UNIVERSITA' DEGLI STUDI DI VERONA

DEPARTMENT OF NEUROSCIENCES, BIOMEDICINE AND MOVEMENT SCIENCES

PhD SCHOOL OF HEALTH AND LIFE SCIENCES

PhD IN NEUROSCIENCE, PSYCHOLOGY AND PSYCHIATRY

XXX CYCLE /YEAR 2014

Acute progressive hypoxia: effects on endurance performance and its physiology

S.S.D. M-EDF/02

Coordinator: Prof. Leonardo Chelazzi

Tutor: Dr. Barbara Pellegrini

Co-Tutor: Dr. Gianluca Vernillo

PhD candidate: Aldo Savoldelli

ABSTRACT

Introduction

Hypoxia is defined as a reduction in the amount of oxygen (O_2) available to any cell, tissue, or organism (Semenza, 2009). Research examined the effects of this reduction on endurance performance [see (Fulco et al., 1998) for review] and the benefits deriving from the exposure (and training) in hypoxia on the sea level endurance performance [see (Millet et al., 2010) for review]. However, endurance and ultra-endurance performances can also be performed in hypoxic environments. This is an intrinsic feature of those performances that either start from an altitude level and finish at a higher one or where the altitude profile changes during the race (e.g. uphill cycling time trial, running vertical kilometres, etc.). Accordingly, to date less is known about the determinants of performance where the severity of hypoxia changes during the trial (i.e. Progressive Hypoxia, PH). Therefore, the aim of this project was to investigate the effects of acute progressive hypoxia on the endurance performance and fatigue. A secondary aim was to determine the main physiological responses during these tasks in PH at different intensities of effort.

Study I

There are competitions like the mass start events (e.g. running and ski mountaineering vertical kilometres, cycling stages with uphill arrival, etc.) that take place in hypoxia, or even in progressive hypoxia, with the final high intensity effort at a higher altitude compared with the starting one. The aim of the study was to understand if a different hypoxic stimulus, during a submaximal cycling exercise (50% of relative Peak Power Output, PPO) impairs the high intensity performance of the final high intensity effort. After a maximal ramp test to obtain the PPO and baseline measurements of endurance performance (time to exhaustion, TTE) in a non-fatigued state (both in normoxia and in hypoxia) 8 subjects completed an 1-hour cycling protocol in normoxia (at 50% of PPO obtained in normoxia, N), constant hypoxia and progressive hypoxia (FiO_2 = 13.4%, and FiO_2 starting from 16.25 to 13.4%, at 50% of PPO obtained in hypoxia,

Hcost and HH, respectively). TTE duration was reduced both after the N and Hcost session (-27.9% P=0.03 and -21.6% P=0.007, respectively) with no effect after HH. Higher oxygen saturation (SpO_2) was observed during cycling exercise in N compared to the other two conditions. Hcost resulted in a lower SpO_2 compared to HH, until the end of the 1-h bout, where Hcost and HH presented similar SpO_2 due to similar altitude levels reached. Oxygen consumption was similar during the HH and Hcost condition, but Hcost is lower than in N (P=0.03). Rate of perceived exertion was similar in the three conditions. The primary finding of this study was that an impairment of ~25% in the endurance performance (tested through a TTE test and compared to a non-fatigued trial at baseline) was observed after both a normoxic (P=0.03) and a constant hypoxic (P=0.007) task; no effects after 1-h in HH. A possible explanation to the different effect of HH and Hcost on TTE performance can be related to the hypoxic dose (5.25 VS 3.75 kilometres/hour in Hcost VS HH, respectively).

Study II

There are competitions that take place in progressive hypoxia at a submaximal intensity throughout the entire duration with the final part of the race at a higher altitude compared to the starting one. The aim of this study was to investigate the effects of an 1-h exposure at different cycling submaximal intensities at progressive hypoxia on fatigue and endurance performance (tested through a Time To Exahustion, TTE). Peak power output (PPO) and baseline duration in a TTE were obtained in a non-fatigued state (both in normoxia and in hypoxia) in 11 subjects. Subsequently, in three separated days, they completed an 1-h protocol under the same progressively hypoxic stimulus (FiO_2 starting from 16.25 to 13.4%, simulating an increase in altitude from 2000 to 3500 m) at different intensities: no effort (H_NoPO), 50% of the PPO in hypoxia (HH) and 50% of the PPO in normoxia (HN). Oxygen consumption, heart rate, blood lactate, cerebral blood flow and pulse oxygen saturation were monitored during each session. Neuromuscular fatigue was assessed pre and post the 1-h intervention as well as after the subsequent TTE. We observed a reduced duration of TTE only after 1-h HN, when compared to baseline and H_NoPO (-37.2% P<0.001 and -30.8% P=0.016). One of the

reason of this impairment in performance can be the higher blood lactate accumulation and the higher RPE during 1-h HN. The general reduction in SpO2 during the three interventions may be one of the causes of the reduction in voluntary activation, as an index of central fatigue, even though cerebral blood flow increased with time without any differences between conditions. The novelty of this study was to investigate the acute effects on performance and fatigue at different submaximal intensities when athletes are exposed to a progressively increased hypoxia. The main finding was that the endurance performance (assessed by means of TTE, that can be considered as the final high intensity effort at a higher altitude compared with the starting one) was only compromised after 1-h of cycling at 50% of the absolute peak power output obtained in normoxia. Therefore, it can be a good practice to test athletes that need to perform at altitude, in simulated condition.

General conclusion

Progressive hypoxia is a condition encountered during several endurance and ultra endurance performances. The understanding of the effects driven by a PH exposure at different intensities on a subsequent endurance performance can be useful for coaches and athletes that need to plan and pace their efforts in similar environments. We need to be conscious that in altitude, and especially in PH, the threshold between choosing the correct intensity of an effort and the intensity that can results in a subsequent impairment during an endurance performance (TTE) is really thin. Therefore, it can be a good practice to test athletes that need to perform at altitude, in a similar condition.

Finally, we can conclude that a small step forward in the understanding of efforts during a progressive hypoxic stimulus has been provided. More work is needed, and the next step could be to study PH in field performances.

1 Introduction

Research on the effects of hypoxia has its roots roughly 100 years ago and it has received new emphasis in the last two decades. Researchers described hypoxia not only as an abnormal stress associated with injury and disease, but also as a physiologic mechanism modulating different biological processes. This has led to extensive studies of the many and varied molecular signalling pathways, as well as cellular responses, triggered or modulated by the exposure to hypoxia. Moreover, the effects of hypoxia on endurance performance are well-know, with an overall impairment systematically observed. Considering the huge amount of people who ascend by cable car, walking, running or cycling to medium or high altitude locations every year, there are consistent information concerning the effects of hypoxia on numerous physiologic variables (Figure 1).

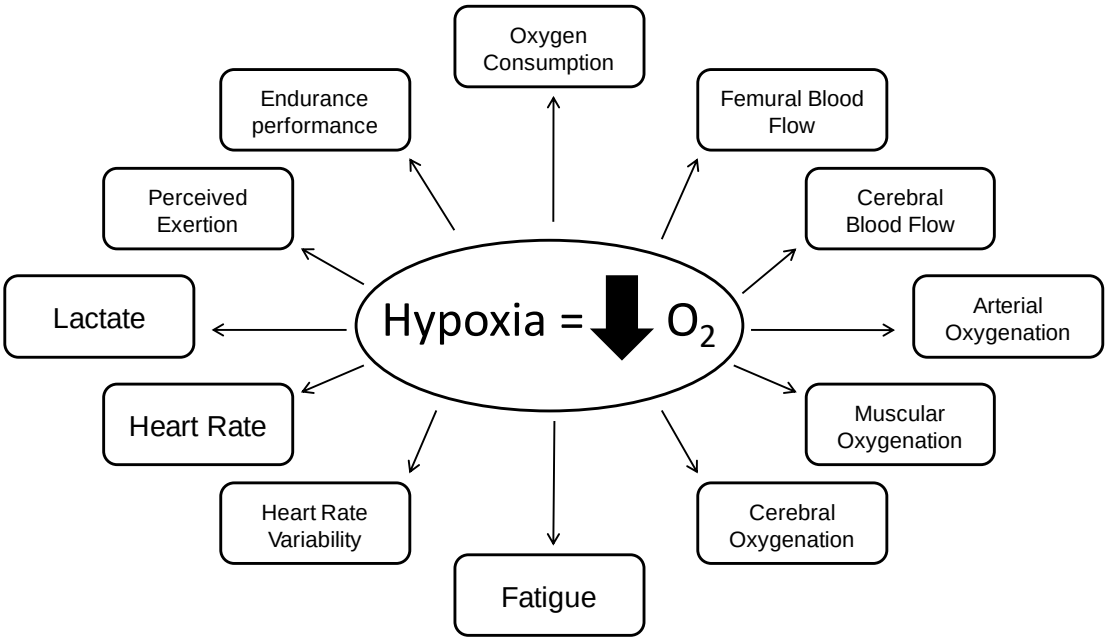

Figure 1 The main physiological variables affected by acute exposure to hypoxia

Hypoxia can be defined as a reduction in the amount of oxygen (O_2) available to any cell, tissue, or organism (Semenza, 2009). This reduction impairs endurance

performance and maximal oxygen consumption (VO_{2max}) (Calbet et al., 2003) at different altitude levels, both in normobaric (constant barometric pressure, reduced fraction of inspired oxygen) (Peltonen et al., 1995) and hypobaric (typically, natural altitude) (Cerretelli, 1976) regimens that will be better described below. Several works investigated the effects of Normobaric/Hypobaric hypoxia with the aim to explain and predict hypoxic performances [e.g. (MacInnis et al., 2015; Peronnet et al., 1991; Townsend et al., 2017; Wehrlin and Hallen, 2006)]. Overall, these studies reported a hypoxia-induced reduction in the endurance performance that can often be related to the subjects' physical fitness level (see paragraph 2.1 for details).

Firstly, the attention on hypoxia was dedicated to the challenge to reach the Mt. Everest even with (Hillary and Norgay, 1953) or without (Messner and Habeler, 1978) an external O_2 supply (West et al., 1983).

After that, and especially after the Olympic games held in Mexico City in 1968 (where no new world records were established in events lasting longer than 2.5 minutes), researchers' attention moved to new methods and devices able to enhance performance through altitude training. Specifically, the focused attention was if a prolonged exposure to an hypoxic environment would have brought some positive effects on future endurance performances (Levine and Stray-Gundersen, 1992, 1997). Usually this kind of research was designed with the aim to improve sea level performances thanks to the positive adaptations obtained during continuous or intermittent exposure to hypoxia (Mounier and Brugniaux, 2012; Peacock, 1998; Stray-Gundersen and Levine, 2008). Most of the altitude/hypoxia training methods, and their combination for peak performance, were reviewed by Millet and colleagues (Millet et al., 2010). To date, a new research line has been added, with the main goal to enhance team sport performances (Brocherie et al., 2015) by means the development of new training methodologies such as repeated-sprint training in hypoxia (Brocherie et al., 2017) (Figure 2).

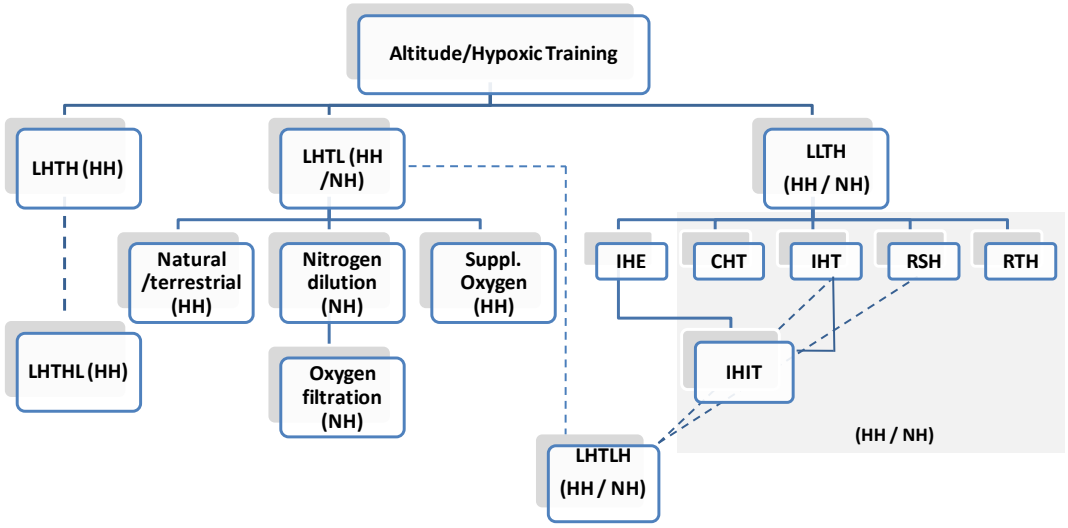

Figure 2 Panorama of the different hypoxic/altitude training methods used by athletes in 2015. LH – live high; TH – train high; TL – train low; LHTLH – live high, train low and high (different intensities); LL – live low; IHE – intermittent hypoxic exposure during rest; CHT – continuous hypoxic training; IHT - intermittent hypoxic exposure training; RSH – repeated sprint training in hypoxia; RTH – resistance training in hypoxia; IHIT - intermittent hypoxic exposure during interval training; HH – Hypobaric Hypoxia; NH – Normobaric Hypoxia (redrawn by Millet and Girard, pp 94, (Seifert, 2016) **)**

Acute or chronic exposure to hypoxia may lead to different responses of the human body (Calbet et al., 2009). Especially, as described before, people can experience a reduction in their endurance performance if acutely exposed to hypoxic environments (MacInnis et al., 2015).

The magnitude of acute hypoxia's ergolytic effect is dependent on the type of exercise and the duration. For instance, 1-hr distance records on the velodrome are often attempted at altitude to take advantage of reduced air resistance. One may find performances in events of short duration and high power requirements are improved at altitude while events beyond 800 m generally show a decline (Wyatt, 2014).

It seems that this detrimental effect can be attenuated after a prolonged period in a hypoxic environment that firstly leads to acclimatization and subsequently to adaptation in the human body (Peacock, 1998).

Moreover, it seems that when the effects of a prolonged sojourn in hypoxia are combined with different hypoxic training methods, they can play an important role in peak performance of multiple sports (Millet et al., 2010).

Nowadays, hypoxia is a common environment in the endurance and ultra-endurance performances (Clark et al., 2007), with an increasing number of competition that take place in altitude. For example, cyclist can reach 2800 meters of Stelvio pass (Italy) during the Giro d'Italia; runners can perform a "vertical kilometer" (VK) or try to conquer a peak as fast as possible ("Enervit Vitesse" or "Summit of my life" projects); ski mountaineers can arrive at 4200 m of Mt. Castore during Mezzalama Trophy (Italy); mountaineers can reach the top of Mount Elbrus at 5640 m during the Red Fox Elbrus race (Russia) or the Pikes Peak at 4260 m (North America) and alpinists can overpass these altitudes for reaching one of the 8000 meters mountains. In these performances there is a common peculiarity: athletes start from an altitude and finish to a higher one. Furthermore, the increase in altitude during these efforts is to a certain extent linear.

Accordingly, the performance determinants when the severity of hypoxia changes during the trial (Progressive Hypoxia, PH) is still unclear.

Therefore, the aim of our project was to investigate the main physiological responses during an acute exposure to PH and the effects of simulated performances in PH both on the development of fatigue and on the endurance performance.

Before starting with a review of the literature about the effects of hypoxia it must be acknowledged that despite much of the hypoxia research has been carried out at high altitude in a hypobaric hypoxia environment, many research teams sought to replicate high-altitude conditions at lower altitudes in either hypobaric hypoxic conditions or normobaric hypoxic laboratories. The main difference between Hypobaric Hypoxia and Normobaric Hypoxia is the partial pressure of oxygen [i.e. the partial pressure of oxygen in moist inspired air can be express as: $PIO_2 = FiO_2$ x (Barometric Pressure-47)], which is commonly presumed to be the principal physiological stimulus to adaptation at high altitude. Coppel and colleagues in their systematic review included

13 studies. Several studies reported many variables [e.g. minute ventilation and Nitric Oxide (NO) levels] that were different between the two conditions, supporting the notion that true physiological differences are present. However, the presence of confounding factors such as time spent in hypoxia, temperature, humidity, and the limited statistical power due to small sample sizes, limit the conclusions that can be drawn from these findings. They concluded that a proper standardization of the study methods and reporting may aid interpretation of future studies. They underlined the importance to improve the quality of data aiming to improve the understanding of hypoxia tolerance in both normobaric and hypobaric hypoxia, both in altitude and in the clinical settings (Coppel et al., 2015). However, the popularity of using normobaric hypoxia to investigate and to obtain some early adaptations compared to the hypobaric hypoxia seems to be actual. For example, Netzer and colleagues (Netzer et al., 2017) compared the effect on SpO_2 and heart rate measured during a real hike on Mount Mauna Kea (4205 m) with a simulated effort in a normobaric hypoxic chamber. Both the trials were performed at a constant speed of 1.6 km/h. They reported the general trend of SpO2 throughout the entire duration of the two 7 hours trials with a general decline from ~93% to 72-77% with an always higher saturation during the normobaric hypoxic trial compared to the hypobaric hypoxic one. Therefore, this information is essential since there is a need to understand the determinants of an efforts in a hypoxic setting as standardized as possible. Practically, it can be useful to train athletes involved in training and competition in an environment where the altitude increases during the competition (the FiO_2 decrease). Furthermore, it may also help to advise the athletes with the right volume/intensity of training or suggest them the right pacing.

2 Review of the literature and aim of the project

The following subchapters contain a review of the main topics related to the effects of the hypoxia on exercise performance.

2.1 Maximal performance in hypoxia

Several studies have examined the effect of increasing altitude on VO2max reporting either a linear decrease (Clark et al., 2007; Wehrlin and Hallen, 2006), a curvilinear decrease (Bassett Jr. and Howley, 2000; Peronnet et al., 1991), or a curvilinear interaction between altitude and sea level VO2max (MacInnis et al., 2015) (**Figure 3**).

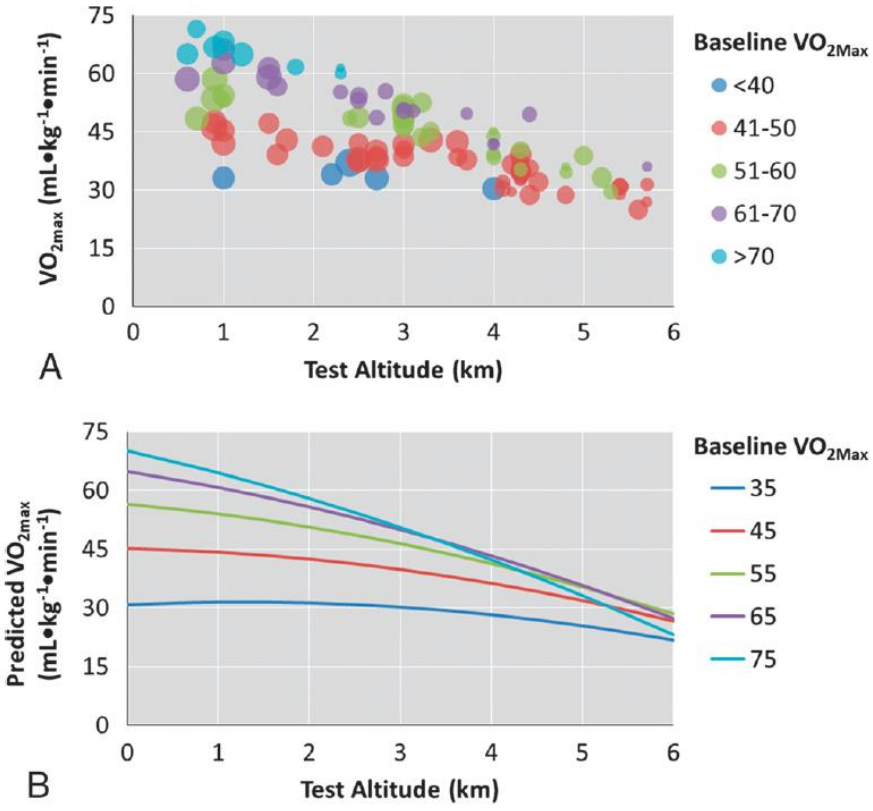

Figure 3 A. Observed VO_{2max} at high-altitude test (circles) for each independent participant group as a function of average baseline VO_{2max} and test altitude. The size of each circle is inversely proportional to the variance of each study. B. Predicted VO_{2max} at high altitude test as a function of test altitude and baseline VO_{2max} (shown as separate lines). These predictions are based on the curvilinear model (MacInnis et al., 2015)**.**

Another concept related to the endurance performance is critical power (CP) that describe the relationship between sustainable power output and duration for severe-intensity exercise. Recently, Townsend and colleagues, examined the dose-response effect of increasing altitude on both CP and W' (the total work accumulated above CP until task failure), and thereafter to develop a prediction equation enabling W'_{BAL} computation in hypoxia (Townsend et al., 2017). They found a decrease in CP at altitude described by a 3rd order polynomial function while W' decreased only at 4250 m asl (Figure 4). This last finding is in line with a previous study which reported a large reduction in W' at high altitude (5,050 m) (Valli et al., 2011), whereas another study found no changes at simulated altitude equivalent to 3,800m (Simpson et al., 2015).

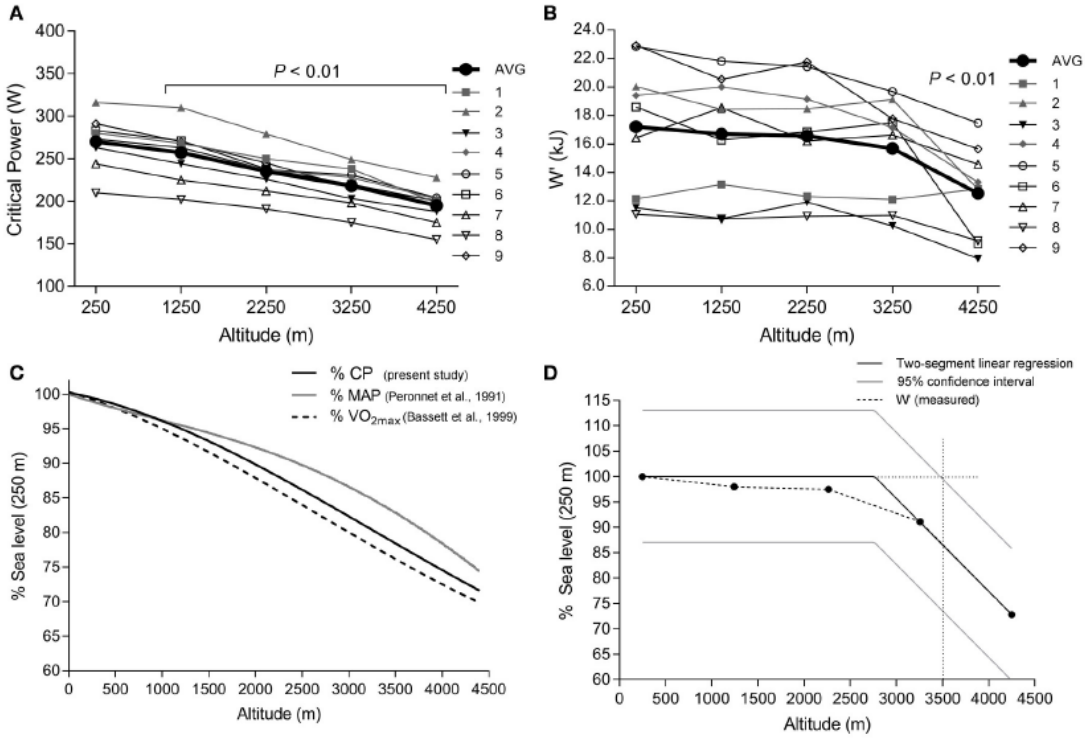

Figure 4 Effect of increasing altitude on group mean and individual subject critical power (A) and W′ (B). Model predicted critical power also showing comparison to maximal aerobic power (MAP) and ˙VO2max (C), and W′ (D), expressed as percent of sea level measured values. In (D) light gray solid lines represent 95% CI. Intersection of the dotted lines indicates predicted altitude where a statistically significant decline in W′ would occur. P < 0.05 indicates significant difference compared to 250 m (Townsend et al., 2017)**.**

The degree to which performance and maximal oxygen consumption are impaired during endurance exercise at altitude is not uniform between athletes, and inter-individual variability can be substantial. The maintenance of pulse oxyhemoglobin saturation (SpO_2) (see subchapter 2.3.1) seems to play a fundamental role especially due to the ventilatory response to hypoxia (Chapman, 2013a). An increase in minute ventilation (VE) during exercise at altitude drives to a higher alveolar partial pressure of oxygen that is related to SpO_2 and seems to be beneficial to physical effort. We know by Chapman and colleagues (Chapman et al., 1998) that in some athletes it can happen an expiratory flow limitation (expiratory flow-limited athletes, FL) that leads to an inability to increase VE during an effort with a negative impact on SpO_2. Despite the mechanical reserve to increase VE in non-expiratory flow-limited athletes (non-FL) they allow SpO_2 to fall during heavy and maximal exercise in mild hypoxia. This is likely due to a sub-conscious regulation of VE during hypoxic exercise with the aim to optimize the work of breathing and/or dyspnea around some critical threshold that the athlete is willing to tolerate (Chapman et al., 1998). For example, at the end of a 5km TT even if a similar VE has been found between FL group and non-FL subjects, the non-FL reported a significantly increased dyspnea rating in hypoxia compared to normoxia resulting in a more reduced ability to produce power on the bike in hypoxia, both compared with normoxia and FL group (Weavil et al., 2015). This kind of sub-conscious regulation of VE seems to be "depressed" when athletes who suffer from exercise-induced hypoxemia take 8 mg/kg of caffeine (Chapman and Stager, 2008). The ventilatory response seems to be fundamental also in extreme altitude climbers. During Everest-K2 Italian Expedition, Bernardi and co-workers tested a group of alpinists concluding that the most successful (the ones who reached the top without oxygen supply) had smaller ventilatory responses to hypoxia during acclimatization to 5,200 m, but, as a result, had greater available reserve for the summit. They stated that a less sensitive hypoxic response and a greater ventilatory efficiency might increase ventilatory reserve and allow sustainable ventilation in the extreme hypoxia to the summit (Bernardi et al., 2006)

2.2 Submaximal performance in hypoxia

Submaximal oxygen uptake and efficiency for a particular exercise activity at a specific power output is similar at sea level and altitude (Clark et al., 2007). But because VO_{2max} progressively decline with increasing elevation (Wehrlin and Hallen, 2006), the relative inability of exercising at a specific power output will progressively increase with increasing elevation. Even if maximal and submaximal exercise ability in hypoxia seems to be closely linked it is difficult to predict from the VO_{2max} decrement in hypoxia the magnitude of an individual's submaximal exercise impairment maybe due to the different proportions of aerobic and anaerobic processes related to the various intensities and durations of exercise (Fulco et al., 1998). In their review Fulco and colleagues summarize the finding of several studies obtaining the decrements in performance as a function of increased altitude (Figure 5).

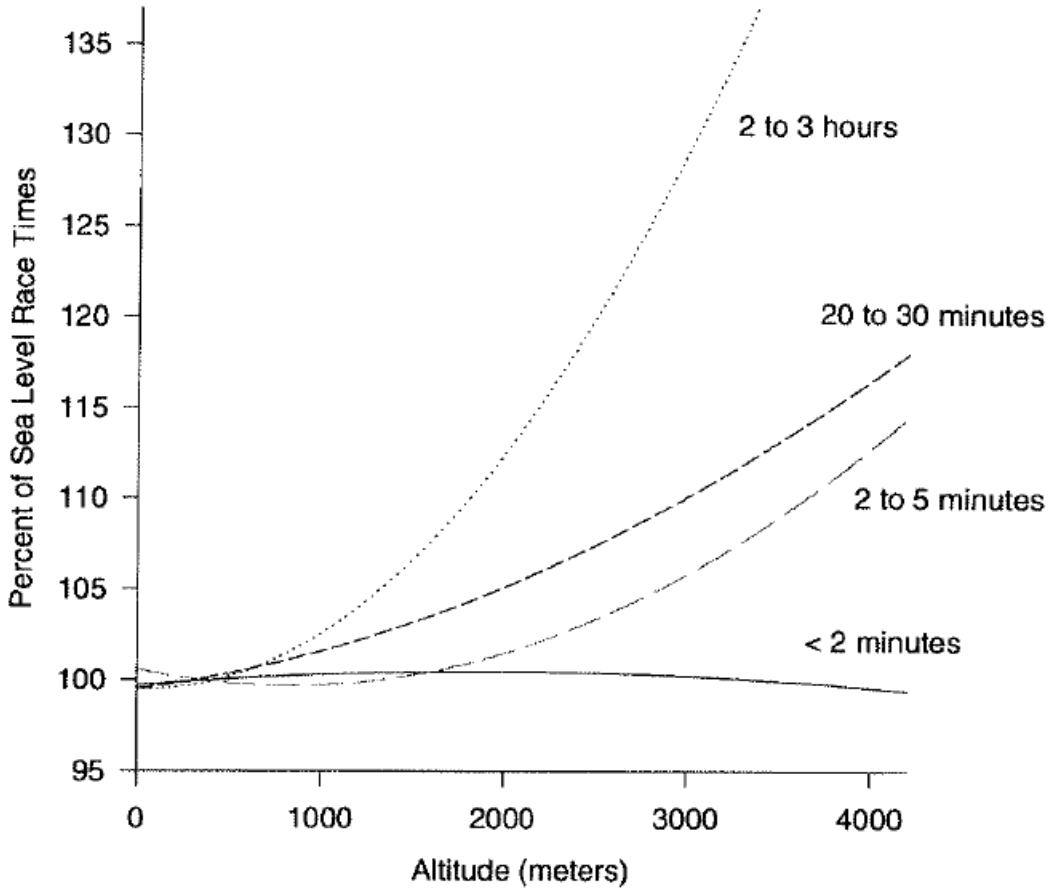

Figure 5 Performance decrements as a functions of event duration and elevation after 10 days of altitude training (modified from Fulco, 1998).

When Clark and colleagues asked to 11 subjects to cycle at different target workloads in normoxia and at different altitudes they observed what is reported in Figure 6. As we will discuss below (paragraph 2.3.1) also in their experiment a significant decrease in SpO_2 was observed during the hypoxic sessions (panel F). On the contrary VCO_2 (panel B), VE (panel C), Lactate (panel D) and heart rate (panel E) observed during their study were higher when altitude was increased (Figure 6). This can be related to the choice to ask for an effort at an absolute target power output. In fact, Friedmann and colleagues during 1 hour exercise at the relative individual anaerobic threshold intensity found a similar lactate concentration and an even higher heart rate and rate of perceived exertion in normoxia compared to hypoxia (Friedmann et al., 2004).

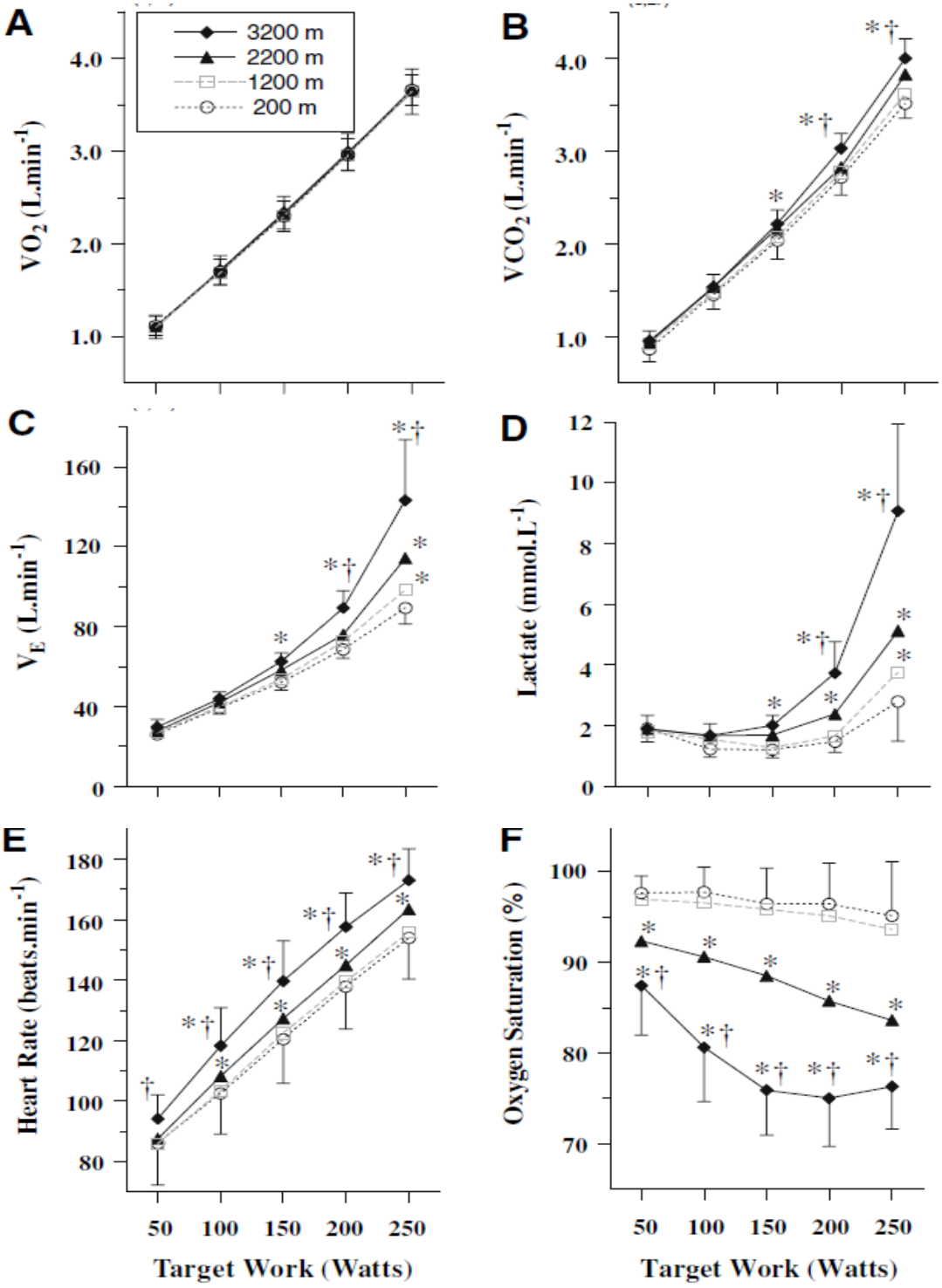

Figure 6 Submaximal VO_2 (a), VCO_2 (b), VE (c), Lactate (d), Heart rate (e) and Oxygen saturation (f) at four simulated moderate altitudes in well-trained male cyclists and triathletes (modified from Clark, 2007).

2.3 Pulse, muscle and cerebral oxygenation in hypoxia

2.3.1 Pulse oxymetry

The behaviour of pulse oxymetry (SpO_2) once people are involved in a physical activity at sea level and in altitude has been observed by several researchers and here we reviewed the work of Smith in 2007 (Smith, 2007). Even during a low power output (30 or 60 Watt) effort he observed an abrupt decrease in SpO_2 once physical activity was commenced (Figure 7). This was small at sea level (1%) and 610 m (2.2%), however, the SpO_2 fell by 4.3% at 2134 m and 5.5% at 2743 m (to SpO_2 88.1% and 85.7%, respectively). SpO_2 returned to near-resting values within 3 min of stopping exercise (Smith, 2007).

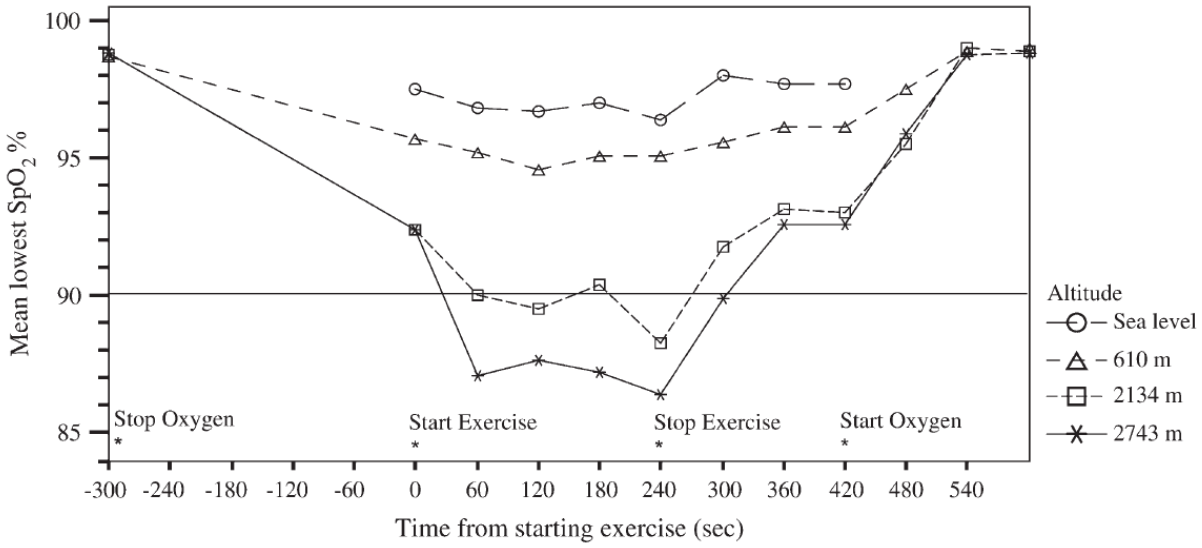

Figure 7 SpO_2 related to physical activity at different altitudes. The line through SpO_2 90% approximates a typical person resting at 3048 m (modified from Smith, 2007).

Another important finding from this study is that subjects who reported symptoms of hypoxia, and in particular symptoms suggesting neurocognitive impairment, were more desaturated than those who did not (Figure 8). The author concluded alerting people working at altitude (such as helicopter aircrew) that even a light to moderate physical activity at an altitude as low as 2134 m can produce hypoxemia and symptoms of

hypoxia similar to that which would normally be expected in a person resting at approximately ~3700 – 4600 m.

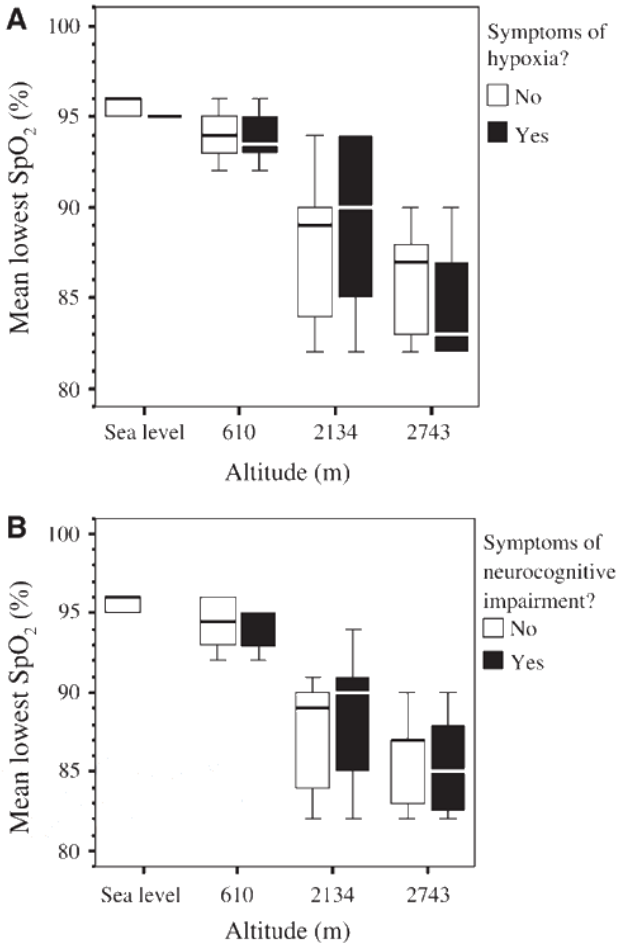

Figure 8 SpO_2 of subjects reporting symptoms of hypoxia (panel A) and symptoms suggesting neurocognitive impairment (panel B) (modified from Smith, 2007).

Monitoring pulse oximetry in hypoxic environments seems to be of interest not only from a performance perspective, indeed, it was also investigated to evaluate the relationship between the individual SpO_2 after a 20-to-30 minutes of exposure to hypoxia and the acute mountain sickness (AMS) susceptibility based on repeated observations (Burtscher et al., 2004). Prediction of the development of AMS in individuals going to high altitudes is still a matter of debate. AMS is a syndrome of nonspecific symptoms including headache, lassitude, dizziness and nausea and it is one

of the three forms of acute altitude illness (Luks et al., 2017). Burtscher and colleagues found that SpO_2 values after 20-to-30 min of hypoxia were on average 4.9% lower in subjects susceptible to AMS than in those who were not (Figure 9), concluding that SpO_2 values after 20-to-30 min of exposure to normobaric or hypobaric hypoxia represent a useful tool to detect subjects highly susceptible to AMS.

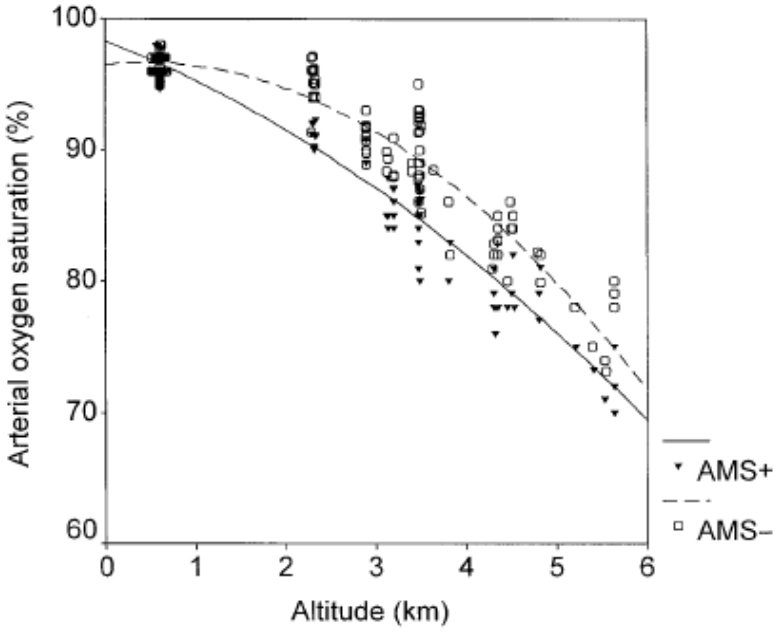

Figure 9 Altitude dependent SpO2 values in AMS-susceptible (AMS+) and nonsusceptible (AMS-) subjects (modified from Burtscher, 2004).

The pulse oxymetry utilization and interpretation is not without any limits (Constantini et al., 2017). In fact, highly trained athletes often also experience a greater degree of arterial oxyhaemoglobin desaturation (SpO_2) both at altitude and sea level (Gaston et al., 2016) (Figure 10), and it has been shown that considerable reductions in SpO_2 at sea level are associated with greater reductions in VO_{2max} and running performance at altitude (Chapman et al., 2011; Gavin and Stager, 1999). Measuring SpO_2 during heavy exercise at sea level could therefore be a useful tool for screening athletes before altitude training and making appropriate altitude training recommendations on an individual basis. For example, while training at an altitude of 1250 m is likely low

enough to induce positive training adaptations in most athletes (Levine and Stray-Gundersen, 1997), those who show substantial reductions in SpO_2 and are more prone to experience declines in training abilities should likely train at an even lower altitude (Chapman et al., 2011). Thus, they recommended measuring SpO_2 during heavy exercise at sea level to detect for a reduction of more than 4% from resting values and/or a drop below 92% in SpO_2 (the shoulder of the oxyhemoglobin dissociation curve), which would indicate that the athlete is likely to have impaired workouts at altitude, especially during the first week of exposure (Chapman, 2013; Chapman et al., 2011).

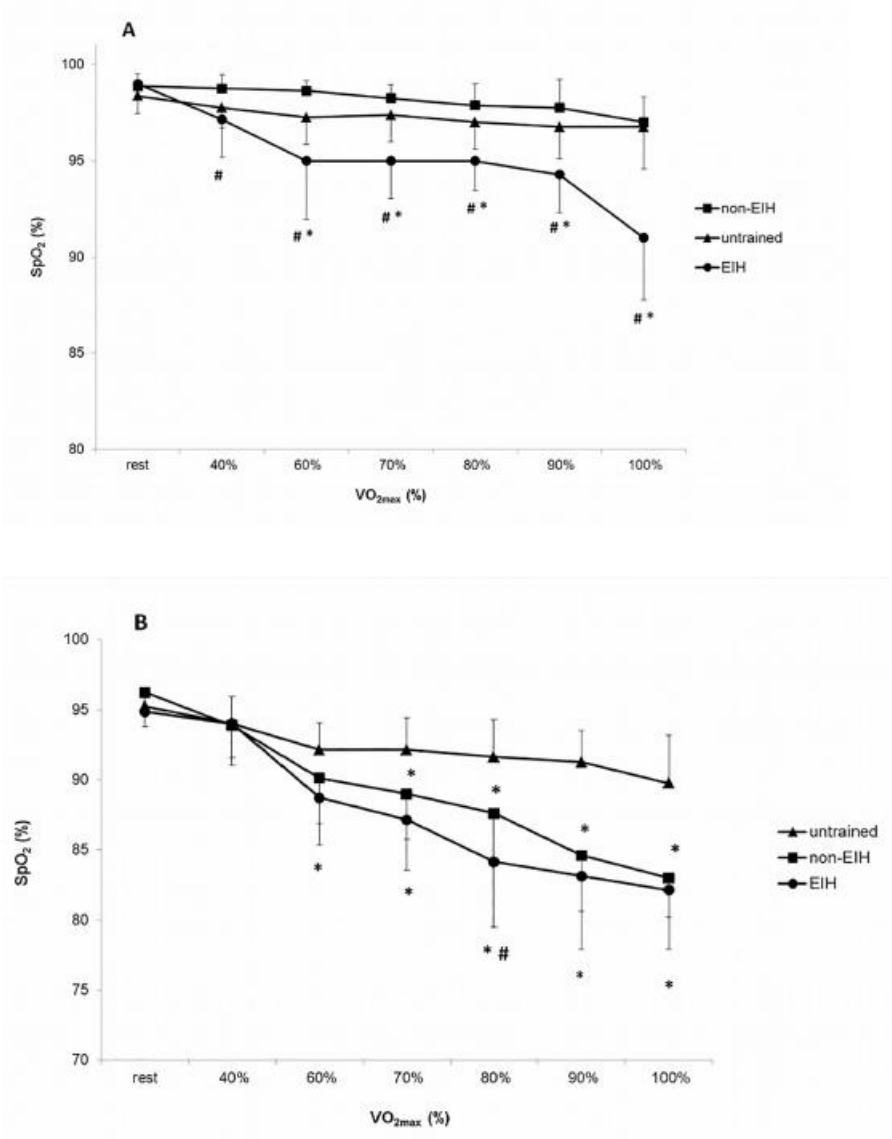

Figure 10 Kinetics of pulse oxymetry (SpO2) during rest and incremental maximal exercise at sea level (A) and altitude (B). * Significantly different from untrained (p < 0.05); # Significantly different from non-EIH (p < 0.05). Note that, at all intensities, SpO2 values at altitude are significantly different from sea level in the three groups (p < 0.05). (modified from Gaston, 2016)

2.3.2 Muscle and cerebral oxygenation

Near-infrared spectroscopy (NIRS) is a non-invasive, continuous, and direct method to determine oxygenation and hemodynamics in tissue. It enables to study the local differences in muscle oxygen consumption and delivery and it is considered an

appropriate tool to provide these kind of information (Van Beekvelt et al., 2001). The NIRS devices usually provides absolute concentrations (μmol/L) for deoxy-[Hb+Mb] and oxygenated-[hemoglobin+myoglobin] (oxygenated-[Hb+Mb]), which sum to determine total-[hemoglobin+myoglobin] (total-[Hb+Mb]), as well as a tissue oxygenation index (TOI= %Saturation of [hemoglobin+myglobin]).

By means of a NIRS device the effects of sustained hypoxic exposure on cerebral and muscle oxygenation and cardiorespiratory function at rest have been investigated (Rupp et al., 2013). In their work, Rupp and colleagues asked to the subject to inhale a normobaric hypoxic (FiO2 = 12%) or normoxic (FiO2 = 21%) gas mixture for 4 h at rest, on two separated blinded sessions monitoring continuously oxygenation of quadriceps muscle and prefrontal motor cortex by a NIRS device. The effects of the first 50 minutes of exposure are represented in Figure 11. The results showed a time to plateau of skeletal muscle oxygenation of ~10 minutes while ~30-40 minutes are needed to the prefrontal cortex values to plateau. Deoxygenation was more pronounced in the cerebral cortex compared to the muscle (+2.1 ± 2.3 μMol of deoxygenated-hemoglobin), and NIRS-derived tissue perfusion index showed distinct profiles between the muscle (hypoperfusion) and the brain (hyperperfusion) with prolonged hypoxia. Pulse oxygen saturation and deoxyhemoglobin at prefrontal motor cortex revert to baseline levels during the 15 minutes of normoxic washout at the end of the trial; it is not the same for what happen inside the muscle that is also characterized by a higher variability of response to hypoxia exposure compared to cerebral oxygenation (Rupp et al., 2013). Reduced cerebral oxygenation may reflect an imbalance between oxygen supply and extraction indicating a potential risk for cellular disfunction, although it doesn't necessarily indicate tissue damage. An irreversible neurologic damage may depend on duration and severity of the hypoxic stimulus (Acker and Acker, 2004). Moreover, the human body seems to be able to react to this reduced cerebral oxygenation increasing cerebral blood flow (Ainslie and Subudhi, 2014).

Cerebral and muscular oxygenation have also been studied during a ramp incremental test both in normoxia and in hypoxia (Figure 12). Subudhi and colleagues reported

larger changes in cerebral oxygenation during the ramp incremental test in hypoxia compared to normoxia. Within the muscle, oxygenation decreased progressively throughout exercise in both normoxia and hypoxia (decrease in oxyhemoglobin and increase in both deoxyhemoglobin and total hemoglobin), although deoxyhemoglobin was unchanged between 75 and 100% of the peak power output. They also found greater changes in muscle oxygenation in hypoxia compared to normoxia. On the basis of these findings they concluded that, it is unlikely that changes in cerebral oxygenation limit incremental exercise performance in normoxia, yet it is possible that such changes play a more pivotal role in hypoxia (Subudhi et al., 2007).

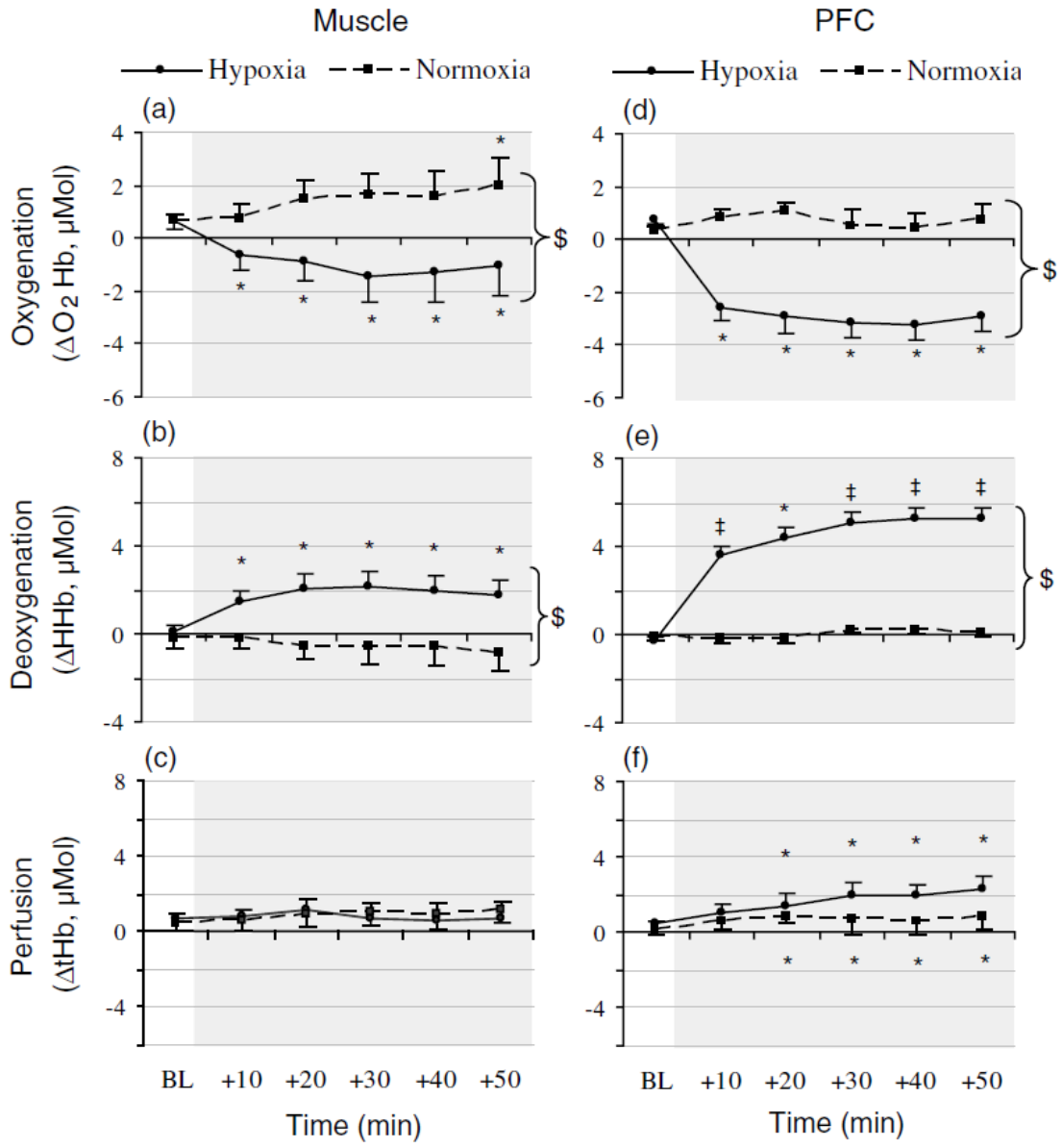

Figure 11 Mean changes (SE) in oxy-([O2Hb]), deoxy-([HHb]), and total-hemoglobin ([tHb]), during the first hour of exposure to 12% O2 hypoxia Data are shown for the vastus lateralis muscle [panels (a) to (c)], for the prefrontal cortex [PFC, panels (d) to (f)]. BL, baseline. *P < 0.05 versus BL; ‡P < 0.05 versus BL and þ20 min; $P < 0.05 versus normoxic session (modified from Rupp, 2013).

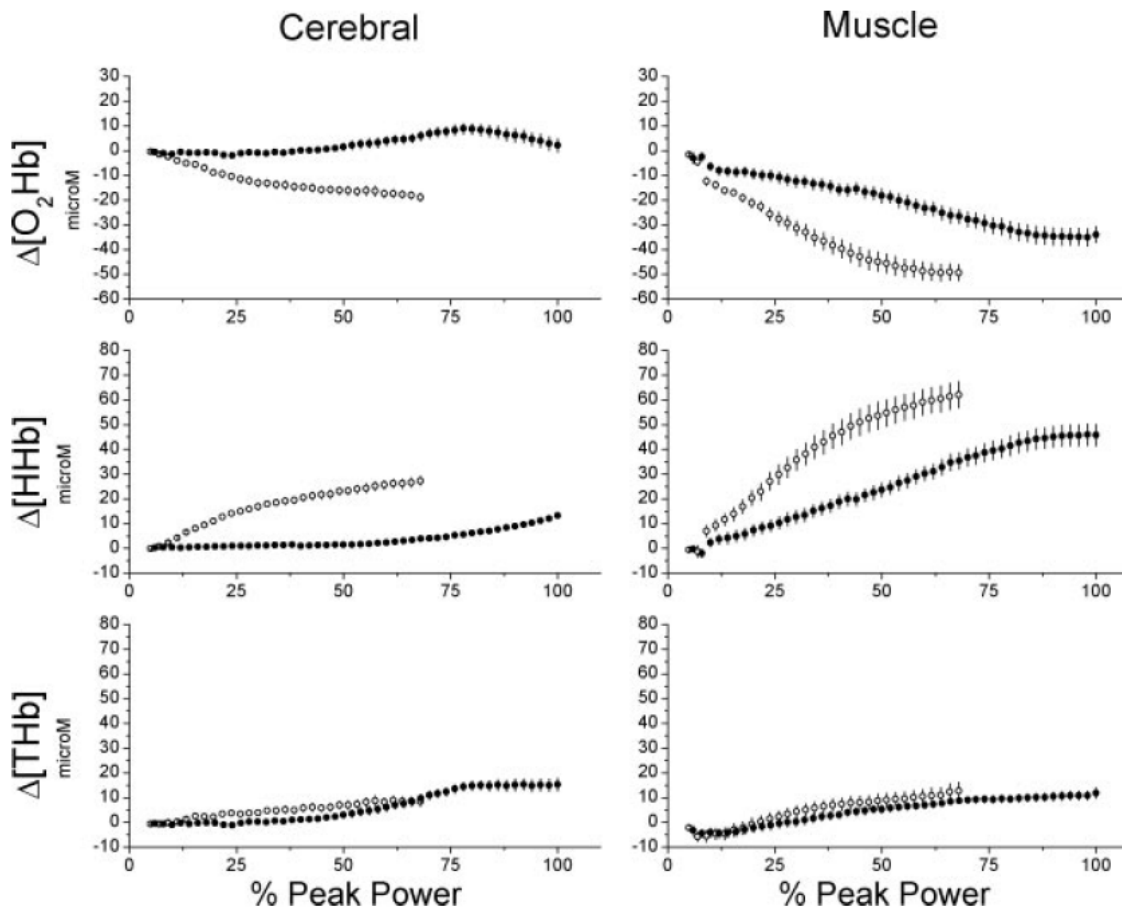

Figure 12 NIRS values during incremental exercise in normoxia (●) and hypoxia (○). All values are expressed relative to the peak power output achieved in normoxia (modified from Subudhi, 2007).

Therefore, during maximal exercise, exacerbated at exhaustion and in hypoxia, the circulatory system is challenged to facilitate oxygen delivery to working tissues through cerebral autoregulation which influences fatigue development (see paragraph 2.5) and muscle performance (see previous paragraph 2.1). Considering the wide spreading of new training methods in hypoxia, such as repeated sprint training (RSH) (Brocherie et al., 2017), Willis and colleagues recently evaluated the effects of different levels of normobaric hypoxia on the changes in peripheral and cerebral oxygenation and performance during repeated sprints to exhaustion. They concluded that performance in hypoxia is limited by continually decreasing oxygen saturation, even though exercise can be sustained despite maximal peripheral deoxygenation maybe

thank to a cerebral autoregulation of increased perfusion accounting for the decreased arterial oxygen content and allowing for task continuation, as shown by the continued cerebral deoxygenation (Willis et al., 2017).

Even though a lot of studies already showed the important role and the peculiarity of tissue oxygenation we don't know the effects of progressive hypoxia exposure on these indexes. So, considering our interest in the competitions that take place in progressive hypoxia, we aimed to better understand the pulse, muscle and cerebral oxygenation during a constant load effort in constant and progressive hypoxia with special emphasize on their effects on fatigue and endurance performance.

2.4 Cerebral blood flow and hypoxia

The degree of change in cerebral blood flow (CBF) at high altitude is influenced by many variables summarized by Ainslie and Subudhi (Ainslie and Subudhi, 2014) in terms of the relative strengths of four key integrated reflexes:

1) hypoxic cerebral vasodilatation;

2) hypocapnic cerebral vasoconstriction;

3) hypoxic ventilatory response;

4) hypercapnic ventilatory response

A more detailed scheme of variables acting on the regulation of CBF in hypoxia is presented in Figure 13.

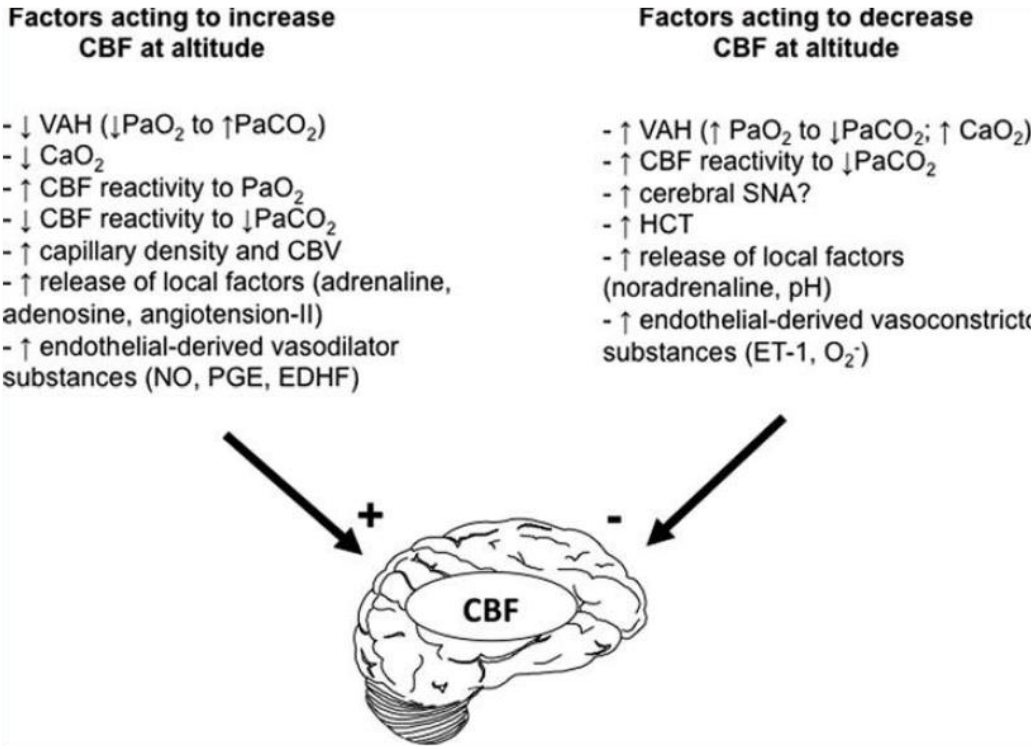

Figure 13 Summary of the major factors acting to increase (plus) and decrease (minus) CBF during exposure to hypoxia. Cao2, arterial oxygen content; CBV, cerebral blood volume; EDHF, endothelium-derived hyperpolarizing factor; ET-1, endothelin-1; HCT, hematocrit; NO, nitric oxide; O_2^-, superoxide; PGE, prostaglandins; SNA, sympathetic nerve activity; VAH, ventilatory acclimatization to hypoxia/altitude (Ainslie and Subudhi, 2014)**.**

Relative to its size, the brain is the most oxygen dependent organ in the body, but many environmental processes may either cause or result in changes to its oxygen supply. As such, studying blood flow to the brain at high altitude is an appropriate model to investigate both acute and chronic effects of hypoxemia on cerebrovascular function. A clear time-dependent changes in CBF during acclimatization to high altitude was reported by Severinghaus and colleagues which first reported a ~24% CBF increase within the first 6–12 hours of arrival at 3810m before decreasing to ~13% above sea level values by day 3–5 (Severinghaus et al., 1966). Severinghaus in a recent review reported an historical background on this matter but, primarily focused on early-hypoxia studies (Severinghaus, 2016). The apparent dose dependency in the CBF response to high altitude has been confirmed in two recent studies (Willie et al., 2014;

Wilson et al., 2011), yet it is unclear if the rate of ascent affects the CBF response. One study revealed a peak CBF increase of 60% following slow ascent to 5050m (Willie et al., 2014), while rapid ascent to 5260m (on oxygen), resulted in a peak CBF increase of 70% (Subudhi et al., 2014).

2.4.1 Acute and chronic effects on CBF

As reported in different studies and reviewed by Ainslie and Subudhi, the increase in CBF is closely matched to the reduction in arterial oxygen content (CaO_2); cerebral oxygen delivery is therefore well maintained across acclimatization (Figure 14). Whether mechanisms to maintain cerebral oxygen delivery evolved to protect the brain from hypoxemia is unknown (Ainslie and Subudhi, 2014).

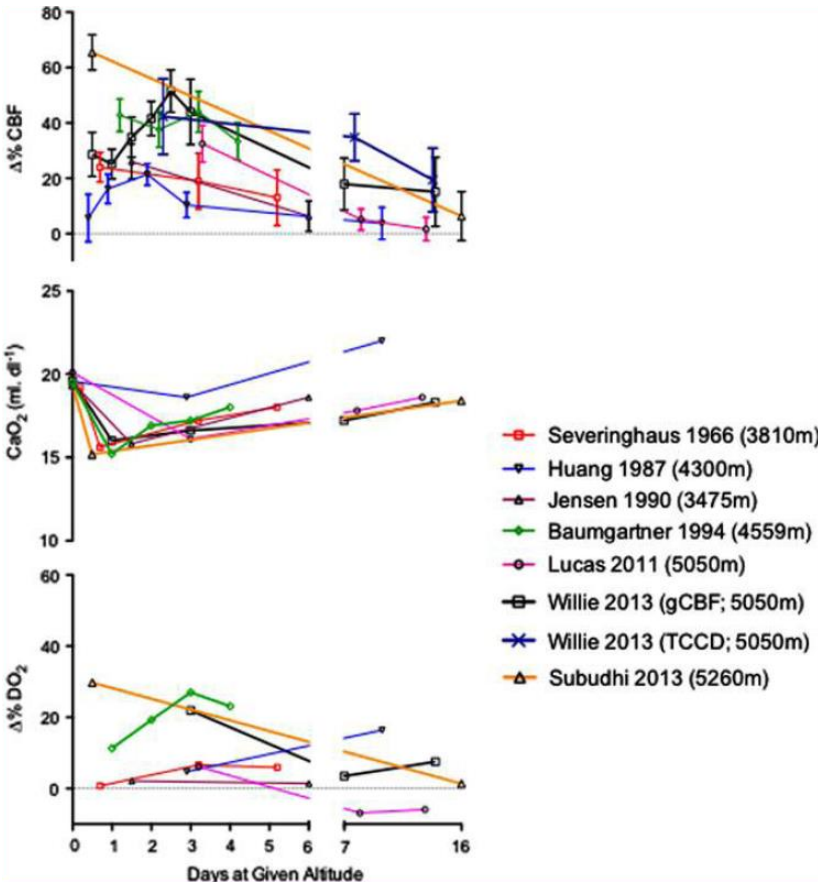

Figure 14 Percent changes in cerebral blood flow (D%CBF, top graph), arterial oxygen content (Cao2, in the middle), and cerebral oxygen delivery (CDO2, bottom graph) with time at high-altitude from seven studies at various altitudes and durations (Ainslie and Subudhi, 2014)

Transcranial Doppler is a widely used noninvasive technique for assessing cerebral artery blood flow. Only two studies (Willie et al., 2014; Wilson et al., 2011) have measured serial changes in CBF during progressive ascent to high altitude. Wilson and colleagues were the first who measured the vessel diameter during their study, while all previous high-altitude studies assessing CBF in the field by using Doppler to measure arterial blood velocity have assumed unaltered vessel diameter. They measured a ~24% dilation of the Middle Carotid Artery (MCA) occurred at 6400 m. Dilation of the MCA further increased to ~90% at 7950m and was rapidly reversed with oxygen supplementation (Figure 15). Cerebral oxygen delivery and oxygenation were maintained by commensurate elevations of CBF even at these extreme altitudes. They showed also how the increases in CBF via cerebral vasodilation were adequate to maintain oxygen delivery, even at these extreme altitudes (Wilson et al., 2011).

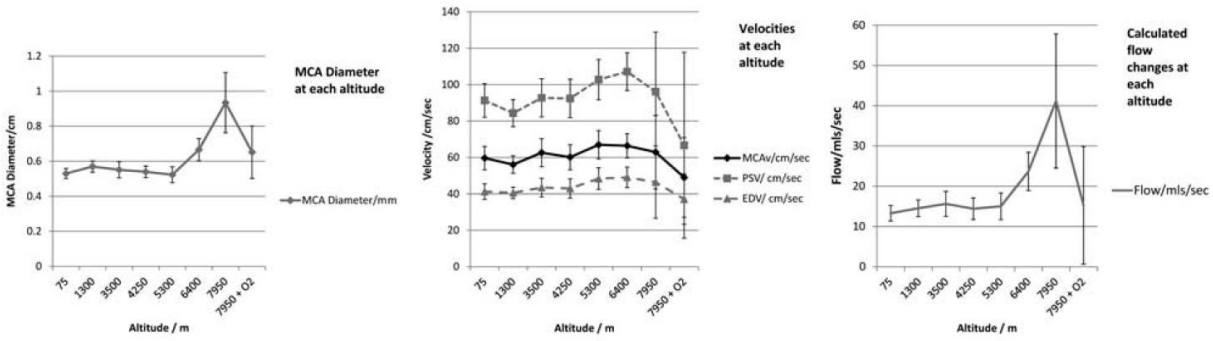

Figure 15 Middle cerebral artery (MCA) diameter (MCADiam), MCA velocity (MCAv) and calculated MCA flow (MCAFlow) (Wilson et al., 2011)

Whether high altitude populations exhibit cerebrovascular adaptations to chronic levels of hypoxia or if changes in CBF are related to the development of acute mountain sickness are currently unknown; yet overall, the integrated CBF response to high altitude appears to be sufficient to meet the brain's large and consistent demand for oxygen.

The well-known within- and between-individual variability in these factors likely highlights the variability in changes in CBF upon initial arrival at altitude and

difficultly in isolating independent effects of the respective mechanisms. The subsequent onset of ventilatory acclimatization coincides with the fall in CBF due to an increase in PaO_2 (and hence CaO_2) and decrease in $PaCO_2$ due to hyperventilation (Dempsey and Forster, 1982).

With acclimatization the influence of the PaO_2-induced threshold for cerebral vasodilation is attenuated and the degree of hypocapnia is accentuated; both factors, along with progressive elevations in hematocrit and CaO_2 over roughly 2 weeks seem to act attenuating the initial rise in CBF. In addition to these reflex responses, which most likely adjust during the acclimatization process, CBF is also influenced by several other hypoxic-induced changes such as capillary density (angiogenesis), adenosine, nitric oxide, blood viscosity/hematocrit, hypoxia-inducible factor, vascular endothelial growth factor, and free radicals (Ainslie and Subudhi, 2014).

There are still some concerns regarding CBF at altitude. As reported above we know about the sensitivity of the brain to different level of hypoxia and to "middle-to-long term" progressive hypoxia. To the best of our knowledge less is known about the effects on CBF to an acute exposure to progressive hypoxia. The aim of our project is to better understand CBF during acute progressive hypoxia without any physical effort and at different intensities (as a % of relative and absolute Peak Power output). We also aimed to determine whether these findings influenced the ones regarding fatigue, rate of perceived exertion and muscle and cerebral oxygenation and endurance performance.

2.5 Neuromuscular function and Hypoxia

Neuromuscular function can change under different conditions such as ageing, training/detraining, long-term spaceflight, environmental conditions (e.g. hypoxia, hyperthermia), diseases, therapy/retraining programs and also with the appearance of fatigue. Neuromuscular fatigue can be defined as any exercise-induced decrease in maximal voluntary force or power produced by a muscle or muscle group (Gandevia,

2001). Fatigue is generally quantified as a decrement in maximal isometric strength that develops soon after exercise onset. Fatigue is a complex, multifactorial phenomenon whose mechanisms are influenced by the characteristics of the task being performed (i.e. type and duration of the exercise, speed and duration of the muscle contraction) (Enoka and Stuart, 1992). The latter can be modified by the investigator aiming to impose fatigue in a standardized context or by a trainer seeking to change the characteristics of the athlete's training session. The reduction in force/power generating capacity involved in both whole body and single joint exercise of sufficient duration and intensity is determined by a peripheral and a central component of fatigue (Enoka and Duchateau, 2016).

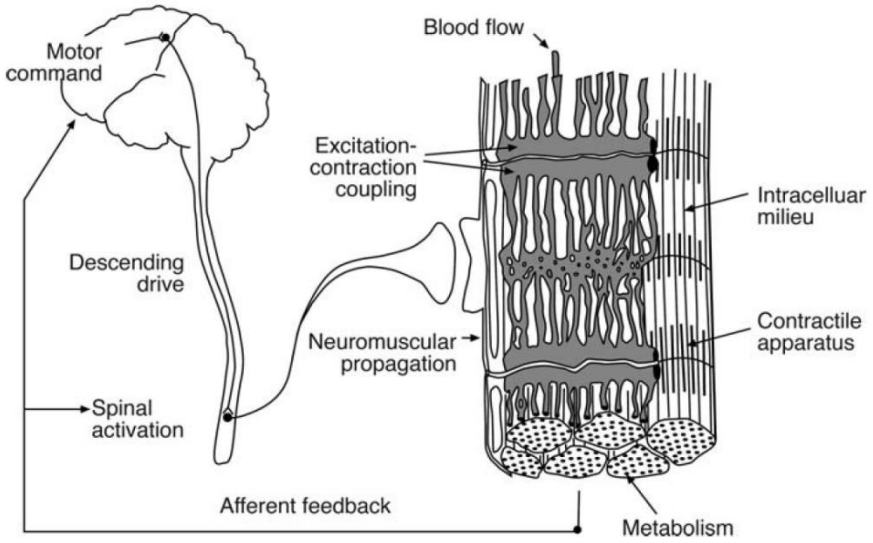

Figure 16 The physiological processes that can contribute to fatigue are classically categorized into two domains, those that establish the level of muscle activation (central) and those that influence contractile function (peripheral). (modified from Enoka, 2016)

Peripheral fatigue encompasses biochemical changes within the contracting muscle leading to an attenuated force/power response to neural excitation (Boyas and Guével, 2011). Central fatigue, structurally including the brain and the spinal cord (Figure 16), refers to the decrease in force/power secondary to a reduction in descending motor drive and/or the efficacy of the afferent pathways which combined result in a decrease

in the output from spinal motoneurons and thus voluntary muscle activation (Enoka and Duchateau, 2016).

Peripheral fatigue is classically identified as a decrease in twitch or tetanic force evoked by peripheral nerve stimulation on relaxed muscles (Millet et al., 2011). The most common method to characterize central fatigue is the maximal voluntary activation level (VA) calculated by the twitch interpolation technique (Merton, 1954). This technique consists of superimposing peripheral electrical stimulation during a maximal voluntary contraction (MVC), which results in a superimposed twitch (SIT). The greater the SIT relative to a reference peripheral nerve stimulation (PNS) on a relaxed muscle, the lower the VA. This observed activation deficit may be due to the inability of the motor units to fire quickly enough and/or to a failure in their spatial recruitment (Gandevia, 2001). Any decrease in VA with exercise characterizes the presence of central fatigue, which may originate anywhere at or above the site of the stimulation. The use of other techniques is, therefore, helpful to identify the different failure sites proximal to the neuromuscular junction, which may be spinal and/or supraspinal. For example, transcranial magnetic stimulation (TMS) has grown in popularity. Single and double pulse TMS (e.g. motorevoked potential; cortical silent period; short and long intracortical inhibitions) is extensively used to understand and characterize fatigue etiology in sport performance (Goodall et al., 2014a).

A decrease in systemic O_2 transport affect muscular performance and the rate of development of both central and peripheral fatigue. Amann and Calbet (Amann and Calbet, 2008) summarize in a review the effects of convective oxygen transport and its effects on fatigue and exercise (Figure 17).

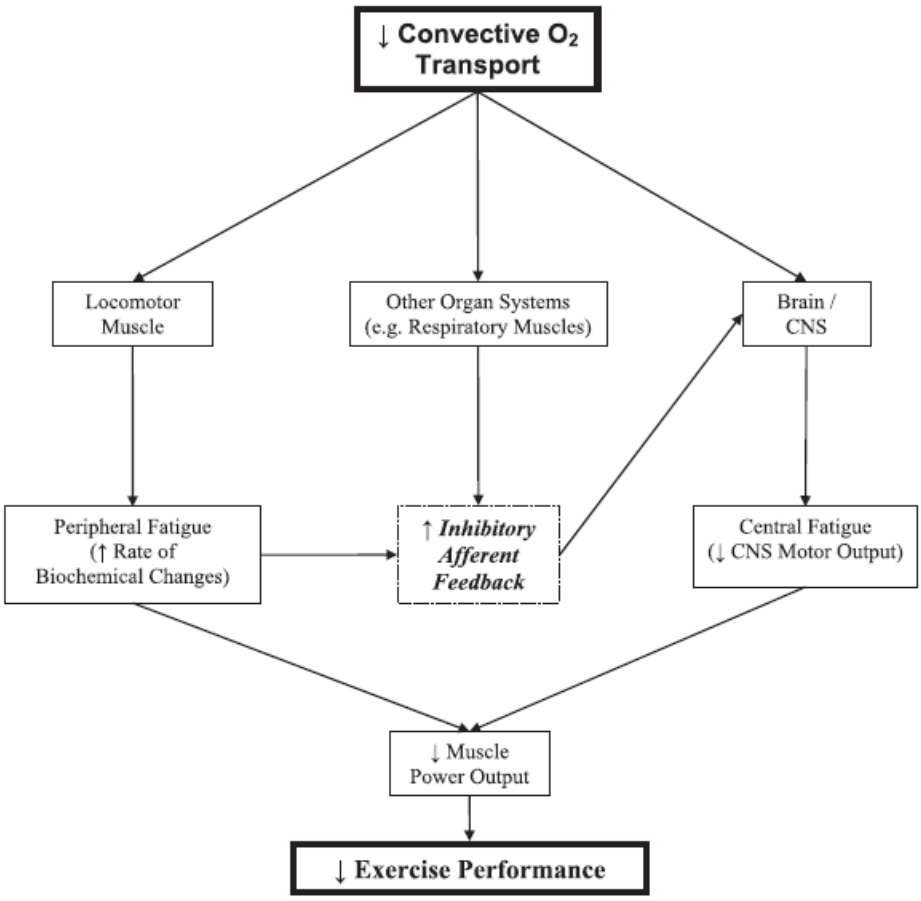

Figure 17 The scheme reviewed by Amann and Calbet explaining the role of convective transport of oxygen on exercise performance. (modified from Amann and Calbet, 2008)

Perrey and Rupp reviewed the effects of acute or prolonged exposure to hypoxia on human skeletal muscle performance and contractile proprietiesThey also proposed a scheme (Figure 18) to show the possible mechanisms underlying the impairment of the excitation–contraction coupling in human skeletal muscle with exposure to acute hypoxia (Perrey and Rupp, 2009). In fact, observations on humans indicate that hypoxia (during simulated ascent or prolongued exposure) exerts modest influences on the membrane propagation of the muscle action potentials during voluntary contractions. Overall in humans, in physiological conditions including climbing the Mt. Everest, there are extraordinarily little changes regarding the maximal force-

generating capacity (Figure 19). Interestingly, it appears that the adaptations to chronic hypoxia minimize the effects on skeletal muscle dysfunction (i.e. impairment during fatigue resistance exercise and in muscle contractile properties) that may occur during acute hypoxia for some isolated muscle exercises. Only sustained isometric exercise exceeding a certain intensity (30% MVC) and causing substantial and sustained ischemia is not affected by acute hypoxia (Figure 20).

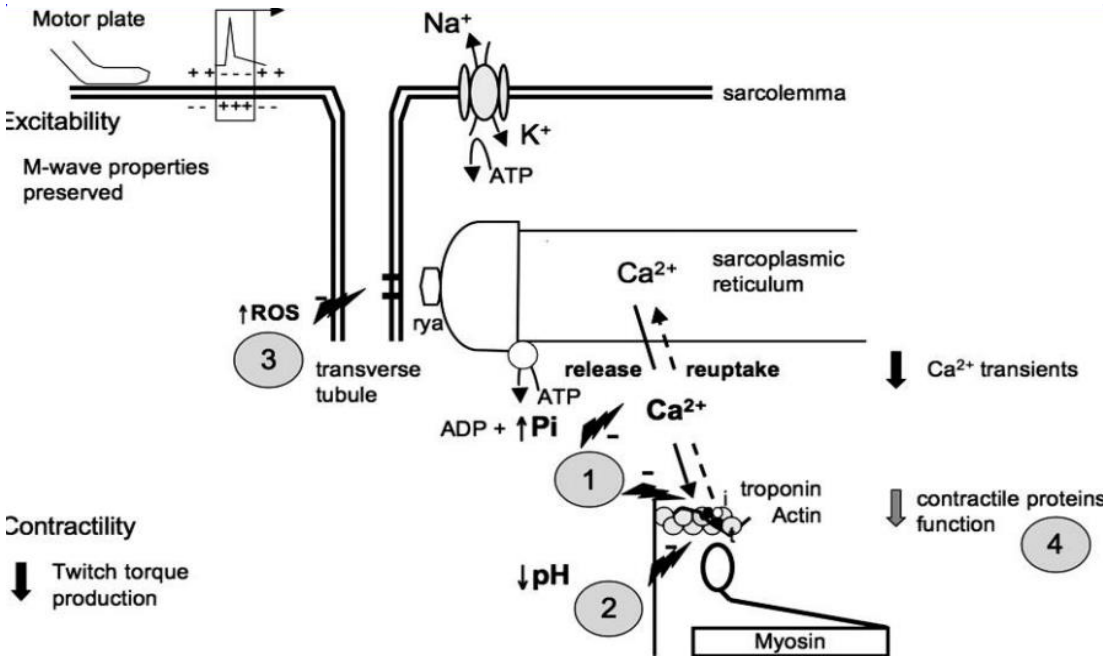

Figure 18 – A scheme to show the possible mechanism underlying the impairment of the excitation-contraction coupling in human skeletal muscle with exposure to hypoxia. For further details see Perrey and Rupp (Perrey and Rupp, 2009)

Muscle group	Experimental protocol	Hypoxic conditions: Type	Hypoxic conditions: Degree (m)	Results in MVC	References
Knee extensors	43-day stay; hypobaric	Chronic	5050	=	Esposito et al., 2003
	Short-term hypobaric; simulated altitude	Acute	~3700	=	Bowie and Cumming, 1971
	Short-term hypobaric; simulated altitude	Acute	4300	=	Fulco et al., 1996
	Normobaric gas mixture; F_{IO_2} = 10%–11%	Acute	~4500 to 5000	=	Eiken and Tesch, 1984; Katayama et al., 2007; Millet et al., 2008
	Normobaric gas mixture; F_{IO_2} = 12%	Acute	~4000 to 4500	=	Degens et al., 2006

Acute: short-term hypoxia (≤24 h); F_{IO_2}, inspired fraction of oxygen. =, no changes, ↑, increase; ↓, decrease.

Figure 19 Table summarizing the effects of acute and chronic hypoxia on maximal force-generating capacity of knee extensors isolated muscle group in resting state in humans (modified from Perrey and Rupp, 2009). Only data of knee extensors group muscle because they are typically involved in the process of evaluation of neuromuscular function and they are also involved in activities like walking and cycling (both upright and recumbent) (Bouillon et al., 2016).

Muscle group	Experimental protocol	Hypoxic conditions: Type	Hypoxic conditions: Degree (m)	Results	References
Knee extensor	Normobaric gas mixture, F_{IO_2} = 12%; static endurance test	Acute	~4000 to 4500	↓Sustained 30% MVC, = sustained 70% MVC	Degens et al., 2006
	48-h sojourn at simulated altitude; strength endurance exercise (repeated isokinetic contractions)	Chronic	4572	=	Young et al., 1980
	Normobaric gas mixture, F_{IO_2} = 11%; 60 MVC + sustained static contraction at 27% MVC until exhaustion	Acute	~4000 to 4500	Faster decline in peak torque, = average peak torque, ↓static endurance time (−20%)	Eiken and Tesch, 1984
	Short-term hypobaric hypoxia; one-leg dynamic contractions (1 Hz) at 60% peak work rate until exhaustion	Acute	4300	↓Time to exhaustion(−56%)	Fulco et al., 1996
	Normobaric gas mixture, F_{IO_2} = 11%; three sets of 9 intermittent, isometric, unilateral contractions at 62% MVC	Acute	~4000 to 4500	Faster rate of decline of MVC during fatiguing exercise	Katayama et al., 2007
	Normobaric gas mixture, F_{IO_2} = 11%; one-leg intermittent isometric contractions (10 sec/10 sec) at 50% MVC until exhaustion	Acute	~4000 to 4500	↓Repetitions to exhaustion (−13%)	Millet et al., 2008

Acute: short-term hypoxia (≤24 h); F_{IO_2}, inspired fraction of oxygen; MVC, maximal force-generating capacity. =, no change; ↓, decrease.

Figure 20 Table summarizing the effects of acute and chronic hypoxia on endurance time to fatiguing dynamic or static contraction of knee extensors muscle group in humans (modified from Perrey and Rupp, 2009)

Moreover, Goodall and colleagues (Goodall et al., 2012) asked whether central processes of fatigue would be increased after strenuous exercise in environments where oxygen availability is reduced (hypoxia) compared to the same absolute exercise intensity at sea-level. Their main finding was that the contribution of central processes to fatigue was increased after exercise in hypoxia (equivalent to ~3800 m above sea-level). The greater amount of central fatigue in hypoxia was due to suboptimal neural output from the brain and was associated with reductions in oxygen availability. The findings provide a plausible mechanism for why exercise performance is impaired at high altitude and might help our understanding of exercise limitation in patients with reduced oxygen delivery to the brain.

Goodall et al. (Goodall et al., 2014b) also investigated whether acclimatization to chronic hypoxia attenuates the level of supraspinal fatigue that is observed after locomotor exercise in acute hypoxia. They provide data indicating that the exacerbated supraspinal fatigue after exercise in acute hypoxia is attenuated after 14 days of acclimatization to altitude. The reduced development of supraspinal fatigue in cronic hypoxia may have been attributable to increased corticospinal excitability, consequent to an increased cerebral O2 delivery.

In 2014, Girard and Racinais conducted a study in order to better understand the effects of a combination of heat stress and moderate hypoxia on cycling time to exhaustion performance. They concluded that moderate hypoxia in combination with heat stress reduces cycling time to exhaustion without modifying neuromuscular fatigue characteristics. Impaired oxygen delivery and/or increased cardiovascular strain due to an increasing relative exercise intensity, may have also contributed to earlier exercise cessation, without modifying neuromuscular fatigue characteristics (Girard and Racinais, 2014). The use of absolute power output in studies comparing the effects of hypoxia on neuromuscular function could be a limitation.

Hypoxic environments are part of most of the endurance and ultra-endurance performances (Clark et al., 2007; Peinado et al., 2018) and the processes regarding

neuromuscular function are still debated. Moreover, to the best of our knowledge, the effects on fatigue of a performance where the severity of hypoxia changes during the trial (Progressive Hypoxia) are still unclear.

2.6 Hypoxia and Rate of Perceived Exertion and Acute Mountain Sickness

As reported above, several studies have clearly demonstrated that hypoxic conditions affect endurance performance and imply different physiological responses (Calbet et al., 2003; Cerretelli, 1976; Peltonen et al., 1995). These environmental conditions also appear to alter subjective feelings and perceptions of exertion during exercise, but few studies have investigated this hypothesis.

Horstman and colleagues (Horstman et al., 1977) compared short term and prolonged work of equal relative intensities (i.e., % VO_{2max}), at 4300 m to those at sea level. Twenty subjects cycled for six minutes at 60, 80 and 95% of their relative VO_{2max} and to exhaustion at 85% VO_{2max}. At 4300 m, VO_{2max} was reduced by 19%, while Ventilation (VE_{max}) and respiratory exchange ratio (R_{max}) increased by 17 and 8%, respectively; Heart $Rate_{max}$ and rate of perceived exertion (RPE_{max}) was unchanged. At 4300 m, RPE at the lower intensities and early during prolonged work were significantly less than at sea level. These differences were reduced and finally eliminated as work intensity increased toward maximal or as prolonged work continued to exhaustion. Endurance time to exhaustion at 4300 m was not different from sea level (Horstman et al., 1977).

In a study comparing an exercise at sea level and at 4300 m asl, different RPE (including an overall rating as well as a local muscular rating and a central or cardiopulmonary rating) were collected during a 30 minutes cycling at 85% of the VO_{2max} in a group of Low Altitude Residents. Despite reduced absolute exercise intensity during acute (less than 2 hours) high altitude exercise, local (e.g. legs) RPE were unchanged from sea level values. Chronic (18 days) high altitude exercise, however, was associated with a significant reduction in local (e.g. the muscles

involved) RPE (Young et al., 1982). Thus, differences can be found when perceived effort is monitored in acute and/or chronic conditions.

Recently, to examine the physiological strain associated with hypoxic high intensity interval training (HHIT), 8 highly trained young runners (age, 18.6 years) randomly performed, 5 x 3-minute intervals in either normoxic (N, 90% of the velocity associated with VO_{2max}, vVO_{2max}) or hypoxic (H, simulated 2,400-m altitude, 84% of vVO_{2max}) conditions. Although perceived as harder, they concluded that HHIT is not associated with an exaggerated physiological stress in highly trained young athletes (Buchheit et al., 2012).

Mellor and colleagues (Mellor et al., 2014), showed a link between the subject's RPE (monitored with the version of the scale with a rating ranging from 6, no exertion at all, to 20, maximal exertion) and the development of acute mountain sickness (AMS) during a field study. AMS is the most common form of acute altitude illness and typically occurs in un-acclimatized people ascending to altitudes >2500 m (especially for real altitudes, less typical in normobaric hypoxia), although it can develop at lower altitudes in highly susceptible individuals. Established risk factors include rate of ascent, altitude reached, and individual predisposition (Roach et al., 2018). In Mellor's study, subjects who reported a higher level of perceived exertion were much more likely to experience AMS. While the data should be viewed as pilot ones, they raised the possibility for using perceived exertion scales as a way to influence behaviour and reduce the incidence of AMS. These results support to the view that hard exercise during a trekking in altitude is a trigger for AMS. We need to take into consideration these results because, as the authors reported, one potential criticism of their study is that the investigation was not designed specifically to look at the relationship of exercise intensity to AMS and they did not control the pace of individual subjects. They concluded stating that this makes the finding that increased perceived exertion leads to AMS more intriguing and opens the possibility that controlling the exercise intensity (i.e. performing a correct and specific hypoxic dose-related fitness evaluation) could reduce AMS rates. They also postulated that efforts to reduce the perception of exertion

per se, through better education, fitness training, or reducing anxiety may lead to reductions in AMS. Finally, it must be considered that it seems that there is a different response in groups of low altitude natives (LAN) and moderate altitude natives, especially when they perform at 4270 meters asl. At this altitude it is possible to find a relationship between RPE and AMS in LAN (Maresh et al., 1993).

Using the Borg RPE score during a "real world" exercise stimulus is a logical and attractive proposition. Firstly it requires no specialist equipment and is highly reproducible [at least at sea level, (Borg and Kaijser, 2006)] secondly, and importantly, multiple other factors can have an impact on perceived exertion during an acute/chronic exposure to hypoxia (such as AMS, poor sleep, viral illness, etc.). This would not alter the work rate required to perform a test at a fixed proportion of VO_{2max} (even if altitude specific) but would alter perceived exertion and those other conditions could predispose to AMS.

Considering our interest in the competitions that take place in progressive hypoxia and as a consequence of highly reproducibility of this tool, we aimed to better understand the RPE during a constant load effort in constant and progressive hypoxia as a first observational study with the final aim to use RPE production as a tool to set the right intensity at altitude.

Even though those studies used perceived exertion, to the best of our knowledge there are few data about the validations of this scales in hypoxic conditions. At sea level conditions it is possible to find a comparison between three rating scales for perceived exertion and two different work tests (Borg and Kaijser, 2006) and another experiment on perceived exertion, where Category Ratio 100 (CR100) was compared with Absolute Magnitude Estimation (AME) (Borg and Borg, 2002). Therefore, a side part of our project aim is to verify the validity of session-RPE as a measure of training load at different altitudes.

Moreover, as already well-known, training quantification is essential to evaluate an endurance athlete's responses to the training loads, ensure adequate stress/recovery

balance and determine the relationship between training and performance. Among subjective methods of quantification the RPE stands out because of its wide use (Mujika and Iñigo, 2017). Therefore, a more detailed knowledge about RPE in hypoxic environments should be of great interest in the future. This is particularly relevant when considering the use of this tool in order to better monitor training load during altitude training camps both for endurance and team sport athletes that nowadays can use new hypoxic training methods such as the above mentioned repeated sprint ability in hypoxia.

2.7 Acute Progressive Hypoxia

Sojourns to terrestrial high altitudes have grown in popularity in recent years, with the World Health Organisation reporting that approximately 35 million people visit terrains greater than 3000 m every year. And it is estimated that more than 100 million people per year travel to the hypoxic environments found at altitudes above 2500 m (Netzer et al., 2013). Given that people often reach altitude in a brief period of time, researchers have focused on safety and exercise tolerance which have been tested after acute high-altitude exposure, at different ascent rates, in patients with coronary artery disease (Schmid et al., 2006) as well as in patients with chronic heart failure (Schmid et al., 2015). In both cases they tested their subjects after a travel from Bern (Switzerland, 540 m) to Jungfraujoch (Switzerland, 3454 m) with a rate of ascent of 0.23 meters/second. When arrived at the top, they asked to the subjects to wait 1-2 hours before starting the testing procedure. Once considered the gain in altitude, they found typical decreases in VO_{2max} (~20%) and in SpO_2 (~7%) and an increase in resting heart rate (~7 bpm). With a similar approach Bradwell and colleagues investigated the effect of acetazolamide [Acetazolamide is a drug that causes mild diuresis and increases renal excretion of bicarbonate, causing a mild metabolic acidosis which in turn increases respiratory rate (improving oxygenation)(Williamson et al., 2018)] on exercise performance during early acclimatization to altitude, testing their subjects

after an 1-h travel by cable-car starting from Cervinia (Italy, 2050 m) to Rifugio Testa Grigia (Italy, 3459 m), with an ascent rate of ~0.4 m/s.

Zhang and colleagues reported that when human body at rest is exposed to a rapid (10 minutes) and "important" (sea level to 3600 m) exposure to a progressive increase in altitude heart rate increased from 78.59 ± 3.73 (bpm) at SL to 86.93 ± 5.55 (bpm) at 3600 m (Figure 21). Conversely, SpO_2 decreased from 97.75 ± 0.87 % at SL to 88.33 ± 2.54 % at 3600 m (Figure 22). The rate of ascent used for this study was 6 m/s (Zhang et al., 2015).

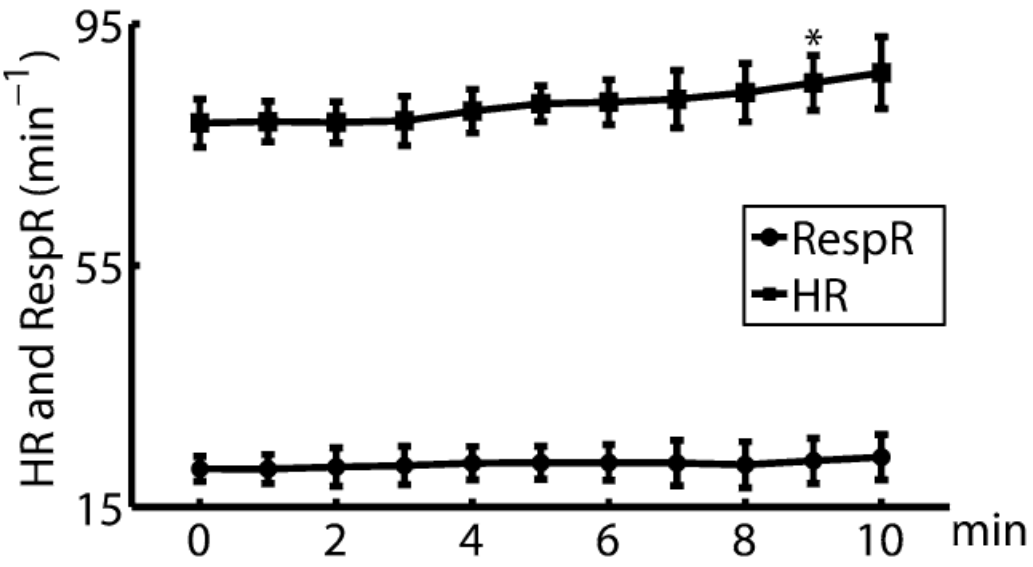

Figure 21 Heart rate monitored during 10 minutes exposure to a progressively increase in altitude from sea level to 3600 m. It was 85.24 ± 4.28 at 3240 m or the 9th min and it was significantly faster than that at SL (* indicates p = 0.0011). Respiratory rate (RespR) was not changed. (modified from Zhang, 2015).

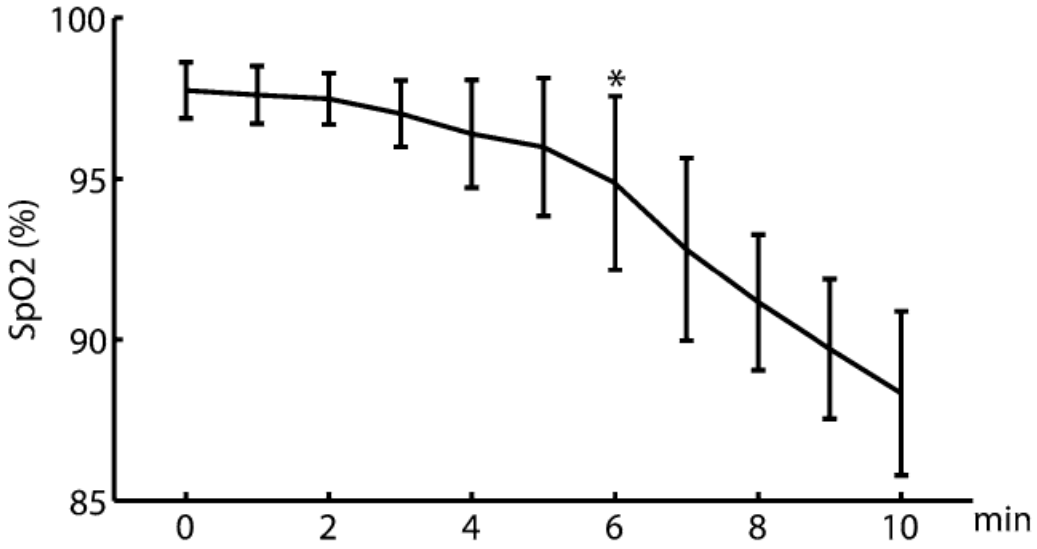

Figure 22 Pulse oximetry monitored during 10 minutes exposure to a progressively increase in altitude from sea level to 3600 m. SpO_2 was 94.87 ± 2.70 at the 6th min corresponding to 2160 m and decreased significantly to SL (* indicates p = 0.0077) (modified from Zhang, 2015).

Also other variables, such as balance, has been investigated immediately after exiting the cable car at 3200 m in eighty-nine subjects where postural stability was immediately reduced, particularly in subjects above the age of 40 years with an expertise in mountain climbing of less than 20 days/year (Bruyneel et al., 2017). This information, for example can have an important application for alpine skiers. In this case the ascent rate was not reported, but we can consider a mean value calculated from other famous cable-car ascent rates, typically ranging from 2.3 to 4.2 meters/second.

Furthermore, there is a greater prevalence of altitude and hypoxic training camps amongst elite athletes in preparation for major competition. Moreover, as already previously reported, hypoxic environments are part of most of the endurance and ultra-endurance competitions (Clark et al., 2007). Moreover, in 2013, our lab had a request by an elite athlete to help him in the training and pacing of the Red Fox Elbrus race that is characterized by an only uphill route starting from 2330 metres to the top of the mountain (0.25 meters/second of ascent rate for the winner). We set up a testing

protocol with 3 sessions, one in normoxia and two in hypoxia at 3400 and 5400 meters of simulated altitude. We were able to give him the right advices in order to pace the competition in a correct way and win it with the record of the race. But, even if this experience had a happy ending we were aware that more knowledge is needed especially when athletes need to perform against a more or less constant increase in altitude (i.e. progressive increase in hypoxia). In most of the competitions and performances performed in hypoxia there is a common peculiarity: athletes start from an altitude and finish to a higher one. Furthermore, the increase in altitude during these efforts is more or less linear.

Even some studies already showed the trend of different physiological response as well as safety and exercise tolerance when exposed to acute hypoxia during exercise and at rest, we don't know the effects of a progressive exposure hypoxia on these indexes. So, considering our interest in the competitions that take place in progressive hypoxia, we aimed to observe these variables during a constant load effort in constant and progressive hypoxia with a special remark on their subsequent effects on fatigue and endurance performance.

2.8 Aim of the project

As previously reported, it is well known the physiological demands at different altitudes. However, the determinants of a performance where the severity of hypoxia changes during the trial (Progressive Hypoxia, (PH)) is still unclear. Also the interaction between hypoxia and exercise intensity needs to be better investigated.

The aim of this thesis was to go deeper in the knowledge of the determinants of endurance performance in hypoxic conditions with a special emphasize to the responses associated with progressive hypoxia. The investigation was divided in two studies. The protocol has been designed in order to obtain information both on pre to post effect of the interventions and on the physiological responses observed during the

1-hour intervention. Where possible, the protocol aimed to determine both the central and peripheral effects.

2.8.1 Aim of the two studies

Aim of Study I: This study was designed to investigate the effects of submaximal cycling during an acute exposure to a constant and a progressive hypoxia on the high intensity performance of a hypothetical subsequent final effort tested through a TTE. This could have a practical application deriving from the interest to know if after an endurance effort in progressive hypoxia, our ability to perform at high intensity is impaired.

More in detail, we aimed to determine the effects of exposure to constant (FiO_2 13.4% - reproducing and altitude of 3500 m asl, Hcost) vs progressive hypoxia during an 1-h of constant exercise at 50% (HH) of the relative (adapted for condition's FiO_2) Peak Power Output (PPO) on fatigue and endurance performance tested through a TTE. We ran a comparison with a normoxic condition (N). We also aimed to observe the main physiological responses during the 1-hour interventions.

Hypothesis of study I: Regarding the exposure in constant condition of FiO_2 (hypoxia vs normoxia; Hcost vs N), we hypothesized, due to the choice of same relative intensities of effort, a similar impairment in performance. Considering the different hypoxic dose exposure, we hypothesised a minor impairment in performance in HH compared to Hcost, even with similar oxygen consumption during the 1-h intervention and similar effects on neuromuscular function. According to the three different fractions of inspired oxygen, the hypothesis is to obtain a response in pulse oxygenation characterized by the same trend of FiO_2, related to altitude.

Aim of Study II: The present study was planned to investigate the effects of an acute exposure to progressive hypoxia (PH). To answer the research question subjects rested and exercised at two different intensities during 1-h exposure in PH. This is an

observational study regarding the effects on a subsequent high-intensity endurance performance, as well as on the neuromuscular function, but with the aim also to investigate the physiological responses during the three exposures. The findings could be an help considering the increasing number of competitions in hypoxic environments and characterized by a linear gain in altitude during the course of the race, and the need of more knowledge to train athletes involved in this kind of events.

More in detail we aimed to determine the effects of the 1-h interventions at different work intensities to PH on fatigue and endurance performance. A comparison between NO physical effort (resting on the bike, H_NoPO), a relative intensity (e.g. 50% of the PPO obtained in a ramp incremental test performed at FiO_2 13.4%, HH) and an absolute intensity of effort (e.g. 50% of PPO in Normoxia, HN) has been conducted. The last two sessions were planned also with a secondary aim to understand the possible role of performing an endurance task in hypoxia at relative intensities determined during tests at the same hypoxic condition, in order to have more ecological (i.e. closer to the real-world scenario) conditions. We also aimed to observe the main physiological and perceived responses during the 1-hour interventions.

Hypothesis of study II: Regarding the effects on endurance performance, the hypothesis is that only after the HH and HN conditions there will be impairments related to the previous submaximal task. Central aspects of fatigue will be influenced by the increase in altitude from pre to post 1-h measurement, while peripheral aspects will be influenced only by the intensity of the subsequent TTE tests. We hypothesized a different physiological and perceived responses collected during the 1-h interventions due to intensity of effort.

3 Materials and Methods

3.1 Subjects

Thirteen healthy men have been enrolled for this project. The main characteristics of the eligible subjects are given in Table 1. According to De Pauw and colleagues (De Pauw et al., 2013) the participants were categorized in the performance level 2 and 3, being considered recreationally trained and endurance trained subjects (see maximal oxygen consumption in Table 5). Before starting the projects all of them underwent a general physical assessment by a physician. None of the participants involved had clinical evidence of cardiovascular, neuromuscular, or musculoskeletal problems. One of them reported knee joint pain during the familiarization trial after the second test session and he was therefore excluded from the rest of the project. Table 2 and Table 3 report the main characteristics of subjects involved in study I and study II. They were instructed to avoid caffeine, alcohol and high-intensity exercise during the 24-h preceding each test session. Before data collection, all participants were properly informed about the experimental protocols, they voluntarily accepted to participate and gave their written informed consent for the studies. The experimental protocols and procedures were approved by the institutional ethics committee of the Department of Neurosciences, Biomedicine and Movement Sciences of the University of Verona and they were performed according to the ethical standards laid out in the 2013 revision of the Helsinki Declaration for experimentation on human subjects.

Table 1 Main characteristics of the 13 subjects involved in the initial part of the project

	Age	Weight	Height	Body fat	Training	
	(y)	(kg)	(cm)	(%)	(h/week)	(sessions/wk)
mean	34.1	69.1	175.3	11.2	7.6	4.8
s.d.	9.7	6.0	4.6	3.1	3.0	1.0

Table 2 Main characteristics of the 8 subjects involved in the study I

	Age	Weight	Height	Body fat	Training	
	(y)	(kg)	(cm)	(%)	(h/week)	(sessions/wk)
mean	28.9	67.5	174.1	10.8	8.1	5.0
s.d.	3.4	6.8	3.7	3.7	3.1	0.7

Table 3 Main characteristics of the 11 subjects involved in the study II

	Age	Weight	Height	Body fat	Training	
	(y)	(kg)	(cm)	(%)	(h/week)	(sessions/wk)
mean	32.1	69.2	175.5	11.3	7.9	4.7
s.d.	6.8	6.6	4.1	3.2	2.7	0.9

3.2 Materials

3.2.1 Hypoxic chamber

The normobaric 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). The mean barometric pressure at the altitude of the chamber (204 m asl) was 750 mmHg. With the aim to conduct all the tests under controlled laboratory conditions, an automatic system (Albafrigor, Italy) was used to keep temperature and humidity constant (18 °C, ~50% relative humidity). A CO_2 scrubber ran continuously to remove the carbon dioxide expelled by the body while exercising in the chamber. Manipulating FiO_2 we can simulate altitude as shown in Figure 23.

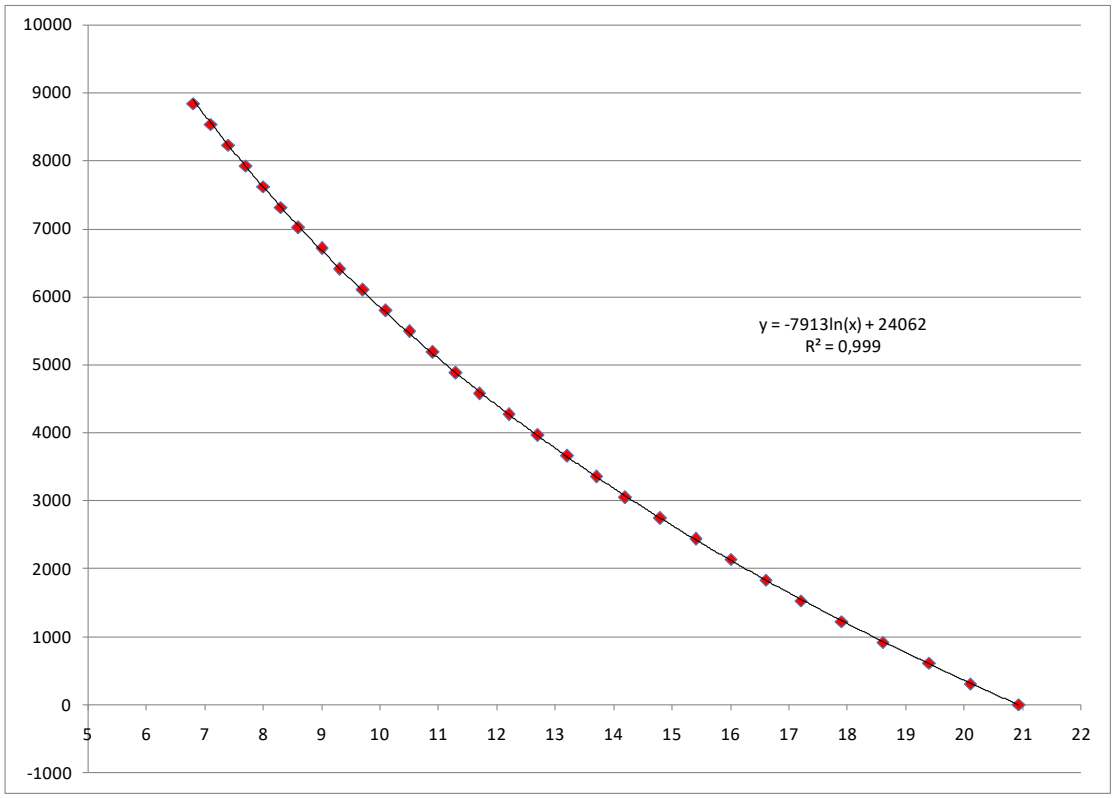

Figure 23 The relationship between FiO2 and altitude simulated in the hypoxic laboratory

3.2.2 Cycle ergometer

All the evaluations were performed on a reclined cycle ergometer (E1200, Cosmed Srl, Rome, Italy, Figure 24) tilted to 50° from the vertical line. This ergometer has been chosen due to its ability to recline at a position able to allow an easier evaluation of femoral blood flow during cycling exercise. The power output on the ergometer was set up manually both during the ramp incremental test and the steady-state interventions.

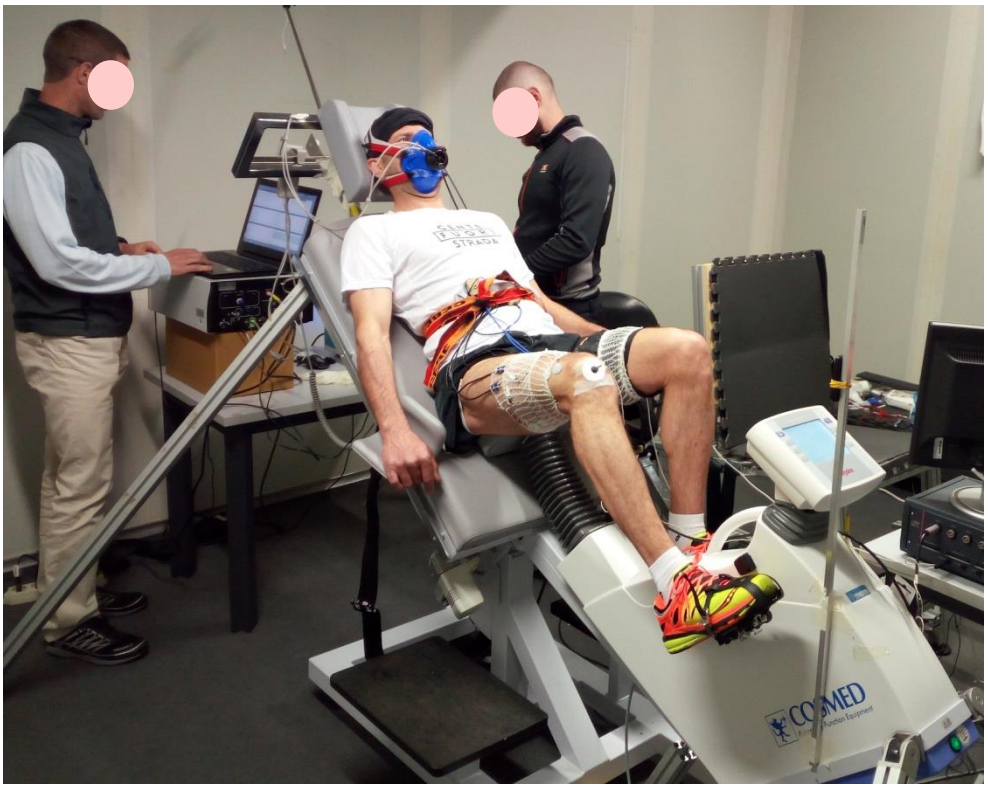

Figure 24 Experimental setup with the recline cycle ergometer, E1200, Cosmed Srl, Rome, Italy.

3.2.3 EchoDoppler

Measurements of arterial blood velocity and vessel diameter were performed with Logiq S7pro ultrasound system (General Electric Medical Systems, Milwaukee, WI). The system was equipped with 12- to 14-MHz linear array transducers for femoral blood flow (FBF) while with a 3Sc-RS cardiac probe for CBF. Common femoral artery diameter was determined at a 90° angle along the central axis of the scanned area. Blood velocity (v) was measured using the same probe at a frequency of 5 MHz. Measurements of v were obtained second by-second with the probe positioned to maintain an insonation angle of 60° or less and the sample volume was centred and maximized according to vessel size. After arterial diameter and mean v (v_{mean}) assessment, FBF was calculated using the equation suggested by Venturelli and

colleagues (Venturelli et al., 2017). All scanning and blinded analyses were performed by experienced and skilled sonographers. This approach has been used to obtain:

- FBF in the right limb, distal to the inguinal ligament and proximal to the deep, superficial femoral bifurcation
- CBF with the system equipped with a non-linear array (3Sc-Rs cardiac probe) using a colordoppler approach for the determination of the CBF in the right Middle Carotid Artery (MCA).

The EchoDoppler measurements were collected by the same researcher throughout the entire project. The researcher is a medical Doctor and was trained due to previous utilization of the machine. According to Menegatti and colleagues the inter observer agreement between trained operators was much more reliable that one of non-trained operators (K coefficient 0.80 95% CI 0.59-1.01). Moreover, the intra observer variability rate in trained operators was 0.93, (95% CI 0.80-1.06) confirming a highly satisfactory agreement (Menegatti et al., 2010).

3.2.4 Near infra-red spectroscopy (NIRS) and pulse oxymetry

Muscle (Mox) and cerebral (Cox) oxygenation were monitored by means of Near-InfraRed Spectoroscopy (NIRS). NIR-determined hemodynamics reflect the dynamic balance between O_2 demand and O_2 supply in the tissue microcirculation. Local muscle oxygenation of the vastus lateralis muscle was measured with near-infrared spectroscopy (NIRS, Nimo-Nirox, Brescia Italy). The natural transparency of the skin and the muscular tissue to near-infrared light (from 650 to 1000 nm) allowed measuring the change in oxy- and deoxyhemoglobin concentration in the muscle tissue. The interaction of radiation-tissue is regulated by 2 physical characteristics: the first is the absorption, which is the attenuation of the intensity of the NIRS signal through the tissue and it is due to water, lipids, oxyhemoglobin (HbO_2), and deoxyhemoglobin (HHb); the second is the scattering that consisted of a deviation from straight trajectory by the nonuniformity characteristic of tissue. This technique, furthermore, performs accurate optical absorption measurements at specific wavelengths that are converted

into quantitative hemoglobin concentration (HbO_2 and HHb) using a proprietary algorithm. The probe was an active emitter unit that comprised a laser diode source (wavelengths from 670 to 980 nm) and a multiplexer (optomechanical switch). NIRS provide also an index of absolute oxygenation, called Tissue Oxygen Index (TOI, %) calculated as $[HbO_2]/([Hb]+[HbO_2])*100\%$. The sampling frequency was 40 Hz. The skin was carefully shaved and cleaned before each test. A skinfold thickness at the site of application of the NIRS probe on the vastus lateralis was determined before the warm-up using a caliper and the value of the subcutaneous adipose tissue was inserted into the Nimo-Nirox software. The probe was firmly attached to the skin overlying the distal third of vastus lateralis muscle (15 cm from the knee joint, kept constant between each session by means of permanent marker signs) of the left limb, parallel to the major axis of the thigh, by a biadhesive tape (Quaresima et al. 2004). A bandage was used to cover the probe from the external light sources.

PreFrontalCortex (PFC) Oxygenation was measured by means of the same device but with a probe only able to obtain variations in the concentrations of the main variables starting from a baseline measurement. PFC synthesizes information from a wide range of brain systems and exerts control over cognitive and executive behaviour (e.g., decision-making, movement planning, pacing strategies), so that these associative areas play a central role in the orchestration of thoughts and actions in accordance with internal goals (Rupp et al., 2013). The distance between the electrodes was fixed at 3.0 cm and the probe holder was secured to the skin with double-sided tape and maintained with an elastic black headband aiming to cover the probe from the external light sources. It is possible to obtain TOI also on the PreFrontalCortex (PFC) NIRS but not elsewhere on the head.

Basically, the parameters gathered by the Nimo-Nirox NIRS device are (quantitative for the Mox and variations from baseline for the Cox) $[HbO_2]$, [HHb], [tHb] (HbO_2 + HHb) and TOI. Because [HHb] is closely associated with changes in venous O_2 content and is less sensitive to [tHb] than $[HbO_2]$, [HHb] provides a highly sensitive measure of changes in muscle deoxygenation status due to O_2 extraction, while $[HbO_2]$ seems

to be the most sensitive indicator of regional CBF modifications. Furthermore [tHb] reflects the changes in tissue blood volume within the illuminated area so that it is used as an index of tissue perfusion (Rupp et al., 2013).

In order to reduce any problem deriving from inter-observer variability only one of the researcher was in charge to place the NIRS probes. Moreover he considered the useful list of practical reccomandations wrote by Ferrari and colleagues (Ferrari et al., 2011).

Peripheral arterial oxygenation (pulse oxymetry, SaO2) was measured by portable pulsoximeter (Nonin Medical, Minneapolis, MN, USA) at a sampling frequency of 1.0 Hz. Measurements were made on the left index finger. SaO_2 was monitored throughout the entire duration of the sessions.

3.2.5 Neuromuscular function assessment

Before and after the Baseline TTE tests and the 1-h intervention's sessions a neuromuscular function (NMF) assessment was performed. It consisted of determining the isometric Maximal Voluntary Contraction (MVC) of Knee Extensors (KE) to provide a global index of fatigue. Maximal voluntary activation (VA) levels of KE was assessed using a high-frequency stimulation (100 Hz) superimposed on MVC. This technique was adapted from the twitch interpolation technique (Merton, 1954) and consisted in superimposing a high frequency stimulation at supramaximal intensity on the MVC isometric plateau. The control high-frequency stimulation was delivered to the relaxed muscle 2 s after the end of the 5-s contraction. VA was calculated from the equation (%VA = [1 - superimposed high frequency stimulation / resting high frequency stimulation] * 100). Finally, stimulations were delivered to the relaxed muscle in a potentiated state to determine the extent and origin of peripheral fatigue. The stimulations were performed with a Peripheral Nerve Stimulation (PNS) on the femoral nerve for the KE. The entire procedure consisted of a MVC with a 100 Hz superimposed stimulation, followed by a high-frequency (100 Hz) doublet, a low-frequency (10 Hz) doublet and a single twitch performed to the relaxed muscle. Moreover, the low frequency fatigue (LFF) has been investigated. LFF can be defined

as a preferential decrease in the force elicited with electrical stimulation at a low-frequency (10 Hz), compared to a high frequency (100 Hz) (Jones, 1996). The entire procedure (Figure 26) was repeated two times at pre, interspersed with 2 minutes of recovery and two times at post with 10 seconds of recovery. For each subject, the end of the task on the reclined cycle ergometer and the start of the NMF assessment were separated by 60 seconds. During all the MVCs, the subjects were strongly encouraged. For NMF the subjects were seated in a custom-built ergometer and Velcro straps were strapped across the chest and hips to avoid lateral and frontal displacements (Figure 25).

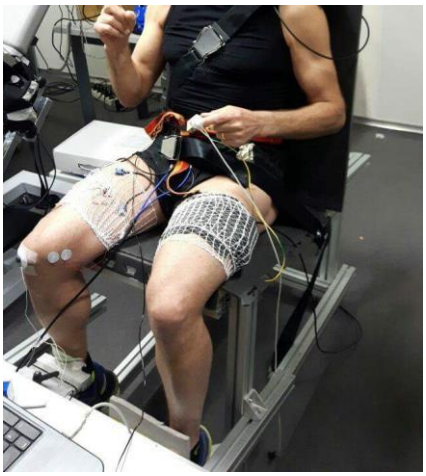

Figure 25 The custom-built ergometer used for NMF evaluation

Subjects were also instructed to cross their arms at chest level handling the straps. The KE muscles mechanical response was recorded with a strain gauge (Deltatech 150 kg, Italy) located in front of the distal part of the tibia bone. The height of the strain gauge has been chosen during the familiarization by each subject and reported throughout the protocol. Torque values were obtained from force measured by the strain gauge multiplied by the lever arm, i.e. knee-strain gauge distance. All measurements were taken from the subject's right leg, with the knee and hip flexed at 90 degrees from full extension.

Single stimuli were delivered incrementally in the relaxed muscle state until M-wave and twitch amplitudes plateaued. A stimulus intensity of 130% of the intensity to elicit

maximal M-wave area and maximal twitch responses was used throughout the rest of the experiment. Stimulus intensity was determined at the start of each session.

Bipolar Ag/AgCl surface EMG electrodes (SpesMedica, Battipaglia, Italy), with 2-cm inter-electrode distance, were placed over *Vastus Medialis, Vastus Lateralis, Rectus Femoris, and Biceps Femoris* of the right leg. The EMG signals were amplified, sampled at 2048 Hz, and converted to digital datausing multichannel amplifier with a 12-bit A/D converter (EMG-USB, OT Bioelettronica, Turin, Italy). Signals were visualised during acquisition and then stored on a personal computer using OT BioLab software version 1.8 (OT Bioelettronica, Turin, Italy) for further analysis. Before the placement of the electrodes, the skin was slightly abraded with abrasive paste and cleaned with water in accordance with SENIAM recommendations (Hermens et al., 2000). The optimal position and orientation of the electrodes were sought for each muscle following guidelines previously described (Beretta Piccoli et al., 2014). A preliminary test was performed to check for cross talk and cable-induced noise and, when needed, electrodes and cables were repositioned. The electrodes and cables were fixed and secured on the body of the participants with an extensible dressing (Fixomull®, Beiersdorf, Hamburg, Germany) to avoid movement artifacts.

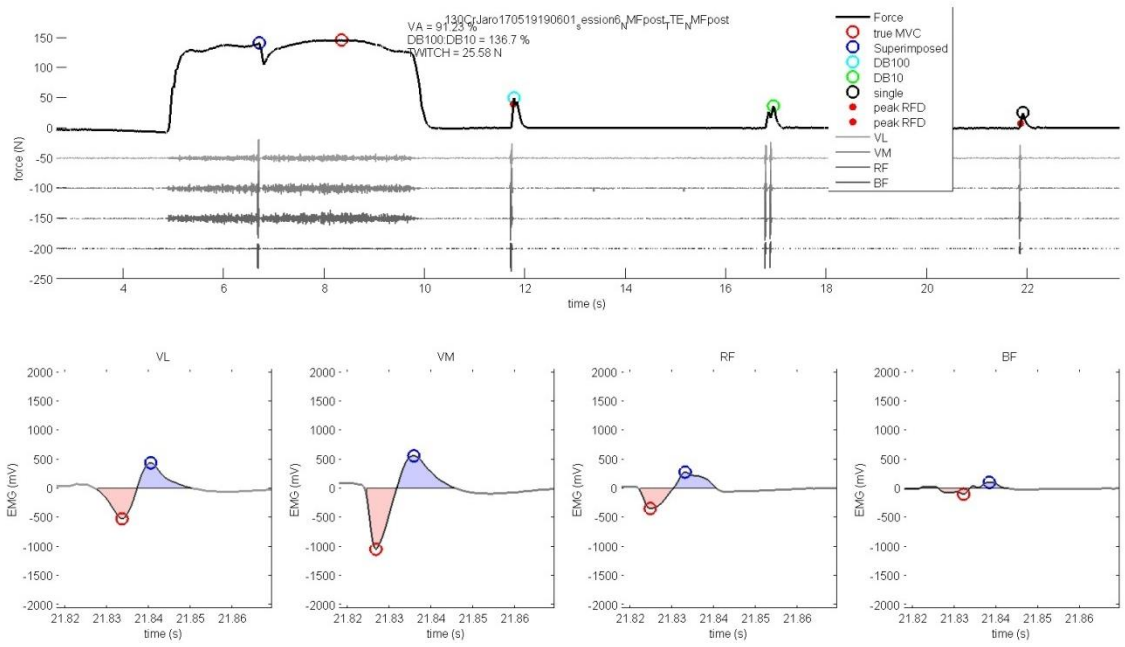

Figure 26 NMF evaluation typical profile. The force signal is the upper one with the four muscle sEMG below. In the lower part of the figure is represented a zoom of the m-waves of the four muscles with the areas underlined with red and blue colours. O SIT, O High-frequency doublet, O Low frequency doublet, O Singlet represent the stimulations of our interest assessed by means of PNS.

3.2.6 Metabolic chart, Lactate analyzer, heart rate monitor, rate of perceived exertion

During both the resting and the exercise periods, cardio-respiratory measures were collected continuously with a 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 analysers were performed before each measurement according to the manufacturer's instructions. The system is able to measure FiO_2 and was used also as a confirmation of the oxygen concentration inside the hypoxic chamber.

Lactate concentration was monitored collecting a blood sample from the earlobe at different time points (resting, 10', 30' and 60' of the 1-h intervention) during the sessions and also during the recovery 3 minutes 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.

Heart Rate was continuously measured during each session via a Polar RS C800 heart rate monitor system (Polar Electro Oy, Kempele, Finland) able to obtain also R-R interval durations. In study I and study II data of HR are presented as a mean value per each 5 minutes (calculated from the last minute mean) during the 1-h intervention sessions.

Rate of perceived exertion (RPE) was monitored by means of a Category Ratio (CR100) scale (Borg and Borg, 2002) each five minutes during the intervention sessions. Differentiated RPE included an overall rating, a local muscular rating and a cardiopulmonary rating (dyspnea) one.

3.3 Methods

3.3.1 Overview of the protocol

All the measurements have been conducted in a hypoxic lab and subjects have been required to exercise on a recumbent bike (described in detail in the chapter 3.2.1 and 3.2.2). The protocol consisted of four baseline preliminary sessions:

- two cardio-pulmonary exercise tests (CPET) both in normoxia and in normobaric hypoxia (FiO_2 13.4%) for the determination of the Peak Power Output (PPO);
- two time to exhaustion tests (TTE) both in normoxia and in normobaric hypoxia as indexes of endurance performance recorded in a non-fatigued state.

The following 5 sessions (Figure 27) have been designed in a similar way and included a resting period at the starting condition selected (e.g. Normoxia or FiO_2 16.25%) followed by a 1-h exercise at a given intensity.

The five 1-h intervention sessions and their characteristics are listed below and represented in Table 4:

- Session I - Condition: Normoxia (N) – exercise conducted at 50% of PPO obtained during normoxic incremental test. This condition is part of study I
- Session II - Condition: Hypoxia, FiO_2 13.4% (Hcost) - reproducing and altitude of 3500 m asl - Exercise conducted at 50% of PPO obtained during hypoxia incremental test. This condition is part of study I
- Session III - Condition: Progressive Hypoxia (H_NoPO), FiO_2 changing during the 1-hour time from 16.25% to 13.4%; reproducing an altitude gain from 2000 to 3500 m asl. Exercise: no exercise. This condition is part of the study II
- Session IV - Condition: Progressive Hypoxia (HN), FiO_2 changing during the 1-hour time from 16.25% to 13.4%; reproducing an altitude gain from 2000 to 3500 m asl. Exercise conducted at 50% of PPO obtained during normoxia incremental test. This condition is part of the study II

- Session V - Condition: Progressive Hypoxia (HH), FiO_2 changing during the 1-hour time from 16.25% to 13.4%; reproducing an altitude gain from 2000 to 3500 m asl. Exercise conducted at 50% of PPO obtained during hypoxia incremental test. This condition is part of the study I and II.

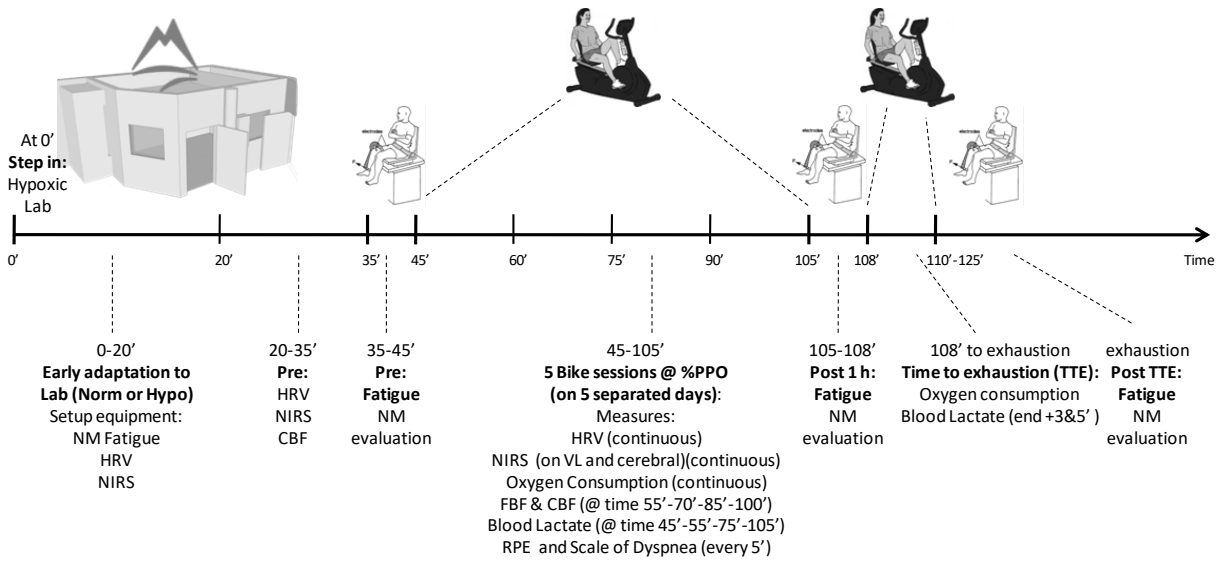

Figure 27 Timeline of the 1-hour protocol used in the 3 studies. This protocol has been done 5 times per each subject with the conditions listed in the paragraph performed in a random order.

As shown in Figure 27, measurements have been conducted PRE and POST exercise but also during exercise. Measurements investigated were gas exchange and ventilation, oxygen consumption, heart rate, blood lactate, tissue oxygenation, cerebral and femoral blood flows rate of perceived exertion. A detailed description can be found in the paragraph 3.3.4.

Table 4. Schematic representation of the combination of conditions and exercise

	Normoxia	Hypoxia	Progressive Hypoxia
No exercise			Session III (H_NoPO)
50% of PPO during Hypoxia		Session II (Hcost)	Session V (HH)
50% of PPO during Normoxia	Session I (N)		Session IV (HN)

The vertical speed chosen (25 m/min, ~0.4 m/s) is in line with the ones people are able to maintain during a cycling competition called "Re Stelvio" which arrives at Stelvio pass that athletes complete in more or less one hour (~01:04:00). This vertical speed can be maintained by more than 100 athletes during the Vertical kilometre de Fully (the fastest on the world, where the winner gains 1000 m of ascent in 28 minutes and 54 seconds). Also Bradwell and colleagues asked to their subjects to move from Cervinia (2050 m) to Rifugio Testa Grigia (3459 m) with a rate of ascent of ~0.4 m/s. Even if the vertical speed chosen is in line with similar task durations, it is way higher compared to one that a middle ranking athlete can perform during a mountain ultra-marathon (Savoldelli et al., 2017).

3.3.2 Maximal cardio-pulmonary exercise test

Before starting the 1-h sessions (intervention), we ran two maximal cardio-pulmonary exercise test (CPET) in order to assess Peak Power Output (PPO) in normoxia and in normobaric hypoxia (FiO2 13.4%, corresponding to ~3500 metres above sea level, asl). The subjects came to the lab in two separated days (at least 48 hours apart) and randomly performed the two tests, after a 30 minutes early acclimatisation to the laboratory aiming to allow a proper set up of the subject and especially to avoid the "super" acute effects of the exposure to hypoxia such as the increase in heart rate (Peacock, 1998) and the acute ventilatory response (Ainslie et al., 2013). This early

acclimatisation has been done also during the normoxic test in order to allow a blind intervention on the subjects. All the evaluations were performed on a recline cycle ergometer (see 3.2.2). Following 30 min of early acclimatisation sitting on the ergometer participants completed: 6 minutes of baseline measurements at rest, 10 minutes of sub-maximal constant load exercise (75 W) and a maximal CPET followed by 5 min of post-exercise recovery assessment. CPET started immediately after the sub-maximal exercise with increments of 25 W every 1 min until participants' volitional exhaustion. We asked to keep a pedalling cadence during the submaximal exercise and the CPET constant at ~90 revolutions/min (rpm), using a monitor that provided to the participants a visual feedback. During resting and exercise period cardio-respiratory measures were collected continuously. Throughout rest, exercise and recovery phases, also beat-to beat heart rate was continuously recorded. Pulse oxygen saturation (SpO_2) was continuously monitored. For a measure of maximal lactate accumulation a blood sample was collected from the earlobe 3 minutes after the end of the test (Goodwin et al., 2007). At the end of the first CPET, the subjects spent more time in the lab with the aim to familiarise with the neuromuscular function evaluations (familiarisation with the MVC and electrical stimulation of the KE muscles. The subjects repeated trials of the procedures until they were able to produce consistent results, for more details see in 3.2.5), while after the second CPET they familiarise with a time to exhaustion test (TTE) performed at the 80% of the PPO obtained during the test (see 3.3.3).

3.3.3 Time to exhaustion test (TTE)

The subjects were asked at day 3 and day 4 to visit the lab and underwent randomly to a TTE both in hypoxia (FiO2 13.4%) and normoxia, respectively.

The TTE, as an index of endurance performance, was done at the begin as a baseline control with the aim to check for the different durations at the end of the intervention sessions (see below). Before and after these evaluations a neuromuscular function (NMF) assessment was assessed. As for the CPET the subjects came to the lab in two separated days (at least 48 hours apart) and randomly performed the two tests, after a

30 minutes early acclimatisation to the laboratory. After 30 min of early acclimatisation sitting on the ergometer participants completed: 6 minutes of baseline measurements at rest (NIRS, HRV, SpO_2). Then they sat down on the NMF chair for the pre-evaluations. Once completed they went back on the cycle ergometer, start a warm up of 10 minutes at 30% of the PPO, 60 seconds rest and they started the TTE. The power output was set at 80% of their relative PPO. We asked to keep a pedalling cadence during the warm up exercise and the TTE constant at ~90 revolutions/min (rpm). An investigator blind to the condition was in charge to encourage the subjects. Once exhausted they were moved (with their help) to the NMF chair because 60 seconds after the end of the TTE we started with the post NMF evaluation.

3.3.4 Overview of the 1-h intervention session protocol used in the two studies

Following these four baseline evaluations, each participant visited our laboratory in five occasions at the same time of the day and completed the experimental protocol within a 3-week period. Participants randomly performed an evaluation sequence in either normoxia (N) or constant normobaric hypoxia (Hcost). Furthermore, the same protocol was done also in 3 different occasions with the subjects exposed to progressive normobaric hypoxia, once resting, without any physical effort, (H_NoPO), once at 50% of the PPO obtained during the CPET in H (HH) and the last performed at 50% of the PPO obtained during the CPET in N (HN). More in detail, participants enter in the chamber end rested for 30 minutes as an early acclimatisation to the laboratory aiming to allow a proper set up of the subject and especially to avoid the "super" acute effects of the exposure to hypoxia such as the increase in heart rate (Peacock, 1998) and the acute ventilatory response (Ainslie et al., 2013). This early acclimatisation has been done also during the normoxic test in order to allow a blind intervention on the subjects. During the acclimatization we prepared the saddle eight of the cycle ergometer (see 3.2.2) and the subjects were set up with the heart rate monitor (see 3.2.6) and the pulse oxymeter (see 3.2.4). Then, surface electromyography and the electrodes for the peripheral nerve stimulations (plus the ground one) were positioned as explained in the

paragraph 3.2.5. After that the NIRS was positioned on the vastus lateralis and on the prefrontal cortex (see 3.2.4). After 30 min of early acclimatisation sitting on the cycle ergometer (see 3.2.2) participants completed: 6 minutes of baseline measurements at rest (NIRS, HRV, cerebral and femoral blood flows, SpO_2). A blood sample were taken for blood lactate accumulation (see 3.2.6) Then they sat down on the Neuromuscular Fatigue chair for the pre evaluations (see 3.2.5). Once completed they went back on the cycle ergometer for a 3 minutes rest with also the metabolic chart on (see 3.2.6), start a 5 minutes warm up at 30% of the PPO and then started the 1 hour intervention at the intensity explained in the paragraph 3.3.1. At the begin of the session III (H_NoPO, see 3.3.1) we didn't ask for a warm up with the only aim to didn't influence the 1 hour of intervention at rest. In session III the warm up was done before the post TTE test. During the 1-h intervention Oxygen consumption, NIRS, HR, SpO_2 and FiO_2 were monitored continuously. Every five minutes an overall index of rate of perceived exertion, one about leg fatigue and one about dyspnea were collected by an investigator. At the begin, at minute 10, 30 and at the end a blood sample was collected. This timing was decided also to have more information regarding the maximal lactate in steady state (MLSS) (see 3.2.6). At minute 10, 25, 40 and 55 cerebral blood flow and femoral blood flow were collected by an expert investigator (see 3.2.3). Once finished the 1-h, they sat down on the Neuromuscular Fatigue chair and 60 seconds after the end of the 1-h intervention they were ready for the post 1-h evaluations (see 3.2.5). Once completed they went back on the cycle ergometer to start the TTE post evaluation Figure 27. This final test was designed to verify how endurance performance is impaired (if any) by the different types of interventions. When the subjects were not able to maintain a cycle cadence above 70 rpm the TTE was terminated and the investigators helped the subjects to move again on the Neuromuscular Fatigue chair and 60 seconds after the end, athletes were ready for the post TTE NMF assessment. A blood sample was also collected at minute 3, 5 and 7 of the recovery when an investigator also asked for the RPE of the three scales regarding the TTE. At this time point the 1-h session finished (Figure 27).

3.3.5 Statistical analysis

All descriptive statistics presented in the text and figures are mean values ± standard deviations. All statistical procedures were completed using the software IBM® SPSS® (version 21.0.0, IBM Corp., Somers, NY, USA). Analysis of variance was assessed for time and condition effects using a two-way ANOVA with repeated measures design. Bonferroni corrected *post-hoc* tests were applied to determine between-means differences if the analysis of variance revealed a significant main effect and an interaction time x condition. Moreover, when of interest according to our aims and hypotheses, an one-way ANOVA with repeated measures was used to verify the effects of time within each condition. Considering the nature of the ceiling effect on the voluntary activation (Gandevia et al., 1998), for this parameter, paired comparisons were done with Wilcoxon test. For all statistical analyses, a P value of 0.05 was accepted as the level of significance. The P value reported in the results of the studies regarding the repeated measures analysis is adjusted thanks to the SPSS Bonferroni-adjusted significance tests, that is a mathematically equivalent of the Bonferroni adjustment. This procedure takes the LSD P value (that is an unadjusted p-value) and multiplies it by the number of comparisons made (IBM SPSS website). Therefore, the uncorrected P value is automatically multiplied by the statistical package by the number of possible comparisons in order to obtain the corrected P value. Effect size was also calculated using Cohen's d for pairwise comparisons for both TTE performances and NMF (pre vs post). A magnitude of d of 0.2 was considered a small effect; 0.5, moderate; 0.8, large and 1.20, very large (Cohen, 1988).

4 Results

4.1 Results of starting (Baseline) evaluations

In Table 5, the results of the ramp incremental test of the participants involved in the projects are reported. In Table 6 and Table 7 the results of the baseline evaluations (ramp incremental test and Time to Exhaustion) on the participants involved in study I and study II are reported.

Table 5 Baseline evaluations with ramp incremental test data on 13 subjects involved at the begin of the project. Data are presented with mean, SD and differences between Normoxia and Hypoxia. Significant differences are reported in red.

Ramp incremental test	Normoxia	Hypoxia	Difference (%)	*P value*
VO_{2max} ($mlO_2 \cdot min^{-1}$)	3894 ± 280	3085 ± 157	-20.8	*<0.001*
VO_{2max}/kg ($mlO_2 \cdot min^{-1} \cdot kg^{-1}$)	56.1 ± 8.4	44.6 ± 4.1		
Heart $Rate_{max}$ (bpm)	178 ± 13	171 ± 12	-3.5	*0.002*
Peak Power Output (watt)	309 ± 42	264 ± 28	-14.6	*<0.001*
VE_{max} ($L \cdot min^{-1}$)	151.9 ± 22.8	156.1 ± 15.9	2.7	*n.s.*
Rate of Perceived Exertion (CR100)	98.1 ± 10.7	98.8 ± 13.1	0.8	*n.s.*
Blood $Lactate_{max}$ (mM)	12.0 ± 2.3	13.1 ± 1.8	8.8	*n.s.*

Table 6 Baseline evaluations with ramp incremental test and Time to Exhaustion data on 8 subjects involved in study I. Data are presented with mean, SD and differences between Normoxia and Hypoxia. Significant differences are reported in red.

Ramp incremental test	Normoxia	Hypoxia	Difference (%)	*P value*
VO_{2max} ($mlO_2 \cdot min^{-1}$)	3963 ± 607	3175 ± 184	-19.9	*0.003*
VO_{2max}/kg ($mlO_2 \cdot min^{-1} \cdot kg^{-1}$)	58.6 ± 8.9	47.1 ± 2.7		
Heart $Rate_{max}$ (bpm)	184 ± 11	176 ± 12	-4.1	*0.032*
Peak Power Output (watt)	313 ± 40	272 ± 22	-12.9	*0.001*
VE_{max} ($L \cdot min^{-1}$)	157.3 ± 24.9	162.4 ± 13.4	3.2	*n.s.*
Rate of Perceived Exertion (CR100)	99.4 ± 9.8	98.7 ± 5.8	-0.6	*n.s.*
Blood $Lactate_{max}$ (mM)	12.4 ± 2.4	13.5 ± 2.0	8.5	*n.s.*
Time to Exhaustion				
PowerOutput (watt)	250 ± 32	218 ± 18	-12.7	*<0.001*
Duration (sec)	690 ± 223	427 ± 99	-38.1	*0.009*
Blood $Lactate_{max}$ (mM)	9.9 ± 1.6	11.2 ± 2.2	13.0	*0.059*
RPE overall (CR100)	91.7 ± 2.2	97.5 ± 7.0	6.2	*n.s.*
RPE leg discomfort (CR100)	94.4 ± 5.6	99.1 ± 6.5	5.0	*n.s.*
RPE dyspnea (CR100)	86.2 ± 6.9	94.6 ± 13.1	9.7	*n.s.*

Table 7 Baseline evaluations with ramp incremental test and Time to Exhaustion data on 11 subjects involved in study II. Data are presented with mean, SD and differences between Normoxia and Hypoxia. Significant differences are reported in red.

Ramp incremental test	Normoxia	Hypoxia	Difference (%)	*P value*
VO_{2max} ($mlO_2 \cdot min^{-1}$)	3996 ± 518	3172 ± 157	-20.6	*<0.001*
VO_{2max}/kg ($mlO_2 \cdot min^{-1} \cdot kg^{-1}$)	57.6 ± 6.3	46.5 ± 3.6		
Heart $Rate_{max}$ (bpm)	180 ± 12	173 ± 12	-3.7	*0.006*
Peak Power Output (watt)	316 ± 36	271 ± 19	-14.2	*<0.001*
VE_{max} ($L \cdot min^{-1}$)	156.6 ± 21.4	160.7 ± 12.4	2.6	*n.s.*
Rate of Perceived Exertion (CR100)	96.4 ± 10.3	97.7 ± 5.6	1.4	*n.s.*
Blood $Lactate_{max}$ (mM)	12.5 ± 2.0	13.3 ± 1.7	7.1	*n.s.*
Time to Exhaustion				
PowerOutput (watt)	253 ± 28	217 ± 16	-14.2	*<0.001*
Duration (sec)	643 ± 210	418 ± 86	-35.0	*0.003*
Blood $Lactate_{max}$ (mM)	10.1 ± 1.5	11.2 ± 1.9	11.9	*0.019*
RPE overall (CR100)	91.1 ± 4.8	95.7 ± 8.3	5.1	*n.s.*
RPE leg discomfort (CR100)	92.7 ± 6.7	96.5 ± 8.4	4.4	*n.s.*
RPE dyspnea (CR100)	86.7 ± 7.4	93.7 ± 12.6	9.2	*n.s.*

4.2 Results of study I

Reminder of the aim of Study 1:

Determine the effects of exposure to constant (FiO_2 13.4% - reproducing and altitude of 3500 m asl, Hcost) vs progressive hypoxia during an 1-h of constant exercise at 50% (HH) of the relative (adapted for condition's FiO_2) Peak Power Output (PPO) on fatigue and endurance performance tested through a TTE. We ran a comparison with a normoxic condition (N). We also aimed to observe the main physiological responses during the 1-hour interventions.

The data of eight out of thirteen subjects over the three different sessions (Table 2) were suitable for being analysed. One of the subjects reported knee joint pain during the familiarization trial after the second test session and was then excluded from the

rest of the studies, and 4 subjects were removed from this study as their measurements were corrupted by some problems. Baseline results presented in Table 6 included values of the 8 subjects involved in this study. The mean FiO_2 registered during the 60 minutes of interventions in the 1-h interventions in N, HH and Hcost. Are presented in Figure 28.

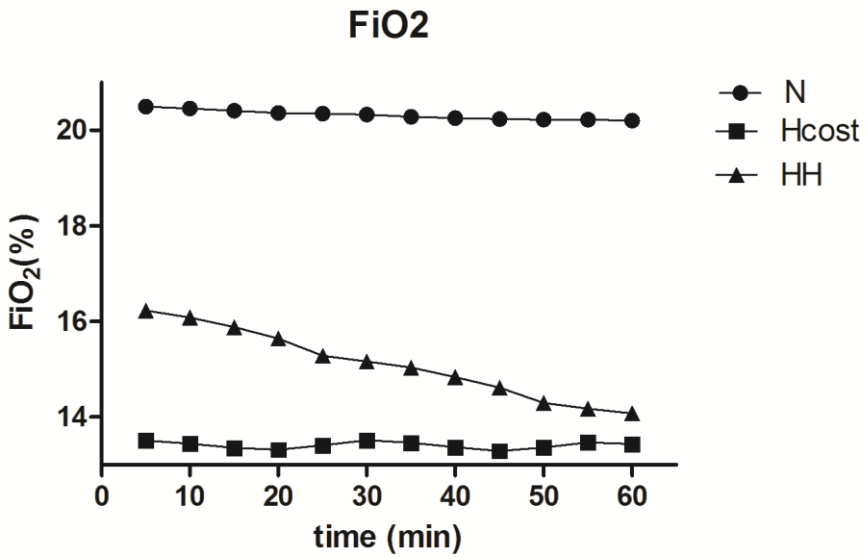

Figure 28 Mean values of fraction of inspired oxygen (FiO2) during the three interventions.

Effects on neuromuscular function

No effects of condition were found in force signal during the Maximal Voluntary Contractions (MVC), only a general effect of time ($P<0.001$, $F=26.551$). MVC declined significantly both in N ($P=0.024$) by -5.8%, Hcost ($P=0.002$) by -8.5% and HH ($P=0.001$) by -10.5% Post 1-h intervention when compared to pre, respectively. MVC declined significantly both in N ($P=0.007$) by -11.9%, Hcost ($P=0.002$) by -8.7% HH ($P=0.025$) by -15.7% Post TTE intervention when compared to pre, respectively. A significant difference was found in N condition between post 1-h and post TTE measurements (-0.3%, $P=0.021$) (Figure 29, panel A). Maximal voluntary activation was significantly reduced by the 1-h interventions in the N ($P=0.013$) and HH

(P=0.022) conditions (Figure 29, panel B). We found an effect of time (P<0.001, F=139.976) and time*condition (P=0.041, F=2.938) in the low frequency fatigue (LFF) index (as the ratio Db10:100Hz). The decrease in this ratio, as an index of LFF, was found between pre and post 1-h interventions (-22.2%, P<0.001, Cohen's d= 2.83; -18.0%, P=0.001, Cohen's d= 1.96 and -18.5%, P=0.001, Cohen's d=1.79, in N; Hcost and HH, respectively). Db10:100 was significantly reduced by the TTE performance in two conditions (-14.4%, P<0.001, Cohen's d= 1.68 and -13.2% P=0.002, Cohen's d= 1.01; in N, Hcost and HH, respectively) (Figure 29, panel C). NO significant differences were found in the EMG derived data (areas, RMS/peak to peak amplitude).

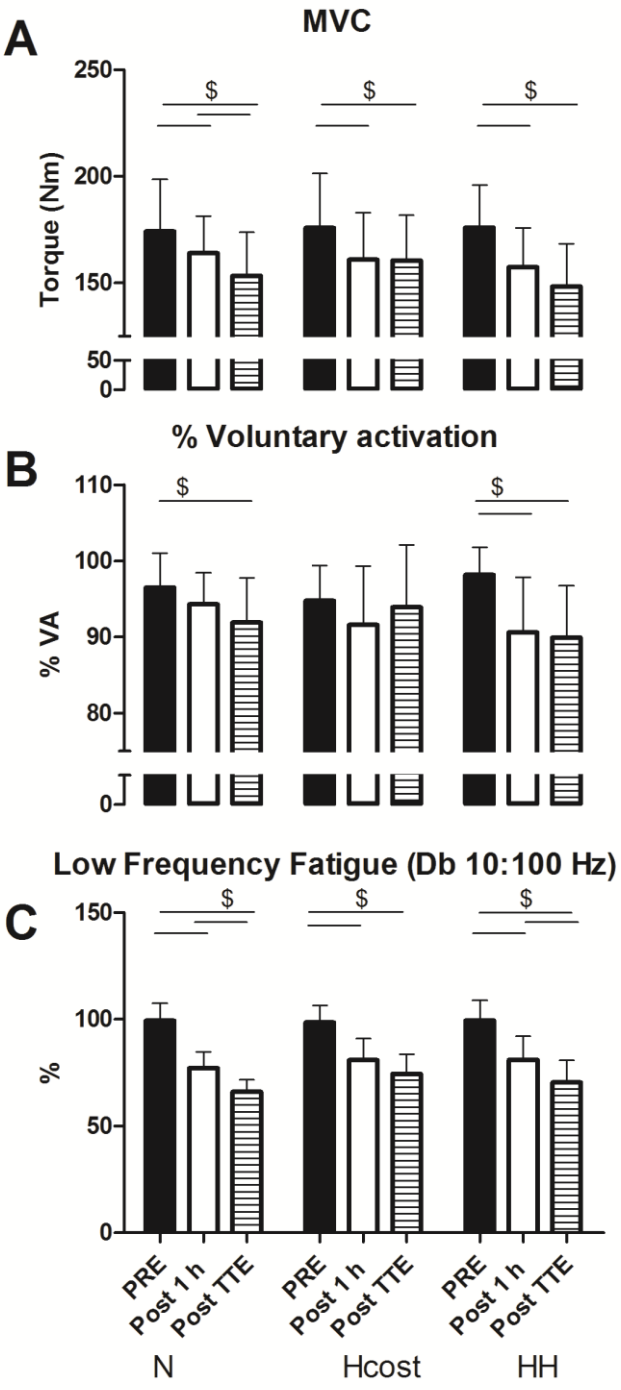

Figure 29 Main NMF effect of the three 1-h interventions. Maximal voluntary contraction, MVC (panel A), % of voluntary activation, %VA (panel B), low frequency fatigue, as the low-to-high-frequency doublet ratio, 10:100 ratio (panel C). $: P<0.05 on time.

Effects on performance

TTE test outcome decreased significantly post 1-h interventions when compared to baseline measurements in normoxia (-27.9%, P=0.03, Cohen's d=0.89) and in hypoxia when compared to post Hcost condition (-30.7%, P=0.007, Cohen's d=1.64). No significant decrease in duration post 1-h PH at HH has been found (Figure 30). Also when the durations of the two TTE post 1-h interventions (both after the hypoxic sessions, HH and Hcost) are compared, a significant difference was found with an even lower duration in Hcost (-21.6%, P=0.038, Cohen's d=0.85). Even if the effort was matched (80% of the relative PPO obtained in the two conditions, normoxia and hypoxia) the duration of the TTE test in hypoxia at baseline was lower compared to the normoxic condition (-34.7%, P=0.03, Cohen's d=1.40). And, even if there is only a tendency, the 1-h intervention intensity was matched at 50% of the relative PPO, a shorter duration in the TTE was observed in the Hcost condition when compared to N (-37.3%, P=0.058, Cohen's d=1.15).

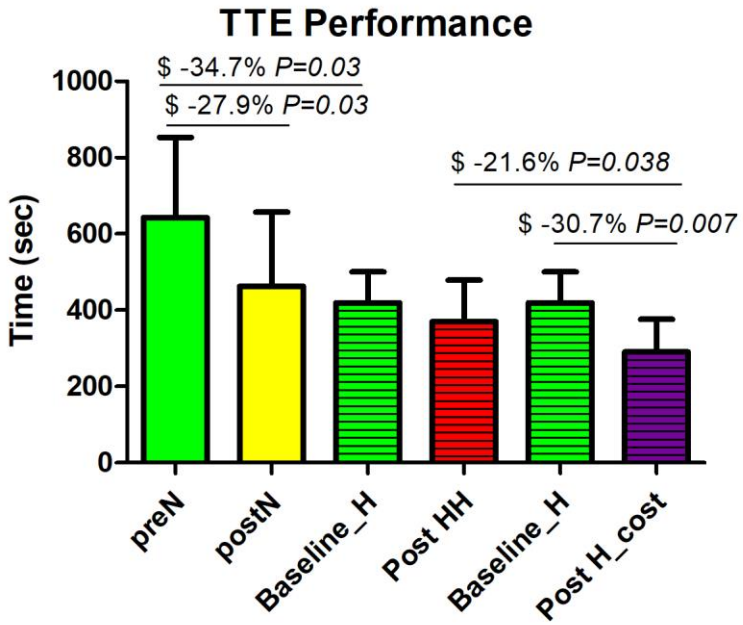

Figure 30 Durations of TTE test at baseline (both in Normoxia and Hypoxia) and after the three 1-h interventions.

During the interventions

Pulse oxygen saturation (SpO_2) during the three different 1-h interventions is presented in Figure 31. A significant effect of condition (P<0.001; F=170.131), time (P<0.001; F=21.958) and an interaction condition*time effect (P<0.001; F=13.449) was found. An effect of time (with 12 times point) was found for every condition, N (P=0.001, F=5.257), Hcost (P=0.002, F=2.987) and HH (P<0.001, F=57.318). Once excluded from the analysis the first time point (5' after the start of exercise) considered as a first adaptation to the effort required, an effect of time on SpO2 was found only in the HH session (P<0.001, F=38.134).

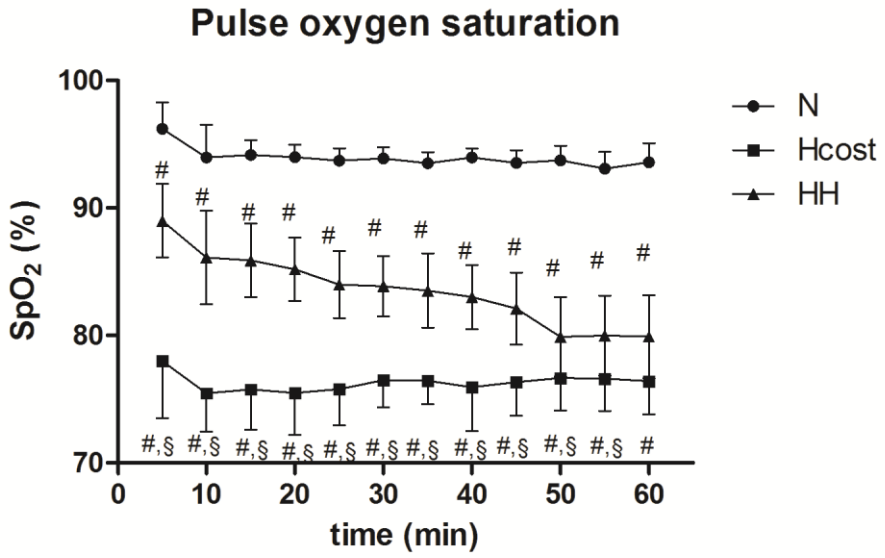

Figure 31 Pulse oxygen saturation during the 1-h interventions. # significantly different from N, § significantly different from HH.

NIRS data are shown in Figure 32. Tissue Oxygen Index (on Vastus lateralis, VL) during the three interventions changed significantly with an effect of time (P=0.002, F=9.946). A significant interaction time*condition effect was also found (P=0.020, F=4.421) but without any significant effects of the condition. No effects of condition were found for oxyhemoglobin and deoxyhemoglobin (VL) while for both a general effect of time was observed (P=0.017, F=6.966; P=0.047, F=1.959, respectively). The same behaviour for oxyhemoglobin and deoxyhemoglobin at the prefrontal cortex level

(PFC) (P=0.049, F=4.790); P<0.001, F=5.294, respectively). An interaction time*condition effect was also observed both for VL and PFC deoxyhemoglobin (P<0.001, F=11.424; P=0.004, F=19.166, respectively).

A general effect of time was found for Cerebral blood flow (CBF) (P<0.001, F=14.027), but neither an effect of condition nor an interaction time*condition effect. An effect of time was found for every condition, N (P<0.001, F=12.360), Hcost (P=0.037, F=5.45) and HH (P=0.009, F=6.372). Significant effect of condition (P=0.041, F=4.930), time (P<0.001, F=106.609) and an interaction condition*time (P=0.046, F=4.507) were found for the FBF (panel B). An effect of time was found for N (P<0.001, F=80.319), Hcost (P=0.007, F=18.541) and HH (P<0.001, F=130.902).

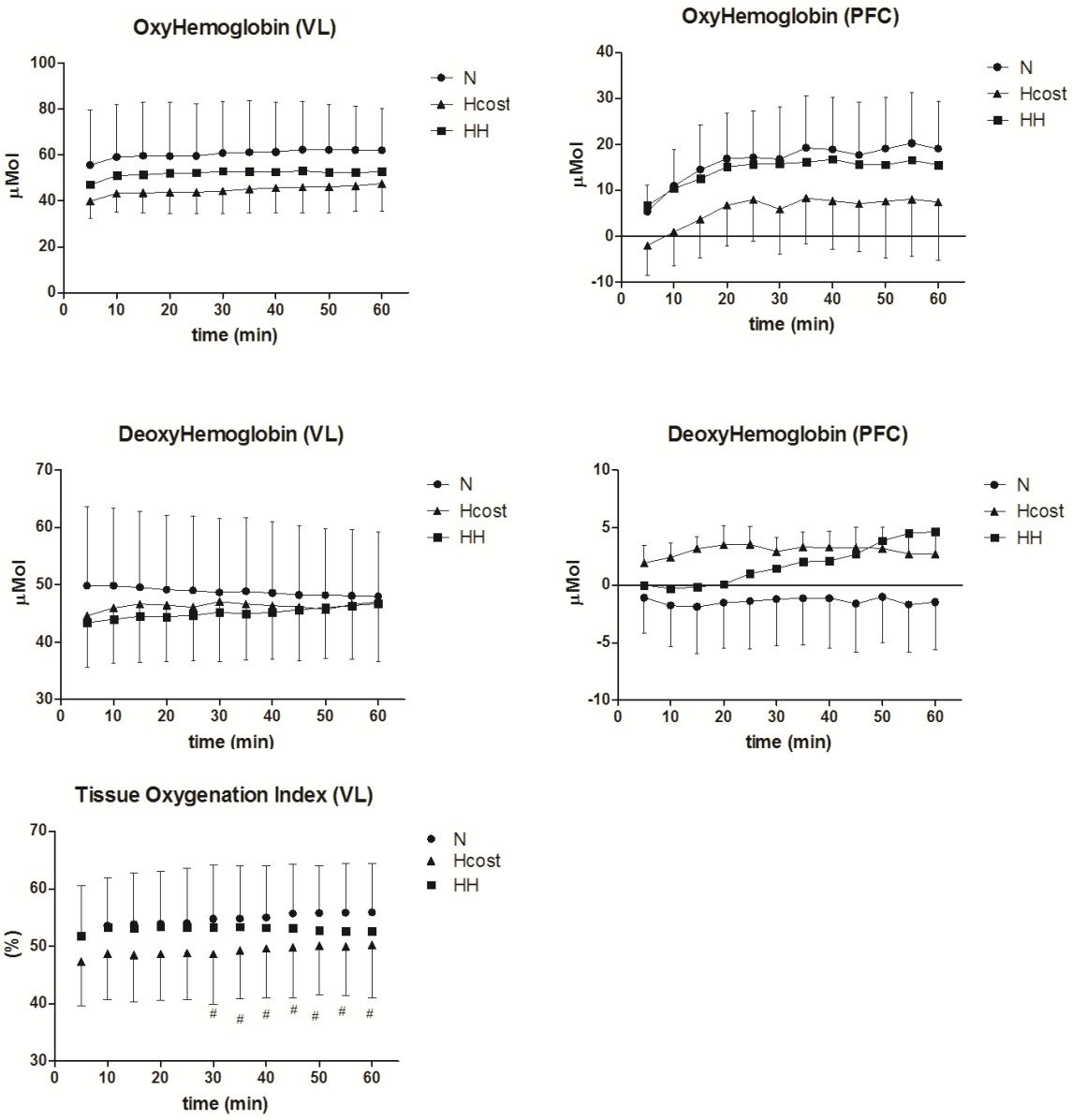

Figure 32 NIRS data on the vastus lateralis (VL, left side) and PreFrontal cortec (PFC, right side) during the 1-h interventions. # significantly different from N

Rate of perceived exertion was collected with three focuses (Figure 33) every 5 minutes. Subjects reported firstly an overall index of RPE (panel A) which changed significantly with an effect of time ($P<0.001$, $F=98.482$). No significant effects of condition and neither an interaction condition*time effect were found. An effect of time was found for every condition, N ($P<0.001$, $F=74.740$), Hcost ($P<0.001$, $F=51.334$) and HH ($P<0.001$, $F=53.774$).

The RPE legs, (see panel B) changed significantly with a general effect of time ($P<0.001$, $F=46.285$). Neither a significant effect of condition nor an interaction condition*time effect was found. An effect of time was found for every condition, N ($P<0.001$, $F=33.610$), Hcost ($P<0.001$, $F=27.862$) and HH ($P<0.001$, $F=53.77$).

Finally, subjects reported the RPE of their dyspnea which also increased significantly with a general effect of time ($P<0.001$, $F=58.896$). Neither a significant effect of condition nor an interaction condition*time effect was found. An effect of time was found for every condition, N ($P<0.001$, $F=45.413$), Hcost ($P<0.001$, $F=31.088$) and HH ($P<0.001$, $F=45.577$).

Pairwise comparisons between conditions at each time point are indicated in Figure 33.

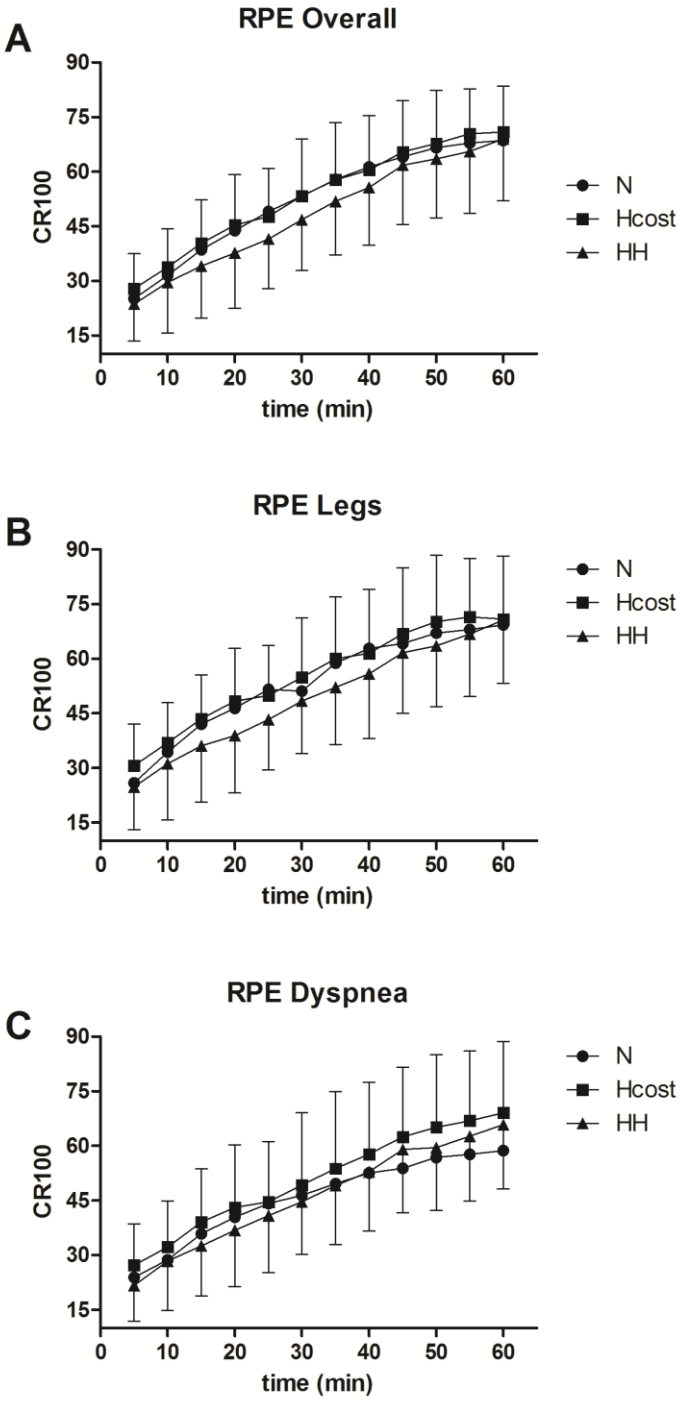

Figure 33 Rate of perceived exertion during the 1-h interventions. Overall perception (panel A), leg perception of fatigue (panel B), perception of dyspnea (panel C). # significantly different from Hcost, § sign. different from N.

Blood lactate was collected at minute 10 and 30 as a double check for MLSS (Figure 34). An effect of condition (P<0.001, F=36.141), time (P<0.001, F=18.317) and an interaction condition*time effect (P<0.001, F=12.759) was found. An effect of time was found for condition, HH (P<0.001, F=17.41) and HN (P<0.001, F=28.441). In H_NoPO no effect of time was observed (P=0. 331, F=1.187). Significant differences between conditions at each time point have been reported on Figure 44.

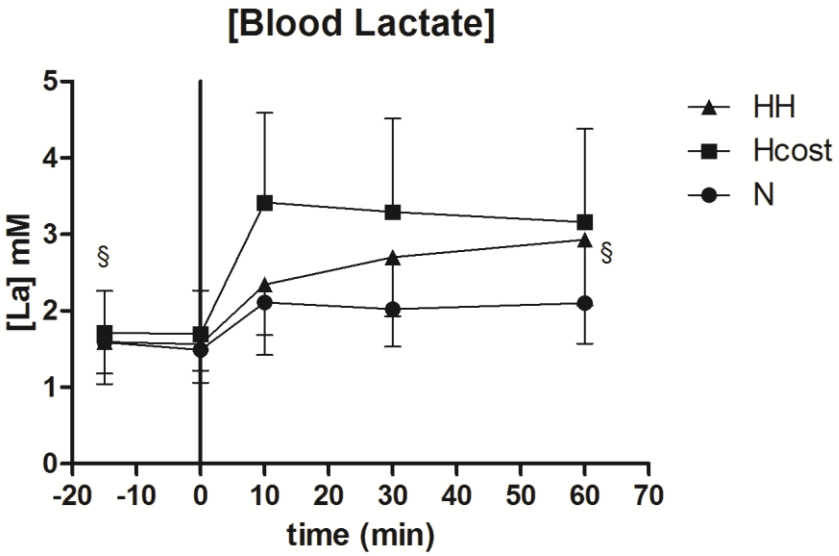

Figure 34 Blood lactate concentration at fixed time points during the 1-h interventions. § significantly different from N (§ refers to Hcost at time -15 minutes, HH at time 60 minutes).

Also heart rate (HR) was monitored throughout the entire duration of the interventions (Figure 35). An effect of time (P<0.001, F=44.812) and an interaction condition*time effect (P=0.004, F=5.760) was found but without any significant effects of conditions. An effect of time (with 12 time points) was found for every condition, N (P<0.001, F=42.293), Hcost (P=0.001, F=20.873) and HH (P<0.001, F=58.374). Once excluded the first time point (5' after the start of exercise) considered as a first adaptation to the effort required, an effect of time on HR was still found for every condition, N (P<0.001, F=27.685), Hcost (P=0.004, F=11.273) and HH (P<0.001, F=48.648). Pairwise comparisons between conditions at each time point are shown in Figure 35.

Ventilation (VE) during the 1-h interventions is presented in Figure 36. A significant effect of condition (P=0.038; F=4.333), time (P=0.001; F=21.750) and an interaction condition*time effect (P=0.003; F=2.203) was found. An effect of time was found for every condition, N (P<0.001, F=26.917), Hcost (P=0.025, F=7.054) and HH (P<0.001, F=39.267). Pairwise comparisons between conditions at each time points are shown in Figure 36.

Oxygen consumption during the 1-h interventions are presented in Figure 37. An effect of condition (P=0.002, F=11.185), time (P<0.001, F=43.830) but no significant interaction condition*time effect (P<0.001, F=15.902) were found. Hcost differs from N (P=0.03) but no differences were found between the other conditions. An effect of time was found for every condition, N (P<0.001, F=34.180), Hcost (P<0.001, F=22.756) and HH (P<0.001, F=43.725).

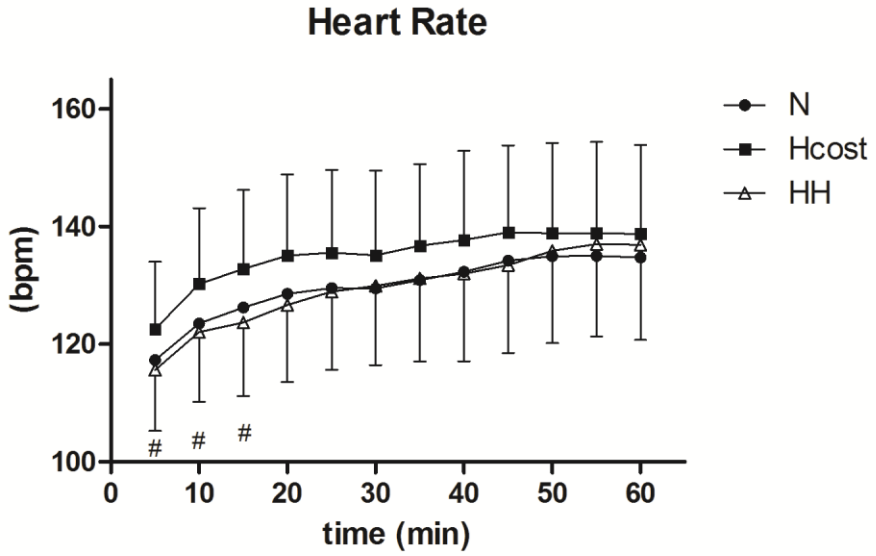

Figure 35 Heart rate during the 1-h interventions. # significantly different from Hcost.

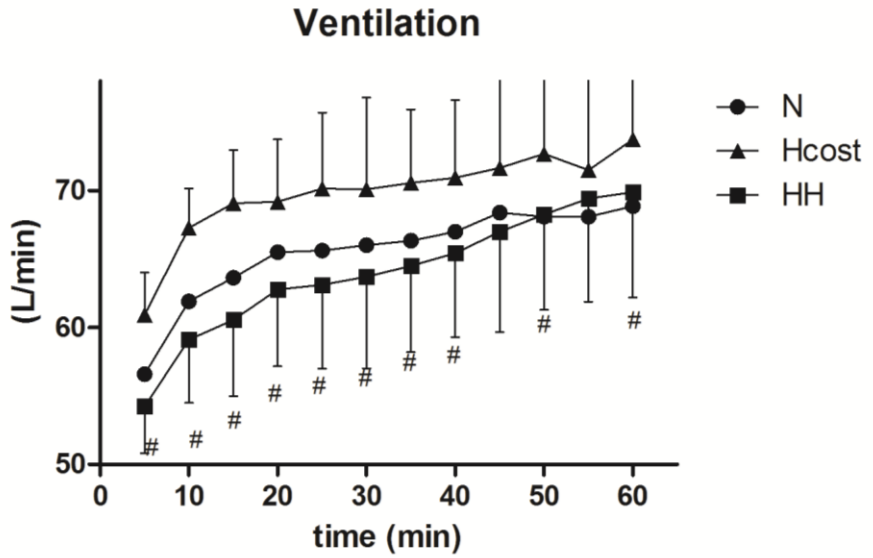

Figure 36 Ventilation (L/min) during the 1-h interventions. # significantly different from Hcost, § significantly. different from N

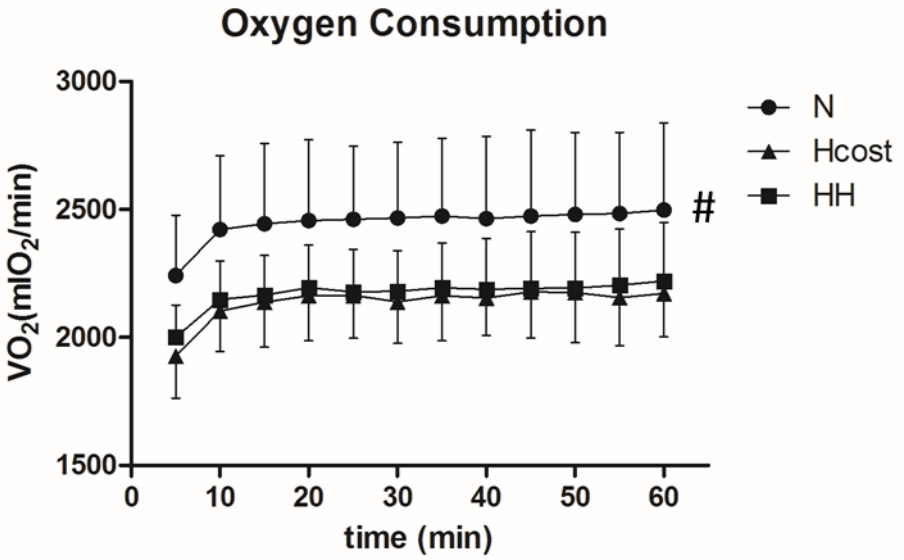

Figure 37 Oxygen consumption during the 1-h interventions. # significantly different from Hcost.

4.3 Results of study II

Reminder of the aim of Study II:

Determine the effects of the 1-h interventions at different work intensities to PH on fatigue and endurance performance. A comparison between NO physical effort (resting on the bike, H_NoPO), a relative intensity (e.g. 50% of the PPO obtained in a ramp incremental test performed at FiO_2 13.4%, HH) and an absolute intensity of effort (e.g. 50% of PPO in Normoxia, HN) has been conducted. We also aimed to observe the main physiological and perceived responses during the 1-hour interventions.

Eleven subjects out of thirteen enrolled for the entire project were able to complete the three different sessions. Of the two excluded, one of them reported knee joint pain during the familiarization trial after the second test session and was then excluded from the rest of the studies while the other one was not able to complete the entire protocol properly. In Table 7 are reported the results of the baseline evaluations (ramp incremental test and TTE). Figure 38 reports the mean FiO_2 registered during the 60 minutes of interventions.

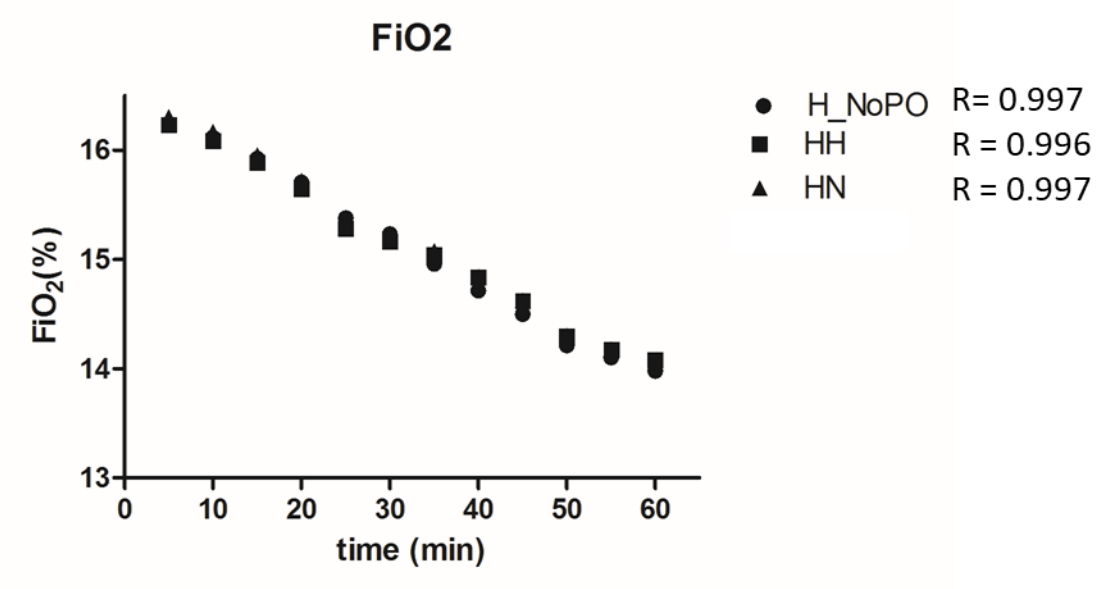

Figure 38 Mean values of fraction of inspired oxygen (FiO_2) during the three PH interventions. R for linear fit is reported.

Once verified this assumption we can go through the pre to post intervention results.

Effects on neuromuscular function

No effects of condition were found in force during the Maximal Voluntary Contractions (MVC), only a general effect of time was recorded ($P<0.001$, $F=19.763$). MVC did not decline in the H_NoPO condition. MVC declined significantly both in HH ($P=0.001$) by -9.1% and HN ($P=0.008$) by -10.9% Post 1h intervention, respectively. MVC declined significantly both in HH ($P=0.001$) by -13.0% and HN ($P=0.008$) by -14.4% Post TTE, respectively. No significant differences were found between post 1 h and post TTE measurements (Figure 39, panel A). Maximal voluntary activation was significantly reduced by the 1-h exposure to PH in the three conditions ($P=0.033$, $P=0.013$ and $P=0.01$ in H_NoPO, HH and HN, respectively) (Figure 39, panel B). We found an effect of condition ($P=0.011$, $F=5.925$), time ($P<0.001$, $F=137.751$) and time*condition ($P<0.001$, $F=8.021$) in the low frequency fatigue (LFF) index (as the ratio Db10:100Hz). The decrease in this ratio, as an index of LFF, was found between pre and post 1 h cycling in Progressive Hypoxia (-17.6%, $P<0.001$, Cohen's d= 1.78 and -17.1%, $P=0.003$, Cohen's d=1.33, in HH and HN, respectively). Db10:100 was significantly reduced by the TTE performance in all three conditions (-25.8%, $P<0.001$, Cohen's d= 2.79; -12.2%, $P=0.003$, Cohen's d= 0.98; and -8.6% $P=0.029$, Cohen's d= 1.08; in H_NoPO, HH and HN, respectively) (Figure 39, panel C). NO significant differences were found in the EMG derived data (areas, RMS/peak to peak amplitude).

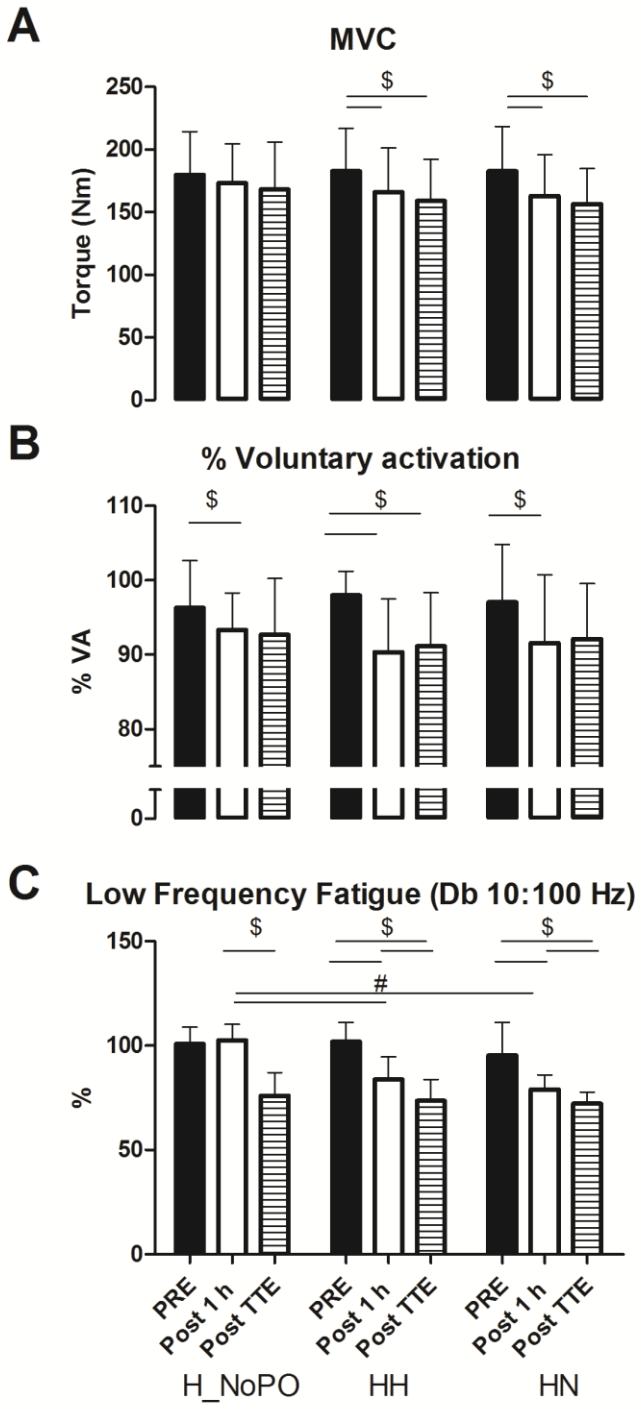

Figure 39 Main NMF effect of PH. Maximal voluntary contraction, MVC (panel A), % of voluntary activation, %VA (panel B), low frequency fatigue, as the low-to-high-frequency doublet ratio, 10:100 ratio (panel C). $: P<0.05 on time. #: P<0.05

Effects on performance

Endurance performance, tested with the TTE decreased significantly only after the HN condition by -37.2% (P<0.001, Cohen's d=1.78) when compared to the test duration obtained in the baseline condition (Figure 40). Test duration of TTE at the end of HN intervention was lower compared to the one performed after the H_NoPO intervention (-30.8%, P=0.016, Cohen's d=1.21).

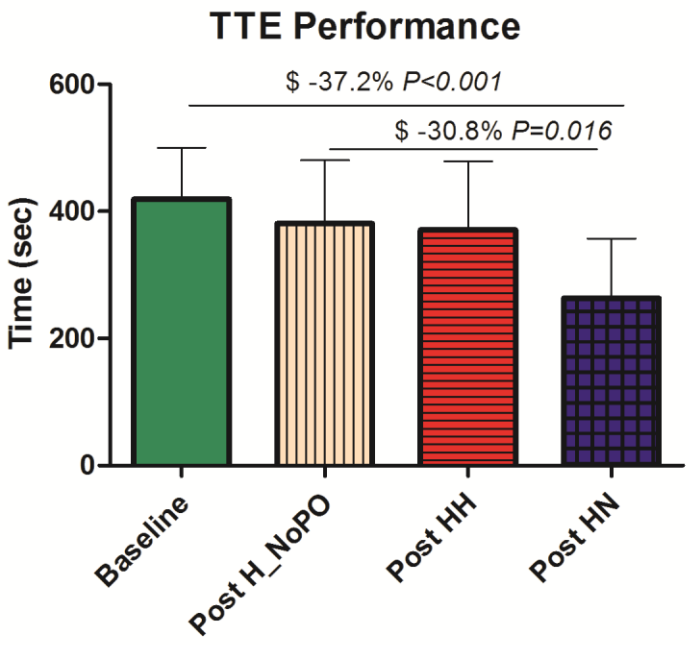

Figure 40 Durations of TTE test at baseline and after the three 1-h progressive hypoxia interventions.

During the interventions

Pulse oxygen saturation (SpO_2) during the three different PH interventions are presented in Figure 41. Significant effect of condition (P<0.001, F=42.806), time (P<0.001, F=47.731) and an interaction condition*time effect (P=0.005, F=4.923) was found. An effect of time was found for every condition, HH (P<0.001, F=57.318) and HN condition (P<0.001, F=35.835) and H_NoPO (P=0.001, F=4.210). Tissue Oxygen Index (on Vastus lateralis) decreased significantly with an effect of time (P=0.012,

F=5.055). An effect of condition was also found (P=0.006, F=16.098) but without any significant interaction time*condition effect. On the contrary deoxyhemoglobin (on Vastus lateralis) increased significantly with an effect of time (P<0.001, F=18.571). An effect of condition was also found (P<0.001, F=25.001) but without any significant time*condition interaction.

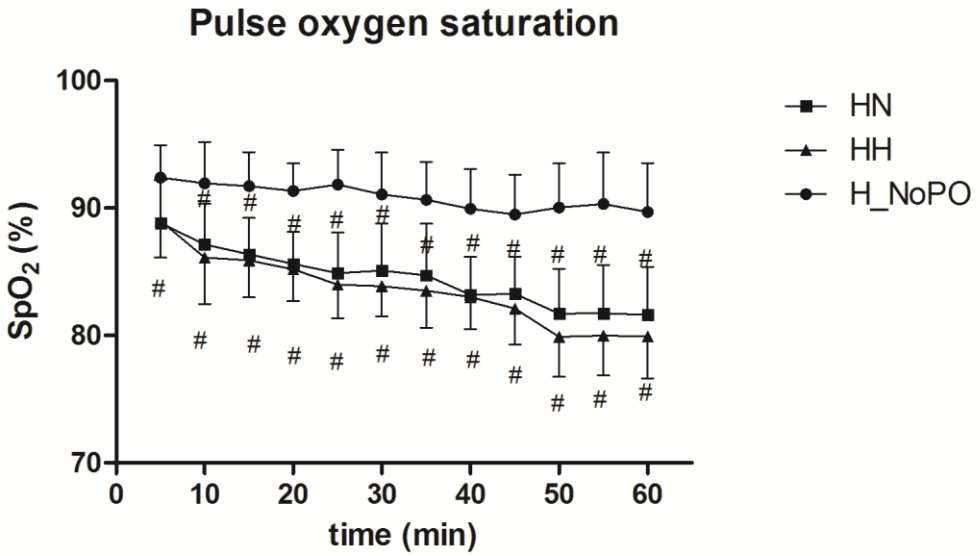

Figure 41 Pulse oxygen saturation during the 1-h PH interventions. # significantly different from H_NoPO

Cerebral (CBF) and femoral (FBF) blood flow during the three different PH interventions are presented in Figure 42. A general effect of time was found for CBF (P<0.001, F=20.595), but neither an effect of condition nor an interaction time*condition effect was recorded. An effect of time was found for every condition, H_NoPO (P=0.023, F=4.834), HH (P=0.009, F=6.372) and HN condition (P=0.034, F=4.375). A significant effect of condition (P<0.001, F=120.622), time (P<0.001, F=188.191) and an interaction condition*time effect (P=0.005, F=57.187) was found for the FBF (panel B). An effect of time was found for HH (P<0.001, F=130.902) and HN (P<0.001, F=91.932). No effects of time on H_NoPO have been recorded.

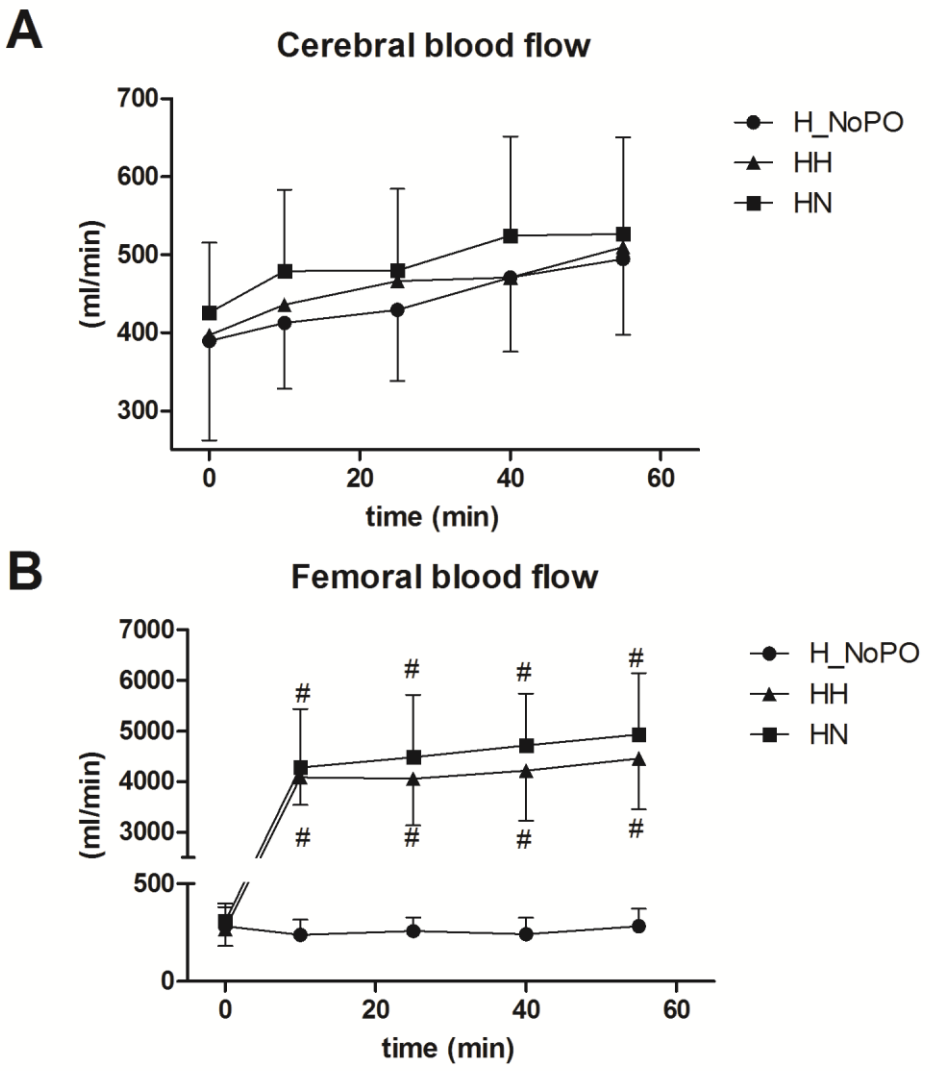

Figure 42 Cerebral (panel A) and Femoral blood flow during the 1-h PH interventions. # significantly different from H_NoPO

Rate of perceived exertion was collected with three focuses (Figure 43) every 5 minutes. Subjects reported firstly an overall index of RPE (panel A) which changed significantly with an effect of condition ($P<0.001$, $F=151.216$), time ($P<0.001$, $F=100.273$) and an interaction condition*time effect ($P<0.001$, $F=29.819$). An effect of time was found for every condition, H_NoPO ($P=0.018$, $F=2.21$), HH ($P<0.001$, $F=53.774$) and HN ($P<0.001$, $F=86.321$). Pairwise comparisons between conditions at each time point are indicated in Figure 43, panel A.

Subjects furthermore reported RPE of their legs (see panel B) which changed significantly with an effect of condition ($P<0.001$, $F=153.015$), time ($P<0.001$, $F=100.135$) and with an interaction condition*time effect ($P<0.001$, $F=37.619$). An effect of time was found for condition, HH ($P<0.001$, $F=53.77$) and HN ($P<0.001$, $F=86.38$). In H_NoPO no effect of time was seen ($P=0.451$, $F=1.00$). Pairwise comparisons between conditions at each time point are indicated in Figure 43, panel B.

Finally, subjects reported the RPE of their dyspnea which also increased significantly with an effect of condition ($P<0.001$, $F=136.527$), time ($P<0.001$, $F=86.163$) and with an interaction condition*time effect ($P<0.001$, $F=40.124$). An effect of time was found for every condition, HH ($P<0.001$, $F=45.577$), HN ($P<0.001$, $F=85.117$) and H_NoPO ($P<0.001$, $F=4.639$). Pairwise comparisons between conditions at each time point are shown in Figure 43, panel C.

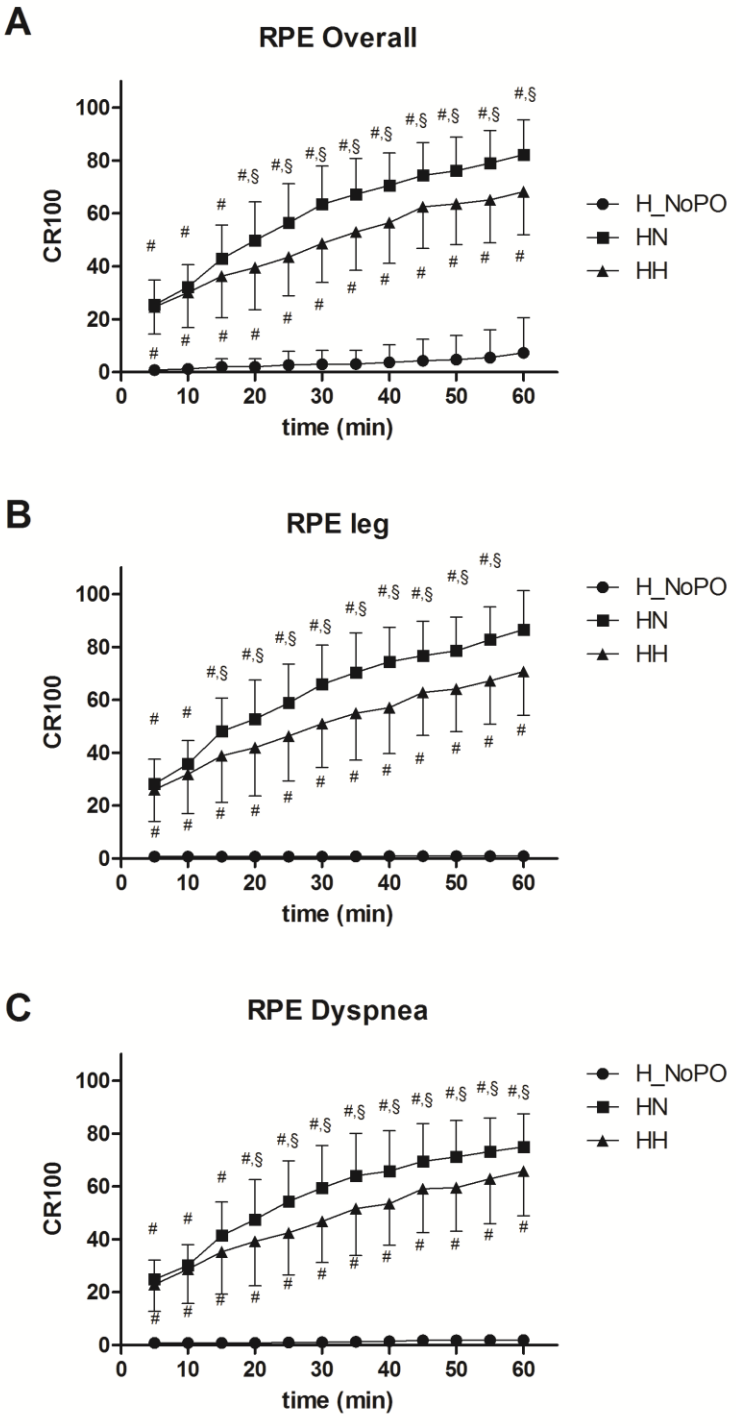

Figure 43 Rate of perceived exertion during the 1-h PH interventions. Overall perception (panel A), leg perception of fatigue (panel B), perception of dyspnea (panel C). # significantly different from H_NoPO, § sign. different from HH.

Blood lactate was collected at minute 10 and 30 as a double check for MLSS (Figure 44). An effect of condition (P<0.001, F=36.141), time (P<0.001, F=18.317) and an interaction condition*time effect (P<0.001, F=12.759) was found. An effect of time was found for condition, HH (P<0.001, F=17.41) and HN (P<0.001, F=28.441). In H_NoPO no effect of time was seen (P=0. 331, F=1.187). Significant differences between conditions at each time point have been reported on Figure 44.

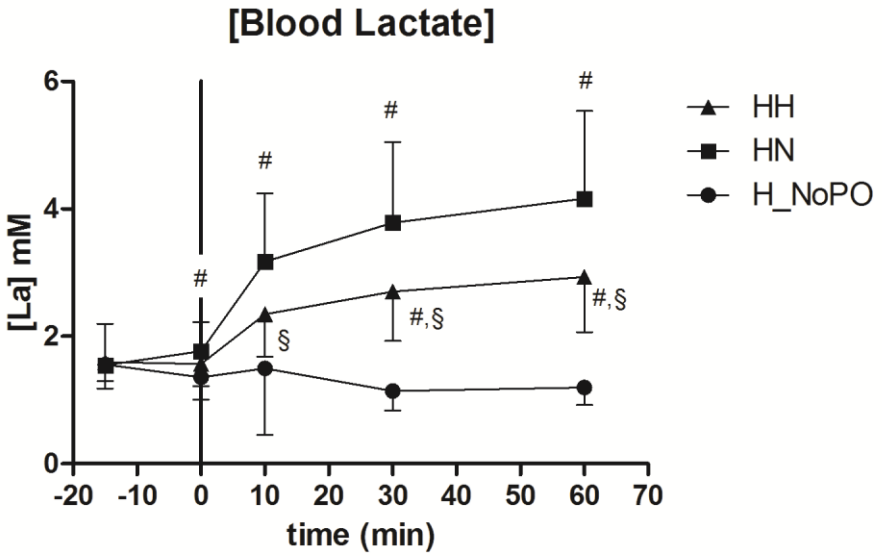

Figure 44 Blood lactate concentration at fixed time points before and during the 1-h PH interventions. # significantly different from H_NoPO, § sign. different from HH.

The heart rate was monitored throughout the entire duration of the interventions (Figure 45). An effect of condition (P<0.001, F=467.470), time (P<0.001, F=74.278) and an interaction condition*time effect (P<0.001, F=17.037) was found. An effect of time was found for every condition, H_NoPO (P=0.001, F=4.16), HH (P<0.001, F=58.374) and HN condition (P<0.001, F=46.011). Pairwise comparisons between conditions at each time point are indicated in Figure 45.

Data of oxygen consumption monitored during the entire 1-h interventions are presented in Figure 46. An effect of condition (P<0.001, F=1247.206), time (P<0.001, F=32.612) and an interaction condition*time effect (P<0.001, F=15.902) was found. An effect of time was found for HH (P<0.001, F=43.725) and HN condition (P<0.001,

F=26.465). For H_NoPO condition, no effect of time was found (P= 0.309, F = 1.186). Pairwise comparisons between conditions at each time point are indicated in Figure 46. Also Ventilation (VE) followed a similar trend with a significant effect of condition (P<0.001, F=399.430), time (P<0.001, F=54.196) and an interaction condition*time effect (P<0.001, F=22.569). An effect of time was found for every condition, H_NoPO (P=0.001, F=3.185), HH (P<0.001, F=19.032) and HN condition (P<0.001, F=26.870).

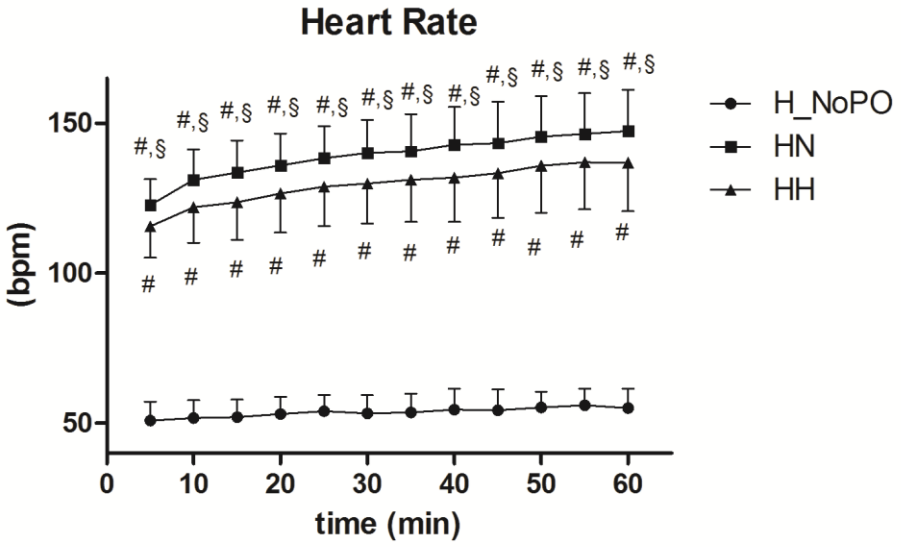

Figure 45 Heart rate during the 1-h PH interventions. # significantly different from H_NoPO, § sign. different from HH.

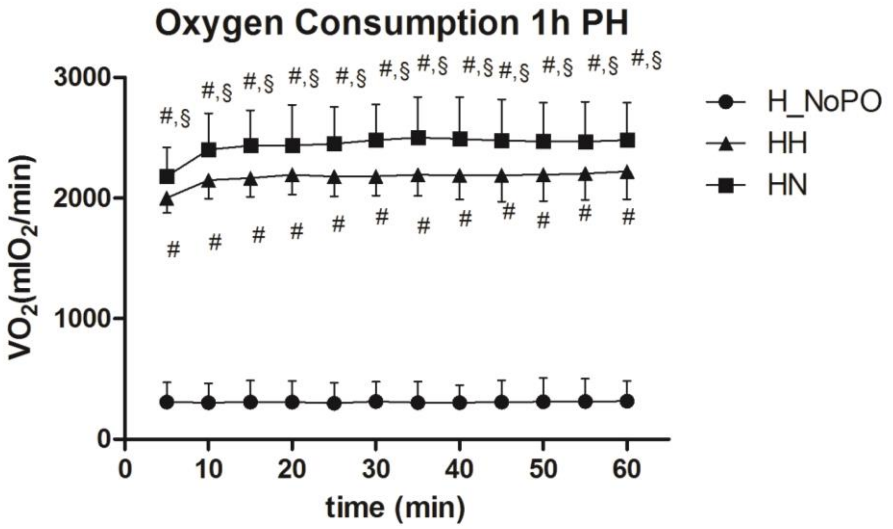

Figure 46 Oxygen consumption during the 1-h PH interventions. # significantly different from H_NoPO, § sign. different from HH.

5 Discussion

5.1 Discussion of study I

With this study we aimed to determine the effects of exposure to acute progressive hypoxia during an 1-h of constant exercise at 50% of the relative Peak Power Output, when compared to constant hypoxia and normoxia on endurance performance.This study was designed to try to understand if different hypoxic doses of exposure during a submaximal exercise influence (impairs) the high intensity performance of a hypothetical final high intensity effort.

Information about this topic can have a practical application deriving from the interest to know if after an endurance effort at a moderate intensity our ability to perform at high intensity is changed (reduced). This can be the real situation of a mass start competition which take place at submaximal intensity throughout the entire duration, but a high intensity effort need to be sustained to try to win the competition (50 km XCskiing, cycling stage, etc.). Moreover, there can be the case of a submaximal exercise which take place in an environment where the athlete is exposed to hypoxia (Hcost) or to a progressive hypoxia (HH) with the final end high intensity effort at a higher altitude compared with the starting one (cycling mountain stage with an uphill arrival or an uphill cycling time trial (Peinado et al., 2018)).

According to the data collected in our study one of the main results regarding the effects on performance is that after both a normoxic and constant hypoxic cycling effort at 50% of the relative PPO the endurance performance, tested through a TTE test, is impaired by roughly -25%. This reduced performance when compared to a baseline TTE test done in a non-fatigued state (Figure 30) was not observed after a progressive increase in hypoxia during the 1-h submaximal effort.

This different impairment, happened even if the final altitude was matched with the Hcost one and the SpO2 measured at minute 60 was not different between Hcost and HH condition (Figure 31). For this reason, we cannot ascribe to the pulse oxygen saturation value the different effect on performance of the two interventions. The

matching of SpO_2 reflect the trend observed in the FiO_2 (Figure 28) and confirm the ability of the hypoxic chamber to reproduce the FiO_2 target and, subsequently, the goodness of the entire setup built also to verify the effects on neuromuscular function. This is of crucial importance when people are involved in a physical activity. In fact, even during low power output (30-60 Watt) efforts a reduction in SpO_2 was observed both in normoxia and at different degree of hypoxia (Smith, 2007). The importance of similar SpO_2 is also underlined by Deb and colleagues which pointed out that exercise performance is moderated also by SpO_2 with a mean reduction of roughly -2% per 1000 meters of altitude gain (Deb et al., 2018). During the HH condition SpO_2 reflected the trend monitored at rest by Zhang and colleagues where subjects were exposed to a 10 minutes of progressive hypoxia (Zhang et al., 2015).

A lower SpO_2 at Hcost can justify the lower Tissue Oxygenation Index found from minute 30 to 60 when compared to normoxia. One may argue that a lower TOI value may reflect a higher anaerobic contribution (Grassi et al., 1999) that is in line with our finding of an increased concentration of blood lactate during the Hcost compared to the N trial even if performed at the same relative power output. This occurs despite lower oxygen consumption is needed in Hcost compared to N (Figure 37).

Indeed, even if a similar oxygen consumption during the two hypoxic interventions was observed, a significantly difference ($P=0.03$) with the N condition was registered. The similar results in HH and Hcost are in line with what previously reported about gross efficiency and the effects of hypoxia on this index (Clark et al., 2007; Wyatt, 2014) even if there is not a clear consensus about the effects of simulated altitude on gross efficiency and, more in general on oxygen cost (Noordhof et al., 2013). Despite a similar oxygen consumption Hcost results in a higher heart rate compared to HH. This data confirmed previous studies reporting an increase in heart rate at rest or at similar target work rate in hypoxia (Clark et al., 2007; Zhang et al., 2015) where also a higher ventilation (L/min) was observed.

Another important finding is that these differences were not perceived by the subjects that reported a RPE increasing with time but without any differences between conditions. This is in contrast with the results of Friedmann and colleagues that reported higher values of RPE in normoxia when they compared to 1-h of exercise at the same relative Individual Anaerobic Threshold (Friedmann et al., 2004) and maybe because we chose an intensity relative to the peak power output. An interesting point on RPE can be seen when Hcost and HH are compared. In fact, despite a higher simulated altitude at the beginning of the 1-h effort in Hcost, we did not observe an increase neither in the overall, nor legs or dyspnea RPE indexes compared to the HH condition performed at the same power output. We found a similar effect of time on RPE in all the three conditions (N, Hcost and HH) that is similar to what found by Hopker and colleagues during a 120 minutes constant load cycling (Hopker et al., 2017). The same effect of time was found also by Girard and colleagues in athletes involved in 10 4-seconds sprints in both normoxia and hypoxia. On the contrary with our findings, they also found an effect of the condition itself, with higher rate of perceived exertions even with lower power output in hypoxia. This can probably be imputed to the different physical task duration (Girard et al., 2017). Our findings on perceptions didn't help to predict the different effects on the subsequent TTE performance.

A possible explanation to the different effect of HH and Hcost on TTE performance can be related to the hypoxic dose. To be more precise we can try to apply the same model proposed by Garvican-Lewis and colleagues (Garvican-Lewis et al., 2016b) (e.g the model is termed "kilometer hours" and is defined as km·h = (m/1,000) x h, where m indicates elevation of exposure in meters and h indicates total duration of exposure in hours; 5.25 in Hcost VS 3.75 for HH) even if we are analysing a shorter duration of exposure compared to the ones hypothesized by the authors (Garvican-Lewis et al., 2016a; Millet et al., 2016b). Otherwise we can consider, as a possible explanation, that we choose the relative intensity in HH as the 50% of the PPO obtained at the final altitude of the HH (FiO_2 13.4%) even if part of the intervention session was performed

at lower altitude, so at a hypothetical lower percentage of PPO. This can be considered a limitation and might have brought a positive effect on the subsequent TTE compared to Hcost.

Furthermore, even the effects on neuromuscular function does not seem to be the cause of the different response in TTE. In fact, as we know, the magnitude and the etiology of fatigue following whole-body exercises at different altitudes remain equivocal owing to the actual degree of hypoxemia (SpO2) achieved by the subjects and the consideration of relative vs absolute workloads. We already discussed the effects of SpO_2 and we already considered the choice of a relative intensity contrary to previous studies such as the one of Amann and colleagues (Amann et al., 2007). And our results on NMF evaluations seems to confirm that SpO_2 is related to the magnitude and the etiology of fatigue with a reduction in the % of voluntary activation Figure 29, as an index of central fatigue, that confirms the change in altitude between the pre and the post 1-h evaluation (Figure 31).

Concluding, the similar impairment in performance found after the two conditions where the athletes are involved in a task in constant condition of hypoxia (N and Hcost) and the minor impairment in performance in HH compared to Hcost confirm our hypothesis. This can be a first consideration for a practitioner, athletes or coach involved in the training process of athletes competing at altitude, but further investigations need to be planned to better investigate the effects of progressive hypoxia.

5.2 Discussions of study II

As described above, in most of the competitions and performances in hypoxic environments there is a common peculiarity: athletes start from an altitude and finish to a higher one. Furthermore, the increase in altitude during these efforts is more or less linear. The present study was planned to investigate the effects of an acute exposure to PH. To answer the research question subjects rested and exercised at two different intensities during a 1-h exposure. We aimed to understand the effects on a subsequent high-intensity endurance performance and on the Neuro Muscular Function, but with the aim also to investigate the physiological responses during the three exposures.

Contrary to our hypothesis to find an impairment both after HH and HN, one of the main findings is that only after 1-h cycling at 50% of the absolute peak power output obtained in normoxia, HN, we observed an impairment of endurance performance. In fact, a -37.2% time reduction of the HN TTE was found when compared to the baseline test performed in a non-fatigued state at the same simulated altitude. This reduction can be observed also when the TTE after HN is compared to the H_NoPO's one (-30.8%). No effects on performance were found after HH and H_NoPO when compared to the baseline test. This can maybe be justified by a higher RPE during the HN 1-h intervention compared to HH and H_NoPO, and a higher blood lactate concentration and oxygen consumption even if SpO_2, known to be responsible of changes in aerobic exercise capacity (Amann and Kayser, 2009), was not reduced at a major extent compared to HH.

In all the three sessions a decrease post 1-h of the voluntary activation, as an index of central fatigue, can be the consequence of a reduced oxygen availability (Siebenmann and Rasmussen, 2016). On the contrary the LLF, as an index of peripheral fatigue, was impaired more from the intensity itself than form the altitude gain. In fact, differences were found between the evaluations post 1-h and the post TTE ones. Moreover, differences have been found also between post 1-h at H_NoPO and Post 1-h at HH and HN supporting the idea of a major role of effort rather than hypoxia (Figure 39, panel C). So, peripheral fatigue in PH is related to the exercise intensity. In fact, muscles

were mostly impaired after the TTE due to the accumulation of metabolites production at high intensity known to cause the failure of the excitation-contraction coupling within the muscle fibres (Amann and Calbet, 2008).

During the 1-h interventions, as shown in Figure 38, the hypoxic chamber was able to decrease linearly the fraction of inspired oxygen. This reduction influenced SpO_2 and we observed an effect of time in all the conditions. This decrease was already found in literature by Zhang and colleagues, during a 10 minutes rest exposure to a progressive increase in altitude from sea level to 3600 m (Zhang et al., 2015) and by Netzer and colleagues during a comparison between a real hike on Mount Mauna Kea and a simulated one (Netzer et al., 2017). In our study, the reduction in SpO_2 was most pronounced when athletes were exercising and also this finding is in line with the literature (Smith, 2007). Importantly, there was not a different reduction in SpO_2 due to different exercise intensity.

This reduction is counteracted by an increase in cerebral blood flow in all the conditions considered in this study and also this results are in line with what was reported in literature when investigating CBF at different altitudes (Ainslie and Subudhi, 2014).

Considering the decrease in Voluntary Activation occurred from pre to post 1-h and considering the role of oxygen within the brain in order to allow a good central motor drive (Siebenmann and Rasmussen, 2016) we can argue that an acute exposure to PH induced a decrease in SpO_2 that is not reversed sufficiently in the brain by an increase of CBF, inducing a decrease in %VA. It seems that, even we know that a higher CBF may be seen as an adaptation useful to increase oxygen availability to the brain level (Ainslie and Subudhi, 2014), this increase could be not enough in an acute PH condition with a 0.42 m/s vertical speed to prevent from central fatigue.

On the contrary the femoral blood flow increased with an effect of time in HH and HN without any differences related to the exercising intensity but, interestingly, FBF did not increase in the H_NoPO condition at rest. So, we can sustain that intensity rather than hypoxia influences with major extent the FBF.

During the resting condition (H_NoPO), such as for FBF, also leg RPE was not affected by time (i.e. decrease in FiO_2) while an increase in this index was found in HH and a further increase is related to the higher effort intensity in HN. All the other results on RPE are influenced by time and intensities. The increase in RPE during 1 hour exercise at the same intensity was also observed by Friedmann and colleagues both in normoxia and constant hypoxia (Friedmann et al., 2004). Interestingly, athletes' perception of effort is in line with all the physiological parameters presented in the study where a significant difference between condition was registered and can be related to the intensity of the effort and this can be an interesting when a coaches need a tool to monitor training load in hypoxic environements.

The findings could bring some practical application considering the increasing number of competitions in hypoxic environments and characterized by a linear gain in altitude during the course of the race, and the need of more knowledge to train athletes involved in this kind of events.

5.3 General discussion and conclusion

As already observed elsewhere the results of our maximal tests obtained during the baseline measurements reported a decrease in maximal oxygen uptake related to hypoxia (MacInnis et al., 2015; Peronnet et al., 1991; Wehrlin and Hallen, 2006). Rate of perceived exertion at the end of the CPET are similar and in line with Horstmann and colleagues (Horstman et al., 1977). Contrary to what expected and even if performed at the same relative PPO, the TTE performed in hypoxia lasted less than the one in normoxia (see Table 7 and Figure 30). Elsewhere, and for longer durations, an intensity related to the individual anaerobic threshold has been chosen (Friedmann et al., 2004) and could be that our findings has been influenced by the use of a non-individualized selection of the intensity. But, in the last study mentioned, they found the individual anaerobic threshold at the same percentage of maximal oxygen consumption tested in hypoxia and normoxia, respectively. According to this point we choose non-individualized selection of intensity but we used both a relative vs absolute way to choose exercise intensity contrary to Amann and colleagues (Amann et al.,

2007) that found at 2700 m an obvious reduction of duration at ~81.4% of the PPO obtained in normoxia.

Considering the effects on TTE performance obtain after the intervention session of study I and study II we can suggest to people involved in PH competitions to adapt properly the intensity of the uphill as long as they would be ready to perform a high intensity effort and to stuggle for the victory. Based on this, we suggest to coaches to test their athletes at the altitude of the competitions in order to have a personalized response to hypoxia of the subject or at least consider the formula according to which we can expect a ~6% of reduction in maximal oxygen consumption per 1000 m altitude elevation (Deb et al., 2018).

We reported a decline in SpO2 related to the exercise (Smith, 2007) and the progressive increase in hypoxia (Zhang et al., 2015). This can be the cause of the reduction in percentage of voluntary activation in post 1 hour NMF evaluations compared to the pre ones in the study II (PH) (Siebenmann and Rasmussen, 2016). The Low frequency fatigue reported can be related to the increase in acidosis driven by the intensity of effort itself (Amann and Calbet, 2008). Exercise duration and intensity plays an important role in fatigue, and it will be interesting in the future to understand the recovery of central and peripheral neuromuscular fatigue after exercise (Carroll et al., 2017).

Cerebral blood flow during the PH interventions increase with an effect of time. This is a first insight on this aspect during an acute exercise in PH. This mechanism seems to be related to the decrease on SpO_2, but without any effects of the intensity of effort. Seems to be depending only on the hypoxic stimulus (Ainslie and Subudhi, 2014).

So, even if we know that aerobic exercise capacity is highly sensitive to changes in SpO_2 (Amann and Kayser, 2009), and that the lack of oxygen is the obvious cause of a reduced capacity, the exact mechanisms by which hypoxia leads to an earlier disengagement from an exercise challenge remain unclear (Kayser, 2005). Fan and Kayser listed in their recent review various factors that can contribute toward the

development of fatigue, but the cessation of exercise is ultimately initiated by the brain (Fan and Kayser, 2016). From this perspective, the endpoint of increasing fatigue (i.e. exhaustion) is of central nervous system origin. This is in line with the finding of study I in which a reduced ability to perform after Hcost has been found compared to HH, explained by a longer exposure to an oxygen desaturated condition. This can only partially be in line with the data obtained during our study II, where pulse oxygen saturation was reduced by hypoxia and with a more extent by intensity (Smith, 2007). It has been suggested that the feed-forward regulation of voluntary exercise performance is based on one's RPE (Tucker and Noakes, 2009), that we know is defined as the conscious manifestation of the degree of strain experienced during physical work (Borg, 1982). Although several theories exist regarding the physiological and psychological determinants of RPE, research suggests that it is an integration of multiple sources of information including corollary discharge of the efferent output from the motor cortex, afferent feedback from the periphery, as well as psychological factors such as previous experience and knowledge of exercise duration, motivation, positive and negative affect and awareness (Farra et al., 2017). According to this point we can justify the reduction in TTE performance after HN intervention due to a higher RPE reported by the subjects during the 1-h cycling that influenced the subsequent high-intensity exercise.

In light of our results it is difficult to conclude if RPE is more affected by exercise intensity or by hypoxia per se. In fact, when athletes performed under the same hypoxic stimulus the RPE was affected by the intensity of the effort itself (study II). On the contrary at the same power output (relative or absolute) but different hypoxic stimulus, a similar rate of perceived exertion was recorded (study I). It seems that for submaximal intensities relative to the PPO obtained in the different conditions, the perception of effort is similar. This can be an interesting information for coaches that need to monitor training camps in altitude (Mujika, 2017). Moreover, these results could be useful in sedentary people in which usually a less engagement in physical activity is an important refrain from an active lifestyle (Figard-Fabre et al., 2010). Hopefully, according to the

renewed interest on hypoxic exposure (plus exercise) as a therapy in this kind of population (Millet et al., 2016a), this findings can help in a more consciousness utilization of this method.

Concluding, progressive hypoxia is part of a lot of endurance performances. The knowledge of the effects driven by a PH exposure at different intensities on a subsequent endurance performance can be useful for coaches and athletes that need to plan and pace their efforts in similar environments. We need to be conscious that the threshold between choosing the correct intensity of effort and an intensity that can results in a subsequent impairment during an endurance performance is really thin. Therefore, it can be a good practice to test athletes that need to perform at altitude, in a similar condition.

Finally, we hope that a small step forward in the knowledge of efforts during a progressive hypoxic stimulus has been provided. More work is needed, and the next step will be to obtain useful information also from field studies.

5.4 Limitations

Considering the popularity of using normobaric hypoxia to prepare for hypobaric hypoxia experienced during mountaineering activities at real altitude, Netzer and colleagues (Netzer et al., 2017) compared the effect on SpO_2 and heart rate measured during a real hike on Mount Mauna Kea (4205 m) with a simulated effort in a normobaric chamber. Both the trails were done at a constant speed of 1.6 km/h. They reported the general trend of SpO2 throughout the entire duration of the two 7 hours trials with a general decline from ~93% to 72-77% with an always higher saturation during the normobaric hypoxic trial and a recovery of 4-5% point of saturations during the three breaks at 3100, 3500 and 3900 m of altitude. According to this point it can be questionable to try to study the effect of hypobaric hypoxia in a normobaric chamber.

But, considering the novelty of the topic and the need to understand the determinants of these efforts, we can start studying PH from the more standardized environment as possible in order to provide an help with more evidences to athletes involved in training and competition in an environment where the altitude increase during the competition (the FiO_2 decrease) and give them the right amount of training or suggest them the right pacing. Moreover the range of altitude chosen for the intervention sessions has been decided taking into account this slightly different physiological response attributable to the difference between normobaric and hypobaric hypoxia and we decided to finish the tasks at an altitude slightly higher than the ones easily accessible on the Alps [for example Stelvio pass (~2800 m asl), or the end point of a lot of Vertical kilometre competition (Cervino VK, Triple vertical Kilometer, Italy)].

Also the fixed power output during the 1 hour intervention can be pointed out as a limitation, especially during the progressive hypoxia sessions. We decided to keep the power output constant even if the constant change in altitude during the sessions changed the % of relative intensity according to what describes in the introduction at chapter 1. We choose this approach with the idea to exclude another dependent variable.

The subjects involved in the study are only males. This can be considered a limitations but there are evidence supporting the idea that despite several baseline differences between men and women, the cardiopulmonary effects of acute NH are consistent between men and women (Boos et al., 2016).

5.5 Parallel projects

I. Hypoxia acts as a stimulus for an increased sympathetic activity (Amann and Kayser, 2009) and a reduced parasympathetic cardiac control (Buchheit et al., 2004), that can turn in a slower recovery of HR and HRV indices in the post-exercise period (Al Haddad et al., 2012). 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. This can certainly limit the evaluation of recovery from hypoxic exercise both when used for health assessment or training load estimation purposes (Ward et al., 2017).The aim of this I parallel project is to investigate the effects of acute hypoxic exposure on the post-exercise cardiac autonomic modulation following maximal cardio-pulmonary exercise testing(CPET). To answer this question we monitored the recovery phase of the CPETs both in normoxia and in hypoxia with a heart rate monitor able to assess heart rate variability (Fornasiero et al., 2018).

II. Respiratory compensation point has been determined also by means of NIRS in healthy adults (Fontana et al., 2015). To date we don't know if it can be done also in hypoxic condition and especially if the VO_2 associated with the onset of a plateau in NIRS-derived deoxyhemoglobin occurs in coincidence with the VO_2 at respiratory compensation point such as in normoxia. To answer this question, we monitored the entire duration of CPETs both in normoxia and in hypoxia. This answer can be interesting in light of the new and always cheaper NIRS portable devices. We can maybe consider this kind of tool in the future as useful to monitor physical efforts in hypoxic environments.

III. As already mentioned in paragraph 2.1, some studies used perceived exertion in hypoxia, to the best of our knowledge there are few data about the validations of this scales in hypoxic conditions. At sea level it is possible to find a comparison between three rating scales for perceived exertion and two different work tests (Borg and Kaijser, 2006) and another experiment on perceived exertion, where Category Ratio 100 (CR100) was compared with Absolute

Magnitude Estimation (AME) (Borg and Borg, 2002). Therefore, a side part of the present project will aim to verify the validity of CR100 in hypoxia. To answer this question, we asked at the end of each step of CPETs both in normoxia and in hypoxia a RPE on CR100. As already well-known, training quantification is basic to evaluate an endurance athlete's responses to the training loads, it ensures adequate stress/recovery balance and determines the relationship between training and performance. Among subjective methods of quantification the RPE is the most important because of its wide use (Mujika Iñigo, 2017). A more detailed knowledge about RPE and hypoxia should be of great interest also in the future use of this tool in order to better monitor training load during altitude training camps.

IV. Endothelium derived nitric oxide (NO) is crucial in preserving vascular tone. Several investigations show that NO bioavailability and utilization is regulated by oxygen (O_2) concentration (Trinity et al., 2012). However, there are still insufficient experimental data to show how $O2$ concentration affects NO-dependent endothelial function of the vasculature. Therefore, it could be interesting to investigate the role of O_2 concentration on NO-dependent endothelial function. To answer this question, we performed two single passive limb movement (sPLM) tests. Data obtained from this procedure are known to be an index of NO-dependent systemic endothelial function. The tests were performed at rest, after 30 minutes of early acclimatisation, both in normoxia and in hypoxia. We hypothesized that different O_2 concentration would have elicited different responses during sPLM test and preliminary data goes in this direction.

5.6 Perspectives

Probably, as suggested by Fan and Kayser (Fan and Kayser, 2016) novel approaches are clearly needed to tackle the complex matter of hypoxia and the role of oxygen into that matter. Employing alternative neuroimaging techniques may help researchers gain

better insights to the “origin” of central fatigue, especially in moderate-to-severe hypoxic environments. Identifying the sites involved in central fatigue will bring us one step closer toward clarifying the role of cerebral oxygenation in exercise performance in condition of reduced oxygen.

Considering my actual knowledge, my interest in practical applications, and my passion in outdoor activities I hope that in the future we will be able, at least for cycling, to suggest how to adjust intensity of effort as a function of maximal mean power output ability obtained at different altitudes. Then, this kind of applicative final aim can be moved into running/skiing uphill performances maybe implementing the ability to calculate external power output of the athletes on commercial GPS monitors as a new tool to monitor exercise intensity with less (or known) influence by hypoxia (e.g. compared to HR). To do that we can start analysing cycling field power data stratified for altitudes and parallelly work on a tool (e.g. a smartwatch) able to measure external power generated by runners and alpinists in uphill performances.

Acknowledgments

I started visiting CeRiSM in Rovereto (at that time the name was CeBiSM) back in 2007. The idea to join Rovereto was born after the first class in Verona with prof. Schena, he was teaching aerobic training methods wearing a t-shirt of the cross-county skiing national team, talking about G. Di Centa at Torino 2006 Olympic Games and just the year before I quit from XCskiing competitions (low level). I'm thanks to Prof. Schena and to most of the opportunities I had since that day. Since the begin I met the unconditioned support of Barbara (that is now my supervisor) and Lorenzo. In that period Nicolas and Helene were at CeRiSM for a post-doc position, and thanks to shared passions I spent a lot of time with Nicolas who help me a lot on my bachelor thesis on D-max mod during XC-skiing testing protocols. I learned a lot from him. I grew side by side with Alfredo…call him a colleague, is reductive. Moving forward I'm grateful to the time spent with the other guys at CeRiSM… Chiara, Roberto, Spyros, Andrea, Gilli, Stella, Laura, Gennaro, Alessandro R., Alessandro L. and Franco Impellizzeri who was my supervisor at the master thesis. A special thanks to Alessandro, I helped him for his master thesis and now he's running faster than me. Back to this thesis, I'm proud to move in a basically new field that is Progressive Hypoxia but, without the critical approach of Gianluca (my co-supervisor) probably I didn't have the chance to run the protocol as it is now. Also Prof. Laurent Mourot and Millet GY helped me in being more critic. Barbara helped me a lot in defining the protocol and revising the thesis. Spyros and Alessandro spent a lot of time in the hypoxic chamber (we also had some improvements in performance due to hypoxic exposure 😉). Gennaro, Anna (she was also in the lab half of the times), Massimo and Stella helped me in the data analysis. Lorenzo provide the ergometer for the NMF evaluations and the SW for the PNS. Basically, everyone at CeRiSM was a little bit involved.

After that, I need to switch to Italian to give my general acknowledgments.

Per prima cosa devo ringraziare i miei genitori, Battista e Franca, che mi hanno dato la possibilità di studiare con tranquillità e di appassionarmi allo sport. Oltre a loro i miei

fratelli Roberta, Fabio e Monica; insieme a loro Ivan, Luisa e Christian e i miei nipoti Valeria, Virginia, Lorenzo, Luca, Alessia, Chiara, Davide, Emma e Pietro. Oltre a loro, devo ringraziare chi negli ultimi anni riesce a sopportarmi più di tutti…non so come faccia, sinceramente..., Federica. Chissà se finita questa avventura sarò meno irascibile 😉.

Stare al CeRiSM per me, significa vivere a Rovereto. Qui ho avuto modo di condividere molto (casa, passioni, qualche birra) con colleghi e amici come Spyros, Genna, Alfred, Gian, Zigno, Star, Ale, Michele, Della… Gennaro e Spyros poi hanno avuto la sfortuna di essere anche miei coinquilini, ma sono sopravvissuti. Grazie. Poi un grazie va anche a chi è "collega" e amico extraCeRiSM…Jo, Mirco, Luca F….

Poi ci sono gli amici di sempre, soprattutto di Clusone…come farei senza di voi, grazie.

Ultimi ma so che non si offendono, un enorme grazie ai ragazzi che sono venuti "volontariamente" per 9 volte nei lab del CeRiSM a farsi torturare per la scienza: Maurizio, Jacopo, Dacroix, Dema, Della, Grisa, Simone, Soni, Re Giorgio, Trentini, Giorgio, Zando e Ema.

Un ringraziamento speciale devo farlo anche a Dario Broccardo, discutere di ciclismo con lui è quasi come avere l'enciclopedia a disposizione.

References

Acker, T., and Acker, H. (2004). Cellular oxygen sensing need in CNS function: physiological and pathological implications. *J. Exp. Biol.* 207, 3171–88. doi:10.1242/jeb.01075.

Ainslie, P. N., Lucas, S. J. E., and Burgess, K. R. (2013). Breathing and sleep at high altitude. *Respir. Physiol. Neurobiol.* 188, 233–256.

Ainslie, P. N., and Subudhi, A. W. (2014). Cerebral Blood Flow at High Altitude. *High Alt. Med. Biol.* 15, 133–140. doi:10.1089/ham.2013.1138.

Al Haddad, H., Mendez-Villanueva, A., Bourdon, P. C., and Buchheit, M. (2012). Effect of acute hypoxia on post-exercise parasympathetic reactivation in healthy men. *Front. Physiol.* 3, 289. doi:10.3389/fphys.2012.00289.

Amann, M., and Calbet, J. A. L. (2008). Convective oxygen transport and fatigue. *J. Appl. Physiol.* 104, 861–870.

Amann, M., and Kayser, B. (2009). Nervous System Function during Exercise in Hypoxia. *High Alt. Med. Biol.* 10, 149–164. doi:10.1089/ham.2008.1105.

Amann, M., Romer, L. M., Subudhi, A. W., Pegelow, D. F., and Dempsey, J. A. (2007). Severity of arterial hypoxaemia affects the relative contributions of peripheral muscle fatigue to exercise performance in healthy humans. *J. Physiol.* 581, 389–403. doi:10.1113/jphysiol.2007.129700.

Bassett Jr., D. R., and Howley, E. T. (2000). Limiting factors for maximum oxygen uptake and determinants of endurance performance. *Med Sci Sport. Exerc* 32, 70–84. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=10647532.

Beretta Piccoli, M., Rainoldi, A., Heitz, C., Wüthrich, M., Boccia, G., Tomasoni, E., et al. (2014). Innervation zone locations in 43 superficial muscles: toward a standardization of electrode positioning. *Muscle Nerve* 49, 413–21. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24741685 [Accessed December 22, 2017].

Bernardi, L., Schneider, A., Pomidori, L., Paolucci, E., and Cogo, A. (2006). Hypoxic ventilatory response in successful extreme altitude climbers. *Eur. Respir. J.* 27, 165–171. doi:10.1183/09031936.06.00015805.

Boos, C. J., Mellor, A., O'Hara, J. P., Tsakirides, C., and Woods, D. R. (2016). The Effects of Sex on Cardiopulmonary Responses to Acute Normobaric Hypoxia. *High Alt. Med. Biol.* 17, 108–115. doi:10.1089/ham.2015.0114.

Borg, E., and Borg, G. (2002). A comparison of AME and CR100 for scaling perceived exertion. *Acta Psychol* 109, 157–175. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=11820425.

Borg, E., and Kaijser, L. (2006). A comparison between three rating scales for perceived exertion and two different work tests. *Scand. J. Med. Sci. Sports* 16, 57–69.

Borg, G. A. (1982). Psychophysical bases of perceived exertion. *Med. Sci. Sports Exerc.* 14, 377–81. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7154893 [Accessed August 8, 2018].

Bouillon, L., Baker, R., Gibson, C., Kearney, A., and Busemeyer, T. (2016). COMPARISON OF TRUNK AND LOWER EXTREMITY MUSCLE ACTIVITY AMONG FOUR STATIONARY EQUIPMENT DEVICES: UPRIGHT BIKE, RECUMBENT BIKE, TREADMILL, AND ELLIPTIGO®. *Int. J. Sports Phys. Ther.* 11, 190–200. Available at: http://www.ncbi.nlm.nih.gov/pubmed/27104052 [Accessed July 19, 2018].

Boyas, S., and Guével, A. (2011). Neuromuscular fatigue in healthy muscle: Underlying factors and adaptation mechanisms. *Ann. Phys. Rehabil. Med.* 54, 88–108. doi:10.1016/J.REHAB.2011.01.001.

Brocherie, F., Girard, O., Faiss, R., and Millet, G. P. (2017). Effects of Repeated-Sprint Training in Hypoxia on Sea-Level Performance: A Meta-Analysis. *Sport. Med.* 47, 1651–1660. doi:10.1007/s40279-017-0685-3.

BROCHERIE, F., MILLET, G. P., HAUSER, A., STEINER, T., RYSMAN, J., WEHRLIN, J. P., et al. (2015). "Live High–Train Low and High" Hypoxic Training Improves Team-Sport Performance. *Med. Sci. Sport. Exerc.* 47, 2140–2149. doi:10.1249/MSS.0000000000000630.

Bruyneel, A.-V., Humbert, A., and Bertrand, M. (2017). Comparison of balance strategies in mountain climbers during real altitude exposure between 1.500 m and 3.200 m: Effects of age and expertise. *Neurosci. Lett.* 657, 16–21. doi:10.1016/j.neulet.2017.06.010.

Buchheit, M., Kuitunen, S., Voss, S. C., Williams, B. K., Mendez-Villanueva, A., and Bourdon, P. C. (2012). Physiological strain associated with high-intensity hypoxic intervals in highly trained young runners. *J Strength Cond Res* 26, 94–105. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=22158261.

Buchheit, M., Richard, R., Doutreleau, S., Lonsdorfer-Wolf, E., Brandenberger, G., and Simon, C. (2004). Effect of Acute Hypoxia on Heart Rate Variability at Rest and During Exercise. *Int. J. Sports Med.* 25, 264–269. doi:10.1055/s-2004-819938.

Burtscher, M., Flatz, M., and Faulhaber, M. (2004). Prediction of susceptibility to acute mountain sickness by SaO2 values during short-term exposure to hypoxia. *High Alt. Med. Biol.* 5, 335–40. doi:10.1089/ham.2004.5.335.

Calbet, J. A., Boushel, R., Radegran, G., Sondergaard, H., Wagner, P. D., and Saltin, B. (2003). Determinants of maximal oxygen uptake in severe acute hypoxia. *Am J Physiol Regul Integr Comp Physiol* 284, R291-303. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12388461.

Calbet, J. A., Radegran, G., Boushel, R., and Saltin, B. (2009). On the mechanisms that limit oxygen uptake during exercise in acute and chronic hypoxia: role of muscle mass. *J Physiol* 587, 477–490. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=19047206.

Carroll, T. J., Taylor, J. L., and Gandevia, S. C. (2017). Recovery of central and peripheral neuromuscular fatigue after exercise. *J. Appl. Physiol.* 122, 1068–1076. doi:10.1152/japplphysiol.00775.2016.

Cerretelli, P. (1976). Limiting factors to oxygen transport on Mount Everest. *J Appl Physiol* 40, 658–667. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=931890.

Chapman, R. F. (2013a). The individual response to training and competition at altitude. *Br. J. Sports Med.* 47, i40–i44. doi:10.1136/bjsports-2013-092837.

Chapman, R. F. (2013b). The individual response to training and competition at altitude. *Br. J. Sports Med.* 47, i40–i44. doi:10.1136/bjsports-2013-092837.

Chapman, R. F., Emery, M., and Stager, J. M. (1998). Extent of expiratory flow limitation influences the increase in maximal exercise ventilation in hypoxia. *Respir. Physiol.* 113, 65–74. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9776552 [Accessed December 20, 2017].

CHAPMAN, R. F., and STAGER, J. M. (2008). Caffeine Stimulates Ventilation in

Athletes with Exercise-Induced Hypoxemia. *Med. Sci. Sport. Exerc.* 40, 1080–1086. doi:10.1249/MSS.0b013e3181667421.

CHAPMAN, R. F., STAGER, J. M., TANNER, D. A., STRAY-GUNDERSEN, J., and LEVINE, B. D. (2011). Impairment of 3000-m Run Time at Altitude Is Influenced by Arterial Oxyhemoglobin Saturation. *Med. Sci. Sport. Exerc.* 43, 1649–1656. doi:10.1249/MSS.0b013e318211bf45.

Clark, S. A., Bourdon, P. C., Schmidt, W., Singh, B., Cable, G., Onus, K. J., et al. (2007). The effect of acute simulated moderate altitude on power, performance and pacing strategies in well-trained cyclists. *Eur J Appl Physiol* 102, 45–55. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17882451.

Cohen, J. (1988). *Statistical power analysis for the behavioral sciences*. doi:10.1234/12345678.

CONSTANTINI, K., TANNER, D. A., GAVIN, T. P., HARMS, C. A., STAGER, J. M., and CHAPMAN, R. F. (2017). Prevalence of Exercise-Induced Arterial Hypoxemia in Distance Runners at Sea Level. *Med. Sci. Sport. Exerc.* 49, 948–954. doi:10.1249/MSS.0000000000001193.

Coppel, J., Hennis, P., Gilbert-Kawai, E., and Grocott, M. P. (2015). The physiological effects of hypobaric hypoxia versus normobaric hypoxia: a systematic review of crossover trials. *Extrem. Physiol. Med.* 4, 2. doi:10.1186/s13728-014-0021-6.

De Pauw, K., Roelands, B., Cheung, S. S., de Geus, B., Rietjens, G., and Meeusen, R. (2013). Guidelines to classify subject groups in sport-science research. *Int J Sport. Physiol Perform* 8, 111–122. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=23428482.

Deb, S. K., Brown, D. R., Gough, L. A., Mclellan, C. P., Swinton, P. A., Andy Sparks, S., et al. (2018). Quantifying the effects of acute hypoxic exposure on exercise performance and capacity: A systematic review and meta-regression. *Eur. J. Sport Sci.* 18, 243–256. doi:10.1080/17461391.2017.1410233.

Dempsey, J. A., and Forster, H. V (1982). Mediation of Ventilatory Adaptations. *Physiol. Rev.* 62, 262–346. doi:10.1152/physrev.1982.62.1.262.

Enoka, R. M., and Duchateau, J. (2016). Translating Fatigue to Human Performance. *Med Sci Sport. Exerc* 48, 2228–2238. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=27015386.

Enoka, R. M., and Stuart, D. G. (1992). Neurobiology of muscle fatigue. *J. Appl. Physiol.* 72, 1631–48. doi:10.1152/jappl.1992.72.5.1631.

Fan, J.-L., and Kayser, B. (2016). Fatigue and Exhaustion in Hypoxia: The Role of Cerebral Oxygenation. *High Alt. Med. Biol.* 17, 72–84. doi:10.1089/ham.2016.0034.

Farra, S. D., Cheung, S. S., Thomas, S. G., and Jacobs, I. (2017). Rate dependent influence of arterial desaturation on self-selected exercise intensity during cycling. *PLoS One* 12, e0171119. doi:10.1371/journal.pone.0171119.

Ferrari, M., Muthalib, M., and Quaresima, V. (2011). The use of near-infrared spectroscopy in understanding skeletal muscle physiology: recent developments. *Philos. Trans. A. Math. Phys. Eng. Sci.* 369, 4577–90. doi:10.1098/rsta.2011.0230.

Figard-Fabre, H., Fabre, N., Leonardi, A., and Schena, F. (2010). Physiological and perceptual responses to Nordic walking in obese middle-aged women in comparison with the normal walk. *Eur J Appl Physiol* 108, 1141–1151. Available at:

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=20091181.

Fontana, F. Y., Keir, D. A., Bellotti, C., De Roia, G. F., Murias, J. M., and Pogliaghi, S. (2015). Determination of respiratory point compensation in healthy adults: Can non-invasive near-infrared spectroscopy help? *J. Sci. Med. Sport* 18, 590–595. doi:10.1016/j.jsams.2014.07.016.

Fornasiero, A., Savoldelli, A., Skafidas, S., Stella, F., Bortolan, L., Boccia, G., et al. (2018). Delayed parasympathetic reactivation and sympathetic withdrawal following maximal cardiopulmonary exercise testing (CPET) in hypoxia. *Eur. J. Appl. Physiol.* doi:10.1007/s00421-018-3945-5.

Friedmann, B., Bauer, T., Menold, E., and Bartsch, P. (2004). Exercise with the intensity of the individual anaerobic threshold in acute hypoxia. *Med. Sci. Sports Exerc.* 36, 1737–1742.

Fulco, C. S., Rock, P. B., and Cymerman, A. (1998). Maximal and submaximal exercise performance at altitude. *Aviat. Space. Environ. Med.* 69, 793–801. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9715971 [Accessed January 13, 2018].

Gandevia, S. C. (2001). Spinal and Supraspinal Factors in Human Muscle Fatigue. *Physiol. Rev.* 81, 1725–1789. doi:10.1152/physrev.2001.81.4.1725.

Gandevia, S. C., Herbert, R. D., and Leeper, J. B. (1998). Voluntary activation of human elbow flexor muscles during maximal concentric contractions. *J. Physiol.* 512 (Pt 2), 595–602. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9763647 [Accessed August 13, 2018].

Garvican-Lewis, L. A., Sharpe, K., and Gore, C. J. (2016a). Last Word on Viewpoint: Time for a new metric for hypoxic dose? *J. Appl. Physiol.* 121, 359–359. doi:10.1152/japplphysiol.00457.2016.

Garvican-Lewis, L. A., Sharpe, K., and Gore, C. J. (2016b). Time for a new metric for hypoxic dose? *J. Appl. Physiol.* 121, 352–355. doi:10.1152/japplphysiol.00579.2015.

Gaston, A.-F., Durand, F., Roca, E., Doucende, G., Hapkova, I., and Subirats, E. (2016). Exercise-Induced Hypoxaemia Developed at Sea-Level Influences Responses to Exercise at Moderate Altitude. *PLoS One* 11, e0161819. doi:10.1371/journal.pone.0161819.

Gavin, T. P., and Stager, J. M. (1999). The effect of exercise modality on exercise-induced hypoxemia. *Respir. Physiol.* 115, 317–23. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10424361 [Accessed January 13, 2018].

Girard, O., Billaut, F., Christian, R. J., Bradley, P. S., and Bishop, D. J. (2017). Exercise-related sensations contribute to decrease power during repeated cycle sprints with limited influence on neural drive. *Eur. J. Appl. Physiol.* 117, 2171–2179. doi:10.1007/s00421-017-3705-y.

Girard, O., and Racinais, S. (2014). Combining heat stress and moderate hypoxia reduces cycling time to exhaustion without modifying neuromuscular fatigue characteristics. *Eur J Appl Physiol* 114, 1521–1532. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=24748530.

Goodall, S., Gonzalez-Alonso, J., Ali, L., Ross, E. Z., and Romer, L. M. (2012). Supraspinal fatigue after normoxic and hypoxic exercise in humans. *J Physiol* 590, 2767–2782. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=22473785.

Goodall, S., Howatson, G., Romer, L., and Ross, E. (2014a). Transcranial magnetic stimulation in sport science: a commentary. *Eur. J. Sport Sci.* 14 Suppl 1, S332-40. doi:10.1080/17461391.2012.704079.

Goodall, S., Twomey, R., Amann, M., Ross, E. Z., Lovering, A. T., Romer, L. M., et al. (2014b). AltitudeOmics: exercise-induced supraspinal fatigue is attenuated in healthy humans after acclimatization to high altitude. *Acta Physiol* 210, 875–888. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=24450855.

Goodwin, M. L., Harris, J. E., HernÃ¡ndez, A., and Gladden, L. B. (2007). Blood lactate measurements and analysis during exercise: a guide for clinicians. *J. diabetes Sci. Technol.* 1, 558.

Grassi, B., Quaresima, V., Marconi, C., Ferrari, M., and Cerretelli, P. (1999). Blood lactate accumulation and muscle deoxygenation during incremental exercise. *J. Appl. Physiol.* 87, 348–355. doi:10.1152/jappl.1999.87.1.348.

Hermens, H. J., Freriks, B., Disselhorst-Klug, C., and Rau, G. (2000). Development of recommendations for SEMG sensors and sensor placement procedures. *J. Electromyogr. Kinesiol.* 10, 361–374. doi:10.1016/S1050-6411(00)00027-4.

Hopker, J. G., O'Grady, C., and Pageaux, B. (2017). Prolonged constant load cycling exercise is associated with reduced gross efficiency and increased muscle oxygen uptake. *Scand. J. Med. Sci. Sports* 27, 408–417. doi:10.1111/sms.12673.

Horstman, D. H., Weiskopf, R., and Robinson, S. (1977). The nature of the perception of effort at sea level and high altitude. DTIC Document.

IBM SPSS website, S. IBM The calculation of Bonferroni-adjusted p-values - Italia. Available at: https://www-01.ibm.com/support/docview.wss?uid=swg21476685 [Accessed July 31, 2018].

Jones, D. A. (1996). High- and low-frequency fatigue revisited. *Acta Physiol. Scand.* 156, 265–270. doi:10.1046/j.1365-201X.1996.192000.x.

Kayser, B. (2005). Why is endurance performance decreased at high altitude?

Schweizerische Zeitschrift fur Sport. und Sport. Available at: https://www.mendeley.com/research-papers/endurance-performance-decreased-high-altitude/?utm_source=desktop&utm_medium=1.18&utm_campaign=open_catalog&userDocumentId=%7Ba137319e-fa80-3c6d-8cbf-2988afd6bb60%7D [Accessed August 8, 2018].

Levine, B. D., and Stray-Gundersen, J. (1997). "Living high-training low": effect of moderate-altitude acclimatization with low-altitude training on performance. *J. Appl. Physiol.* 83, 102–112. doi:10.1152/jappl.1997.83.1.102.

Levine, B., and Stray-Gundersen, J. (1992). A Practical Approach to Altitude Training. *Int. J. Sports Med.* 13, S209–S212. doi:10.1055/s-2007-1024642.

Luks, A. M., Swenson, E. R., and Bärtsch, P. (2017). Acute high-altitude sickness. *Eur. Respir. Rev.* 26, 160096. doi:10.1183/16000617.0096-2016.

MacInnis, M. J., Nugent, S. F., MacLeod, K. E., and Lohse, K. R. (2015). Methods to Estimate V˙O2max upon Acute Hypoxia Exposure. *Med. Sci. Sports Exerc.* 47, 1869–76. doi:10.1249/MSS.0000000000000628.

Maresh, C. M., Deschenes, M. R., Seip, R. L., Armstrong, L. E., Robertson, K. L., and Noble, B. J. (1993). Perceived exertion during hypobaric hypoxia in low- and moderate-altitude natives. *Med Sci Sport. Exerc* 25, 945–951. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=8371656.

Mellor, A. J., Woods, D. R., O'Hara, J., Howley, M., Watchorn, J., and Boos, C. (2014). Rating of Perceived Exertion and Acute Mountain Sickness During a High-Altitude Trek. *Aviat. Space. Environ. Med.* 85, 1214–1216. doi:10.3357/ASEM.4083.2014.

Menegatti, E., Genova, V., Tessari, M., Malagoni, A. M., Bartolomei, I., Zuolo, M., et

al. (2010). The reproducibility of colour Doppler in chronic cerebrospinal venous insufficiency associated with multiple sclerosis. *Int. Angiol.* 29, 121–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20351668 [Accessed August 7, 2018].

Merton, P. A. (1954). Voluntary strength and fatigue. *J. Physiol.* 123, 553–64. Available at: http://www.ncbi.nlm.nih.gov/pubmed/13152698 [Accessed January 15, 2018].

Millet, G. P., Debevec, T., Brocherie, F., Malatesta, D., and Girard, O. (2016a). Therapeutic Use of Exercising in Hypoxia: Promises and Limitations. *Front. Physiol.* 7, 224. doi:10.3389/fphys.2016.00224.

Millet, G. P., Roels, B., Schmitt, L., Woorons, X., and Richalet, J. P. (2010). Combining hypoxic methods for peak performance. *Sport. Med* 40, 1–25. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=20020784.

Millet, G. P., Wehrlin, J. P., Peltonen, J. E., Constantini, K., Kumar, V. H. S., Schmidt, W. F. J., et al. (2016b). Commentaries on Viewpoint: Time for a new metric for hypoxic dose? *J. Appl. Physiol.* 121, 356–358. doi:10.1152/japplphysiol.00460.2016.

Millet, G. Y., Martin, V., Martin, A., and Vergès, S. (2011). Electrical stimulation for testing neuromuscular function: from sport to pathology. *Eur. J. Appl. Physiol.* 111, 2489–500. doi:10.1007/s00421-011-1996-y.

Mounier, R., and Brugniaux, J. V (2012). Counterpoint: Hypobaric hypoxia does not induce different responses from normobaric hypoxia. *J Appl Physiol* 112, 1784–1786. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=22589489.

Mujika, I., and Iñigo (2017). Quantification of Training and Competition Loads in Endurance Sports: Methods and Applications. *Int. J. Sports Physiol. Perform.* 12, S29–S217. doi:10.1123/ijspp.2016-0403.

Netzer, N. C., Rausch, L., Eliasson, A. H., Gatterer, H., Friess, M., Burtscher, M., et al. (2017). SpO2 and Heart Rate During a Real Hike at Altitude Are Significantly Different than at Its Simulation in Normobaric Hypoxia. *Front. Physiol.* 8, 81. doi:10.3389/fphys.2017.00081.

Netzer, N., Strohl, K., Faulhaber, M., Gatterer, H., and Burtscher, M. (2013). Hypoxia-Related Altitude Illnesses. *J. Travel Med.* 20, 247–255. doi:10.1111/jtm.12017.

Noordhof, D. A., Schoots, T., Hoekert, D. H., and De Koning, J. J. (2013). Is gross efficiency lower at acute simulated altitude than at sea level? *Int. J. Sports Physiol. Perform.* 8, 319–322.

Peacock, A. J. (1998). ABC of oxygen: oxygen at high altitude. *BMJ* 317, 1063–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9774298 [Accessed December 19, 2017].

Peinado, A. B., Romero-Parra, N., Rojo-Tirado, M. A., Cupeiro, R., Butragueño, J., Castro, E. A., et al. (2018). Physiological Profile of an Uphill Time Trial in Elite Cyclists. *Int. J. Sports Physiol. Perform.*, 1–6. doi:10.1123/ijspp.2016-0768.

Peltonen, J. E., Rantamaki, J., Niittymaki, S. P., Sweins, K., Viitasalo, J. T., and Rusko, H. K. (1995). Effects of oxygen fraction in inspired air on rowing performance. *Med Sci Sport. Exerc* 27, 573–579. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=7791589.

Peronnet, F., Thibault, G., and Cousineau, D. L. (1991). A theoretical analysis of the effect of altitude on running performance. *J. Appl. Physiol.* 70, 399–404. doi:10.1152/jappl.1991.70.1.399.

Perrey, S., and Rupp, T. (2009). Altitude-induced changes in muscle contractile properties. *High Alt Med Biol* 10, 175–182. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=19519224.

Roach, R. C., Hackett, P. H., Oelz, O., Bärtsch, P., Luks, A. M., MacInnis, M. J., et al. (2018). The 2018 Lake Louise Acute Mountain Sickness Score. *High Alt. Med. Biol.* 19, 4–6. doi:10.1089/ham.2017.0164.

Rupp, T., Leti, T., Jubeau, M., Millet, G. Y., Bricout, V. A., Levy, P., et al. (2013). Tissue deoxygenation kinetics induced by prolonged hypoxic exposure in healthy humans at rest. *J. Biomed. Opt.* 18, 095002. doi:10.1117/1.JBO.18.9.095002.

Savoldelli, A., Fornasiero, A., Trabucchi, P., Limonta, E., La Torre, A., Degache, F., et al. (2017). The Energetics during the World's Most Challenging Mountain Ultra-Marathon—A Case Study at the Tor des Geants®. *Front. Physiol.* 8, 1003. doi:10.3389/fphys.2017.01003.

Schmid, J.-P., Nobel, D., Brugger, N., Novak, J., Palau, P., Trepp, A., et al. (2015). Short-term high altitude exposure at 3454 m is well tolerated in patients with stable heart failure. *Eur. J. Heart Fail.* 17, 182–6. doi:10.1002/ejhf.227.

Schmid, J.-P., Noveanu, M., Gaillet, R., Hellige, G., Wahl, A., and Saner, H. (2006). Safety and exercise tolerance of acute high altitude exposure (3454 m) among patients with coronary artery disease. *Heart* 92, 921–5. doi:10.1136/hrt.2005.072520.

Seifert, L. (2016). *Science of Climbing and Mountaineering.* Taylor and Francis Available at: https://books.google.it/books?hl=it&lr=&id=riQlDwAAQBAJ&oi=fnd&pg=PP1&dq=the+science+of+climbing+and+mountaineering&ots=FJtfX6Tz1b&sig=muNTUmelqpfIGC8smobctbCo8Ac#v=onepage&q=the science of climbing and mountaineering&f=false [Accessed April 9, 2018].

Semenza, G. L. (2009). Regulation of oxygen homeostasis by hypoxia-inducible factor 1. *Physiol.* 24, 97–106. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=19364912.

Severinghaus, J. W. (2016). HIGH LIFE: High altitude fatalities led to pulse oximetry. *J. Appl. Physiol.* 120, 236–43. doi:10.1152/japplphysiol.00476.2015.

Severinghaus, J. W., Chiodi, H., Eger, E. I., Brandstater, B., and Hornbein, T. F. (1966). Cerebral blood flow in man at high altitude. Role of cerebrospinal fluid pH in normalization of flow in chronic hypocapnia. *Circ. Res.* 19, 274–82. Available at: http://www.ncbi.nlm.nih.gov/pubmed/5914844 [Accessed January 8, 2018].

Siebenmann, C., and Rasmussen, P. (2016). Does cerebral hypoxia facilitate central fatigue? *Exp. Physiol.* 101, 1173–1177. doi:10.1113/EP085640.

Simpson, L. P., Jones, A. M., Skiba, P. F., Vanhatalo, A., and Wilkerson, D. (2015). Influence of hypoxia on the power-duration relationship during high-intensity exercise. *Int. J. Sports Med.* 36, 113–9. doi:10.1055/s-0034-1389943.

Smith, A. M. (2007). Acute hypoxia and related symptoms on mild exertion at simulated altitudes below 3048 m. *Aviat. Space. Environ. Med.* 78, 979–84. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17955948 [Accessed January 12, 2018].

Stray-Gundersen, J., and Levine, B. D. (2008). Live high, train low at natural altitude. *Scand. J. Med. Sci. Sports* 18, 21–28.

Subudhi, A. W., Dimmen, A. C., and Roach, R. C. (2007). Effects of acute hypoxia on cerebral and muscle oxygenation during incremental exercise. *J. Appl. Physiol.* 103, 177–183. doi:10.1152/japplphysiol.01460.2006.

Subudhi, A. W., Fan, J.-L., Evero, O., Bourdillon, N., Kayser, B., Julian, C. G., et al. (2014). AltitudeOmics: effect of ascent and acclimatization to 5260 m on regional

cerebral oxygen delivery. *Exp. Physiol.* 99, 772–81. doi:10.1113/expphysiol.2013.075184.

Townsend, N. E., Nichols, D. S., Skiba, P. F., Racinais, S., and Périard, J. D. (2017). Prediction of Critical Power and W′ in Hypoxia: Application to Work-Balance Modelling. *Front. Physiol.* 8, 180. doi:10.3389/fphys.2017.00180.

Trinity, J. D., Groot, H. J., Layec, G., Rossman, M. J., Ives, S. J., Runnels, S., et al. (2012). Nitric oxide and passive limb movement: a new approach to assess vascular function. *J. Physiol.* 590, 1413–1425. doi:10.1113/jphysiol.2011.224741.

Tucker, R., and Noakes, T. D. (2009). The physiological regulation of pacing strategy during exercise: a critical review. *Br. J. Sports Med.* 43, e1–e1. doi:10.1136/bjsm.2009.057562.

Valli, G., Cogo, A., Passino, C., Bonardi, D., Morici, G., Fasano, V., et al. (2011). Exercise intolerance at high altitude (5050 m): critical power and W'. *Respir. Physiol. Neurobiol.* 177, 333–41. doi:10.1016/j.resp.2011.05.014.

Van Beekvelt, M. C., Colier, W. N., Wevers, R. A., and Van Engelen, B. G. (2001). Performance of near-infrared spectroscopy in measuring local O(2) consumption and blood flow in skeletal muscle. *J. Appl. Physiol.* 90, 511–9. doi:10.1152/jappl.2001.90.2.511.

Venturelli, M., Cè, E., Limonta, E., Bisconti, A. V., Devoto, M., Rampichini, S., et al. (2017). Central and peripheral responses to static and dynamic stretch of skeletal muscle: mechano- and metaboreflex implications. *J. Appl. Physiol.* 122, 112–120. doi:10.1152/japplphysiol.00721.2016.

Ward, S. A., Grocott, M. P. W., and Levett, D. Z. H. (2017). Exercise Testing, Supplemental Oxygen, and Hypoxia. *Ann. Am. Thorac. Soc.* 14, S140–S148. doi:10.1513/AnnalsATS.201701-043OT.

Weavil, J. C., Duke, J. W., Stickford, J. L., Stager, J. M., Chapman, R. F., and Mickleborough, T. D. (2015). Endurance exercise performance in acute hypoxia is influenced by expiratory flow limitation. *Eur. J. Appl. Physiol.* 115, 1653–1663. doi:10.1007/s00421-015-3145-5.

Wehrlin, J. P., and Hallen, J. (2006). Linear decrease in .VO2max and performance with increasing altitude in endurance athletes. *Eur J Appl Physiol* 96, 404–412. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16311764.

West, J. B., Lahiri, S., Maret, K. H., Peters, R. M., and Pizzo, C. J. (1983). Barometric pressures at extreme altitudes on Mt. Everest: physiological significance. *J. Appl. Physiol.* 54, 1188–1194.

Williamson, J., Oakeshott, P., and Dallimore, J. (2018). Altitude sickness and acetazolamide. *BMJ* 361, k2153. doi:10.1136/BMJ.K2153.

Willie, C. K., Smith, K. J., Day, T. A., Ray, L. A., Lewis, N. C. S., Bakker, A., et al. (2014). Regional cerebral blood flow in humans at high altitude: gradual ascent and 2 wk at 5,050 m. *J. Appl. Physiol.* 116, 905–10. doi:10.1152/japplphysiol.00594.2013.

Willis, S. J., Alvarez, L., Millet, G. P., and Borrani, F. (2017). Changes in Muscle and Cerebral Deoxygenation and Perfusion during Repeated Sprints in Hypoxia to Exhaustion. *Front. Physiol.* 8, 846. doi:10.3389/fphys.2017.00846.

Wilson, M. H., Edsell, M. E. G., Davagnanam, I., Hirani, S. P., Martin, D. S., Levett, D. Z. H., et al. (2011). Cerebral artery dilatation maintains cerebral oxygenation at extreme altitude and in acute hypoxia--an ultrasound and MRI study. *J. Cereb. Blood Flow Metab.* 31, 2019–29. doi:10.1038/jcbfm.2011.81.

Wyatt, F. (2014). Physiological Responses to Altitude: A Brief Review. *J. Exerc.*

Physiol. 17. Available at: https://www.asep.org/asep/asep/JEPonlineFEBRUARY2014_Wyatt.pdf [Accessed August 6, 2018].

Young, A. J., Cymerman, A., and Pandolf, K. B. (1982). Differentiated ratings of perceived exertion are influenced by high altitude exposure. *Med Sci Sport. Exerc* 14, 223–228. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=7109891.

Zhang, D., She, J., Yang, J., and Yu, M. (2015). Linear and nonlinear dynamics of heart rate variability in the process of exposure to 3600 m in 10 min. *Australas. Phys. Eng. Sci. Med.* 38, 263–70. doi:10.1007/s13246-015-0354-5.