobbins et al. 2017). Nowadays, hypoxic exercise is implemented within training programs of different populations (Millet et al. 2016; Pramsohler et al. 2017; Hobbins et al. 2017; De Groote et al. 2018). For instance, hypoxic exercise is adopted to increase the physiological load while reducing the mechanical load both in obese patients (Girard et al. 2017) and in older adults (Pramsohler et al. 2017). Additionally, hypoxic exercise has been shown to improve glucose tolerance and weight loss in obese subjects (Hobbins et al. 2017), further representing a promising strategy for insulin resistance and type 2 diabetes prevention (De Groote et al. 2018).

Despite its promise, acute hypoxic exercise can result in altered physiological responses (Mollard et al. 2007a; Calbet et al. 2009; Mourot 2018) and in increased exercise-induced homeostatic perturbation (Koelwyn et al. 2013; Fornasiero et al. 2018), that must be taken into account when prescribing exercise training. Particularly, both external (e.g. exercise load) and internal (e.g. heart rate) markers, commonly used for setting exercise intensity (Garber et al. 2011), are affected by acute hypoxia, making exercise prescription more challenging.

At rest, acute hypoxic exposure leads to ventilatory and hemodynamic changes due to increase in sympathetic activation arising from arterial chemoreceptor stimulation (Dinenno 2016) and to decrease in baroreflex sensitivity (Bourdillon et al. 2017). Alongside, hypoxia alters ANS function

during sub-maximal exercise (Favret and Richalet 2007; Amann and Kayser 2009) (i.e. increased sympathetic activation and withdrawal of parasympathetic cardiac activity (Fisher 2015)) and can lead to greater ANS disturbance and to a delayed recovery of autonomic balance after exercise (Al Haddad et al. 2012; Koelwyn et al. 2013; Fornasiero et al. 2018).

During acute hypoxic exercise arterial oxygen saturation is further decreased (Mollard et al. 2007a, b), whilst heart rate (HR) and cardiac output are increased at any same absolute submaximal exercise intensity and reduced at maximal exercise intensities (Grataloup et al. 2007; Mollard et al. 2007a; Mourot 2018), as well as maximal exercise capacity (i.e. VO_{2max} and peak exercise intensity). Similarly, ventilation and blood lactate levels are increased in hypoxia at the same absolute submaximal exercise load (Sheel et al. 2010) due to the greater relative exercise intensity and the increased anaerobic energy contribution (Mazzeo 2008). In addition, the exercise-induced decrease in arterial saturation is particularly aggravated during maximal hypoxic exercise intensities (Mollard et al. 2007a), further producing amplified post-exercise physiological outcomes, such as increased post-exercise ventilation and delayed recovery of HR and HRV indices (i.e. delayed parasympathetic activation and sympathetic withdrawal), denoting increased and prolonged homeostatic perturbation (Fornasiero et al. 2018). It has been reported that HRV can be depressed (i.e. cardiac autonomic recovery reduced) up to 24h after high-intensity hypoxic exercise in subjects reaching low arterial saturation levels (ΔSpO_2>10% compared to normoxic exercise) (Koelwyn et al. 2013).

To date, evidence of altered cardiac autonomic modulation responses and greater ANS disturbance mainly refers to high-intensity hypoxic exercise (Koelwyn et al. 2013; Fornasiero et al. 2018). It is currently unknown whether less demanding moderate hypoxic exercises, which are more pertinent for health and rehabilitation purposes, cause greater overall homeostatic stress and ANS disturbance. Moreover, the method used to set exercise intensity plays a crucial role on the physiological responses and the homeostatic stress induced by hypoxic exercise, which can further influence cardiac autonomic recovery responses (Al Haddad et al. 2012; Fornasiero et al. 2018). Indeed, a clear relationship has been shown between the impact of the exercise-induced homeostatic perturbation

(Mann et al. 2014) (in terms of increased exercise intensity (Michael et al. 2017a), duration (Michael et al. 2017b), or the presence of more stressful environmental conditions (Al Haddad et al. 2012; Fornasiero et al. 2018)) and the delay observed in cardiac autonomic recovery.

Interestingly, during submaximal exercise performed at a similar relative intensity (same HR), acute hypoxic exercise seems to induce ventilatory and metabolic responses similar to normoxic exercise (Zupet et al. 2009; Chacaroun et al. 2018). However, the cardiac autonomic modulation responses to an exercise performed at a similar relative intensity in hypoxia have not yet been investigated.

The purpose of this study was to examine the effect of hypoxic exercise performed at the same absolute load (Power-matched exercise) or same relative load (HR-matched exercise), on the exercise and recovery cardiac autonomic modulation and physiological responses. Based on the current literature, we hypothesised that moderate HR-matched hypoxic exercise would be associated with similar responses when compared to normoxic exercise, supporting the prescription of hypoxic exercise in this range of moderate intensity based on this physiological marker.

2.2 Materials and methods

Participants

Thirteen moderately aerobically trained healthy men (age 28±6 years, height 176±6 cm, weight 70.2±5.3 kg) volunteered for this study. None of the participants 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. They were instructed to avoid caffeine, alcohol and high-intensity exercise during the 24-h preceding each test session. The experimental protocol was approved by the institutional Ethics Committee of the University of Verona (Italy, n°138232) and was in accordance with international ethical standards (Harriss et al. 2017).

Protocol

Each participant visited the laboratory on 4 occasions (1 preliminary evaluation + 3 experimental sessions) at the same time of the day and completed the experimental protocol within a 4-week period. All tests were conducted in a climatic chamber under controlled laboratory conditions (21°C, 50% relative humidity). For the experimental sessions, the hypoxic environment was created by reducing the inspired fraction of oxygen (FiO_2) by means of an oxygen dilution system based on the Vacuum-Pressure Swing Adsorption principle (B-Cat, Tiel, The Netherlands). For hypoxic conditions the FiO_2 was set at 14.2% to simulate an altitude of ≈3000m above sea level (a.s.l.). All exercises were performed on a cycle ergometer (Excalibur Sport, Lode BV, Groningen, The Netherland) and pedalling cadence was kept constant at 90 revolutions/min, using a monitor that provided participants with visual feedback.

During the preliminary session participants were asked to perform a maximal cardiopulmonary exercise test (CPET) under normoxia to assess peak power output (PPO) and maximal oxygen uptake ($\dot{V}O_{2max}$). The CPET started after a 10-min warm-up at 75 W with increments of 25 W every minute until participants reached volitional exhaustion.

During the next three visits participants underwent three different interval exercise protocols: normoxic exercise at 80% of the PO associated with the first ventilatory threshold (Sugawara et al. 2001) (PO@VT1) (N), hypoxic exercise with the same absolute power as during N (HPO, matched-power exercise) and hypoxic exercise with the same absolute HR recorded during N (HHR, matched-heart rate exercise). N was always performed first. Then HPO and HHR sequences were randomized. During each session participants completed 5 min of baseline measurements at rest, 5 min of sub-maximal constant load exercise (50% PO@VT1) and an interval training session consisting of five 5-min intervals interspersed by 1-min of passive recovery (i.e. 5 x (5-min work: 1-min rec)). Recovery periods were introduced in the exercise protocol to permit the investigation of post-exercise cardiac

autonomic modulation and physiological responses, as well as the impact of exercise duration on these responses (Michael et al. 2017b).

Throughout exercise and recovery phases, beat-to beat HR was continuously recorded using a Polar RS800CX HR monitor (Polar, Kempele, Finland). During rest 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 calibration of flow sensors and gas analyzers was 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 immediately before the end of each exercise bout (Goodwin et al. 2007). 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 each exercise bout using Borg Category Ratio Scale (CR100) (Borg and Borg 2002).

Data Analysis

PPO was determined according to the equation: PPO (W) = power output last stage completed (W) + [*t* (s)/stage duration (s) * stage increment (W)], where *t* is the time of the uncompleted stage. $\dot{V}O_{2peak}$ and other maximal cardio-respiratory variables were defined as the highest 20-s average.

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 a minimum protection zone of 6 bpm (Buchheit et al. 2007a). All the time series of R-R intervals showed low noise (identified errors <5%).

Post-exercise heart rate recovery (HRR) and HRV indices were calculated with a customized script in Matlab (Matlab, Mathworks Inc., USA). HRR indices were measured from the absolute differences between $HR_{bout,}$ calculated as the mean of the last 30s of exercise, and the HR values at 30s (HR30) and 60 s (HR60) of recovery (HRR30 and HRR60, respectively) in the post-exercise period (averaged

over 5s) (Buchheit et al. 2007a). HRR was also calculated as the relative decline in HR expressed as a percentage of HR_{bout} (%HRR=HRR/HR_{bout}×100), i.e. normalised HRR (nHRR30 and nHRR60). T30, the short-time constant of HRR, was calculated as the negative reciprocal of the slope of the regression line of natural-logarithmic transformed HR during the first 30 s of recovery (Pecanha et al. 2017). Additionally, the time-varying vagal-related index RMSSD (Root mean square of successive differences of R–R intervals) was also calculated in the last 30-s of the bout and for each of the two 30-s segments of recovery (Pecanha et al. 2017). The excess post-exercise oxygen consumption magnitude ($EPOC_{MAG}$) was determined as the time integral of $\dot{V}O_2$ recovery curve values (Mann et al. 2014; Fornasiero et al. 2018). Similarly, excess post-exercise ventilation (ExcessVE) was also calculated during recovery periods, as the time integral of $\dot{V}E$. Pulse oxygen saturation values in the last 30 s of exercise (SpO_2) and at 30 s (SpO_230) and 60 s (SpO_260) of recovery were also calculated (Fornasiero et al. 2018).

Statistical Analysis

Data are presented as means ± standard deviations (SD). Data were tested for normal distribution with Shapiro–Wilk test. If data were not normally distributed, natural logarithm transformation (Ln) was applied to obtain a normal distribution and allow parametric statistical comparisons. Cardiorespiratory, HRR, HRV indices in the post-exercise period were compared using a two-way ANOVA with repeated measures, with "condition" (N, HHR, HPO) and "time" (number of bout) as within factors. When statistical significance was identified, a Sidak post hoc test was used to further delineate differences between conditions or time. Statistical analysis was completed using statistical software (SPSS Inc, Chicago, Illinois, USA). Repeated measures correlations between parasympathetic indices and physiological variables were conducted using the R package labelled "rmcorr" (Bakdash and Marusich 2017). The level of statistical significance was set at $p<0.05$.

2.3 Results

Preliminary evaluation

Results from the preliminary assessment and participants' characteristics are presented in Table 2.1. Power output used during training sessions was 183±19 W in N and HPO and 144±18 W in HHR, with a decrement of -21.1±9.3%.

Table 2.1 Participants' Characteristics

Age	(years)	28	(6)
Anthropometry			
Body mass	(kg)	70.2	(5.3)
Height	(cm)	176	(6)
BMI	(kg/m^2)	22.6	(1.6)
Body fat	(%)	11.5	(5)
Fat-free mass	(kg)	62.1	(5.4)
Fat mass	(kg)	8.1	(3.7)
CPET			
HRmax	(bpm)	185	(6)
$\dot{V}O_{2max}$	(L/min)	4.16	(0.31)
$\dot{V}O_{2max}$/kg	(mL/min/kg)	59.3	(3.5)
$\dot{V}E$	(L/min)	179	(19)
PPO	(W)	358	(33)
PPO/kg	(W/kg)	5.1	(0.5)
[La]	(mmol/L)	11.68	(1.46)

Values are Mean ± (SD). HRmax: maximal heart rate; $\dot{V}O_2$max: maximal oxygen consumption; $\dot{V}E$: ventilation; PPO: peak power output; [La]: blood lactate accumulation.

Exercise Physiological Responses

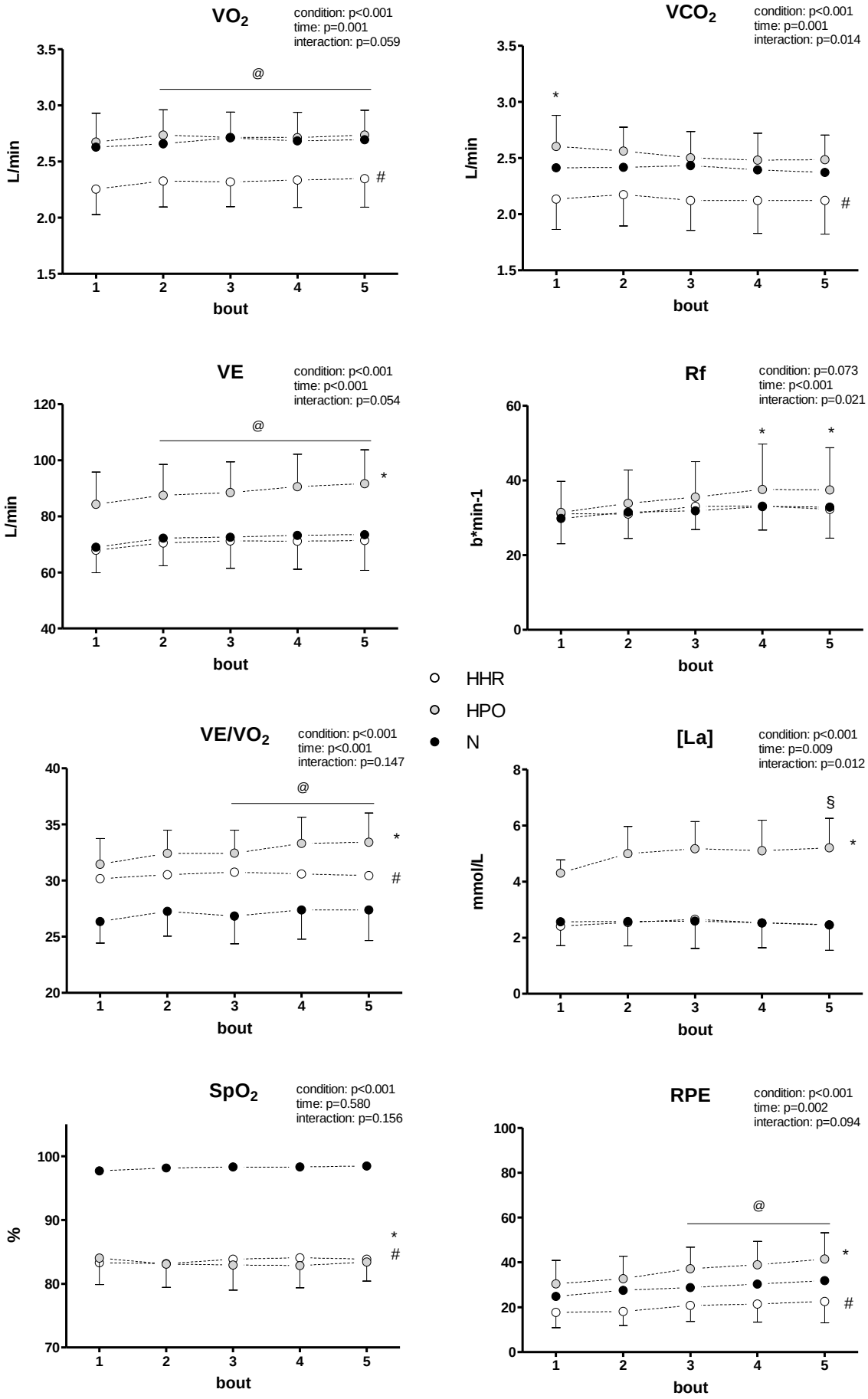
VO2
condition: p<0.001
time: p=0.001
interaction: p=0.059
L/min
bout
VCO2
condition: p<0.001
time: p=0.001
interaction: p=0.014
L/min
bout
VE
condition: p<0.001
time: p<0.001
interaction: p=0.054
L/min
bout
Rf
condition: p=0.073
time: p<0.001
interaction: p=0.021
b*min-1
bout
HHR
HPO
N
VE/VO2
condition: p<0.001
time: p<0.001
interaction: p=0.147
bout
[La]
condition: p<0.001
time: p=0.009
interaction: p=0.012
mmol/L
bout
SpO2
condition: p<0.001
time: p=0.580
interaction: p=0.156
%
bout
RPE
condition: p<0.001
time: p=0.002
interaction: p=0.094
bout

Figure 2.1. Physiological exercise responses during the three exercise protocols. Black, white and grey circles represent normoxic exercise (N), heart rate-matched hypoxic exercise (HHR) and power-matched hypoxic exercise (HPO), respectively. VO_2: oxygen consumption, VCO_2: carbon dioxide production, VE: ventilation, Rf: respiratory frequency, VE/VO_2: ventilatory equivalents for oxygen [La]: blood lactate accumulation, SpO_2: pulse oxygen saturation; RPE: Rate of Perceived Exertion; *: HPO ≠ N; #: HHR ≠ N; @: time effect: bouts ≠ 1st bout; §: bout ≠ 1st bout in HPO

Complete physiological responses during the three exercise sessions (N, HHR and HPO) are reported in Figure 2.1. When compared to N (98.2±1.2%) SpO_2 was reduced in a similar extent both in HPO (83.2±3.6%, p<0.01) and HHR (83.6±3.5%, p<0.01). $\dot{V}O_2$ was increased during the 2nd, 3rd, 4th and 5th compared to 1st bout (time effect p<0.05). Mean $\dot{V}O_2$ was similar in N and HPO (2.68±0.23 and 2.71±0.23 L/min, respectively, p=0.80) and decreased in HHR (2.32±0.24 L/min, condition effect p<0.01). $\dot{V}E$ was similar in N and HHR (72.1±7.7 and 70.4±9.3 L/min, p=0.81) but increased in HPO (88.5±11.4, p<0.01). In addition, $\dot{V}E$ was higher during the 2nd, 3rd, 4th and 5th bouts compared to 1st bout (time effect p<0.05). Respiratory frequency (Rf) was significantly higher in HPO compared to N (p<0.05) and HHR (p<0.05) during the 4th and 5th bouts. A significant effect of condition (p<0.001), time (p<0.001) without interaction (p=0.147) was reported on VE/VO_2 ratio. VE/VO_2 ratio was increased in HHR compared to N (p=0.001) (mean value during the session 30.5± 2.8 vs 27.0±2.4, for HHR and N, respectively), and was significantly increased during the 4th and 5th bouts compared to the first three bouts. Blood lactate accumulation was similar in N and HHR (p=0.99) and higher during HPO (p<0.01). In addition, blood lactate was significantly higher during the 5th bout compared to the 1st bout only in HPO (interaction effect p=0.03). Compared to N (28.6±9.9) mean RPE during the five exercise bouts was significantly higher in HPO (36.1±10.5, p<0.018) and lower in HHR (20.1±7.5; p<0.010). Moreover, RPE was higher during the 3rd, 4th and 5th bouts compared to 1st and 2nd bouts (time effect p<0.05).

Table 2.2 Recovery Physiological Responses

		N					**HHR**					**HPO**					RM ANOVA			N vs H	
Intervals	n	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	condition	time	interaction	N vs HHR	N vs HPO
$EPOC_{MAG}$	L	1.13	1.12	1.16	1.13	1.13	0.99	1.01	1.03	1.04	1.07	1.32	1.32	1.34	1.34	1.35	**<0.001**	0.101	0.335	**0.035**	**<0.001**
		(0.14)	(0.11)	(0.11)	(0.12)	(0.08)	(0.15)	(0.17)	(0.2)	(0.2)	(0.19)	(0.2)	(0.16)	(0.12)	(0.17)	(0.16)					
ExcessVE	L	46.9	46.6	47.3	47.6	46.7	42.5	43.7	44.5	44.2	44.6	54.7	55.0	56.3	57.0	57.5	**<0.001**	0.053	0.405	0.086	**<0.001**
		(7.5)	(6.6)	(7.2)	(6.7)	(6.8)	(6)	(6.7)	(7.9)	(7.5)	(7)	(7.5)	(7.2)	(5.7)	(7.4)	(6.6)					
$SpO_2$30	(%)	98.0	98.3	97.9	98.4	98.2	83.3	84.1	85.0	85.2	85.2	84.1	84.3	84.6	85.5	85.5	**<0.001**	**0.020**	**0.016**	**<0.001**	**<0.001**
		(0.9)	(0.7)	(1.3)	(0.5)	(1.3)	(4.2)	(4.2)	(4.4)	(3.4)	(4.1)	(3.5)	(3.4)	(3.4)	(2.7)	(2.7)					
$SpO_2$60	(%)	98.4	98.7	97.7	98.8	98.8	88.4	89.4	89.4	89.1	89.6	89.2	89.9	90.1	91.0	90.9	**<0.001**	0.052	0.381	**<0.001**	**<0.001**
		(1.6)	(0.5)	(4.3)	(0.4)	(0.3)	(2.8)	(3.1)	(2.7)	(1.4)	(2.6)	(3)	(3.6)	(3.2)	(2.6)	(3)					

Values are Mean ± (SD). RPE: Rate of Perceived Exertion; $EPOC_{MAG}$: excess of post-exercise oxygen consumption; Excess Ve: excess ventilation; SpO_2: pulse oxygen saturation at 30 and 60 s during recovery periods

Recovery physiological responses

When compared to N (1.53±0.15 L), $EPOC_{MAG}$ was significantly increased in HPO (1.73±0.18 L, $p<0.01$) and similar in HHR (1.44±0.20 L, $p=0.07$). Similarly, when compared to N (47±7 L), ExcessVE was increased in HPO (56±6 L, $p<0.01$) but similar in HHR (44±7 L, main effect for condition $p=0.08$). $SpO_2$30 was reduced in HPO (84.8±3.1%, $p<0.01$) and HHR (84.6±4.1%, $p<0.01$) compared to N (98.1± 0.9%) (condition effect $p<0.05$). In addition, $SpO_2$30 was significantly increased during the 3rd, 4th and 5th bout compared to the 1st bout only in HHR (interaction effect $p=0.03$). In contrast, when compared to N (98.5±1.4%), $SpO_2$60 was decreased to a similar extent ($p=0.70$) both in HPO (90.2±3.1 %, $p<0.01$) and in HHR (89.2±2.5%, $p<0.01$).

Cardiac autonomic modulation responses during exercise and recovery

Table 2.3 Heart Rate and Heart Rate Variability Recovery Indices

		N					**HHR**					**HPO**					RM ANOVA			N vs H	
Intervals		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	condition	time	interaction	N vs HHR	N vs HPO
HRR30	(bpm)	43	41	39	38	39	40	37	36	37	38	35	36	33	31	32	**0.018**	**0.008**	0.139	0.624	**0.033**
		(11)	(10)	(7)	(9)	(8)	(7)	(7)	(7)	(8)	(8)	(11)	(11)	(9)	(9)	(10)					
HRR60	(bpm)	55	57	56	54	56	57	53	53	54	55	55	57	54	53	56	0.864	0.284	0.558	0.900	0.995
		(12)	(13)	(8)	(10)	(10)	(8)	(9)	(11)	(9)	(9)	(12)	(11)	(10)	(10)	(10)					
nHRR30	(%)	32.4	30.0	28.4	27.7	27.9	29.8	27.2	26.5	26.8	27.5	24.0	23.7	21.7	19.9	20.4	**0.001**	**<0.001**	0.098	0.677	**0.005**
		(9)	(8.8)	(7)	(7.9)	(7.7)	(6.8)	(5.8)	(6.3)	(7)	(6.9)	(8.5)	(7.8)	(6.6)	(6.8)	(7.4)					
nHRR60	(%)	41.5	41.5	40.4	38.7	40.1	42.1	38.8	38.6	39.1	39.8	37.7	37.8	34.8	34.2	35.5	**0.049**	**0.005**	0.540	0.960	0.129
		(9)	(10.9)	(7.8)	(8.9)	(8.9)	(8.2)	(8)	(8.6)	(7.7)	(7.1)	(9)	(8.2)	(6.8)	(6.9)	(7.4)					
T30	(s)	82	97	98	104	101	87	96	106	97	103	125	131	139	148	148	**0.003**	**<0.001**	0.304	0.998	**0.031**
		(31)	(54)	(38)	(36)	(35)	(21)	(26)	(27)	(25)	(32)	(62)	(72)	(63)	(67)	(63)					
Ln-RMSSDbout	(ms)	1.24	1.14	1.18	1.19	1.16	1.18	1.12	1.09	1.10	1.07	1.08	1.13	1.14	1.13	1.14	0.493	0.831	0.305	0.481	0.817
		(0.35)	(0.33)	(0.36)	(0.35)	(0.31)	(0.21)	(0.19)	(0.15)	(0.2)	(0.19)	(0.18)	(0.32)	(0.32)	(0.34)	(0.37)					
Ln-RMSSD30	(ms)	2.36	2.03	2.05	1.94	1.91	2.11	1.94	1.85	1.91	1.90	1.75	1.63	1.54	1.41	1.44	**<0.001**	**<0.001**	0.349	0.568	**0.005**
		(0.65)	(0.6)	(0.6)	(0.73)	(0.68)	(0.57)	(0.5)	(0.48)	(0.53)	(0.51)	(0.63)	(0.54)	(0.55)	(0.35)	(0.39)					
Ln-RMSSD60	(ms)	2.98	2.80	2.69	2.54	2.70	2.91	2.60	2.49	2.61	2.63	2.52	2.25	2.18	2.04	2.00	**0.004**	**<0.001**	0.530	0.915	**0.025**
		(0.59)	(0.74)	(0.66)	(0.87)	(0.87)	(0.67)	(0.52)	(0.59)	(0.56)	(0.56)	(0.8)	(0.63)	(0.58)	(0.67)	(0.75)					

Values are Mean ± (SD). HRR: Heart rate recovery, T30: Short-term time constant of heart rate recovery; Ln: Natural-logarithm transformation; nHRR: normalized HRR = (HRR/HRBout)*100; RMSSD: Root mean square of successive differences of R–R intervals.

Complete HR and HRV responses during the three exercise sessions (N, HHR and HPO) are presented in Table 2.3. Mean HR during the five exercise bouts was significantly higher in HPO (154±11 bpm) compared to N (139±10 bpm; $p<0.001$) and HHR (138±9 bpm; $p<0.001$). In addition, a significant increase in HR throughout the exercise bouts was reported (time effect $p<0.05$). HR30 was significantly higher at each time point in HPO condition compared to N and HHR (interaction effect $p<0.05$). In addition, HR30 after the 5th bout was significantly higher in all the conditions when compared to the 1st bout ($p<0.05$).

When compared to N, HR60 was on average significantly higher in HPO ($p<0.01$) but not in HHR ($p=0.99$). In addition, starting from the 3rd bout onward, HR60 was significantly higher compared to the 1st bout ($p<0.05$).

When compared with N (40±9 bpm), mean HRR30 during the exercise session was reduced in HPO (33±9 bpm, $p<0.03$) but not in HHR (38±7 bpm, $p=0.62$). In addition, post-hoc analysis showed a decrease in HRR30 after the 4th and 5th bout compared to the 1st bout ($p<0.05$). Similarly, nHRR30 was decreased after the 2nd, 3rd, 4th and 5th bout, compared to the 1st bout ($p<0.05$). Furthermore, when compared with N (29.2±7.8 %), mean nHRR30 was on average reduced in HPO (21.9±7.1 %, $p=0.005$) but not in HHR (27.5±6.3 %, $p=0.67$).

T30 was significantly increased in HPO (138±64 s, $p=0.03$) but not in HHR (98±25 s, $p=0.99$) compared with N (96±36 s). Furthermore, starting from the 3rd bout mean T30 was significantly decreased compared to the 1st bout (time effect $p<0.01$).

Regarding HRV indices, we did not find any effect of condition ($p=0.49$), time ($p=0.83$) or interaction ($p=0.31$) on RMSSD during exercise. Differently, significant reductions in both RMSSD30 and RMSSD60 in HPO, but not in HHR, when compared to N were reported ($p<0.05$). Post-hoc analysis revealed a decrease in both RMSSD30 and RMSSD60 after the 2nd, 3rd, 4th and 5th compared to the 1st bout (time effect $p<0.05$).

Repeated measures correlations

Complete repeated measures correlations results are reported in Table 2.4. Overall, HRR30, RMSSD30 and RMSS60 were negatively correlated ($p<0.05$) to exercise metabolic and cardiorespiratory parameters (HRbout, V'E, [La] and SpO_2), while T30 was positively associated ($p<0.05$) with these variables. Also, HRR30, RMSSD30 and RMSS60 were negatively associated ($p<0.05$) with $EPOC_{MAG}$ and ExcessVE and positively associated ($p<0.05$) with SpO_230 and SpO_260. T30 was positively associated ($p<0.05$) with $EPOC_{MAG}$ and ExcessVE and negatively associated ($p<0.05$) with SpO_230 and SpO_260. HRR60 was significantly associated with $EPOC_{MAG}$ and ExcessVE ($p<0.05$).

Table 2.4 Repeated Measure Correlations between recovery parasympathetic reactivation indices and exercise and recovery physiological responses

Exercise

	HRbout					$\dot{V}$E					[La]					SpO_2				
	N	*HHR*	*HPO*	*Overall*		*N*	*HHR*	*HPO*	*Overall*		*N*	*HHR*	*HPO*	*Overall*		*N*	*HHR*	*HPO*	*Overall*	
HRR30	-0.19	-0.09	**-0.37**	**-0.51**	large	-0.21	**-0.31**	**-0.41**	**-0.29**	small	0.06	-0.23	**-0.33**	**-0.46**	moderate	0.10	-0.10	-0.20	**0.37**	moderate
HRR60	0.12	-0.02	-0.01	0.00	trivial	-0.01	0.23	0.01	0.24	small	0.04	0.03	0.14	-0.01	trivial	-0.16	0.12	0.17	0.10	small
T30	**0.33**	**0.29**	**0.54**	**0.67**	large	0.19	0.22	**0.48**	**0.48**	moderate	-0.05	0.07	**0.41**	**0.59**	large	-0.10	0.14	**0.30**	**-0.30**	moderate
RMSSD30	**-0.55**	**-0.41**	**-0.48**	**-0.57**	large	**-0.35**	**-0.28**	**-0.27**	**-0.34**	moderate	0.07	**-0.30**	-0.12	**-0.48**	moderate	0.05	0.00	0.04	**0.39**	moderate
RMSSD60	**-0.47**	**-0.43**	**-0.44**	**-0.49**	large	**-0.32**	-0.24	**-0.32**	**-0.24**	small	-0.01	-0.16	-0.17	**-0.46**	moderate	-0.09	0.03	0.12	**0.37**	moderate

Recovery

	$EPOC_{MAG}$					ExcessVE					$SpO_2$30					$SpO_2$60				
	N	*HHR*	*HPO*	*Overall*		*N*	*HHR*	*HPO*	*Overall*		*N*	*HHR*	*HPO*	*Overall*		*N*	*HHR*	*HPO*	*Overall*	
HRR30	-0.24	**-0.32**	**-0.30**	**-0.34**	moderate	-0.23	**-0.47**	**-0.44**	**-0.31**	moderate	0.05	-0.21	**-0.33**	**0.35**	moderate	0.16	-0.26	-0.25	**0.34**	moderate
HRR60	-0.21	-0.03	-0.07	**0.15**	small	-0.15	-0.07	-0.11	**0.20**	small	-0.06	0.00	0.06	0.05	trivial	-0.14	-0.03	-0.02	0.08	trivial
T30	0.11	**0.45**	0.17	**0.45**	moderate	0.17	**0.38**	**0.33**	**0.47**	moderate	-0.06	0.03	**0.44**	**-0.30**	moderate	-0.05	0.17	**0.34**	**-0.29**	small
RMSSD30	-0.04	**-0.33**	-0.13	**-0.37**	moderate	-0.06	**-0.38**	**-0.32**	**-0.36**	moderate	-0.04	-0.04	-0.17	**0.39**	moderate	0.05	-0.08	-0.21	**0.32**	moderate
RMSSD60	**-0.32**	-0.26	0.18	**-0.25**	small	-0.13	**-0.34**	-0.02	**-0.15**	small	-0.06	-0.20	0.00	**0.37**	moderate	0.03	-0.07	-0.14	**0.32**	moderate

Bold characters denote significant correlations ($p<0.05$). HRR: Heart rate recovery; T30: Short-term time constant of heart rate recovery; RMSSD: Root mean square of successive differences of R–R intervals; $\dot{V}E$: ventilation, [La]: blood lactate accumulation; SpO_2: pulse oxygen saturation; $EPOC_{MAG}$: excess of post-exercise oxygen consumption; ExcessVE: excess ventilation.

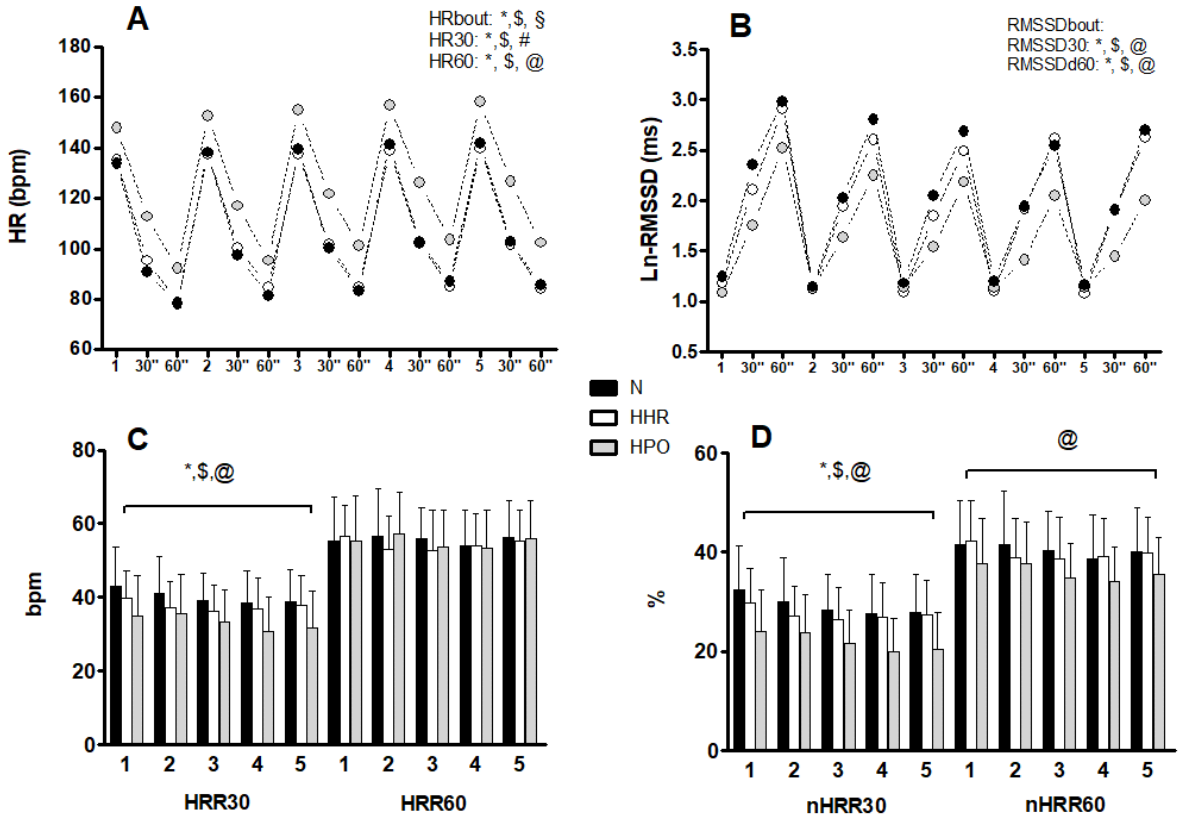

Figure 2.2 Cardiac autonomic modulation responses during the three interval exercise sessions (5x (5-min exercise bout; 1-min passive recovery)). Black, white and grey circles and bars represent normoxic exercise (N), heart rate-matched hypoxic exercise (HHR) and power-matched hypoxic exercise (HPO), respectively. Fig 2.2.A: Heart Rate (bpm); *: HPO ≠ N; $: HPO ≠ HHR; # time effect: 3rd, 4th and 5th ≠ 1st and 2nd bout in N and HPO, not in HHR; @: time effect: 2nd, 3rd, 4th and 5th ≠ 1st bout; §: time effect: each bout ≠ from previous bout; Fig 2.2.B: Natural-logarithm transformation of the root mean square of successive differences of R–R intervals (Ln-RMSSD); *: HPO ≠ N; $: HPO ≠ HHR; @: time effect: 2nd, 3rd, 4th and 5th ≠ 1st bout; Fig 2.2.C: Heart rate recovery (bpm) at 30 and 60 s of recovery; *: HPO ≠ N; $: HPO ≠ HHR; @: time effect: 4th and 5th ≠ 1st bout; Fig 2.2.D: Normalized Heart Rate Recovery (nHHR) at 30 and 60 s of recovery calculated as (%HRR=HRR/HR_{bout}×100); *: HPO ≠ N; $: HPO ≠ HHR; @: time effect: 4th and 5th ≠ 1st bout.

2.4 Discussion

In the present study, we investigated the effects of performing hypoxic exercise (FiO_2=14.2%, ≈3000 m a.s.l) at the same absolute and relative normoxic exercise intensity on exercise and recovery cardiac autonomic modulation and physiological responses. The key finding of this study is that, in accordance with our hypothesis, when the exercise intensity was matched with the reference normoxic HR, no significant differences in cardiac autonomic responses were observed in hypoxic exercise compared to normoxic exercise. On the contrary the same absolute exercise intensity in hypoxia was associated with amplified physiological responses and delayed cardiac autonomic recovery.

Cardiac autonomic modulation responses during exercise and recovery

At rest, a hypoxic stimulus increases sympathetic activity and reduces parasympathetic regulation (Amann and Kayser 2009), elevating HR and decreasing HRV (Oliveira et al. 2017). In addition, during exercise HR is higher at any similar sub-maximal exercise intensity and decreased ad maximal intensities (Mazzeo 2008; Mourot 2018). It has been shown that, for sufficiently high exercise intensities (>50%$\dot{V}O_{2max}$), hypoxia does not exert an additional influence upon HRV (Buchheit et al. 2004), due to the already substantial exercise-induced vagal withdrawal and sympathetic activation (White and Raven 2014). In line with these observations, in our study mean HR was higher in HPO (≈+15 bpm) compared to N during each exercise bout. Moreover, we did not observe any difference in HRV vagal activity index (RMSSD) during exercise in the three experimental conditions (>50% $\dot{V}O_{2max}$) (Table 2.3, Figure 2.2 b).

ANS responsiveness to an exercise stimulus can be assessed by investigating HR and HRV indices during recovery periods (Pecanha et al. 2017; Michael et al. 2017a). It has been shown that higher exercise intensities, as well as challenging environmental conditions (Al Haddad et al. 2012; Fornasiero et al. 2018), cause higher homeostatic disruptions and slower post-exercise recovery of HR and HRV indices (Terziotti et al. 2001; Buchheit et al. 2007a; Michael et al. 2017a).

For instance, cardiac autonomic recovery is delayed both after maximal hypoxic exercise (FiO_2=13.4%, ≈3500 m, mean SpO_2 ≈ 77 %) (Fornasiero et al. 2018), as well as after hypoxic exercise (FiO_2=15.4%, ≈2400 m) performed at the intensity associated with the first ventilatory threshold (Al Haddad et al. 2012). HRR30 and HRR60 are reduced respectively by ≈6 and ≈12 bpm (≈-25% reduction) after a maximal exercise at ≈3500 m (Fornasiero et al. 2018). Similarly, HRR60 was also found to be reduced by ≈8 bpm (≈-14%) after an exercise performed at the first ventilatory threshold intensity at a simulated altitude of ≈2400m (Al Haddad et al. 2012), mirroring a delayed parasympathetic reactivation (Michael et al. 2017a).

In line with these results we found that parasympathetic reactivation indices were delayed in response to power-matched hypoxic exercise (FiO_2=14.2%, ≈3000 m). However, no significant differences were observed in response to HR-matched hypoxic exercise (Table 2.3). For instance, HRR at 30s of recovery was significantly reduced in HPO session both when express as bpm (HRR30, mean reduction ≈7 bpm, ≈-17%) (Figure 2.2.c) and when normalized for exercising HR (nHRR30, mean reduction ≈7 %) (Figure 2.2.d). Similarly, we observed an increased short-term time constant of HRR (T30, ≈+44%), showing a reduced parasympathetic reactivation in HPO session. Equally, HRV indices of parasympathetic reactivation (Ln-RMSSD)(Goldberger et al. 2006) were reduced in HPO session both at 30 and 60s of recovery (Figure 2.2.b), but not in HHR.

Moreover, as previously suggested (Michael et al. 2017b), we reported that most of the cardiac autonomic recovery indices are influenced (i.e. decreased) by exercise duration, although without apparent interactions with hypoxia. Altogether, these results underlined that parasympathetic reactivation is impaired during moderate power-matched but not HR-matched hypoxic exercise.

Exercise and recovery physiological responses

Due to the reduced arterial saturation and the increased chemoreflex stimulation, the same absolute exercise intensity in hypoxia is associated with an increased ventilatory response (Mazzeo 2008; Sheel et al. 2010). In contrast, when hypoxic exercise is performed at the same relative intensity as normoxic exercise, similar ventilatory responses have been reported (Zupet et al. 2009; Chacaroun et

al. 2018). For instance, in a recent study Chacaroun et al. (2018) showed that matching HR at an intensity of 75% of maximal HR (mean PO decrement of -21%), the ventilatory responses were similar in hypoxic and normoxic exercise. In accordance with these observations, the mean power decrement in our study (21.1±9.3%) at a similar relative exercise intensity (75% of HRmax) induced similar ventilatory responses in N and HHR (≈72 vs ≈70 L/min), whereas HPO induced higher mean responses (≈89 L/min) (Mazzeo 2008) (Table 2.3). Moreover, this latter condition was further associated with increased respiratory frequency in the last two exercise bouts.

In line with the existing literature, blood lactate accumulation and anaerobic metabolic contributions were higher during HPO but not during HHR (Fig. 2.1). Moreover, HPO condition was associated with a progressive increase in blood lactate accumulation (Fig. 2.1). Accordingly, higher metaboreflex activation and increased sympathetic activation (Romero et al. 2017) were likely experienced by participants in HPO condition, which in turn reduced cardiac autonomic recovery and may explain the delayed parasympathetic reactivation (Fisher et al. 2013; Peçanha et al. 2016).

Decreased SpO_2 levels in hypoxia can result in reduced cardiac autonomic recovery due to a direct effect on carotid chemoreflex (Favret and Richalet 2007). However, this reduction was observed only in HPO and not in HHR sessions despite similar SpO_2 levels (Fig. 2.1). Other mechanisms, including greater contribution of central command (Michael et al. 2017a) could have accounted for the differences observed. For instance, it is worth noting that, when compared to N, RPE was reduced in HHR and increased in HPO, respectively. Accordingly, a lower contribution of central command can be hypothesized in HHR sessions, thus rebalancing the possible decrease occurring in cardiac autonomic recovery. Similarly, the decreased power output in HHR may have mitigated parasympathetic inhibition during exercise by means of a reduced muscle mechanoreceptors stimulation (Fisher 2014).

The investigation of post-exercise physiological responses can help to explain the homeostatic stress induced by an acute exercise stimulus (Mann et al. 2014) and its impact on HR and HRV recovery. For instance, in the study of Fornasiero et al. (2018) the delayed cardiac autonomic recovery

observed after hypoxic exercise was associated with amplified post-exercise physiological responses, indicating increased exercise-induced metabolic stress (Mann et al. 2014).

In the present study physiological recovery responses were amplified only during HPO and not during HHR (Table 2.2). In fact, we reported higher ExcessVE and $EPOC_{MAG}$ (i.e. higher oxygen consumption during recovery), mirroring increased metabolic stress. Taken together, these markers may further explain the delayed cardiac autonomic recovery responses observed in HPO sessions.

As suggested by repeated measures correlations analysis (Table 2.4), parasympathetic reactivation indices were related to the degree of cardiac and metabolic stress induced by exercise, noticeable in other physiological markers (i.e. HR, [La], $\dot{V}E$, and SpO_2) both during exercise and recovery periods. We reported that the higher were the cardiac stress (i.e. HR) and the anaerobic contribution (i.e. [La]) during exercise the lower were the parasympathetic reactivation indices ("moderate" to "large" negative correlations) in the immediate post-exercise recovery period (i.e. reductions in HRR30 and recovery RMSSD). Equally, higher reductions in parasympathetic recovery indices were related to greater increases in exercise ventilatory responses ("moderate" negative correlations). Furthermore, parasympathetic reactivation indices were also significantly related to indices reflecting exercise-induced metabolic stress during recovery (i.e. $EPOC_{MAG}$ and ExcessVE) (Mann et al. 2014; Fornasiero et al. 2018) (Table 2.4). Also in this case, negative correlations ("moderate"), showing reduced parasympathetic reactivation indices in the presence of amplified post-exercise metabolic and ventilatory responses, were reported.

Overall, our study results suggest that, in the moderate exercise intensity domain, cardiac autonomic recovery and physiological responses, denoting homeostatic stress, are altered during hypoxic exercise only when performed at the same absolute, but not at the same relative normoxic exercise intensity (based on HR). Moreover, these responses did not seem to depend on the duration of the exercise (no interaction effect), although cardiac autonomic recovery is progressively decreased with exercise duration (Tab 2.3). Indeed, for most of the investigated indices we observed the same

trends when comparing the mean responses, the responses at the end of the exercise or when comparing successive bouts within an exercise session among the different conditions.

Limitations

Firstly, in the present study we did not investigate blood pressure responses, therefore we could not infer about the impact of the different protocols on this variable. Secondly, the individual assessment of respiratory and leg muscles perceived exertion may have helped to clarify the reduction observed in the RPE values in HHR, and the role played by the decrease in power output and metabolic demand in this latter condition (Aliverti et al. 2011). Thirdly, a fourth condition performed at the same relative intensity of HPO session in normoxia would have provided a better understanding of the independent effects of both hypoxia and exercise intensity on cardiac autonomic responses.

The present findings were obtained in healthy active subjects, and, therefore, further experimental research investigating the impact of hypoxic exercise on different special populations is required (Millet et al. 2016).

2.5 Conclusions

Moderate HR-matched hypoxic exercise triggers similar cardiac autonomic and physiological responses to normoxic exercise with a reduced mechanical load. On the contrary, the same absolute intensity exercise in hypoxia is associated with increased exercise-induced physiological stress and delayed cardiac autonomic recovery.

3. Study 3

Post-exercise cardiac autonomic and cardiovascular responses to heart rate matched and work rate matched hypoxic exercise

Based on the article

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Abstract

This study investigated the effect of performing hypoxic exercise at the same heart rate (HR) or work rate (WR) as normoxic exercise on post-exercise autonomic and cardiovascular responses. Thirteen men performed three interval-type exercise sessions (5 x 5-min; 1-min recovery): normoxic exercise at 80% of the WR at the first ventilatory threshold (N), hypoxic exercise (FiO_2 =14.2%) at the same WR as N (H-WR) and hypoxic exercise at the same HR as N (H-HR). Autonomic and cardiovascular assessments were conducted before and after exercise, both at rest and during active squat-stand manoeuvres (SS). Compared to N, H-WR elicited a higher exercise intensity (≈83% vs ≈75%HRmax, $p<0.001$) and H-HR a reduced exercise work rate (-21.1±9.3%, $p<0.001$). Cardiac parasympathetic indices were reduced 15 min after exercise and recovered within 60 min in N and H-HR, but not after H-WR ($p<0.05$). H-WR altered cardiac baroreflex sensitivity (cBRS) both at rest and during SS (specifically in the control of blood pressure fall during standing phases) in the first 60 min after the exercise bout ($p<0.05$). Post-exercise hypotension did not occur in H-HR ($p>0.05$) but lasted longer in H-WR than in N ($p<0.05$). Moderate heart rate matched hypoxic exercise does not affect cBRS and does not blunt cardiac autonomic recovery during the early post-exercise recovery phase. However, post-exercise hypotension is limited or absent and may relate to the reduced WR. Conversely, WR matched hypoxic exercise delays cardiac autonomic recovery, temporarily decreases cBRS and evokes prolonged post-exercise hypotension.

3.1 Introduction

In the past few years, hypoxic exercise (i.e. exercise combined with hypoxic stress) has been repeatedly highlighted as a promising nonpharmacological therapeutic intervention (Millet et al. 2016; Millet and Girard 2017; Lizamore and Hamlin 2017; Brocherie and Millet 2020). By reducing the mechanical load needed for adequate cardiovascular stimulation, hypoxic exercise represents a suitable option for obese and elderly patients to help meet exercise recommendations (Haufe et al. 2008; Girard et al. 2017; Pramsohler et al. 2017; Hobbins et al. 2017). Hypoxic exercise also has the potential to improve weight loss and cardio-metabolic health in overweight and obese patients (Netzer et al. 2008; Hobbins et al. 2017; Ramos-Campo et al. 2019), further representing a promising approach for insulin resistance and type 2 diabetes prevention and treatment (Mackenzie et al. 2012; De Groote et al. 2018; Mai et al. 2019). Potential applications of hypoxic training in patients with various cardiovascular diseases, including hypertension, have also been highlighted (Wee and Climstein 2015).

The interest in hypoxic exercise arises from its potential to promote greater physiological and health-related adaptions compared to normoxic exercise (Millet et al. 2016; Girard et al. 2020) in the long-term (i.e. chronic effects), stemming from the markedly different acute physiological responses (i.e. acute effects). At the same absolute work rate (WR), hypoxia-induced arterial chemoreceptor stimulation promotes greater sympathetic activation and withdrawal of parasympathetic activity (Hainsworth et al. 2007; Amann and Kayser 2009; Fisher 2015; Siebenmann et al. 2018), which lead to increased cardiovascular and ventilatory responses (Calbet et al. 2009; Sheel et al. 2010; Fisher 2015; Winkler et al. 2017) to cope with the lower blood oxygen content and to match metabolic demands (Bartsch and Gibbs 2007; Fisher 2015). Compensatory vasodilation of vascular beds facilitating blood and oxygen delivery to the working muscles also occurs (Joyner and Casey 2014; Dinenno 2016). As a result, the same absolute exercise intensity represents a greater physiological challenge in hypoxia (Mazzeo 2008; Fornasiero et al. 2019), which potentially affects post-exercise recovery responses (Luttrell and Halliwill 2015; Romero et al. 2017). An exacerbated increase of

exercise-induced physiological stress of hypoxic exercise, can result in delayed recovery of autonomic balance (i.e. progressive restoration of normal resting balance between parasympathetic and sympathetic activity) and altered post-exercise cardiovascular responses (Romero et al. 2017; Michael et al. 2017a).

Delayed parasympathetic recovery has been observed after hypoxic exercise conducted at similar absolute normoxic exercise intensity (Koelwyn et al. 2013; Fornasiero et al. 2018, 2019). In addition, acute post-exercise blood pressure reduction (i.e. post-exercise hypotension, PEH) (Halliwill et al. 2013) may be intensified after hypoxic exercise (Horiuchi et al. 2016, 2018; Saito et al. 2019), and autonomic blood pressure control (e.g. cardiac baroreflex sensitivity, cBRS) affected, increasing the risk of orthostatic intolerance and hypotension (Halliwill et al. 2014). However, while it is known that hypoxia decreases cBRS at rest and during exercise (Gujic et al. 2007; Bourdillon et al. 2017, 2018), to the best of our knowledge no study has investigated the impact of hypoxic exercise on post-exercise cBRS.

A better understanding of the autonomic nervous system activity during recovery is of high importance especially because hypoxic exercise has been recommended amongst vulnerable populations (i.e., elderly and/or overweight/obese people, or suffering from diabetes or hypertension) who often exhibit altered autonomic cardiovascular control, and particularly cardiac baroreflex impairment (Lanfranchi and Somers 2002; Skrapari et al. 2006; Monahan 2007). Also, post-exercise recovery is a critical phase when sudden cardiovascular events are more frequent and often attributable to autonomic disturbances (Luttrell and Halliwill 2015).

The timeframe for cBRS recovery depends on the physiological exercise intensity (Piepoli et al. 1993; Halliwill et al. 1996; Terziotti et al. 2001; Raczak et al. 2005; Reynolds et al. 2017), which is higher at the same absolute WR in hypoxia. Arguably, both the increased physiological exercise intensity relative to maximal, and hypoxic exercise per se, might negatively affect post-exercise cBRS responses, but this currently remains mere speculation. Interestingly, the hypoxia-induced increase in cardiovascular and ventilatory stimulation may be mitigated by matching to the exercise heart rate

(HR) response (Winkler et al. 2017; Chacaroun et al. 2018; Fornasiero et al. 2019). By adjusting submaximal exercise intensity based on HR during exercise in hypoxia, ventilatory, anaerobic (i.e. blood lactate) and cardiac autonomic responses are similar to normoxic exercise (Zupet et al. 2009; Chacaroun et al. 2018; Fornasiero et al. 2019), and involve reduced mechanical load and aerobic demand (i.e. reduced VO_2 due to the decreased mechanical load). Similarly, blood pressure is reduced during hypoxic compared to normoxic exercise when HR is matched (Winkler et al. 2017). However, whether HR-matched hypoxic exercise leads to the same post-exercise responses is currently unknown.

Thus, the aim of this study was to examine the effect of performing hypoxic exercise at the same relative (same HR) or same absolute (same WR) normoxic exercise intensity on the post-exercise cardiac autonomic and cardiovascular responses, while recovering in normoxic conditions. We hypothesised that moderate HR-matched hypoxic exercise would be associated with similar post-exercise autonomic and cardiovascular responses when compared to normoxic exercise, whilst WR-matched hypoxic exercise would result in delayed autonomic recovery and post-exercise cardiovascular responses dissimilar to normoxic exercise.

3.2 Materials and methods

Participants

Thirteen moderately aerobically trained healthy men (age 28±6 years, height 176±6 cm, weight 70.2±5.3 kg, BMI 22.6±1.6 kg/m^2, $\dot{V}O_{2max}$ 59.3±3.5 mL/min/kg) volunteered for this study. None of the participants 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. They were instructed to avoid caffeine, alcohol and high-intensity exercise in the 24-h preceding each experimental session. The experimental protocol was

approved by the institutional Ethics Committee of the University of Verona (Italy, n°138232) and performed in accordance with the Declaration of Helsinki.

Protocol

Each participant visited the laboratory on 4 occasions (1 preliminary evaluation + 3 experimental sessions) at the same time of the day and completed the experimental protocol within a 4-week period. A detailed description of the preliminary evaluation, including anthropometric and maximal cardiorespiratory fitness assessment as well as further details of the experimental sessions, has been provided elsewhere (Fornasiero et al. 2019). The preliminary evaluation consisted of a maximal cardiopulmonary exercise test (CPET) under normoxia (10 min at 75 W with increments of 25 W every minute until participants' volitional exhaustion) to assess maximal exercise work rate (WR) and maximal oxygen uptake ($\dot{V}O_{2max}$). In the experimental protocol all participants completed three exercise sessions, one in normoxia and two in hypoxia, which were conducted in an environmental chamber under controlled laboratory conditions (21°C, 50% relative humidity). The hypoxic environment was produced by lowering the fraction of inspired oxygen (FiO_2) to 14.2%, simulating an altitude of ≈3000m above sea level (a.s.l.), by means of an oxygen dilution system based on the Vacuum-Pressure Swing Adsorption principle (B-Cat, Tiel, The Netherlands). Exercise sessions were performed on a cycle ergometer (Excalibur Sport, Lode BV, Groningen, The Netherland) and comprised: 5 min of seated rest for baseline assessment, 5 min of sub-maximal constant load exercise (warm-up) at 50% of the work rate associated with the first ventilatory threshold (WR@VT1) and five 5-min intervals interspersed by 1-min of passive recovery of either normoxic exercise at 80% WR@VT1(Sugawara et al. 2001) (N), hypoxic exercise with the same absolute WR as during N (H-WR, WR-matched exercise) or hypoxic exercise with the same absolute HR recorded during N (H-HR, HR-matched exercise). A similar design was adopted to obtain key information about cardiorespiratory and cardiac autonomic responses to interval-type hypoxic exercise, which has been

presented in a previous study (Fornasiero et al. 2019). Experimental session sequence was partially randomized, since H-HR was always performed after N.

Autonomic nervous system and haemodynamic assessments were conducted before (PRE) entering the environmental chamber and 15 and 60 min after exercise (POST-15 and POST-60), in a quiet room under normoxic conditions (23 °C, 50% relative humidity). The setup used in the study, including cardiac autonomic and haemodynamic assessments at rest and during repeated squat-stand manoeuvres, was a modified version of the methodology previously described by Mourot et al. (Mourot et al. 2020). Participants sat quietly for 10 min (Terziotti et al. 2001) and then completed 5 min of repeated squat-stand (SS) manoeuvres with a duty cycle of 10-s squat and 10-s stand (Zhang et al. 2009). Resting phases and SS manoeuvres were separated by 30 min (i.e. SS manoeuvres were performed 45 and 90 min after exercise, POST-45 and POST-90), during which a non-invasive assessment of vascular function in brachial and femoral arteries was performed. Those data were not included in the present investigation. During rest and SS manoeuvres beat-by-beat blood pressure and R-R intervals were measured continuously using Portapres® device (Finapres Medical System, Amsterdam, The Netherlands) and Polar RS800CX HR monitor (Polar, Kempele, Finland), respectively.

In the environmental chamber, cardio-respiratory measures were collected continuously using an automated, breath-by-breath open-circuit gas analysis system (Quark PFT Ergo, Cosmed Srl, Rome, Italy). Careful calibration of flow sensors and gas analyzers was 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 Hz. To measure blood lactate concentration a blood sample was collected from the earlobe immediately before the end of each exercise bout (Goodwin et al. 2007). 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 each exercise bout 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 artefacts were filtered out by means of a moderate error correction filter. All the time series of R-R intervals had low noise (identified errors <5%). HRV analysis was performed using Kubios HRV software (Version 2.1, Biosignal Analysis and Medical Imaging Group, Kuopio, Finland). HRV indices were calculated from the last 5 min of the 10-min resting period. The time-domain HRV indices considered were the square root of the sum of successive differences between adjacent normal R-R intervals squared (RMSSD), the standard deviation of normal-to-normal RR intervals (SDNN) and the percentage of successive R-R intervals differing more than 50ms from the previous R-R interval (Task Force of the European Society of Cardiology 1996). For frequency-domain HRV indices, low frequency spectral power (LF, 0.04-0.15 Hz), high frequency spectral power (HF, 0.15-0.4 Hz), and total spectral power (TP, 0-0.4 Hz) were calculated by Fast Fourier Transform (FFT) (Task Force of the European Society of Cardiology 1996).

Inter-Beat Interval (IBI), beat-to-beat systolic (SAP), diastolic (DAP) and mean (MAP) arterial blood pressure values, as well as other estimated haemodynamic variables (SV, CO and TPR) were extracted using Beatscope Software (TNO-TPD, Biomedical Instrumentation). Haemodynamic data were calculated from the last 5 min of the 10-min period during rest and from the entire 5 min of the squat-stand (SS) manoeuvres. Post-exercise hypotension (PEH) was defined as the absolute difference between SAP at POST and SAP at PRE (PEH = SAP POST – SAP PRE) (Brito et al. 2018).

During repeated SS manoeuvres, maximal (SAP_{max}) and minimal (SAP_{min}) systolic blood pressure values were extracted from each 20s cycle (10s squat-10s stand) and averaged over the 5 min period (15 cycles).

Beat-by-beat SAP and IBI values were used to assess cBRS. Custom written Matlab (Mathworks, Natick, MA, ver. 2018a) scripts were used to conduct the following analyses. SAP and IBI data were

linearly interpolated and resampled at 2 Hz for spectral and Transfer Function Analysis. Under resting conditions, transfer function analysis (TF) of gain, phase, and coherence between spontaneous oscillations in SAP and IBI were calculated in accordance with the work of Zhang et al. (Zhang et al. 2009), i.e. 0.05–0.15 Hz for the low frequency (LF) range. During SS manoeuvres (performed at 0.05 Hz) TF gain, phase, and coherence were calculated across a specific frequency (SF) range (i.e. 0.031–0.078 Hz) (Zhang et al. 2009). cBRS was also assessed with the sequence method (Pinna et al. 2015). The sequence method is based on the identification of at least three consecutive beats (sequence) in which a defined increase (or decrease) in SAP is followed by a defined increase (or decrease) in the IBI. Only sequences with a minimum correlation coefficient of 0.85 were accepted. Positive and negative sequences were averaged to obtain a representative value of cBRS ($cBRS_{seq}$). To better represent blood pressure control in the upward and downward directions, mean gain values of positive ($cBRS_{Seq}+$) and negative ($cBRS_{Seq}-$) sequences were also computed separately.

Statistical Analysis

Data are presented as means ± standard deviations (SD). Data were tested for normal distribution with the Shapiro–Wilk test. If data were not normally distributed, natural logarithm transformation (Ln) was applied to obtain a normal distribution and allow parametric statistical comparisons. Autonomic and haemodynamic variables were compared using a two-way ANOVA for repeated measures, with "condition" (N, H-HR, H-WR) and "time" (PRE-POST15-POST60 and PRE-POST45-POST90 for resting and SS assessments, respectively) as within factors. When statistical significance was identified, a Sidak post hoc test was used to further delineate differences between conditions or time. Statistical analysis was completed using statistical software (SPSS Inc, Chicago, Illinois, USA). The level of statistical significance was set at $p<0.05$.

3.3 Results

Exercise Sessions

Detailed results of the exercise sessions are presented elsewhere (Fornasiero et al. 2019).

Work rate used during training sessions was 183±19 W in N and H-WR and 144±18 W in H-HR, with a mean decrement of -21.1±9.3% ($p<0.001$). Mean HR during exercise phases was significantly higher in H-WR (154±11 bpm, 83±5% HRmax) compared to N (139±10 bpm; 75±4% HRmax; $p<0.001$) and H-HR (138±9 bpm; 75±4% HRmax; $p<0.001$). When compared to N (98.2±1.2%) SpO_2 was reduced to a similar extent both in H-WR (83.2±3.6%, $p<0.01$) and H-HR (83.6±3.5%, $p<0.01$). Mean $\dot{V}O_2$ was similar in N and H-WR (2.68±0.23 and 2.71±0.23 L/min, respectively, $p=0.80$) and was reduced in H-HR (2.32±0.24 L/min, $p<0.01$). Mean $\dot{V}_E$ was similar in N and H-HR (72.1±7.7 and 70.4±9.3 L/min, $p=0.81$) but was increased in H-WR (88.5±11.4 L/min, $p<0.01$). Blood lactate concentration was similar in N and H-HR (2.54±1.02 and 2.52±0.87 mmol/L, respectively, $p=0.99$) and higher during H-WR (4.96±0.91 mmol/L, $p<0.01$). Compared to N (28.6±9.9) mean RPE was significantly higher in H-WR (36.1±10.5, $p<0.018$) and lower in H-HR (20.1±7.5; $p<0.010$).

Cardiac autonomic modulation responses

Complete cardiac autonomic modulation responses to the three exercise sessions (N, H-HR and H-WR) are presented in Table 3.1.

Table 3.1 Cardiac autonomic modulation responses before and after the three exercise sessions

	N									H-HR									H-WR								
	PRE			POST 15 min				POST 60 min			PRE			POST 15 min				POST 60 min			PRE			POST 15 min				POST 60 min			
RR (ms)	1069	±	94	899	±	145	*	991	±	134	*	1058	±	130	918	±	147	*	944	±	121	*	1085	±	128	808	±	115	*,#,$	924	±	108	*
Ln-SDNN (ms)	4.05	±	0.47	3.84	±	0.44	*	4.26	±	0.42		4.04	±	0.49	4.02	±	0.58		4.15	±	0.47		4.16	±	0.37	3.65	±	0.33	*,$	4.17	±	0.45	
Ln-RMSSD (ms)	3.69	±	0.49	3.33	±	0.62	*	3.76	±	0.56		3.75	±	0.49	3.49	±	0.51		3.61	±	0.49		3.89	±	0.46	3.08	±	0.37	*,$	3.60	±	0.49	*
pnn50 (%)	21.3	±	19.2	13.3	±	19.9		23.7	±	21.8		23	±	20	14.9	±	17.1		18.0	±	18.0		28.0	±	21.4	5.1	±	8.3	*,$	18.4	±	16.4	
HF_{peak} (Hz)	0.25	±	0.07	0.22	±	0.07		0.23	±	0.07		0.24	±	0.07	0.23	±	0.09		0.26	±	0.07		0.24	±	0.07	0.24	±	0.08		0.25	±	0.07	
Ln-HF (ms^2)	5.93	±	0.99	5.07	±	1.42	*	6.05	±	1.25		6.13	±	1.02	5.33	±	1.09	*	5.66	±	1.25		6.49	±	1.06	4.72	±	0.84	*	5.81	±	1.05	*
Ln-LF (ms^2)	6.72	±	1.02	6.51	±	1.06		7.41	±	0.89		6.84	±	1.02	6.92	±	0.96		7.15	±	1.03		7.18	±	0.91	6.65	±	0.88		7.13	±	0.93	
Ln-LF/HF	1.21	±	0.42	1.80	±	0.93	*	1.64	±	0.65	*	1.18	±	0.47	1.84	±	0.79	*	1.74	±	0.64	*	1.17	±	0.69	2.10	±	0.51	*	1.62	±	0.64	*
Ln-TP (ms^2)	7.91	±	0.85	7.52	±	0.90		8.45	±	0.89		7.94	±	0.95	8.01	±	1.29		8.18	±	1.02		8.14	±	0.84	7.24	±	0.67	*	8.32	±	0.80	

Values are Mean ± SD. *: ≠ PRE; #: ≠ N; $≠ H-HR; $p<0.05$; RR: R-R interval; SDNN: standard deviation of normal to normal R-R intervals; RMSSD: Root mean square of successive differences of R–R intervals; Ln: natural logarithm transformation; pnn50: percentage of successive normal interbeat intervals greater than 50 ms; HF: High-frequency spectral power; LF: Low-frequency spectral power; TP: Total spectral power.

RR exhibited a significant time x condition interaction ($p<0.001$) with a greater decrease at POST-15 in H-WR compared to N ($p=0.019$) and H-HR ($p=0.005$). Similarly, vagal-related HRV indices (RMSSD and HF) exhibited significant time x condition interactions($p<0.001$). RMSSD was decreased at POST-15 after N ($p=0.041$) and H-WR ($p<0.001$) but not H-HR ($p=0.234$), and at POST-60 only in response to H-WR protocol ($p=0.024$). HF decreased after all the exercise sessions at POST-15 ($p<0.05$), and only remained reduced after H-WR at POST-60 ($p=0.036$). Overall indices of HRV (SDNN and TP) also displayed significant time x condition interactions ($p<0.05$). Specifically, SDNN decreased at POST-15 in N ($p=0.045$) and H-WR ($p<0.001$) but not in H-HR ($p=0.998$). Similarly, TP decreased at POST-15 only in H-WR ($p=0.006$).

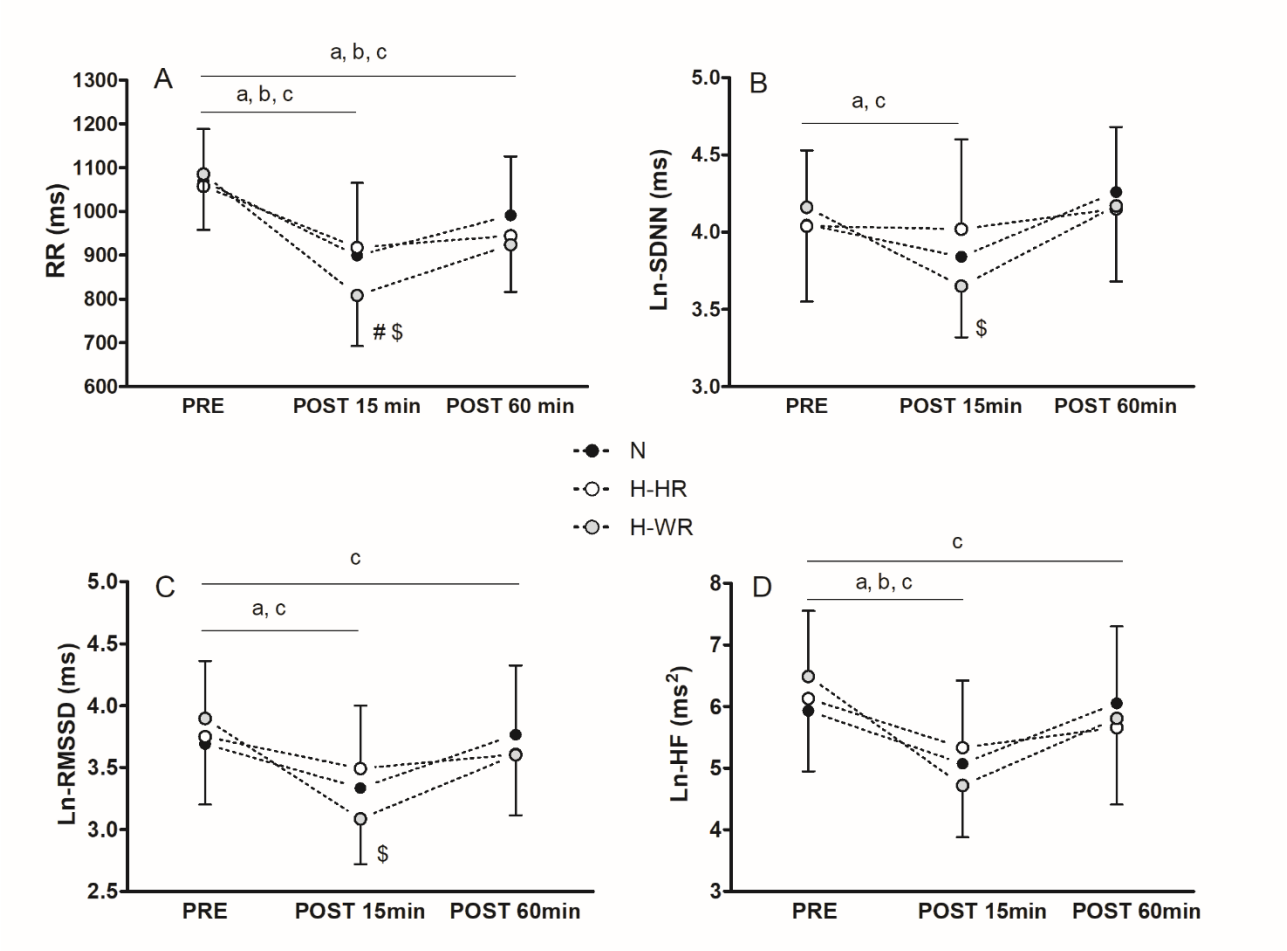

Figure 3.1. Cardiac autonomic activity before and after the three exercise sessions. Black, white and grey circles represent normoxic exercise (N), heart rate matched hypoxic exercise (H-HR) and work rate matched

hypoxic exercise (H-WR), respectively. Error bars represent standard deviation of the mean values; #: H-WR ≠ N; $: H-WR ≠ H-HR, a: N POST ≠ N PRE; b: H-HR POST ≠ H-HR PRE; c: H-WR POST ≠ H-WR PRE; $p<0.05$; Fig 3.1.A: RR interval; Fig 3.1.B: Natural-logarithm transformation of the standard deviation of normal to normal R-R intervals (Ln-SDNN); Fig 3.1.C: Natural-logarithm transformation of the root mean square of successive differences of R–R intervals (Ln-RMSSD); Fig 3.1.D: Natural-logarithm transformation of High Frequency Spectral Power (Ln-HF);

Blood pressure and Baroreflex Sensitivity Responses at rest

Haemodynamic and cBRS responses to the three exercise sessions (N, H-HR and H-WR) are presented in Table 3.2.

Table 3.2 Haemodynamic and cardiac baroreflex sensitivity responses during seated rest

	N									H-HR									H-WR								
	PRE			POST 15 min				POST 60 min			PRE			POST 15 min				POST 60 min			PRE			POST 15 min				POST 60 min			
SAP (mmHg)	122	±	12	113	±	11	*	121	±	13	113	±	11	111	±	14		116	±	10	#	117	±	14	106	±	10	*,#	110	±	12	*,#,$
DAP (mmHg)	71	±	9	69	±	8		75	±	10	65	±	9	67	±	10		72	±	8	*	67	±	10	66	±	8		67	±	9	
MAP (mmHg)	90	±	10	85	±	9	*	91	±	11	83	±	10	83	±	11		87	±	9	*	85	±	12	81	±	9	*	82	±	10	#
CO ($L*min^{-1}$)	5.2	±	0.8	5.3	±	1.0		4.8	±	0.8	*	5.3	±	1.0	5.2	±	1.1		4.9	±	1.2	*	5.2	±	1.1	5.6	±	1.2		4.9	±	1.1	*
SV (mL)	91	±	12	79	±	13	*	79	±	13	*	92	±	12	78	±	15	*	75	±	14	*	92	±	15	75	±	11	*	75	±	12	*
TPR ($mmHg·s·mL^{-1}$)	1.09	±	0.24	1.00	±	0.23		1.19	±	0.27		0.97	±	0.26	1.00	±	0.27		1.15	±	0.33	*	1.07	±	0.32	0.91	±	0.25	*,$	1.07	±	0.33	
Transfer Function (TF)																															
Ln-$cBRS_{TF}$-Gain ($ms*mmHg^{-1}$)	2.35	±	0.35	2.25	±	0.55		2.37	±	0.38		2.38	±	0.34	2.37	±	0.52		2.43	±	0.38		2.39	±	0.36	2.11	±	0.29	*	2.32	±	0.34	
$cBRS_{TF}$-Phase (rads)	-0.43	±	0.20	-0.51	±	0.23		-0.50	±	0.20		-0.49	±	0.24	-0.58	±	0.27		-0.55	±	0.26		-0.37	±	0.14	-0.56	±	0.22		-0.48	±	0.25	
$cBRS_{TF}$-Coherence	0.66	±	0.12	0.67	±	0.08		0.58	±	0.17		0.65	±	0.13	0.62	±	0.17		0.55	±	0.19		0.66	±	0.13	0.67	±	0.11		0.62	±	0.13	
Sequence Method																															
n seq +	13	±	4	19	±	6	*	16	±	4		12	±	3	16	±	4	*	16	±	4		15	±	6	21	±	7	*	17	±	3	
n seq -	14	±	3	18	±	6	*	16	±	4		14	±	4	17	±	4	*	16	±	5		16	±	7	22	±	10	*	17	±	3	
Ln-$cBRS_{seq}$+ ($ms*mmHg^{-1}$)	2.32	±	0.52	2.28	±	0.49		2.54	±	0.54		2.59	±	0.66	2.49	±	0.60		2.46	±	0.49		2.50	±	0.52	2.11	±	0.30	*,$	2.44	±	0.51	
Ln-$cBRS_{seq}$- ($ms*mmHg^{-1}$)	2.33	±	0.60	2.22	±	0.56		2.52	±	0.52		2.32	±	0.56	2.51	±	0.61		2.46	±	0.57		2.40	±	0.46	2.14	±	0.34	*	2.38	±	0.44	
Ln-$cBRS_{seq}$ ($ms*mmHg^{-1}$)	2.34	±	0.54	2.26	±	0.52		2.54	±	0.50		2.50	±	0.57	2.50	±	0.59		2.48	±	0.51		2.45	±	0.47	2.13	±	0.32	*,$	2.41	±	0.47	

Values are means ± SD. Transfer function gain, phase, and coherence values were estimated in the low frequency range (LF) from 0.05 to 0.15 Hz; *: ≠ PRE; #: ≠ N; $≠H-HR; $p<0.05$;
SAP: systolic arterial pressure; DBP: diastolic arterial pressure; MAP: mean arterial pressure; CO: cardiac output; SV: stroke volume; TPR: total peripheral resistance; cBRS: cardiac baroreflex sensitivity; TF: transfer function; Seq: sequence method, +: up- sequences, -: down- sequences

SAP exhibited a significant time x condition interaction (p=0.020). Specifically, compared to PRE (Fig. 2), SAP decreased at POST-15 in N (p=0.005) and H-WR (p=0.001) and not in H-HR (p=0.807), and at POST-60 only in H-WR (p=0.043).

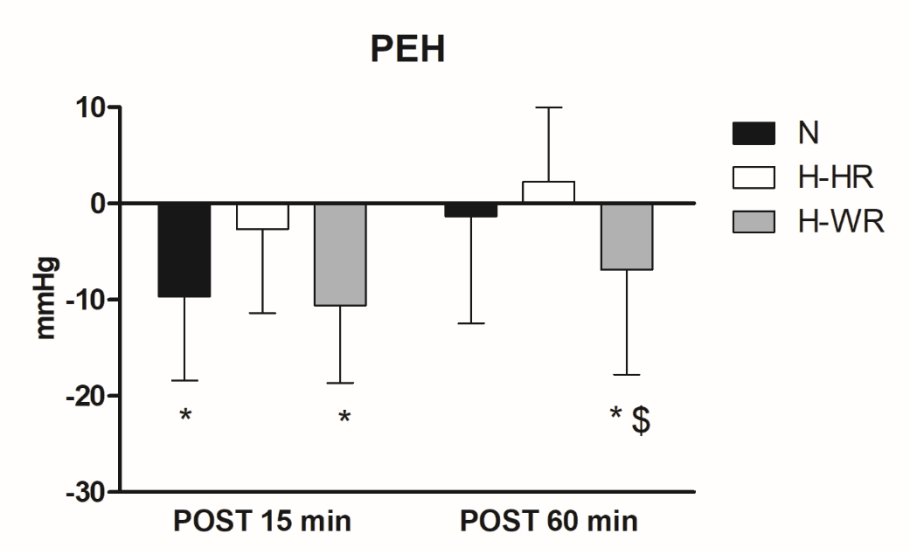

Figure 3.2 Post-exercise hypotension (PEH) responses evoked by the three exercise sessions. Black, white and grey bars represent normoxic exercise (N), heart rate matched hypoxic exercise (H-HR) and work rate matched hypoxic exercise (H-WR), respectively. Error bars represent standard deviation of the mean values. *: ≠ PRE; # ≠ N, $: ≠ H-HR; p<0.05. PEH is defined as the absolute difference between systolic arterial pressure (SAP) at POST and SAP at PRE (PEH = SAP POST – SAP PRE).

Similar to SAP, DAP displayed a significant time x condition interaction (p=0.014). Compared to PRE, DAP was significantly increased at POST-60 in H-HR (p=0.002). Moreover, MAP exhibited a significant time x condition interaction (p=0.026) with a decrease at POST-15 in N (p=0.024) and H-WR (p=0.037), not in H-HR (p=0.955), and increased at POST-60 in H-HR (p=0.017) compared to PRE. At POST-60 MAP was significantly lower in H-WR compared to N (p=0.044).

CO significantly decreased at POST-60 compared to PRE (main effect for time p=0.002) and SV was significantly reduced at POST-15 and POST-60 compared to PRE (main effects for time all p<0.001).

TPR exhibited a significant time x condition interaction (p=0.035) with decreases at POST-15 in H-WR (p=0.009) and increases at POST-60 in H-HR (p=0.003) compared to PRE.

cBRS assessed by means of transfer function analysis ($cBRS_{TF}$) displayed a significant time x condition interaction (p=0.018) with a decreased gain at POST-15 only in H-WR (p=0.004). Similarly, $cBRS_{seq}$ (p=0.020) significantly decreased (time x condition interaction p=0.020) at POST-15 in H-WR (p=0.027) and not in H-HR (p=0.998) nor N (p=0.863). At POST-15 $cBRS_{seq}$ was significantly different in H-WR compared to H-HR (p=0.045). A significant time x condition interaction was also found for $cBRS_{seq}$+ (p=0.027) and $cBRS_{seq}$- (p=0.036), which decreased at POST-15min only in H-WR (p=0.018 and p=0.038, for $cBRS_{seq}$+ and $cBRS_{seq}$-, respectively). The number of positive (n+) and negative (n-) sequences increased at POST-15 after all exercise sessions (time effect, $p<0.05$).

Blood pressure and Baroreflex Sensitivity responses to repeated squat stand test manoeuvres

Blood pressure and cBRS responses to repeated squat stand test manoeuvres in the three exercise sessions (N, H-HR and H-WR) are presented in Table 3.3.

Table 3.3 Haemodynamic and cardiac baroreflex sensitivity responses during repeated squat-stand manoeuvres

	N									H-HR										H-WR								
	PRE			POST 45 min				POST 90 min			PRE			POST 45 min				POST 90 min			PRE			POST 45 min				POST 90 min			
SAP	134	±	14	125	±	11	*	132	±	11	128	±	13	124	±	14		131	±	13		129	±	12	116	±	14	*,#;$	123	±	12	*,#,$
DAP	75	±	9	73	±	9		75	±	10	70	±	9	70	±	8		75	±	9	*	71	±	9	67	±	9	*,#;$	72	±	8	$
MAP	97	±	10	92	±	10	*	96	±	11	91	±	10	89	±	10		96	±	10	*	92	±	10	84	±	11	*,#;$	91	±	9	$
HR	73	±	7	84	±	10	*	82	±	10	* 77	±	8	84	±	10	*	82	±	9	*	75	±	10	89	±	13	*,#;$	85	±	13	*
SAP_{max}-Squat	165	±	17	162	±	14		168	±	12	164	±	14	162	±	13		167	±	15		163	±	13	160	±	12		159	±	18	
SAP_{min}-Stand	111	±	16	99	±	12	*	107	±	10	104	±	16	97	±	17		103	±	15		103	±	15	84	±	17	*,#;$	98	±	18	
Transfer Function																															
Ln-$cBRS_{TF}$-Gain	2.03	±	0.33	2.03	±	0.34		2.00	±	0.30	1.93	±	0.29	1.90	±	0.28		1.93	±	0.22		2.03	±	0.29	1.91	±	0.19		2.07	±	0.25	
$cBRS_{TF}$-Phase	-0.51	±	0.17	-0.49	±	0.22		-0.47	±	0.26	-0.50	±	0.14	-0.68	±	0.29		-0.73	±	0.24		-0.67	±	0.34	-0.76	±	0.28		-0.66	±	0.33	
$cBRS_{TF}$-Coherence	0.66	±	0.09	0.69	±	0.08		0.66	±	0.08	0.68	±	0.09	0.65	±	0.07		0.65	±	0.08		0.71	±	0.07	0.68	±	0.08		0.69	±	0.07	
Sequence Method																															
Ln-$cBRS_{seq}$+	1.96	±	0.25	1.95	±	0.24		1.98	±	0.24	1.91	±	0.21	1.87	±	0.17		1.94	±	0.17		1.94	±	0.29	1.89	±	0.17		1.99	±	0.18	
Ln-$cBRS_{seq}$-	1.75	±	0.30	1.65	±	0.29		1.71	±	0.33	1.65	±	0.28	1.58	±	0.26		1.67	±	0.19		1.69	±	0.35	1.42	±	0.24	*,#	1.67	±	0.33	
Ln-$cBRS_{seq}$	1.86	±	0.27	1.81	±	0.26		1.85	±	0.28	1.79	±	0.23	1.74	±	0.20		1.81	±	0.16		1.82	±	0.31	1.68	±	0.17		1.85	±	0.24	

Values are means ± SD. Transfer function gain, phase, and coherence values were estimated in the squat-stand manoeuvres frequency range (SF) from 0.031 to 0.078 Hz; *: ≠ PRE; #: ≠ N; $≠H-HR; $p<0.05$; SAP: systolic arterial pressure; DAP: diastolic arterial pressure; MAP: mean arterial pressure; HR: heart rate; cBRS: cardiac baroreflex sensitivity; TF: transfer function; Seq: sequence method, +: up- sequences, -: down- sequences

During SS, mean SAP significantly decreased (time x condition interaction p=0.018) at POST-45 in N (p=0.010) and H-WR (p<0.001) but not in H-HR (p=0.307), and to a greater extent in H-WR compared to N (p=0.003). SAP was still decreased at POST-90 only in H-WR (p=0.018) with reduced values compared to N and H-HR (p<0.05). SAP_{min} significantly decreased (time x condition interaction p=0.011) at POST-45 in N (p=0.002) and H-WR (p<0.001) but not in H-HR (p=0.080), with a larger decrease in H-WR compared to N (p<0.001).

Compared to PRE, DAP significantly decreased (time x condition interaction (p=0.013) at POST-45 in H-WR (p=0.035) and increased at POST-90 in H-HR (p=0.017). Similarly, compared to PRE, MAP significantly decreased (time x condition interaction (p=0.038) at POST-45 in N (p=0.031) and H-WR (p=0.001), not in H-HR (p=0.638), and increased at POST-90 only in H-HR (p=0.042). MAP was significantly lower in H-WR compared to N (p=0.003) and H-HR (p<0.001) at POST-45 and compared to H-HR at POST-90 (p=0.018). No significant differences were apparent in cBRS gain, phase and coherence of transfer function analysis during SS manoeuvres across the exercise sessions (p>0.05). Conversely, $cBRS_{seq}$- exhibited a significant time x condition interaction (p=0.032), which decreased at POST-45 only with H-WR (p=0.030).

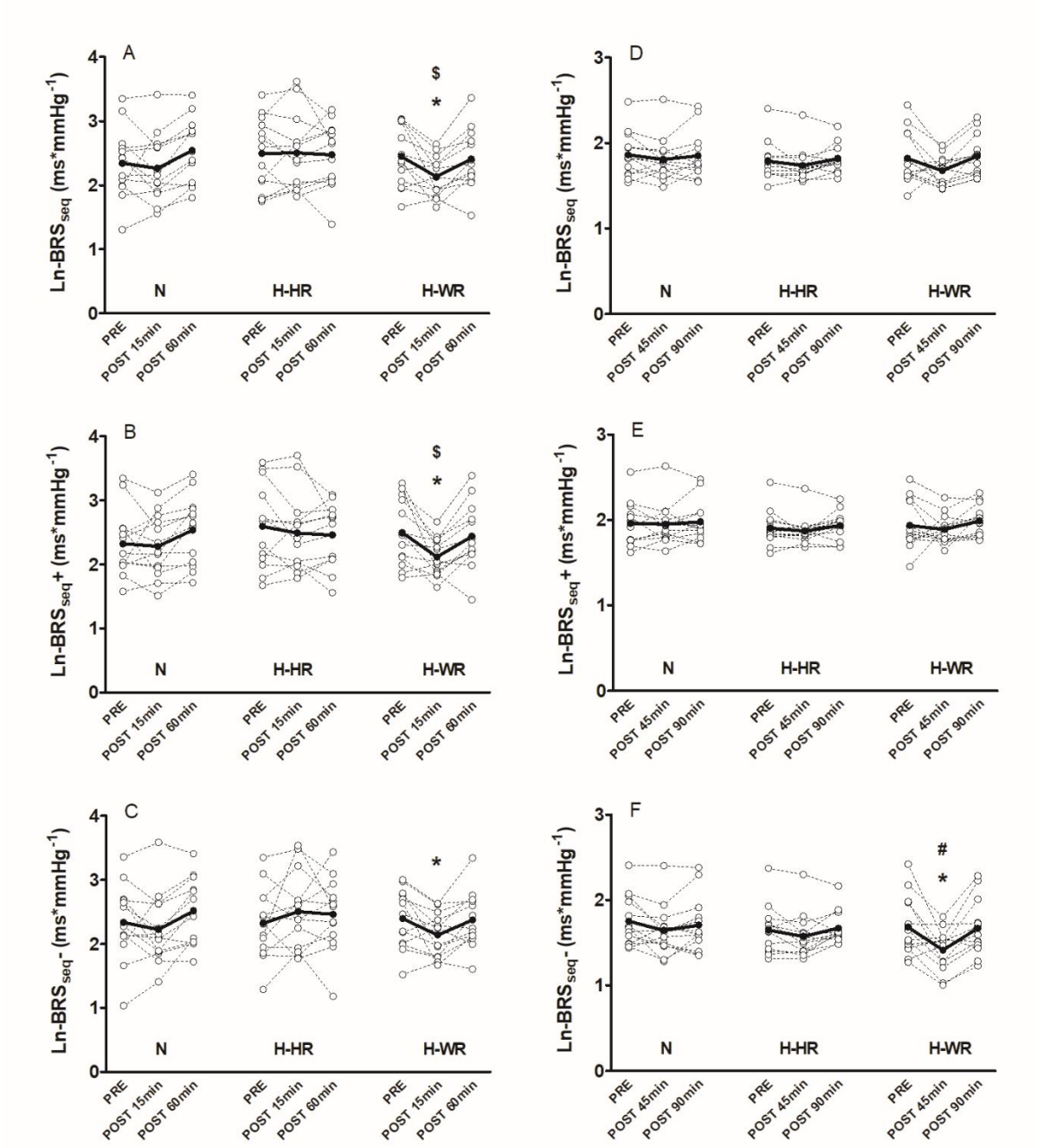

Figure 3.3 Cardiac baroreflex sensitivity (cBRS) responses (sequence method) before and after the three exercise sessions during seated rest and active squat-stand manoeuvres. Individual (white circles) and mean (black circles and lines) responses are shown. *: ≠ PRE; #: ≠ N; $: ≠ H-HR; $p<0.05$. Fig 3.3.A: Mean gain of up- and down- cBRS sequences during seated rest; Fig 3.3.B: Mean gain of up- cBRS sequences during seated rest; Fig 3.3.C: Mean gain of down- cBRS sequences during seated rest; Fig 3.3.D: Mean gain of up- and down- cBRS sequences during squat-stand manoeuvres; Fig 3.3.E: Mean gain of up- cBRS sequences during squat-stand manoeuvres; Fig 3.3.F: Mean gain of down- cBRS sequences during squat-stand manoeuvres;

3.4 Discussion

The purpose of this study was to investigate the effects of performing hypoxic exercise (interval-type exercise, 5 x 5 min exercise with 1 min rest, FiO_2=14.2%) at the same relative (HR-matched) and same absolute (WR-matched) normoxic exercise intensity on post-exercise cardiac autonomic and cardiovascular responses. The key findings were that: 1) post-exercise responses from moderate HR-matched hypoxic exercise (~75% HRmax) were similar to responses during normoxic exercise. cBRS remained unchanged, while cardiac parasympathetic indices decreased and recovered within 60 min after exercise in the two exercise modalities. However, contrary to normoxic exercise, HR-matched hypoxic exercise, associated with a reduced mechanical WR (-21%), did not result in post-exercise hypotension (PEH). 2) WR-matched hypoxic exercise, associated with greater physiological stimulation (~83% HRmax, +15 bpm), delayed cardiac autonomic recovery (parasympathetic indices still decreased 60 min after exercise), decreased cBRS at rest and during repeated squat-stand (SS) manoeuvres (recovered within 45 min after exercise) and evoked longer PEH (still present 60 min after exercise). Additionally, during this dynamic task, wider fluctuations in blood pressure, greater post exercise hypotension and reduction in cBRS were also apparent, specifically during standing phases (i.e. reduction in control of blood pressure fall).

After exercise, a progressive decrease in HR and CO occurs due to cardiac parasympathetic reactivation and sympathetic withdrawal (Michael et al. 2017a). Alongside these alterations, increases in vascular conductance due to a combination of centrally mediated (i.e. decrease in sympathetic outflow) and persistent local vasodilator mechanisms reduce AP below pre-exercise levels, i.e. to post-exercise hypotension (PEH) (Halliwill et al. 2013; Luttrell and Halliwill 2015). PEH has been documented after exercises of different type, intensity and duration (Halliwill et al. 2013; Romero et al. 2017), and was an expected phenomenon in response to the protocol adopted in our study (≈30 min of exercise at moderate intensity). Indeed, PEH [calculated from systolic arterial pressure change (Brito et al. 2018)] was present (-9 mmHg) at rest 15 min after exercise, and during SS manoeuvres

45 min after exercise for N condition. Similarly, in agreement with the existing literature, parasympathetic indices (RMSSD and HF) were completely recovered within 60 min after N (Terziotti et al. 2001; Seiler et al. 2007; Michael et al. 2017b). cBRS is important in maintaining AP, and often post-exercise alterations depend upon the previous exercise intensity (Piepoli et al. 1993; Halliwill et al. 1996; Terziotti et al. 2001; Raczak et al. 2005; Reynolds et al. 2017). For instance, 30 min of exercise at 65% of HRmax leads to cBRS improvement (Raczak et al. 2005). Conversely, high-intensity (>85% HRmax) and maximal aerobic exercises often decrease cBRS with recovery by 60 min after exercise cessation (Somers et al. 1985; Piepoli et al. 1993; Terziotti et al. 2001; Reynolds et al. 2017). Within the moderate intensity range used during N (~75% HRmax), post-exercise cBRS responses have been shown to be more variable (Halliwill et al. 2013). For example, cBRS improvements may occur after moderate exercise at the same intensity as in our study (Halliwill et al. 1996) but with a longer duration (60 min vs 5x5min here). Hence, our results are in line with the documented autonomic and cardiovascular responses following normoxic exercise of moderate intensity.

It has been repeatedly reported that HR (and HRV) and blood pressure monitoring in the post-exercise period provides important non-invasive indices of autonomic function (Luttrell and Halliwill 2015; Romero et al. 2017; Michael et al. 2017a), revealing the impact of the previous exercise stimulus on autonomic disturbance and subsequent recovery. These indices have been further shown to provide meaningful information about the added autonomic disturbance induced by exercising with environmental stressors, such as cold (Sanchez-Gonzalez and Figueroa 2013), heat (Pecanha et al. 2017) and hypoxia (Fornasiero et al. 2018). For the same submaximal work rate in hypoxia greater sympathetic activation and parasympathetic withdrawal is induced by arterial chemoreceptor stimulation (Amann and Kayser 2009; Nobrega et al. 2014; Siebenmann et al. 2018) with further increases in HR, SV, CO, and AP (Calbet et al. 2009; Fisher 2015; Winkler et al. 2017), and greater respiratory involvement (Sheel et al. 2010; Fornasiero et al. 2019). These heightened responses,

coupled with the so-called ‘compensatory vasodilation’ (Joyner and Casey 2014; Dinenno 2016), ensure adequate perfusion of active muscles during hypoxic exercise, but represent additional cardiovascular and autonomic stresses and may translate into delayed post-exercise autonomic and cardiovascular responses (Koelwyn et al. 2013; Horiuchi et al. 2016, 2018; Fornasiero et al. 2018, 2019; Saito et al. 2019).

Accordingly, and in line with our hypotheses, we observed a delayed post-exercise cardiac autonomic recovery after H-WR. Indeed, a larger decrease in resting RR interval compared to N (Fig.1A), without complete parasympathetic recovery (i.e. RMSSD and HF) 60 min after exercise were observed. This agrees with previous investigations suggesting that cardiac autonomic recovery is profoundly influenced by exercise intensity, with higher exercise intensities (above VT) delaying cardiac autonomic recovery (Terziotti et al. 2001; Seiler et al. 2007; Michael et al. 2017b). This delayed recovery of parasympathetic indices was associated to decreased cBRS at rest, and during the dynamic stimulation induced by SS manoeuvres (i.e. wider fluctuations in blood pressure), mirroring impaired autonomic control of blood pressure. At rest, gains of $cBRS_{seq}+$, $cBRS_{seq}-$ and total cBRS sequences were altered, as well as cBRS gain calculated using the transfer function approach ($cBRS_{TF}$). Conversely, during repeated SS manoeuvres, only gain of negative sequences ($cBRS_{seq}-$) was decreased, indicating a specific decrease in the control of decreasing blood pressure (i.e. blood pressure fall during standing phases). These autonomic alterations, together with a probable prolonged vasodilation, as indirectly supported by reduced TPR and SV, likely contributed to the longer PEH observed after H-WR compared to N both at rest and during SS manoeuvres, underlining the long-lasting blood pressure control perturbations induced by H-WR.

The impact of hypoxic exercise on PEH and associated cardiovascular and autonomic responses has been poorly studied. Some studies reported amplified PEH after hypoxic exercise (Horiuchi et al. 2016, 2018; Saito et al. 2019) but it is worth noting that these previous studies differ from the previous investigation either in the recovery modality (hypoxia) (Horiuchi et al. 2016; Saito et al. 2019) or exercise type (i.e. resistance exercise) (Horiuchi et al. 2018). Most current suggestions to use hypoxia

as a training modality (Millet et al. 2016; Millet and Girard 2017; Lizamore and Hamlin 2017; Brocherie and Millet 2020) suggest performing the recovery under normoxic conditions, like in the present study. To the best of our knowledge, no studies have investigated PEH following hypoxic exercise (aerobic) with normoxic recovery, making any comparison with the present investigation difficult. For example, Saito et al. (2019) found greater and longer PEH effects after a maximal exercise (to exhaustion) under hypobaric hypoxia (~2200 m) compared to maximal exercise in normoxia and sub-maximal exercise in normoxia matched for the total volume of hypoxic exercise (~10 min). In that study, reductions in SAP and MAP were still present 60 min after the hypoxic trial only. In addition, Horiuchi et al. (2016) found a more pronounced decrease in MAP (-3 mmHg average during 60 min recovery, -5 mmHg 60 min post-exercise) after 2 h of moderate intensity exercise (4 x 30 min exercise with 15 min recovery) at 50% of altitude-adjusted $\dot{V}O_{2\ max}$ in hypoxia (FiO_2= 14.1%) compared to normoxia. Both the higher intensity (Saito et al. 2019), the longer duration of exercise (Horiuchi et al. 2016), and the recovery modality (hypoxia) might explain the different results from the present study.

Interestingly, although WR-matched exercise exhibited differences between hypoxic and normoxic conditions, H-HR was associated with neither delayed post-exercise cardiac autonomic recovery nor affected cBRS responses. Mean RR interval and vagal-related HRV indices (HF) decreased to a similar extent in H-HR and N 15 min after exercise. Moreover, vagal-related HRV indices were completely recovered 60 min after exercise both after H-HR and N, suggesting similar parasympathetic recovery. This is in accordance with our hypotheses and previous results showing that when cardiorespiratory stimulation is matched (i.e. similar cardiac, ventilatory and blood lactate responses), cardiac autonomic responses are not different in hypoxia compared to normoxia (Fornasiero et al. 2019). Also, H-HR hypoxic exercise did not alter post-exercise cBRS responses compared to N, resulting in unaffected and maintained cBRS. Overall, and for the first time, these findings attest similar post-exercise cardiac autonomic recovery and cBRS responses after hypoxic

and normoxic exercise conducted at a similar moderate HR (~75% of HRmax). However, no PEH was observed after H-HR, and the lack of PEH was accompanied by an increase in DAP and MAP compared to pre-exercise levels 60 min after exercise. These responses might be attributable to the prolonged sitting posture of our participants due to our study design (Halliwill et al. 2013; Brito et al. 2018), which would further explain the decreased SV and CO (Halliwill et al. 2013). According to our results, both the increased stimulation (i.e. physiological) of H-WR and the reduced stimulation (i.e., mechanical, in terms of work rate) of H-HR played a role on the PEH responses evoked. In this study, the greater physiological stimulation of H-WR translated into longer PEH, which seems to be in line with previous observations (Horiuchi et al. 2016; Saito et al. 2019), but with the confounding factor of a higher exercise intensity (Halliwill et al. 2013). On the other hand, the reduced work rate (and total work) of H-HR translated into reduced PEH, that may be relevant for both short- and long-term blood pressure reduction. Indeed, PEH is by itself a beneficial short-term reduction of blood pressure of clinical relevance (Brito et al. 2018), which has been shown to predict blood pressure reduction to chronic exercise training in prehypertensive (Liu et al. 2012) and healthy (Hecksteden et al. 2013; Brito et al. 2018) individuals. According to our findings, a longer exercise duration, matching the total work done (Jones et al. 2007), may be required to evoke PEH after hypoxic exercise performed at a similar HR of normoxic exercise. This may be particularly true for exercise sessions of short duration (as the present investigation, ~30 min) and of moderate intensity. However, our results derive from a group of healthy normotensive individuals and therefore, further research is needed to address this issue in prehypertensive and hypertensive individuals, where PEH magnitude might differ (Brito et al. 2018).

Limitations

Different exercises, in terms of type, intensity and duration, different degrees of hypoxia, as well as different body positions assumed during recovery could result in different autonomic and cardiovascular responses (Halliwill et al. 2013; Michael et al. 2017a). Accordingly, the results

obtained in this study may be limited to the specific exercise and the post-exercise recovery modality (seated recovery in normoxia) performed by the participants. In addition, as we did not directly evaluate muscle sympathetic nerve activity (MSNA) or blood catecholamines responses we cannot properly evaluate sympathetic influence on the observed responses. The specific contribution of the peripheral circulation and vasodilation should also be studied. Moreover, the participants in this study were healthy active men and therefore these findings may not be directly generalizable to sedentary, clinical, or elderly populations.

3.5 Conclusions

Moderate HR-matched hypoxic exercise (~75% HRmax, FiO_2=14.2%) does not affect cBRS and does not blunt cardiac autonomic recovery during the early post-exercise recovery phase. However, post-exercise hypotension is limited or absent and may relate to the reduced WR (-21%). Conversely, WR-matched hypoxic exercise, resulting in greater physiological stimulation (~83% HRmax), delays cardiac autonomic recovery, temporarily decreases cBRS and evokes longer post-exercise hypotension. Post-exercise autonomic and cardiovascular responses to different HR-matched and WR-matched hypoxic exercises warrant further investigation.

4. Study 4

Shortening work-rest durations reduces physiological and perceptual load during uphill walking in simulated cold high-altitude conditions

Based on the article

Fornasiero, A., Savoldelli, A., Stella, F., Callovini, A., Bortolan, L., Zignoli, A., Low, D., Mourot, L., Schena, F. & Pellegrini, B. (2020). Shortening Work-Rest Durations Reduces Physiological and Perceptual Load During Uphill Walking in Simulated Cold High-Altitude Conditions. *High Altitude Medicine & Biology*. 21 (3) 249-257. https://doi.org/10.1089/ham.2019.0136

Abstract

We investigated the effects of two different work-rest durations on the physiological and perceptual responses to a simulated mountain hike in a cold hypoxic environment. Twelve healthy non-acclimatised active men (age 31.3±5.3 years, BMI 22.4±1.5 kg/m^2) completed a 80-min work-matched intermittent exercise on a motorized treadmill (25% incline, fixed self-selected speed), in a simulated mountain environment (-25°C, FiO_2=11%, ≈5000m a.s.l), wearing extreme cold weather gear, once with short (20 x 3 min walking with1 min rest; SHORT) and once with long (10 x 6 min walking with 2 min rest; LONG) work-rest durations. Heart rate (HR), oxygen saturation (SpO_2), Rate of Perceived Exertion (RPE) and Thermal Sensation (TS) were assessed throughout the exercise protocols. Cardiac autonomic modulation was assessed before (PRE) and after exercise (POST) in supine position, as well as during standing resting periods by means of HR recovery (HRR) assessment. SpO_2 and TS were similar ($p>0.05$) in SHORT and LONG protocols. HR and RPE were increased and HRR reduced during LONG compared to SHORT ($p<0.05$). Parasympathetic activity indices were reduced at POST after both protocols ($p<0.05$), but to a lesser extent after SHORT ($p<0.05$). Reduced work-rest durations are associated with improved perceptual responses and less perturbation of cardiac autonomic balance, compared to longer work-rest durations. Shorter exercise periods from more frequent breaks during hikes at high altitude may represent a valid strategy to limit the impact of exercise under extreme environmental conditions.

4.1 Introduction

At high altitude different environmental stressors, such as hypoxia and cold, coexist and affect physiological and perceptual responses to exercise (Castellani and Tipton 2015; Burtscher et al. 2018). Hypoxia limits exercise tolerance (Wehrlin and Hallén 2006) and increases exercise-induced perturbation of homeostasis (Mazzeo 2008), amplifying most of the physiological responses, as well as perceptual strain, for a given absolute sub-maximal exercise intensity (Mazzeo 2008; Fornasiero et al. 2019). Furthermore, hypoxic exercise can cause greater Autonomic Nervous System (ANS) disturbance (Hainsworth et al. 2007; Amann and Kayser 2009; Fisher 2015; Siebenmann et al. 2018) and delayed post-exercise cardiac autonomic recovery (i.e. progressive restoration of normal resting balance between parasympathetic and sympathetic activities) (Al Haddad et al. 2012; Koelwyn et al. 2013; White and Raven 2014; Michael et al. 2017a; Fornasiero et al. 2018). Similarly, cold stress can exacerbate exercise-induced perturbation of homeostasis (Castellani and Tipton 2015; Castellani and Young 2016) and can negatively impact cardiac autonomic recovery (Sanchez-Gonzalez and Figueroa 2013). Increased physiological stress associated with exercise and inadequate autonomic recovery at high altitude may facilitate the development of maladaptive responses to the environment itself (Sutherland et al. 2017; Boos et al. 2018).

Cardiorespiratory fitness is of paramount importance when dealing with the metabolic requirements of mountain ascents (Bärtsch and Swenson 2013; Burtscher et al. 2015). Greater cardiorespiratory fitness has been further associated with a decreased Rate of Perceived Exertion (RPE) during trekking at high altitude (≈5000 m a.s.l) (Rossetti et al. 2017), which has also been related to a lesser impairment of cardiac autonomic balance (Boos et al. 2018) and less Acute Mountain Sickness (AMS) development (Mellor et al. 2014). For the above-mentioned reasons, a moderate exertion during exercise at high altitude has been recommended (Mellor et al. 2014; Boos et al. 2018).

To the best of our knowledge, successful strategies for managing exercise and recovery periods during a mountain hike (i.e. optimal work-to-rest ratio or work-rest duration) have not yet been fully investigated. Intuitively, reducing work-to-rest ratio (i.e. reducing exercise time and/or increasing

recovery time) may improve perceptual responses and reduce cardiac autonomic disturbance by means of decreasing the overall mean intensity (Michael et al. 2017a), but this strategy would also increase the time required to complete an ascent. However, the effect of manipulating the work-rest duration during a time-matched exercise (i.e. same time to complete the ascent) seems to be less predictable.

The purpose of this study was to examine the impact of two different work-rest durations on the physiological and perceptual responses to a simulated mountain hike in a cold and hypoxic environment. We hypothesized that reduced work-rest durations during the ascent (i.e. shorter exercise periods and more frequent breaks) would be associated with ameliorated perceptual responses and less impairment of cardiac autonomic balance when compared to longer work-rest durations.

4.2 Materials and methods

Participants

Twelve healthy active men (age 31.3±5.3 years, height 176±6 cm, weight 69.3±6.4 kg, BMI 22.4±1.5 kg/m^2) volunteered for this study. None of them had been at altitude above 2000m for prolonged periods of time (>12 hours) during the previous 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 measurements. 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 Local Ethics Committee. The protocol was conducted according to the principles of the Declaration of Helsinki.

Protocol

Each participant visited the laboratory on two different occasions at the same time of the day and completed the experimental protocol within a 2-week period. During each visit, participants randomly

performed an intermittent exercise protocol, simulating an 80-min mountain ascent, on a motorized treadmill (RunRace, Technogym, Gambettola, Italy), either with long (10 x 6 min walking with 2 min rest; LONG) or short work-rest durations (20 x 3 min walking with 1 min rest, SHORT) .

The exercise protocols were conducted in an environmental chamber under controlled laboratory conditions (-25°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). FiO_2 was set at 11% to simulate an altitude of ≈5000m a.s.l. To ensure that first short-term physiological responses to the hypoxic environment occurred, participants remained seated quietly in the environmental chamber for 30 min before starting the exercise protocols (Duffin 2007). In the environmental chamber participants wore extreme cold weather clothing (including hat, scarf and mittens) and high-altitude mountaineering boots which remained identical for the two protocols, both during exercise and the first 30-min resting period, during which additional clothes were provided.

Treadmill inclination was kept constant at 25%, whereas speed was gradually adjusted within the first 3 min of exercise during the first visit, so as to elicit an individual rating of perceived exertion (RPE) defined by the participants as “moderate” (25 on the Borg Category Ratio Scale (CR100)) (Borg and Borg 2002). Previous research suggested that RPE is a valid and appropriate method to record sense of effort and perceptual responses to exercise, as well as to prescribe the target exercise intensity (Eston 2012). The selected speed was then maintained constant throughout the session and during the second visit, where the same speed adjustment procedure was performed up to the selected speed of the first visit, in order to match the overall distance covered. Recovery phases were performed in standing position on the treadmill using handrail support.

To investigate the impact of the two exercise protocols on resting cardiac autonomic modulation, HRV assessment was conducted before (PRE) and 5 min after exercise (POST), with the subject lying in supine position for 10 min in a quiet room under normothermic conditions (23 °C, 50% relative humidity).

Throughout rest, exercise and recovery phases, beat-to beat HR was continuously recorded using a Polar RS800CX HR monitor (Polar, Kempele, Finland). Pulse oxygen saturation (SpO_2) was continuously recorded during exercise by ear pulse oximetry (Nonin Medical, Minneapolis, MN, USA) at a sampling frequency of 1.0 Hz. The individual rating of perceived exertion (RPE) was assessed using the CR100 Scale every 6 min of accumulated work (Borg and Borg 2002). Thermal sensation was assessed using a 9-point scale (from −4 (very cold) to +4 (very hot)) (Arens et al. 2006).

Data Analysis

The R-R intervals were uploaded using Polar Precision Performance Software (Polar, Kempele, Finland) and then exported as *.txt* files. Signal artefacts 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%). Heart rate recovery (HRR) indices were calculated with a customized script in Matlab (Matlab, Mathworks Inc., USA). HRR60 was calculated from the absolute difference between $HR_{exercise}$ calculated as the mean of the last 30 s of exercise and the HR value at 60 s of recovery (Buchheit et al. 2007a). HRR was also calculated as the relative decline in HR expressed as a percentage of $HR_{exercise}$ (%HRR=HRR/$HR_{exercise}$ ×100), i.e. normalised HRR60 (nHRR60). This was done for every 8 min of accumulated time (i.e. one cycle for LONG and two cycles for SHORT protocol) in order to obtain 10 comparable points in terms of accumulated exercise duration.

HRV analysis was performed using Kubios HRV software (Version 2.1, Biosignal Analysis and Medical Imaging Group, Kuopio, Finland). HRV indices were calculated considering the last 5 min of the 10-min resting period. The time-domain HRV indices considered were the square root of the sum of successive differences between adjacent normal R-R intervals squared (RMSSD), the standard deviation of normal-to-normal RR intervals (SDNN) and the percentage of successive R-R intervals differing more than 50ms from the previous R-R interval (Task Force of the European Society of Cardiology 1996). For frequency-domain HRV indices, low frequency spectral power (LF, 0.04-0.15

Hz), high frequency spectral power (HF, 0.15-0.4 Hz), and total spectral power (TP, 0-0.4 Hz) were calculated by Fast Fourier Transform (FFT) (Task Force of the European Society of Cardiology 1996). Due to the influence of underlying HR on HRV, normalized HRV indices (highlighted by a "n" in Table 1), according to the methods proposed for time domain and frequency-domain analysis, were also reported (Sacha 2014; Billman et al. 2015). Mean values of HR and SpO_2 were calculated for every 8 min of accumulated time, which included for both protocols 6 min of exercise and 2 min of recovery.

Statistical Analysis

Data are presented as means ± standard deviations (SD). Data were tested for normal distribution with Shapiro–Wilk test. If data were not normally distributed, natural logarithm transformation (Ln) was applied to obtain a normal distribution and allow parametric statistical comparisons. HRV indices were compared using a two-way ANOVA for repeated measures, with "condition" (SHORT and LONG) and "time" (PRE and POST) as factors. HR, SpO_2, Thermal Sensation (TS) and RPE values were compared using a two-way ANOVA for repeated measures, with "condition" (SHORT and LONG) and "time" (10 different time points every 6 min of accumulated exercise) as factors. When statistical significance was identified, a Sidak post hoc test was used to further delineate differences between condition or time (Cunha et al. 2015). Statistical analysis was completed using a statistical software (SPSS Inc, Chicago, Illinois, USA). The level of statistical significance was set at $p<0.05$.

4.3 Results

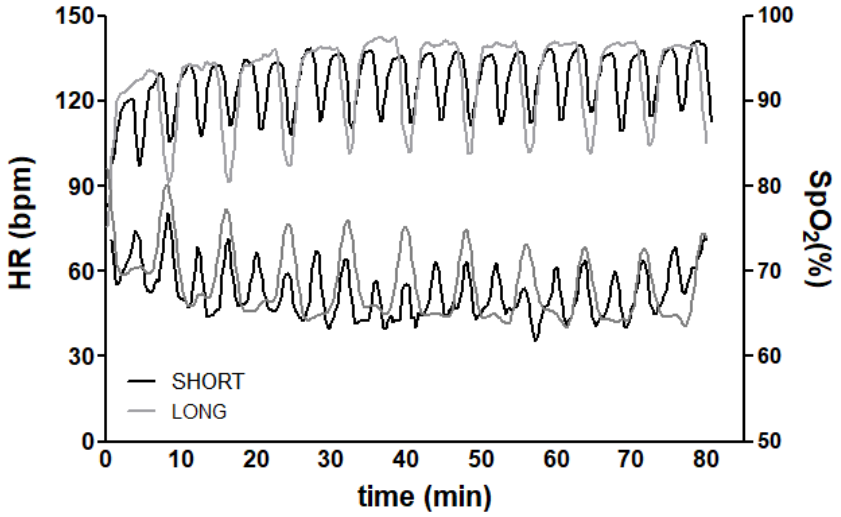

Figure 4.1. Heart rate (HR) (bpm) and Oxygen Saturation (SpO_2) (%) responses during SHORT (3-1 min x 20) and LONG (6-2 min x 10) protocols in a representative participant.

Physiological responses during SHORT and LONG protocol

The average speed in the two protocols was 1.62 ± 0.38 km/h, equal to a mean ascent rate of 404 ± 95 m/h. A significant effect of "condition" ($p=0.028$) and "time" ($p=0.001$) without "interaction" ($p=0.188$) was reported on HR. On average during the whole protocol mean HR was significantly higher in LONG compared to SHORT protocol (136.0 ±11.9 vs 131.6 ±13.9 bpm, respectively). HR gradually increased ($p<0.05$) during the first 18 min of accumulated work and remained stable throughout the remaining time of the two protocols (Figures 4.1 and 4.2). We reported a significant effect of "condition" ($p=0.003$), "time" ($p=0.001$) and "interaction" ($p=0.026$) on RPE. RPE was significantly higher during LONG compared to SHORT protocol after 36 min of accumulated exercise ($p<0.05$) (points 7, 8, 9 and 10). Differently, we did not find any significant effect of condition ($p=0.491$), time, ($p=0.461$) or interaction ($p=0.336$) on Thermal Sensation responses. A significant effect of "time" ($p=0.001$) without "condition" ($p=0.394$) or "interaction" ($p=0.069$) effects was reported on SpO_2. SpO_2 was significantly decreased after 30 min of accumulated exercise

compared to the first 6 min (p=0.049) regardless of the protocol. Overall, mean SpO_2 was similar (p>0.05) in SHORT and LONG protocol (62.4 ± 5.6 vs 63.2 ± 5.4 %, respectively).

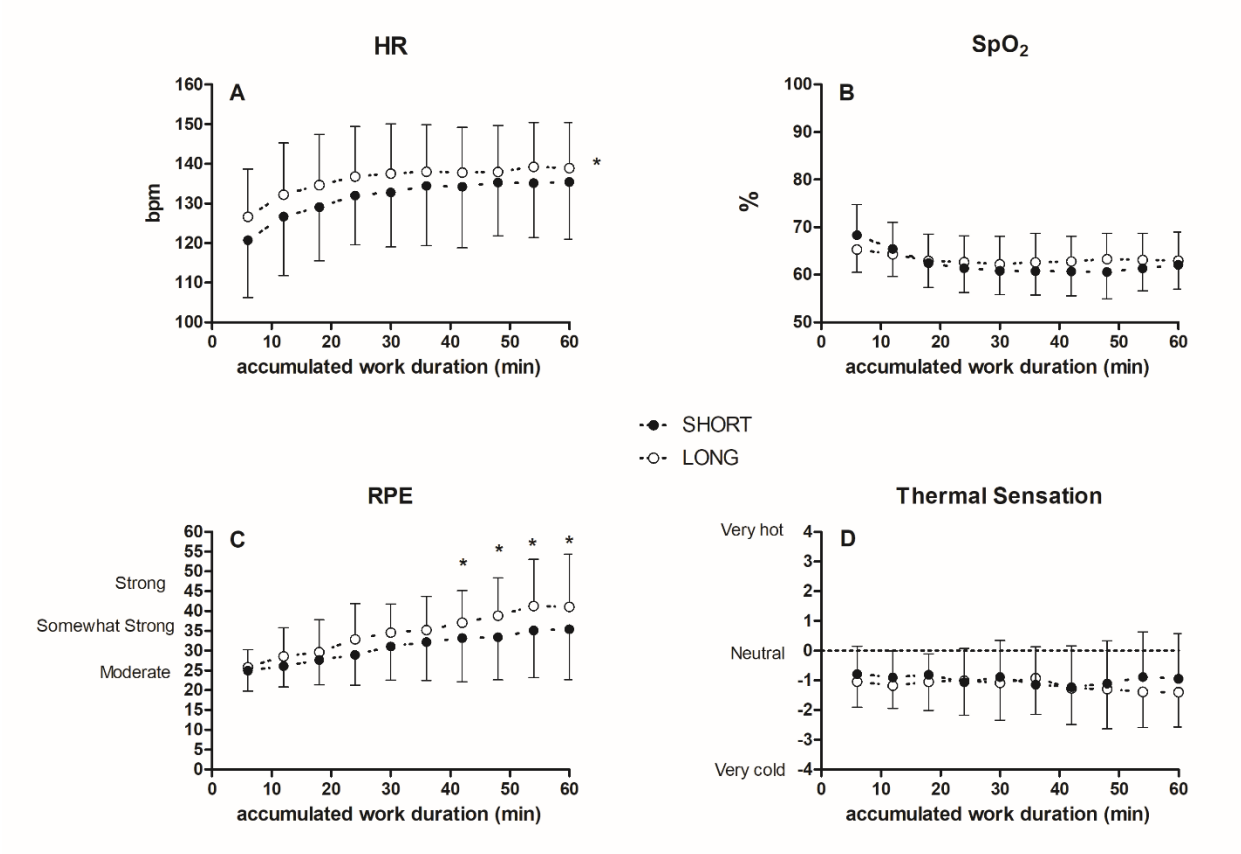

Figure 4.2. Heart Rate (HR), Oxygen Saturation (SpO_2), rate of perceived exertion (RPE) and Thermal Sensation responses during the two exercise protocols. Black and white dots represent SHORT (3-1 min x 20) and LONG (6-2 min x 10) protocols respectively. Error bars represent standard deviation of the mean value; *: LONG ≠ SHORT; p<0.05

Heart Rate Recovery responses during SHORT and LONG protocol

Complete HRR responses during the two protocols are reported in Figure 4.3. A significant effect of "condition" (p=0.002) without "time" (p=0.140) or "interaction" (p=0.840) effects was found on HRR60. Similarly, only a significant effect of "condition" (p=0.012) was reported on nHRR60.

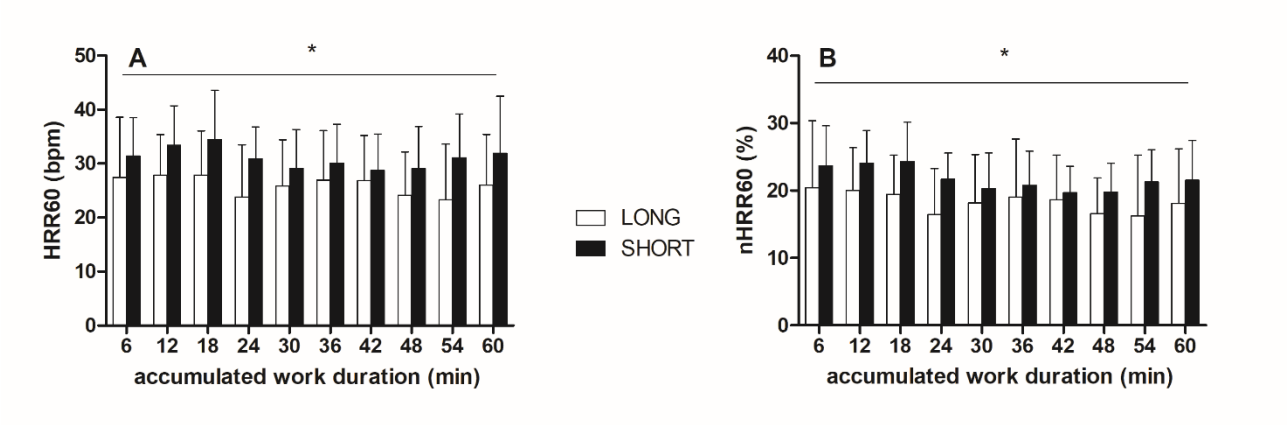

Figure 4.3. Heart Rate Recovery Responses (HRR) during the two exercise protocols. Black and white bars represent SHORT ((3-min ex;1-min rec) x 20) and LONG ((6-min ex;2-min rec) x 10) exercise protocols respectively. Error bars represent standard deviation of the mean value; *: LONG ≠ SHORT general condition effect; $p<0.05$; Fig 4.3A: Heart Rate Recovery at 60 s (HRR60) of recovery; Fig. 4.3B: Normalized Heart Rate Recovery at 60 s of recovery (nHRR60) calculated as (HRR60/HRexercise x 100).

Impact of short and long work-rest durations on resting cardiac autonomic modulation

Complete results from two-way RM ANOVA, showing the impact of SHORT and LONG protocols on cardiac autonomic modulation, are reported in Table 4.1. Mean RR interval was decreased to a greater extent ($p=0.027$) at POST after LONG compared to SHORT protocol. Both time- (RMSSD, pnn50) and frequency-domain (HF) HRV indices reflecting parasympathetic activity showed greater reduction at POST in response to LONG protocol ($p<0.05$). Similarly, other indices of overall variability (SDNN and TP) showed greater reduction at POST after LONG protocol ($p<0.05$).

Table 4.1. Cardiac autonomic modulation indices PRE and POST SHORT and LONG protocols

		SHORT							LONG							RM ANOVA		
		PRE			POST				PRE			POST				time	condition	interaction
RR	(ms)	1040	±	96	913	±	129	#	1069	±	100	847	±	118	#,*	**0.002**	0.459	**0.007**
SDNN	(ms)	58.9	±	18.6	61.4	±	34.8		62.9	±	25.9	44.9	±	27.7	#,*	0.203	0.099	**0.018**
Ln-RMSSD	(ms)	3.79	±	0.48	3.51	±	0.72	#	3.83	±	0.52	3.05	±	0.67	#,*	**0.020**	0.072	**0.004**
Ln-nRMSSD	(-)	3.75	±	0.49	3.61	±	0.63		3.76	±	0.53	3.22	±	0.56	#,*	**0.043**	0.053	**0.007**
pnn50	%	24.3	±	20.1	18.8	±	21.6		28.0	±	21.4	8.6	±	17.7	#	0.063	0.378	**0.010**
Ln-HF	(ms^2)	6.32	±	1.06	5.62	±	1.57	#	6.24	±	1.04	4.72	±	1.42	#,*	**0.014**	**0.045**	**0.026**
Ln-nHF	(-)	0.54	±	0.45	0.46	±	0.53		0.48	±	0.37	0.24	±	0.36	#,*	0.086	0.099	**0.045**
Ln-TP	(ms^2)	8.04	±	0.73	7.96	±	1.21		8.01	±	0.81	7.18	±	1.16	#,*	0.220	**0.021**	**0.032**
Ln-nTP	(-)	1.41	±	0.52	1.60	±	0.79		1.36	±	0.55	1.15	±	0.66	#,*	0.945	0.087	**0.021**

Values are Mean ± SD. #: ≠ PRE ;*: ≠ SHORT; $p<0.05$; RR: R-R interval; SDNN: standard deviation of normal to normal R-R intervals; RMSSD: Root mean square of successive differences of R–R intervals; Ln: natural logarithm transformation; n: HRV parameter normalized for the mean R-R interval, pnn50: percentage of successive normal interbeat intervals greater than 50 ms; HF: High-frequency spectral power; TP: Total spectral power. Normalized HRV data were multiplied by 10^3 for clarity.

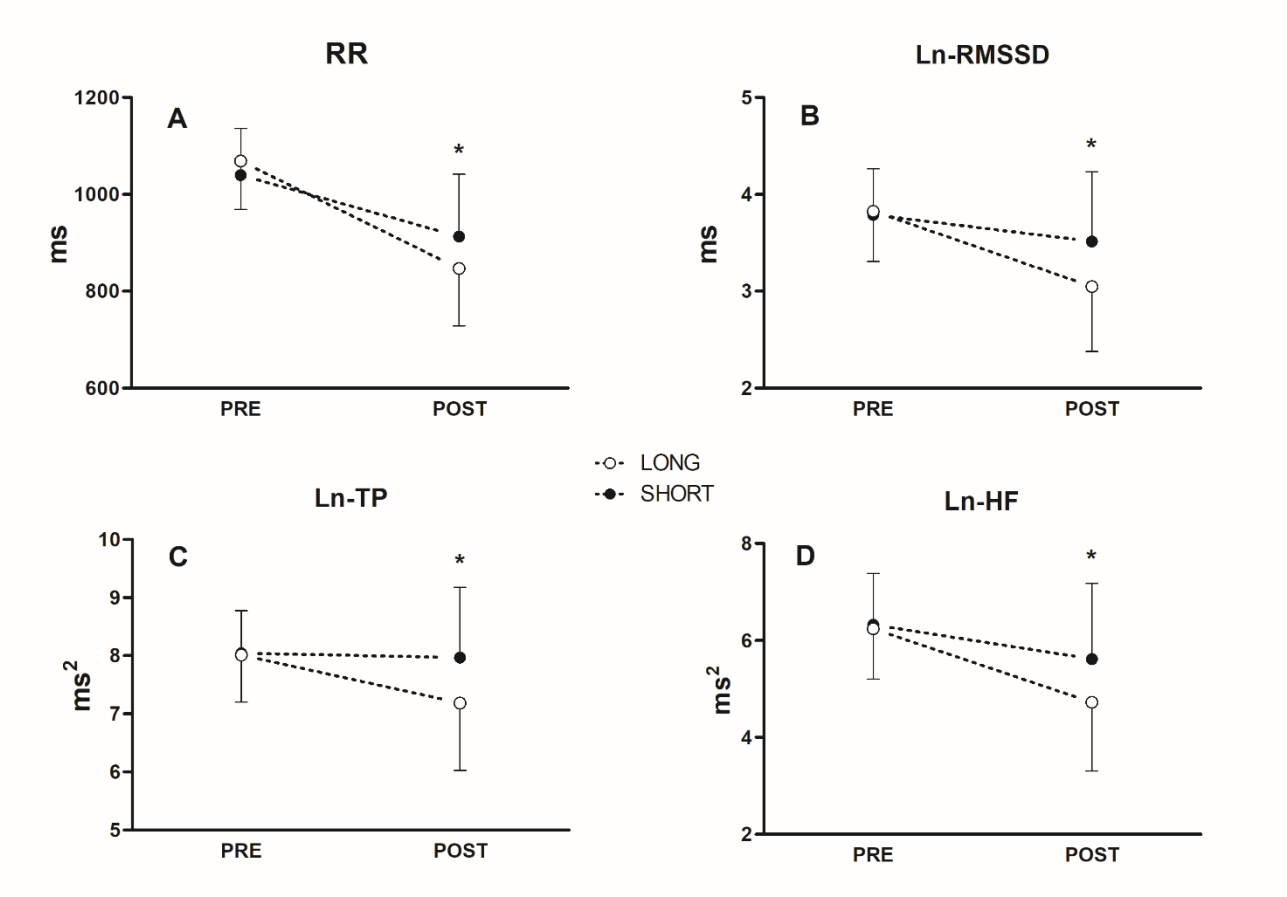

Figure 4.4. Cardiac autonomic modulation responses before (PRE) and after (POST) the two exercise protocols. Black and white dots represent SHORT ((3-min ex;1-min rec) x 20) and LONG ((6-min ex;2-min rec) x 10) exercise protocols respectively. Error bars represent standard deviation of the mean value; *: LONG ≠ SHORT; $p<0.05$; Fig 4.4.A: RR interval (ms); Fig 4.4.B: Natural-logarithm transformation of the root mean square of successive differences of R–R intervals (Ln-RMSSD); Fig 4.4.C: Natural-logarithm transformation of Total Spectral Power; Fig 4.4.D: Natural-logarithm transformation of High Frequency Spectral Power;

4.4 Discussion

In this study we investigated the impact of two different work-rest durations on the physiological and perceptual responses to a simulated mountain ascent performed under extreme environmental conditions (-25°C, FiO_2=11%, ≈5000m a.s.l). The key finding of this study was that shorter work-rest durations were associated with a decreased mean overall physiological strain (i.e. lower mean HR), improved perceptual responses (i.e. reduced RPE) and less perturbation of autonomic nervous

system balance (i.e. lower decrease in vagal-related HRV indices), when compared to longer work-rest durations.

Physiological responses during SHORT and LONG protocol

Increasing human tolerance to high altitude environments is a key goal for mountaineers, athletes and anyone who aims to increase the chance of successfully completing ascents at high-altitude. Manipulating work-to-rest ratio and work-rest duration significantly alters the physiological and perceptual responses to intermittent/interval exercise (Price and Moss 2007; Buchheit and Laursen 2013). At sea level, during work-matched intermittent exercise, longer work-rest durations have been shown to elicit greater physiological and perceptual strain than shorter work-rest durations (Seiler and Sjursen 2004; Price and Halabi 2005; Price and Moss 2007). Similarly, exercise protocols with shorter exercise periods are usually described as more tolerable (Martinez et al. 2015; Farias-Junior et al. 2019). Interestingly, climbing strategies characterized by shorter exercise periods and frequent breaks are spontaneously adopted when facing demanding hikes at extreme altitudes, in order to decrease the mean overall workload in conditions where exercise tolerance is dramatically reduced(West and Wagner 1980).

To the best of our knowledge the present study was the first to investigate the impact of manipulating work-rest duration on the physiological (HR and SpO_2) and perceptual exercise responses (RPE) to a simulated mountain ascent at high altitude. Using an individual "perceptually-regulated exercise" approach via moderate RPE (Rossetti et al. 2017), participants completed the same external workload (same vertical gain (≈400 m) and total work completed) using two different climbing strategies: SHORT and LONG protocols.

In our study, RPE was significant increased during LONG compared to SHORT during the second half of the ascent (Fig. 4.2.C). This is in line with existing literature on interval exercise, reporting improved perceptual responses with shorter work-rest durations (Price and Halabi 2005; Price and Moss 2007). In addition, despite similar mean SpO_2 (Fig. 4.2.B), mean HR was higher in LONG

(≈136 bpm) than in SHORT (≈131 bpm) (general condition effect) (Fig. 4.2.A). A decreased SpO_2 level in hypoxia is a key factor influencing cardiac autonomic modulation due to a direct effect on the carotid chemoreflex (Favret and Richalet 2007). In this case, other influences may have accounted for the difference in HR between the two protocols. Previous studies have suggested that longer exercise durations directly impact cardiac autonomic responses, resulting in reduced parasympathetic activity (Michael et al. 2017a). A similar influence may be hypothesized for the LONG protocol, where the double duration of exercise bouts may have resulted in higher parasympathetic withdrawal. In line with this observation HRR was reduced during LONG protocol (Fig. 4.3-A and Fig.4.3.B), underlining a delayed parasympathetic reactivation during transitions from exercise and recovery periods (Pecanha et al. 2017; Michael et al. 2017a).

These divergent responses to SHORT and LONG were not evident in the Thermal Sensation responses to the two protocols (Fig. 4.1.D). Thermal sensation is governed by core and especially skin temperatures (Filingeri 2016). Given that the ambient temperature of the environmental chamber and clothing were constant between trials and the amount of work (and therefore heat produced at a fixed efficiency) was matched, core and skin temperatures were likely similar in the two protocols, as well as the thermal stress experienced by the participants.

These findings suggest that shorter exercise bouts and more frequent recovery periods during a simulated ascent at high altitude may result in a decreased mean overall physiological stress (as inferred from mean HR) and in ameliorated perceptual responses, representing a convenient climbing strategy while hiking at high altitude.

Impact of short and long work-rest durations on cardiac autonomic recovery

HRV monitoring is a valid tool to investigate the disturbance induced in ANS balance by an acute bout of exercise (Kiviniemi et al. 2007; Michael et al. 2017a). Particularly, vagal-related HRV indices, such as RMSSD and HF, are the most employed indices to investigate post-exercise autonomic recovery (Pecanha et al. 2017; Michael et al. 2017a). During exercise, HR increases and

HRV decreases, with different contributions from parasympathetic withdrawal and sympathetic activation throughout the exercise intensity spectrum (White and Raven 2014). After exercise, HR progressively decreases and HRV indices tend to return to baseline levels (i.e. post-exercise parasympathetic reactivation and sympathetic withdrawal (Pecanha et al. 2017; Romero et al. 2017; Michael et al. 2017a)). A clear relationship has been shown between exercise-induced perturbation of homeostasis and post-exercise cardiac autonomic recovery (Goldberger et al. 2006; Michael et al. 2017a). Higher exercise intensities (Terziotti et al. 2001; Buchheit et al. 2007a; Seiler et al. 2007) and durations (Castrillón et al. 2017; Michael et al. 2017b), as well as the presence of challenging environmental conditions (Al Haddad et al. 2012; Koelwyn et al. 2013; Sanchez-Gonzalez and Figueroa 2013; Fornasiero et al. 2018), increase exercise-induced homeostatic perturbation and lead to a delayed recovery of HR and HRV indices (Michael et al. 2017a).

In line with existent literature, in this study vagal-related HRV indices (HF and RMSSD) were decreased after both exercise protocols (Fig.4.4.B and Fig.4.4.D), indicating incomplete cardiac autonomic recovery 10 minutes after the exercise (Michael et al. 2017a). However, vagal-related HRV indices were decreased to a greater extent in response to the LONG protocol. Similarly, HRV indices of overall variability (SDNN and TP) were significantly decreased at POST only in response to the LONG protocol (Fig. 4.4.C and Tab. 4.1). In addition, RR interval, which is a marker of sympathovagal balance (Medeiros et al. 2018), was decreased to a greater extent at POST in response to the LONG protocol (Fig. 4.4.A), which resulted in a higher mean HR and worsened perceptual responses.

This was consistent with previous studies, showing a positive relationship between the increase in exercise-induced perturbation of homeostasis and the delay observed in cardiac autonomic recovery (Michael et al. 2017a; Fornasiero et al. 2018, 2019). The higher impact of LONG protocol on cardiac autonomic recovery was also evident when HRV indices were normalized for the underlying mean RR interval (Sacha 2014; Billman et al. 2015), which takes into account the influence of HR on HRV measurements (Tab.4.1).

Overall, our results suggest that shorter work-rest durations during the simulated hike were associated with less impairment of cardiac autonomic balance (i.e. smaller reductions in vagal-related HRV indices), compared to longer work-rest durations.

Alterations in ANS activity and particularly in sympathetic/parasympathetic balance associated with exercise stress may play an important role in the development of AMS (Sutherland et al. 2017; Rossetti et al. 2017). Further research is therefore needed to determine the effect of manipulating work-rest duration in real outdoor settings on cardiac autonomic recovery and tolerance to high altitude environments.

Limitations

Some limitations should be considered. As the evaluations were completed at very low ambient temperatures, the assessment of metabolic and ventilatory responses was not possible. Particularly, information regarding respiratory frequency would have helped to explain the higher RPE values reported with a longer work-rest duration, as a close association between respiratory frequency and RPE has been previously suggested (Nicolò et al. 2017). In addition, information regarding exercise-induced metabolite accumulation (e.g. blood lactate concentration $[La]_b$), could have attested a possible sustained metaboreflex and increased sympathetic activation (Romero et al. 2017) in the post-exercise period explaining the worsened cardiac autonomic profile after LONG protocol (Peçanha et al. 2016), as well as the increased HR reported during LONG protocol.

Because the duration of the hypoxic exposure was shorter than 6 hours we didn't ask participants to complete any AMS questionnaire (Hackett and Roach 2001). Some subjects (5/12) experienced a temporary headache after exercise, but without any difference between the two protocols.

We are aware that the approach used in the study does not represent the conventional way of being exposed to this kind of altitudes. However, we intentionally chose an altitude that is often reached also without proper acclimatisation, being many mountain peaks nowadays partially ascended using cableways.

However, different types of hypoxia as well as different statuses of acclimatization have a huge impact on the physiological responses to hypoxic exercise (Netzer et al. 2017; Bhattarai et al. 2018). Accordingly, the results obtained in this study may be limited to the investigated modality of hypoxic exposure/exercise (normobaric hypoxia, 30 min of passive exposure + 80 min intermittent exercise).

Further research, also including acclimatised subjects and a longer post-exercise period assessment under hypoxic conditions, is therefore suggested to generalize these preliminary results to real outdoor situations.

4.5 Conclusions

Reduced work-rest durations during a simulated mountain ascent at high altitude are associated with a decreased overall physiological strain (i.e. lower mean HR), ameliorated perceptual responses (i.e. lower RPE) and less impairment of cardiac autonomic balance (i.e. smaller reductions in vagal-related HRV indices) when compared to longer work-rest durations. Accordingly, shorter exercise periods and more frequent breaks during hiking at high altitude may represent a valid strategy to enhance exercise perceptual responses and limit the impact of exercise on cardiac autonomic recovery under extreme environmental conditions. Further research is needed to generalize these findings to real outdoor settings.

Overview of main outcomes

Study 1:

- Cardiac autonomic recovery is delayed in response to a maximal cardiopulmonary exercise test in hypoxia (FiO_2=13.4%, ≈3500 m);
- The degree of cardiac autonomic recovery impairment is directly related to the increase of physiological stress in hypoxia.

Study 2:

- Moderate heart rate matched hypoxic exercise (≈75% HRmax, FiO_2=14.2%, ≈3000 m) triggers similar cardiac autonomic and physiological responses to normoxic exercise with a reduced mechanical load;
- The same absolute intensity exercise in hypoxia is associated with increased exercise-induced physiological stress and delayed cardiac autonomic recovery.

Study 3:

- Moderate heart rate matched hypoxic exercise (≈75% HRmax, FiO_2=14.2%, ≈3000 m) does not affect cardiac baroreflex sensitivity and does not blunt cardiac autonomic recovery during post-exercise recovery, but does not induce significant post-exercise hypotension;
- Work rate matched hypoxic exercise, resulting in greater physiological stress, delays cardiac autonomic recovery, temporarily decreases cardiac baroreflex sensitivity and evokes prolonged post-exercise hypotension.

Study 4:

- Reduced work-rest durations are associated with improved perceptual responses and less perturbation of cardiac autonomic balance compared to longer work-rest durations in response to a simulated hike at high altitude under extreme environmental conditions (-25°C, FiO_2=11%, ≈5000)

General Conclusion and Future Perspectives

A better understanding of the acute and post-exercise physiological responses to hypoxic exercise is of paramount importance to ensure the adoption of adequate/safe exercise protocols in individuals engaged in hypoxic exercise training and to increase the tolerance to hypoxic stress in people exposed to high altitude environments. In this thesis, we investigated the acute exercise and post-exercise physiological responses evoked by hypoxic exercises of various intensities and nature, with a special focus on the cardiac autonomic responses induced.

In *Study 1* we found that cardiac autonomic recovery was delayed in response to a maximal hypoxic exercise (i.e. increasing intensity to volitional exhaustion) and that the degree of cardiac autonomic recovery impairment was directly related to degree of physiological stress induced by hypoxic exercise when compared to normoxic exercise (i.e. increase in exercise-induced perturbation of homeostasis in hypoxia). The findings from *Study 1* have several implications in the assessment and design of high-intensity hypoxic training sessions, as high-intensity hypoxic exercise is likely to induce greater cardiac autonomic disturbance than normoxic exercise. According to these findings, lower relative exercise intensities as well as different work/rest ratios may be required during high-intensity hypoxic exercise to match the impact of normoxic exercise on subsequent cardiac autonomic recovery. Future studies should therefore assess the impact of different high-intensity hypoxic training sessions on exercise and post-exercise cardiac autonomic responses, also extending post-exercise recovery assessment over longer periods of time (i.e. hours to days). This would help the implementation of high-intensity hypoxic exercises within the training schedules of individuals engaged in hypoxic training, ensuring adequate recovery and optimal long-term adaptations (Kiviniemi et al. 2007).

In *Study 2* we found that moderate heart rate matched hypoxic exercise triggered similar cardiac autonomic and physiological responses to normoxic exercise with a reduced mechanical load, whilst the same absolute exercise intensity in hypoxia was associated with increased exercise-induced

physiological stress and delayed cardiac autonomic recovery. These findings regarding a sub-maximal exercise were in line with the results of *Study 1* showing that when hypoxic exercise is associated with increased exercise-induced physiological stress, this is accompanied by impaired cardiac autonomic responses, and exercise is likely to exert greater impact on cardiac autonomic balance. However, our findings also suggest that the prescription of exercise based on heart rate in hypoxia can help control the increased exercise-induced physiological stress of hypoxic exercise and limit its impact on exercise and recovery cardiac autonomic responses. This highlights the usefulness of heart rate matched hypoxic exercises as a means to control the additional stress imposed by hypoxic stimulus. Future studies should focus on the investigation of how different degrees of hypoxia may affect physiological responses to exercise and on the long-term cardiac autonomic adaptations induced by heart rate matched hypoxic exercises.

In *Study 3* we found that moderate heart rate matched hypoxic exercise (≈75% HRmax) did not affect cardiac baroreflex sensitivity, did not blunt cardiac autonomic recovery during post-exercise recovery but did not induce significant post-exercise hypotension, compared to a similar exercise performed in normoxia. On the other hand, the same absolute exercise intensity in hypoxia, resulting in greater physiological stress, delayed cardiac autonomic recovery, temporarily decreased cardiac baroreflex sensitivity and evoked more prolonged post-exercise hypotension. This study offers a more complete overview of the post-exercise physiological response evoked by a heart rate matched exercise in hypoxia (*Study 2*). In line with *Study 2* similar exercise responses (hypoxia vs. normoxia), in terms of similar within-session cardiac autonomic responses and cardiorespiratory involvement, translated into similar post-exercise autonomic responses (i.e. similar parasympathetic recovery and cardiac baroreflex sensitivity responses after normoxic and heart rate matched hypoxic exercise). However, this latter was not associated with significant post-exercise hypotension, which may relate to the reduced mechanical load adopted during heart rate matched hypoxic exercise. According to these findings both physiological and mechanical stimulation can play a role on the post-exercise

cardiovascular responses evoked by hypoxic exercise. Future studies should therefore focus on the investigation of the interplay between exercise intensity (i.e. physiological and mechanical) and other exercise characteristics (e.g. exercise duration) in inducing (a desired level of) post-exercise hypotension in response to different protocols of hypoxic exercise. This would help anticipate the post-exercise cardiovascular outcomes induced by hypoxic exercise and increase its applicability in a wide range of contexts and clinical populations.

Similarly, as hypoxic exercise, according to *Study 1*, *Study 2* and *Study 3*, can result in greater autonomic disturbance compared to normoxic exercise, further experimental research is needed to investigate the applicability of different hypoxic exercises (i.e. different exercise intensities and hypoxic levels) in various clinical populations.

In *Study 4* we found that reduced work-rest durations were associated with improved perceptual responses and less perturbation of cardiac autonomic balance compared to longer work-rest durations in response to the simulated hike at high altitude. According to our findings, shorter exercise periods and more frequent breaks during hiking at high altitude may represent a valid strategy to enhance exercise perceptual responses and limit the impact of exercise on subsequent cardiac autonomic recovery. Since exercise-induced physiological stress and inadequate cardiac autonomic recovery may facilitate the development of maladaptive responses to high altitude environments (Sutherland et al. 2017; Boos et al. 2018), future studies should investigate optimal climbing strategies and monitor cardiac autonomic responses in real outdoor high altitude settings, relating the observed responses to acclimatization process and tolerance to high altitude.

Again, as our observations only refer to healthy active (male) subjects, further experimental research, investigating the acute exercise and post-exercise physiological responses evoked by different hypoxic exercises in clinical populations, is warranted. Similarly, further research examining the above-mentioned responses in women is required, as the influence of ovarian cycle phases and menopause might impact upon the physiological responses to hypoxic stimulus (Richalet et al. 2020).

Overall, this doctoral thesis wanted to provide new insights into the understanding of the acute exercise and post-exercise physiological responses occurring in response to hypoxic exercise. I hope this doctoral thesis highlights the usefulness of monitoring the exercise and post-exercise physiological responses to hypoxic exercise in various contexts and settings and encourages new research on this topic.

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