

The Effects Of Hyperoxic Recovery Following High Intensity Interval Training In Hypoxia On The Blood Oxygen Transport System And Aerobic Capacities

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FACULTY OF KINESIOLOGY

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FOLLOWING HIGH INTENSITY INTERVAL
TRAINING IN HYPOXIA ON THE BLOOD OXYGEN
TRANSPORT SYSTEM AND AEROBIC CAPACITIES**

DOCTORAL DISSERTATION

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Information on Mentor

Prof. Lana Ružić, M.D., Ph.D.

Professor with tenure in Exercise Physiology, Lana Ruzic, is a medical doctor and scientists holding a position at Department of Sport and Exercise Medicine at Faculty of Kinesiology, Zagreb, Croatia. Currently she is also the Head of the PhD program in Kinesiology. Her work may be recognized through more than 150 various publications among which many published in journals indexed in Web of Science and Current Contents databases and almost 1000 GS citations. She was a collaborator or project lead in several projects dealing with exercise and diabetes, exercise and altitude adaptations, sports endocrinology, GENperform test and she is a reviewer for several scientific journals. Her personal interests are exercise physiology (lactate-based diagnostics), exercise prescription in chronic diseases, especially exercise in altitude and adaptations in winter sports. These topics she also teaches at postgraduate program in Sports and occupational medicine at School of Medicine, University of Zagreb. Her medical background and previous 15 years of experience as a medical advisor for health-related physical activity in several fitness centers provide her an important bond of science and practice.

Apart from her scientific and university duties, the sports background of Professor Lana Ružić is skiing. She is ISIA accredited international ski instructor and for that reason she developed a special interest in exercise at high altitudes.

Abstract

Sports experts and coaches have been continuously looking for new approaches and methods to improve athletic performance across different sports. Out of the methods proposed are the hypoxic and the hyperoxic training methods, which have recently gained popularity. Hyperoxia was previously used in special clinics; however, it has become easily accessible to athletes with the development of 'on-the-spot' equipment. The proposed study aims at determining the potential impact of added hyperoxic conditions during recovery and post-high-intensity interval training (HIIT) in hypoxic conditions. The study posed two hypotheses: a combination of intermittent high intensity hypoxic training and recovery in hyperoxia significantly improves the aerobic capacity than when recovering in normoxia and a combination of intermittent high intensity hypoxic training and recovery in hyperoxia significantly improves the oxygen transport parameters than when recovering in normoxia.

The study's sample included 49 athletes, aged 18 to 20. Athletes were divided into three groups adhering to the intervention protocol as follows: (1) Hypo+Hyper group (N=16) which performed HIIT in hypoxia with a recovery protocol 15 minutes at $FiO_2=0.40$, (2) Hypo group (N=16) which performed HIIT in hypoxia followed by recovery in normoxia, and (3) a control group (N=17) which performed their everyday activities. Each of the four weeks, the intensity of the HIIT and continuous training is increased by 5%. HIIT hypoxia exercise protocol consists of six bouts of one minute at 80 to 95% of HR_{max} , each week at higher rate, respectively, followed by 2 minutes at 60% of HR_{max} . The study adopted a quantitative approach through recording athletes' blood samples pre- and post- intervention to measure red blood cell parameters related to oxygen transport as well as the aerobic capacity measured by the all-out treadmill test. The intervention lasted for four weeks with three sessions of training per week.

The most evident changes recorded among the groups within the aerobic endurance parameters pertain to the hyper-hypo group. At maximal exertion the significant changes were observed in maximal oxygen uptake (VO_{2max}) and the maximal speed reached as Hypo-Hyper group had a larger increase as opposed to control group as expected ($p < 0.05$ and $p < 0.01$, respectively) as well as opposed to Hypo group ($p < 0.01$). Significant changes at anaerobic threshold involved significant

increase in oxygen uptake when Hypo-Hyper group was compared to controls ($P < 0.05$) while in only Hypo group that effect was not confirmed. As for hematological parameters, the most important change pertain to significant hemoglobin increase within the Hypo+Hyper group as opposed to the control group ($p < 0.01$). The significant difference in hemoglobin between the Hypo group and controls was not noted.

The study contributes to the body of knowledge regarding the effects of such a design on aerobic endurance parameters and the effects of HIIT training in hypoxia and hyperoxia recovery and concludes that the optimal training method for short periods like 4 weeks is that of the high intensity interval training group with hyperoxia recovery (HIIT+O₂), accepting both hypotheses proposed by this doctoral dissertation. Indeed, a combination of intermittent high intensity hypoxic training and recovery in hyperoxia significantly improves the aerobic capacity and the oxygen transport parameters than when recovering in normoxia.

Keywords: High-intensity interval training, hyperoxia, normobaric hypoxia, oxygen transport, aerobic performance, athletes

Sažetak

Sportski stručnjaci i treneri kontinuirano traže nove pristupe i metode za poboljšanje atletskih performansi u različitim sportovima. Od predloženih metoda su popularne su od nedavno i hipoksična i hiperoksična metoda treninga, koje. Hiperoksija se ranije koristila u posebnim zdravstvenim institucijama međutim, s razvojem opreme 'na licu mjesta' postala je lako dostupna sportašima. Ovo istraživanje je imalo za cilj utvrditi potencijalni utjecaj intervalnog treninga visokog intenziteta (HIIT) u hipoksičnim uvjetima u slučaju oporavka u hiperoksiji. Postavljene su dvije hipoteze: kombinacija HIIT treninga u hipoksiji s oporavkom u hiperoksiji značajno poboljšava pokazatelje aerobne izdržljivosti u usporedbi s istim protokolom uz oporavak u normoksiji, te također poboljšava hematološke pokazatelje sustava za prijenos kisika.

Uzorak ispitanika uključivao je 49 aktivnih mladih muškaraca u dobi od 18 do 20 godina podijeljenih u tri skupine ovisno o protokolu intervencije kako slijedi: (1) Hypo+Hyper skupina (N=16) koja je izvodila HIIT u hipoksiji s protokolom oporavka 15 minuta pri $FiO_2=0,40n$; (2) Hypo skupina (N=16) koja je izvodila HIIT u hipoksiji nakon čega je uslijedio oporavak u normoksiji i (3) kontrolna skupina (N=17) koja je obavljala svoje svakodnevne aktivnosti. Intervencija je trajala 4 tjedna uz 3 treninga tjedno. U svakom od četiri tjedna, intenzitet treninga povećavao se za 5%. Protokol vježbanja HIIT hipoksije sastoji se od šest sesija trajanja jednu minutu pri 80 do 95% HRmax (ovisno o tjednu), nakon čega slijede 2 minute pri 60% HRmax.

Najvažnije promjene zabilježene među skupinama unutar pokazatelja aerobne izdržljivosti odnose se na Hyper-Hypo skupinu. Pri maksimalnom naporu uočene su značajne promjene u maksimalnom primitku kisika (VO_{2max}) i maksimalnoj postignutoj brzini tračanja i Hypo-Hyper skupina je imala očekivano veći porast u odnosu na kontrolnu skupinu ($p < 0,05$ i $p < 0,01$), kao veći od Hypo grupe ($p < 0,01$). Promjene na anaerobnom pragu uključivale su značajno povećanje primitka kisika u Hypo-Hyper skupini u usporedbi s kontrolom ($P < 0,05$), dok u Hypo skupini taj učinak nije potvrđen. Što se tiče hematoloških parametara, najvažnija promjena odnosila se na značajno povećanje koncentracije hemoglobina u Hypo+Hyper grupi u usporedbi s kontrolnom ($p < 0,01$) dok takva razlika između Hypo skupine i kontrolne nije potvrđena.

Ovo istraživanje pridonosi saznanjima o učincima upravo ovakvog dizajna treninga na pokazatelje aerobne izdržljivosti. Prihvatajući obje hipoteze predložene ovom doktorskom disertacijom zaključuje se da je optimalna metoda treninga od istraživanih, posebno za kratka razdoblja poput 4 tjedna, ona uz HIIT trening u hipoksiji uz oporavak u hiperoksiji.

Ključne riječi: intervalni trening visokog intenziteta (HIIT), hiperoksija, normobarična hipoksija, transport kisika, aerobna izvedba, sportaši

Table of Contents

Information on Mentor	1
Abstract	2
Sažetak.....	4
Table of Contents.....	6
List of Tables.....	8
List of Figures	9
CHAPTER ONE.....	1
1. GENERAL INTRODUCTION	1
1.1 Study's Background	1
1.1.1 Hypoxic Training.....	1
1.1.1.1 Live High-Train High (LHTH).....	2
1.1.1.2 Live High-Train Low (LHTL)	3
1.1.1.3 Intermittent Hypoxic Exposure (IHE).....	3
1.1.1.4 Intermittent Hypoxic Training (IHT).....	4
1.1.1.5 Repeated Spring Training in Hypoxia (RSH).....	4
Hyperoxic Training.....	5
CHAPTER TWO.....	7
2. INTRODUCTION INTO THE PROBLEM AND LITERATURE REVIEW	7
2.1 Hypoxia	7
2.1.1 Hypoxic Effects on Athletes' Training.....	8
2.1.1.1 Advocates of Hypoxic Training	8
2.1.1.2 Critics of Hypoxic Training	10
2.1.2 Hypoxic Training Physiological Implications	12
2.1.2.1 Vascular Health.....	12
2.1.2.2 Changes in Muscle and Cerebral Deoxygenation	13
2.1.2.3 Hemoglobin Mass Response	13
2.1.2.4 Rate of Erythropoietin Formation	15
2.1.2.5 Lactate Paradox	16
2.1.2.6 Muscle Protein Turnover and the Molecular Regulation of Muscle Mass	16
2.1.2.7 Skeletal Muscle Tissue Changes with Hypoxia	16
2.2 Studies in Hyperoxia.....	17
2.2.1 Hyperoxic Effects on Athletes' Training.....	17
2.2.1.1 High Intensity Interval Training.....	17
2.2.1.2 Endurance Exercise	17
2.2.2 Hyperoxic Training Physiological Implications.....	18
2.2.2 Hyperoxic Training and Recovery	19
2.3 LHTH/LHTL training.....	21
2.4 Studies on Protocols of Altitude training.....	25
2.5 Hyperoxia and Hypoxia.....	27
CHAPTER THREE	31
3. PROBLEM AND AIM.....	31
3.1. Problem.....	31
3.2. Objective and Hypotheses.....	31

CHAPTER FOUR	32
4. METHODOLOGY	32
4.1 Approach and Design	32
4.2 Sampling Method	32
4.3 Procedure	33
4.3.1 Initial and Final Testing.....	33
4.3.2. Equipment and Protocols	36
4.3.2.1 HIIT-.....	36
4.3.2.2 HIIT+O2-	37
4.3.2.3 CTR	37
4.4 Data Analysis Procedures	38
4.5 Ethical Considerations	38
CHAPTER FIVE	39
5. RESULTS	39
5.1 Participant Groups and their Characteristics	39
5.2 Parameters at Maximal Exertion	39
5.3 Parameters at Anaerobic Threshold	46
4.4 Hematological Parameters	54
CHAPTER SIX	67
6. DISCUSSION	67
CHAPTER SEVEN 7. CONCLUSION	75
7.1 General Conclusion	75
7.2 Limitations	76
7.3. Scientific Contribution	76
Bibliography	78
Biography of the Author	87

List of Tables

Table 1 Descriptive data by group.....	39
Table 2 Descriptive Statistics of Parameters at Maximal Exertion	40
Table 3. Mean Difference of Parameters at Maximal Exertion for Entire Sample	43
Table 4. Pairwise Comparison of Parameters at Maximal Exertion by groups.....	44
Table 5. Descriptive Statistics of Parameters at Anaerobic Threshold	46
Table 6. Mean Difference of Parameters at Anaerobic Threshold for Entire Sample	50

LIST OF ABBREVIATIONS:

O₂- oxygen
VO₂max- maximal oxygen uptake
Hypo- hypoxia
Hyper-Hyperoxia
ANT- anaerobic threshold
Hb- hemoglobin
HCT- hematocrit

List of Figures

Figures 1a, b, c, d . Recorded Instances of Treadmill Parameters	35
Figure 2. Participants' participation in retrieving treadmill parameters.....	36
Figure 3 Estimated Marginal Means of VO2Max (ml/kg/min) before-1 and after-2 intervention at Maximal Exertion.....	41
Figure 4 Estimated Marginal Means of MaxSpeed (km/h) before-1 and after-2 intervention at Maximal Exertion.....	42
Figure 5 Estimated Marginal Means of HRmax (bpm) before-1 and after-2 intervention at Maximal Exertion.....	43
Figure 6 Estimated Marginal Means of VO2 (ml/kg/min) before-1 and after-2 intervention at Anaerobic Threshold	48
Figure 7 Estimated Marginal Means of Speed (km/h) before-1 and after-2 intervention at Anaerobic Threshold	49
Figure 8 Estimated Marginal Means of HR (bpm) before-1 and after-2 intervention at Anaerobic Threshold.....	50
Figure 9 Estimated Marginal Means of Hb (g/dl) before-1 and after-2 intervention	56
Figure 10 Estimated Marginal Means of HCT before-1 and after-2 intervention.....	57
Figure 11 Estimated Marginal Means of ERT before-1 and after-2 intervention	58
Figure 12 Estimated Marginal Means of MCV before-1 and after-2 intervention.....	59
Figure 13 Estimated Marginal Means of MCHC before-1 and after-2 intervention.....	60
Figure 14 Estimated Marginal Means of MCH before-1 and after-2 intervention.....	61
Figure 15 Estimated Marginal Means of Reticulocyte before-1 and after-2 intervention	62

CHAPTER ONE

1. GENERAL INTRODUCTION

1.1 Study's Background

The sports business has become a prominent, billion-dollar industry across a variety of disciplines. Major investments are being conducted throughout competitions, especially those that include athletics. Competition has never been fiercer. Hence, elite athletes suffer from a considerable amount of stress to perform to their fullest potentials and achieve outstanding results (Barata, Cervaens, Resende, Camacho, & Marques, 2011).

Sport experts and coaches have examined alternatives to illegal doping to legally enhance athletic performance. The late sixties marked an increased attention to the 'altitude training' method and, subsequently, to its opposite, 'hyperoxic training.' Both techniques were said to enhance performance at sea-level and endurance, especially in power sports (Lundby, Millet, Calbet, Bärtsch, & Subudhi 2012). The use of hypoxic and hyperoxic training has not been considered illegal; therefore, throughout the years, these training methods have been devised to guarantee that athletes, across various sports, receive ultimate benefits.

1.1.1 Hypoxic Training

The hypoxic method incorporates training at high altitudes in training camps or in a simulated hypoxic environment (Feriche, Garcia-Ramos, Caldero'nSoto, Drobnic, & Bonitch-Go'ngora, 2014).

Altitude training is a complex procedure, and results may not be achieved easily. Indeed, during the first period of hypoxic exposure, the athlete may be subject to some negative effects, such as jet lag (due to travel), increase in ventilation work, dehydration, and decrease in training capacity, all of which ultimately decrease cardiac output. With time, the benefits of altitude training become visible, and the athlete, among other things, benefits from an increased erythrocyte volume

and capillary density; according to Millet, Roels, Schmitt, Woorons, and Richalet, (2010), some benefits also include an increase in maximal aerobic exercise capacity and muscle buffering capacity as well as exercise economy. Thus, in an effort to attain desired hypoxia effects, an equilibrium between the positives and the negatives should be established. Furthermore, it is worthy to note that although there is a generally positive perception of altitude training, not all athletes benefit equally. In order to obtain maximal benefits from hypoxic training, it is crucial to accomplish haematological adaptations and retain training intensity at the same time.

Most of the athletes who do not benefit from hypoxic training suffer from iron deficiency; without iron, the body cannot produce erythrocytes that are vital to achieve the desired results. Moreover, it seems that untrained subjects are more prone to achieve better results than trained athletes. According to Sinex and Chapman (2015), when trained individuals arrive at higher altitudes, their training intensity diminishes, impacting their general performance.

According to Millet et al. (2010), over the years, hypoxic training developed into multiple protocols, which include the following:

- Live High-Train High (LHTH)
- Live High-Train Low (LHTL)
- Intermittent Hypoxic Exposure (IHE)
- Intermittent Hypoxic Training (IHT)
- Repeated Spring Training in Hypoxia (RSH)

Although each of these five methods have their own characteristics, as elaborated on in the sections to follow, they still share common ground: (1) The ideal altitude at which to conduct training is set between 2200 meters and 2500 meters, (2) exposure ought to last four weeks for twelve hours a day, and (3) one might start noticing results starting 18 days after exposure.

1.1.1.1 Live High-Train High (LHTH)

LHTH technique is considered one of the oldest and most famous forms of altitude training. LHTH is effective when conducted over four stages. The first stage is acclimatization, which lasts between seven to ten days, depending on the subject. During this phase, the

athlete is exposed to high altitude without starting intensive training. The second phase is the primary training, which lasts between two and three weeks. During this phase, the athlete starts increasing the intensity of his/her training progressively; prior to reaching a point at which the intensity is maximal, the athlete gradually decreases the intensity, reaching stage three, the recovery phase, which lasts between two to five days. The fourth and final stage is the return to sea-level phase in which the athletes returned to regular sea-level and, with time, re-adapt to altitude changes. LHTH is meant to increase the red blood cells' volume (RCV) in athletes, which, in turn, increases oxygen transport capacity, boosting athletic performance. Notable experiments were conducted on LHTH; Mellerowicz gathered twenty-two East German police officers and exposed some of them to an altitude of 2020 meters for four weeks while the others lived and trained at sea-level. The results revealed that the group members living at altitude undoubtedly had a great boost in their running performance and their VO_{2max} increased significantly compared to the sea-level group. Mellerowicz was therefore able to prove that the LHTH trial is effective (as cited in Lundby et al., 2012)

1.1.1.2 **Live High-Train Low (LHTL)**

Many experiments based on the LHTL method have been conducted, the most notable being Levine's and Stray-Gundersen's (1997) experiment. This method is favored among others because the athlete is not required to change his/her training regime or intensity. The athlete's regular training at sea-level remains unchanged. Levine and Stray-Gundersen (1997) concluded that LHTL is an effective method for elite athletes. The experiment consisted of athletes living for twenty-seven days at 2500 meters and training at 1250 meters. The results revealed a rise in aerobic performance as well as in oxygen transport capacity. Since that paper was published, there has been a high rise in popularity of that type of altitude training.

1.1.1.3 **Intermittent Hypoxic Exposure (IHE)**

IHE consists of exposure to hypoxic air at rest. Julian et al. (2004) conducted an experiment on IHE; however, the results were disappointing. IHE did not boost any factors related to performance. Other studies, such as a double-blind study, have also concluded the same: IHE is not an effective altitude training method. Nonetheless, more experiments are needed in order to confirm these results.

1.1.1.4 Intermittent Hypoxic Training (IHT)

IHT is also known as live-low train-high and is the exact opposite of LHTL. Athletes live at sea-level but train at higher altitudes. A variation of this technique exists, named Voluntary Hypoventilation. Athletes simulate hypoxia by holding their breaths and keeping their inhalations at low volumes. According to Woorons, Mucci, Aucouturier, Anthierens, and Millet (2017), reducing breathing frequency is effective in boosting anaerobic performance. IHT has various benefits. Compared to actual training in high altitudes, IHT does not force the athlete to change their training environment nor lifestyle; it allows for muscle excitability to remain unchanged at sea-level. After experiments on different groups, it was noticed that IHT increased the performance of swimmers, cyclers, and runners compared to the normoxia training group results. One can thus conclude that IHT boosts performance compared to training in regular sea-level conditions (Faiss, Girard, & Millet, 2013).

1.1.1.5 Repeated Spring Training in Hypoxia (RSH)

The effectiveness of RSH is examined in the following experiment. A total of 32 college females were randomly sampled. Sixteen of which underwent RSH, and the remaining participants, part of a control group, underwent training in normoxia (RSN). The experiment focused on female athletes, contributing to an existing gap in the literature. Indeed, RSH effect might be minimal in females as opposed to males because female arterial O₂ desaturation is less sensitive to hypoxic stimuli. Hence, the experiment begins with familiarizing the athletes with RSN and taking their baseline measurements. The results revealed that the RSN group, which was subject to F_IO₂= 20.9%, had minimal to no

change in their power outputs, in comparison to their pre-training numbers. However, the RSH group ($F_iO_2=14.5\%$, similar to a 3000-meter altitude environment) had a boost in their power output, in comparison to their pre-training figures. Furthermore, RSH group members achieved a longer time to exhaustion (TTE) compared to the RSN group members, who had no variation in their TTE. As for the time to realize results, the RSH group started seeing an increase of the power output in two weeks. The RSN did not benefit from that increase until the fourth week. This experiment demonstrates that RSH has a beneficial effect on female athletes visible through an increase in female athletes' power output (relative to pertaining numbers), an increase in their training stimulus (shorter time to reach desired results), or a prolongation of the time to exhaustion. Given that this experiment was conducted on female athletes, generalizing the results to male athletes is not possible due to biological differences. However, the RSH was included in the weekly training of the athletes and may be introduced into the training schedule of any athlete, male or female. Therefore, it is highly possible that if the subjects of this experiment were to be male athletes, the same results would be noted. Nevertheless, additional tests would be necessary in order to prove this theory (Kasai, Mizuno, Ishimoto, Sakamoto, Maruta, & Goto, 2015).

Hyperoxic Training

The hyperoxic methods involves training with an exposure to air that contains a high oxygen percentage. Hyperoxia has been the focus of several studies as well. One study samples eight male elite athletes, who have undergone five sets of three-minute high intensity cycling followed by a three-minute active recovery period. Some cyclists performed the exercise under hyperoxia ($F_iO_2=0.36$) and the others under normoxic conditions ($F_iO_2=0.21$). The fifth set of exercise was performed until exhaustion, which was reached when the rpm dropped below 85 for more than five seconds. To measure results, time to exhaustion was recorded, and oxygen saturation was measured. The study was debated because of the hypothesis that HIIT lowers S_aO_2 and increases tissue hypoxia. Therefore, the hypothesis is as follows: If the S_aO_2 does not decrease, then performance might be improved. The study, which is a single-blinded, randomized, control trial, concluded that time to exhaustion is longer for the hyperoxic group compared to the normoxic group. Additionally, although the S_aO_2 levels decreased, as expected, for the normoxia group, they

remained the same for the hyperoxia group. Since those levels remained unchanged, one can conclude that hyperoxia increases performance when applied during HIIT (Ohya, 2016).

Another study gathered 400-meter elite runners and hurdlers and exposed them to hyperoxia during intense intermittent training. The objective was to examine whether their oxygen saturation (S_{aO_2}), acidosis, and heart rate recovery are affected compared to training in normoxia. Indeed, the conjecture is that breathing hyperoxic air has an effect on those three factors: S_{aO_2} decrease is prevented, blood acidosis is delayed, and heart rate recovery is improved after the exercise. To prove this theory, the runners were subjected to 3×3×300-meter runs on a treadmill in three different conditions: (1) Normoxia, (2) hyperoxia during exercise and recovery (ERHOX), and (3) hyperoxia during recovery alone (RHOX). To measure the changes of the factors at play, a fingertip blood sample was taken from all the athletes. The results came as follows: Oxygen desaturation was only noted in the NOX and RHOX groups; whereas, the ERHOX maintained the same S_{aO_2} levels. As for the blood pH, blood lactate, and heart rate, they remained unchanged. One can therefore conclude that hyperoxia does prevent a reduction in oxygen saturation. (Nummela, Hamalainen, & Rusko, 2002).

CHAPTER TWO

2. INTRODUCTION INTO THE PROBLEM AND LITERATURE REVIEW

Chapter Two synthesizes relevant research studies that either support or undermine the use of hypoxic and hyperoxic techniques in training. It also presents various topics related to hypoxic and hyperoxic training. It is worth noting that research related to hypoxic and hyperoxic techniques is yet to be built as the number of studies present do not yet provide a clear consensus regarding these modes of training and the implications that they pose.

2.1 Hypoxia

Studies were conducted to assess the efficacy of hypoxic training on athletic performance in various sports and training programs. Moreover, the impact of hypoxic on athlete health has been examined in the literature.

Prior to presenting the literature on hypoxic training, it is worth noting that Chapman et al. (2014) attempted to find the optimal altitude for athletes to benefit from hypoxia in sea level performance. As such, Chapman et al. (2014) hypothesize that higher altitudes yield greater performance enhancements among athletes than lower altitudes. Their study sampled 32 males and 16 females collegiate distance runner. The participants underwent “four weeks of group sea level training and testing” and were then assigned, in a random manner, to four higher altitude living conditions, 1780 meters, 2085 meters, 2454 meters, and 2800 meters. Participants trained at common altitude, ranging from 1250 meters to 3000 meters, and tests were conducted at sea level pre- and post- intervention. EPO was also measured several times. The results post-intervention indicated the following:

- The middle two altitude groups (2085 meters and 2454 meters) improved their times significantly in the 3000-meter time trial.

- EPO was at a significant high in all groups after 24 hours and 48 hours.
- After returning to sea level, erythrocyte volume presented similar, significantly high results.

Therefore, Chapman et al. (2014) conclude that optimal altitudes lie between 2000 meters and 2500 meters.

2.1.1 Hypoxic Effects on Athletes' Training

Generally, the studies presented have not yet established a clear consensus on the efficacy of hypoxic training as some findings support this method while others deem it as uncondusive to the achievement of desirable training outcomes. Moreover, studies acknowledge the benefits of hypoxic training yet note its limitations and recommended further corroboration and/or contribution to the findings on hypoxic training. The studies are presented below and grouped based on their standing regarding the efficiency of hypoxic training.

2.1.1.1 Advocates of Hypoxic Training. Studies have shown that hypoxic training is beneficial to athletes when it comes to their performance. A couple of these studies are presented in this section.

Hendriksen and Meeuwsen (2003) investigate the impact of intermittent training in a hypobaric chamber on the physical exercise of triathletes, training at 2,500 meters. After nine days, the results revealed:

- In hypoxia, a significant increase in the maximal power output, the anaerobic mean power, and the anaerobic peak power was observed.
- In hypoxia, maximal oxygen uptake (VO_{2max}) recorded no changes.
- No change at sea level was evident.

Accordingly, Hendriksen and Meeuwsen (2003) concluded that intermittent hypobaric training improves the aerobic system.

Hamlin, Lizamore, and Hopkins (2017) conducted a systematic literature search within five journal data bases. The groups analyzed were a hypoxic group with natural or stimulated altitude and a control group with sea level. Hypoxic intervention's duration was seven to 28 days. "Results indicated an improvement in high-intensity intermittent running performance post-hypoxic intervention regardless of the altitude training method" (Hamlin et al., 2017). Moreover, the

performance in intermittent and live-high interventions, the dose hypoxia, and the inclusion of training in hypoxia were unclear, creating a literature gap that can be attended to with further research.

Nonetheless, Hamlin et al. (2017) establish that hypoxic intervention improves high-intensity running. Hence, coaches can consider hypoxic training methods to improve performance and customize these methods to best suit training schedules (Hamlin et al., 2017).

Moreover, Park and Lim (2017) explain that swimming performance depends on interchangeable components that include aerobic exercise capacity, anaerobic power, and muscular mechanisms. They argue that hypoxic training method enhance performance as opposed to training at sea-level. Hence, to support their claim, Park and Lim (2017) conducted a six-week study on 20 elite, 10 belonging to the normoxic training group, who trained and resided at sea level, and 10 belonging to the hypoxic training group, who trained at 526 mmHg hypobaric hypoxic condition but resided at sea-level. Findings revealed the following:

- “Muscular function and hormonal response parameters showed significant interaction effects in muscular strength and endurance.”
- The hypoxic training group demonstrated a “significant increase in maximal oxygen consumption, peak anaerobic power, and swimming performances” for both the 50-meter and the 400-meter.

As such, Park and Lim (2017) claimed that hypoxic training is effective in improving muscular strength and endurance in elite athletes as opposed to the normoxic group. Moreover, “despite the changes aerobic exercise capacity (VO₂max), anaerobic power, and swimming performance of 50 m and 400 m,” the researchers argue that there remains uncertainty in how these factors change as opposed to the normoxic training group.

Czuba et al. (2019) assessed the efficacy of intermittent hypoxic training among biathletes. They studied performance and aerobic capacity in 14 male biathletes, assigned into a hypoxic or control groups. “The hypoxic group had trained three times in a week in a normobaric hypoxic environment and had a lactate threshold intensity determined in hypoxia. The control group also

trained three times per week under normoxic conditions and had a lactate threshold intensity determined in normoxia.” The training program was composed of three weekly micro-cycles and were followed by three days of recovery. The results revealed that:

- “Intermittent hypoxic training significantly increased retention time in the target at rest.”
- RT postincremental test was increase 27.4% in normoxia and 26.7% in hypoxia.
- “The capillary oxygen saturation at the end of the maximal effort in hypoxia increased significantly.”

Thus, Czuba et al. (2019) conclude that there exist “beneficial effects of the intermittent hypoxic training protocol on aerobic capacity of biathletes.”

2.1.1.2 Critics of Hypoxic Training. Despite the benefits of hypoxic training mentioned above, some studies have established that hypoxic training does not lead to desirable outcomes or that the evidence presented is insufficient in yielding accurate results. The studies are presented in this section.

In a research study conducted by Shephard, Bouhlel, Vandewalle, and Monod (1988), a total of 16 participants, divided equally among males and females, “performed peak oxygen intake tests on a cycle ergometer breathing ambient air and a mixture of 12% oxygen in nitrogen,” which is equivalent to a 4400-meter altitude. Results revealed a 28% decrease of peak oxygen intake and a slighter decrease in power output as a result of hypoxia. Results also showed a slight decrease in peak heart rate, peak blood pressure, peak ventilation, and peak blood lactate concentration. Upon further analysis, Shephard et al. (1988) conclude that the reduction in arterial oxygen saturation contributes to the impairment in oxygen transport; however, the decrease in heart and in ventilation mildly contributed to this impairment (Shephard et al. 1988).

A number of studies have suggested improving muscle mechanical efficiency requires exposure to a high altitude with acclimatization for more than 60 years. To further investigate these results, Lundby, Saltin, and Hall (2000) revealed the impact of different altitude levels on 153 participants, ranging from sea-level residents to high-altitude ones and from sedentary individuals to world-class athletes, through a review of the literature.

To begin with, living between 20 to 22 hours a day at an altitude of 2,500 meters and training at altitudes between 1,250 meters and 2,800 meters does not cause a difference in running economy.

Moreover, in a second study, sea-level individuals resided for eight weeks at an altitude of 4,100 meter and high-altitude individuals performed cycle ergometer exercise at the same altitude in ambient air and severe hypoxia. Changes were not noted among muscle oxygen uptake and mechanical efficiency between sea-level and acclimatization and between the two groups.

Finally, in a third study, no changes were evident in systemic or leg VO_2 during the cycle ergometer exercise. No changes were detected, as well, during the 21-day exposure to 4300m altitude. Nonetheless, at an altitude of 5,260 meters, after nine weeks of acclimatization, a decrease in the submaximal VO_2 was noted in nine subjects with severe hypoxic exposure. Given that severe hypoxia led to this decrease in VO_2 , reduction is related to the lack of oxygen rather than the anatomical or the physiological adaptations to high altitude.

According to the results of several studies, Lundby, Saltin, and Hall (2000) conclude that “exercise economy remains unchanged after acclimatization to high altitude.” Ventura, Hoppeler, Seiler, Binggeli, Mullis, and Vogt (2003) examined the endurance of 12 trained cyclists under normal training and with hypoxia. Group one trained under hypoxic conditions, corresponding to an altitude of 3200 meters, with an anaerobic threshold. The second group had a same relative intensity at 560 meters. Athletes underwent performance tests under normoxic and hypoxic conditions. Results revealed the following:

- Normoxic and hypoxic $\text{VO}_{2\text{max}}$, maximal power output and hypoxic work-capacity did not improve after six weeks.
- Oxygen saturation and in maximal blood lactate concentration increased in group one.
- Ferritin levels were differently decreased in both groups.
- Both groups had an increase in reticulocyte.

Consequently, Ventura et al. (2003) conclude that the integration of six weeks of high intensity endurance training did not result in improving the performance of the trained athletes whether this training was performed in hypoxic or normoxic conditions.

Rodríguez, Truijens, Townsend, Stray-Gundersen, Gore, and Levine (2007) examine the impact of a four-week resting exposure to intermittent hypobaric hypoxia for three hours/day, five days/week and for a 4000-meter to 5500-meter altitude, as opposed to exposure to normoxia with both cases alongside training at sea-level. Rodríguez et al. (2007) measure performance and maximal oxygen transport among 23 runner and swimmers, randomly assigned to hypobaric hypoxia and normobaric normoxia groups, with 11 and 12 participants respectively. Time trials tests were conducted and $\dot{V}O_{2max}$, $\dot{V}O_{2max}$ at ventilatory threshold, ventilation, and heart rate were measured at week one and week three. In both groups, time trial performance did not improve. And among the two groups, no significant differences have been recorded pertaining to the change in maximal uptake, maximal oxygen uptake at ventilatory threshold, ventilation, and heart rate. Rodríguez et al. (2007) conclude the dose of intermittent hypobaric hypoxia was insufficient to instigate a notable change in either criteria.

Deb, Brown, Gough, Mclellan, Swinton, Sparks, and Mcnaughton, (2017) quantified the impact of severe hypoxia exposure on exercise capacity and performance through employing a systematic review with a three-level mixed effects meta-regression. Through reviewing the literature on exercise performance (Time Trails TTS) and capacity (time to exhaustion test, TTE) with severe hypoxic exposure and a normoxic comparator, Deb at al.'s (2017) findings reveal an overall decrease in TTS and TTE; moreover, less than two-minute exercises did not manifest any ergolytic effect from severe hypoxia.

2.1.2 Hypoxic Training Physiological Implications

Similar to the above, findings on hypoxic training in its direct impact on physiological dimensions are varied and simultaneously tackle one or more aspects of physiology. The sections to follow present the findings per physiological dimension.

2.1.2.1 Vascular Health. Many beneficial adaptations related to vascular health are associated with Exercise Training (ExT), including an increase in skeletal muscle capillarization and vascular dilator function and a decrease in arterial stiffness. Nonetheless, conclusive evidence is yet to be established when it comes to whether hypoxic conditions may trigger enhanced effects when ExT is performed.

Montero and Lundby (2016) review 21 controlled studies on 331 individuals, aged 19 to 57 years, out of which 265 are males. ExT programs were extended from three to ten weeks, mainly consisted of cycling endurance training performed in normobaric hypoxia or normoxia, and were of an intensity similar to the groups trained in hypoxia and normoxia. Results revealed that the skeletal muscle capillarization and the vascular dilator function were enhanced with ExT performed in hypoxia versus normoxia; however, the arterial stiffness was not. Montero and Lundby (2016) conclude that hypoxic ExT increases the power of vascular adaptations related to skeletal muscle capillarization and dilator function.

2.1.2.2 Changes in Muscle and Cerebral Deoxygenation. Willis, Alvarez, Millet, and Borrani. (2017) evaluated “the different levels of normobaric hypoxia on the changes in peripheral and cerebral oxygenation and performance during repeated sprints to exhaustion.” The sample comprised of six males and five females, “participating in three testing visits in conditions of simulated altitude near sea-level.” Each of the sessions began with a 12-minute warm-up and was followed by two 10-second sprints as well as the repeated cycling sprint test until exhaustion. The measurements utilized incorporated “power output, vastus lateralis, and prefrontal deoxygenation, oxygen uptake, femoral artery blood flow, hemodynamic variables, blood lactate concentration, and rating of perceived exertion.” A decrease in performance and pulse oxygen saturation with hypoxia as compared to 400 meters was noted. Moreover, muscle hemoglobin difference and tissue saturation were lower at 3800 meters than at 200 meters and 400 meters so was deoxyhemoglobin at 3800 meters as opposed to 2000 meters. In addition, reduced changes in peripheral total hemoglobin and greater changes in cerebral oxygenation were noted; “changes in cerebral deoxygenation were greater at 3800 meters than at 2000 meters and 400 meters.” The findings to this study corroborate that performance in hypoxia is limited due to the continuous decrease of oxygen saturation. Nevertheless, a cerebral auto-regulation “of increased perfusion accounting for the decreased arterial oxygen content” could exist and allow the continuation of the task (Willis et al., 2017).

2.1.2.3 Hemoglobin Mass Response. McLean, Buttifant, Gore, White, and Kemp (2013) examined the year to year variability in altitude induced (hypoxia) changes in Hb_{mass} in elite athletes. They sampled 12 Australian participants that completed two high altitude, at 2100 meters,

training camps separated by 12 months. An additional nine athletes participated solely in the first 19-day camp and another 11 athletes participated solely in the second 18-day training camp. Carbon monoxide rebreathing was utilized to assess total Hb_{mass} pre- and post-intervention as well as four weeks after the camps. Mclean et al. (2013) concluded that the two preseason camps yielded a 4% mean increase in Hb_{mass} .

The studies reveal that the adaptation to natural or simulated moderate altitude does not stimulate the production of red cells enough to increase red cell volume (RCV) and hemoglobin mass (Hb_{mass}). Nonetheless, according to Hahn and Core (2001), evidence on increasing red cell volume is weak; it is thus improbable for the adaptation to hypoxia to improve sea level VO_{2max} . If red cell volume and hemoglobin mass were elevated, enhancement might be possible.

Neya, Enoki, Kumai, Sugoh, and Kawahara, (2007) examine the impact “of nightly normobaric hypoxia and high intensity training under hypoxic conditions on running economy and hemoglobin mass.” Neya et al. (2007) sampled 25 college long and middle-distance runners, assigned to three groups, namely (1) Hypoxic residential group with 11 hours/night at a 3000-meter simulated altitude, (2) hypoxic training group trained in Tokyo for an altitude of 60 meters with additional high-intensity 30-minute treadmill sessions at 3000 meters for 12 days during the night intervention, and (3) control group. The entire study lasted for 29 nights. Neya et al. (2007) reported the following results: “No significant changes in time to exhaustion in all three groups. No significant changes in VO_2 max in all three groups. No significant changes in total hemoglobin mass in all three groups.” Also, the hypoxic residential group showed around 5% improvement of running economy in normoxia after the intervention.

Neya et al. (2007) concluded that the intermittent hypoxia dose at 3000 meters for 11 hour/night for 29 nights to enhance EPO or VO_2 max was insufficient; nevertheless, this dose improved the running economy at race speed.

Saunders, Garvican-Lewis, Schmidt, and Gore (2013) conducted a study titled “Relationship between changes in hemoglobin mass and maximal oxygen uptake after hypoxic exposure” to examine the relationship between hemoglobin mass and maximal oxygen uptake. The study samples 145 elite endurance athletes. Pre- and post-hypoxic-intervention testing was conducted, particular for Hb_{mass} and VO_{2max} . Saunders et al. (2013) concluded that altitude training increases

VO_{2max} “of more than half the magnitude of the increase in Hb_{mass}.” Therefore, altitude training is recommended for endurance athletes.

Ryan et al. (2014) in “AltitudeOmics: Rapid Hemoglobin Mass Alterations with Early Acclimatization to and De-Acclimatization from 5260 m in Healthy Humans” discussed how sustainable is the increase due to hypoxia with extreme altitudes, greater than 5000 meters. Ryan et al. (2014) aim at identifying how long would the change in Hb_{mass} last. The tests were carried out on healthy male and female participants both at sea level and at 5260 meters, one, seven, and 16 days after this high-altitude exposure. Ryan et al. (2014) also examined participants at an altitude of 1525 meters for seven or 21 days. Findings indicated the following: (1) Hb_{mass} at high altitude remained the same when compared to sea level after one day of exposure, (2) Hb_{mass} increased by 3.765% after seven days of exposure to hypoxia at 5260 meters, (3) Hb_{mass} increased by 7.666% after 16 days of exposure to hypoxia at 5260 meters, and (4) at 1525m, Hb_{mass} decreased after seven days when compared to the tests at 5260 meters at 16 days and was similar to sea level results.

Ryan et al. (2014) conclude that Hb_{mass} increases within seven days of exposure to 5260 meters altitude hypoxia, and this increase is lost following the descent to 1525 meter.

2.1.2.4 Rate of Erythropoietin Formation. Eckardt et al. (1989) examined “the early changes in EPO formation in response to hypoxia.” The studied included around six volunteers that were exposed to 3000 meters and 4000 meters altitude for a duration of 5.5 hours in decompression chambers. Serum samples were withdrawn every 30 minutes during this altitude exposure as well as from two participants after 4000-meter hypoxia. Moreover, EPO from these samples was measured by radioimmunoassay. According to Eckardt, Boutellier, Kurtz, Schopen, Koller, and Bauer (1989), the study yielded the following results:

- The mean EPO values increased from 16.0 to 22.5 mU/ml at 3000-meter simulated altitude.
- The mean EPO values increased from 16.7 mU/ml to 28.0 mU/ml at 4000-meter simulated altitude.
- The increase in EPO above corresponds to 1.8-fold at 3000 meters and 3.0-fold at 4000 meters in the calculated production rate of EPO.
- EPO levels still rose for about 1.5 hours.
- EPO level declined exponentially after 3 hours.
- The average half-life time was 5.2 hours.

2.1.2.5 Lactate Paradox. Lundby, Saltin, and Hall (2000) examine five Danish lowland climbers at sea level during severe exposure to hypoxia and one, four, and six weeks after arrival to the Mt. Everest basecamp to evaluate the “lactate paradox.” Results show that peak blood levels are the same at sea level and under hypoxic conditions. A week after acclimatization to 5400 meters, levels decrease. Four weeks later, the level remained low as opposed to severe hypoxia. Six weeks later, the climbers had a peak lactate level similar to those indicated in severe hypoxia.

Therefore, Lundby, Saltin, and Hall (2000) conclude that the “lactate paradox” is an “impermanent metabolic phenomenon that is reversed during a long period of exposure to severe hypoxia of more than six weeks.”

2.1.2.6 Muscle Protein Turnover and the Molecular Regulation of Muscle Mass. Pasiakos, Berryman, Carrigan, Young, and Carbone (2017) examined “the physiological basis for disrupted skeletal muscle mass during acute and chronic hypoxia as well as the mechanism by which the negative energy balance may modify those effects and worsen the loss of muscle mass in lowlanders that live at high altitude.” The nutritional interventions that “can preserve muscle mass during energy deficit at high altitude” were also discussed.

2.1.2.7 Skeletal Muscle Tissue Changes with Hypoxia. Hoppeler, Mueller, and Vogt (2013) examine the effects of hypoxia during training sessions, canceling the negative effects caused by hypoxia. Results revealed the following:

- An upregulation of the regulatory subunit of the hypoxia-inducible factor – Possibly as a result to this upregulation, an increase was determined in the levels of mRNAs for myoglobin, vascular endothelial growth factor and glycolytic enzymes.
- An increase in mitochondrial and capillary densities
- Positive effects on VO₂max on maximal power output and on lean body mass

Hoppeler et al. (2013) conclude a positive effect on the risk factors pertaining to particular cardiovascular diseases.

In conclusion, the above-mentioned evidence suggests hypoxic effects on athletes' training exercise programs and health.

2.2 Studies in Hyperoxia

Studies have also been conducted in order to assess the efficacy of hyperoxia training on athletic performance in a variety of sports and training programs. Moreover, the impact of hyperoxia on athlete health has been examined in the literature. Data on the hyperoxic method is varied as presented in the sections to follow.

2.2.1 Hyperoxic Effects on Athletes' Training

2.2.1.1 High Intensity Interval Training. Manselin and Södergård (2015) examine the effects of high intensity interval training on the performance of cyclists and triathletes. The tests and trainings were performed at the Swedish School of Sport and Health Sciences (GIH) in the laboratory of applied sport science (LTIV), where 12 athletes conducted HIIT sessions and low intensity sessions over a period of 6 weeks. The athletes were divided into a hypoxia exposed group and a normoxia exposed group. Results indicated that only the group exposed to hypoxic conditions showed improvements in the mean power during the performance test. The training model benefited both groups as $VO_{2\text{mean}}$ increased for all participants. HIIT protocol proved efficient in maximizing performance (Manselin & Södergård, 2015).

2.2.1.2 Endurance Exercise. Przyklenk et al. (2017) sampled eleven male athletes. Over a period of four weeks, the participated performed repeated unipedal cycling EN in hypoxia, hyperoxia and normoxia. Przyklenk et al. (2017) aimed at investigating short-term endurance exercise (EN) in hypoxia to exert decreased mitochondrial adaptation, peak oxygen consumption ($VO_{2\text{peak}}$) and peak power output (PPO) compared to EN in normoxia and hyperoxia. Despite a reduced exercise intensity, increased blood lactate and rate of perceived exertion levels in hypoxia revealed higher metabolic load compared to hyperoxia and normoxia. Moreover, peak power output changed across the groups. An increase in electron transport chain complexes was also evident in the groups but was at its highest in hypoxia. EN-induced mitochondrial adaptability and exercise

capacity remained unchanged in hypoxia and hyperoxia as opposed normoxia; essentially, short term EN under hypoxia may not necessarily weaken the mitochondrial adaptation and exercise capacity, and hyperoxia does not increase adaptation.

2.2.2 Hyperoxic Training Physiological Implications

Keramidas, Kounalakis, Debevec, Norman, Gustafsson, Eiken, and Mekjavic, (2011) examined the normobaric paradox theory through sampling 10 healthy males, exposed to two distinct conditions. The first group was breathing normal air while the second was breathing 100% normobaric oxygen. The period of exposure was two hours, and blood samples were taken to examine changes in EPO concentrations. The results of the blood tests indicated that: (1) EPO increased 8 and 32 hours after the normal air exposure (due to its natural diurnal variation), (2) There was a noticeable 36% EPO decrement three hours after the normobaric exposure, and (3) EPO concentration was higher in normal breathing conditions than in hyperoxic breathing conditions, three, five, and eight hours after the study.

Still, some claimed that hyperoxia does not impact EPO concentration among healthy male athletes. Also, EPO varies in a manner consistent to the natural diurnal variation (Keramidas et al., 2011).

Balestra, Germonpré, Poortmans, and Marroni (2006) “investigated the effect of rebound relative hypoxia after hyperoxia obtained under normobaric and hyperbaric oxygen breathing conditions on serum EPO levels through sampling 16 healthy volunteers.” The tests were conducted pre- and post- intervention and after breathing 100% normobaric oxygen for two hours and a period of breathing 100% oxygen at 2.5 ATA and 1.5 hours. Radioimmunoassay measured serum EPO concentration at various occasions during 24-36 hours after the experiment. The results revealed the following:

- Serum EPO increased by 60% after 36 hours for normobaric oxygen intake.
- Serum EPO decreased by 53% after 24 hours for hyperbaric oxygen.

Nonetheless, the “changes were not related to circadian rhythm of serum EPO” (Balestra et al., 2006). Balestra et al. (2006) conclude a sudden yet sustain “decrease in tissue oxygen tension

may act as a trigger for EPO serum level.” Moreover, this EPO trigger is “not present after hyperbaric oxygen breathing” (Balestra et al., 2006).

2.2.2 Hyperoxic Training and Recovery

MacDonald, Pedersen, and Hughson (1997) examined the effect of supplying extra oxygen to the muscles during the recovery period between exercises. This extra O₂ was delivered through hyperoxic gas breathing masks, where O₂ is concentrated in far larger quantities than that of natural air. The study branches into two sub-studies, each containing seven athletes with one athlete common to both studies. It aims at determining the kinetics of VO₂ through the mean response time, which is the time taken to reach 63% of total change in VO₂, and to calculate oxygen deficit and slow component. Group one was exposed to normoxic and to hyperoxic gas breathing during exercise below and above ventilatory threshold. The second group has the same breathing conditions above ventilatory threshold.

The results of the study indicated that the supply of oxygen to muscle tissues contributes to the control of the VO₂ for high intensity exercises for steps above ventilatory threshold; oxygen transport might not be a limiting factor in light intensity exercises for steps below ventilatory threshold; finally, regulatory mechanisms for the usage and supply of oxygen may differ in on and off transients above ventilatory threshold (Macdonald, Pedersen, & Hughson, 1997).

Pupis, Slizik and Bartik (2013) examine the effects of inhaling concentrated oxygen (hyperoxia) on top level karate and judo players as a means of accelerating recovery time. Fourteen karatekas and nine judokas aged between 22 and 29 participated in the study. The athletes inhaled hyperoxic gas, containing 95 ± 4 % oxygen, or normal placebo air mixture before and after the match. The lactate levels were measured in athletes, three and 10 minutes after finishing the game. The results of the study indicated the following: Directly after the match, the average lactate levels in athletes that inhaled hyperoxic air was 10.44 mmol/L and 10.43 mmol/L in athletes that inhaled placebo air. Thus, this difference is not statistically significant. After the three-minute recovery period, the average lactate level in athletes that inhaled hyperoxic air was 8.53 mmol/L compared to 9.06 mmol/L for those who inhaled placebo air. Thus, a respective 18.12 % decrease in lactate levels compared to 13.4%. After the ten-minute recovery period, the average lactate level was 6.65

mmol/L (36.3% decrease) in those who inhaled hyperoxic gas compared to 7.73 mmol/L (25.86% decrease) in those who inhaled placebo air.

The results prove that the dynamics of lactate metabolism differ when inhaling hyperoxic air and normal air. Thus, hyperoxic air accelerates recovery time after a karate or judo match (Pupiš, Sližik, & Bartík, 2013).

Yokoi, Yanagihashi, Morishita, Fujiwara, and Abe (2014) investigate the effects of normobaric hyperoxia on the recovery of local muscle fatigue. Two protocols were completed by 11 healthy male participants. The first exercise was a single-leg isometric knee extension (for as long as possible) at 70% of the participants' maximum voluntary isometric contraction. After finishing the exercise, each participant was either treated with 20.9% oxygen or 30.0% oxygen for half an hour. Then, an identical isometric task was performed and the extent of recovery was measured. The results were as follows: 30.0% oxygen intake revealed a better recovery rate in the maximum voluntary isometric contraction than that of the 20.9% oxygen intake case. The total hemoglobin after the 30.0% oxygen treatment was of a higher concentration than that of 20.9% oxygen. Thus, the study concludes that normobaric hyperoxic treatments serve a better recovery for muscles than normal conditions (Yokoi et al., 2014).

Kay, Stannard, Morton and North (2008) examine “the effects of hyperoxia during recovery on peak power.” The test considers three different scenarios: 21% oxygen supply, 60% oxygen supply, and 100% oxygen supply for a 4-minute recovery period after 30 seconds of maximal cycling exercise. Twelve male athletes participated in the test, and the results revealed that 100% oxygen breathing improved absolute power output. However, the rate of fatigue also increased. Thus, the usage of such application in physically demanding sports is limited.

Sperlich, Zinner, Krueger, Wegrzyk, Mester, and Holmberg (2011) examine the “ergogenic effect of hyperoxic recovery in elite swimmers performing high-intensity intervals.” Sperlich et al. (2011) argue that breathing oxygen-enriched air (hyperoxia) during the recovery period positively contributes towards peak and mean power. To support their claim, Sperlich et al. (2011) examine 12 elite athletes, belonging either to the hyperoxic group or the normoxic one, during a six-minute

recovery period post five repetitions of high-intensity bench swimming with a maximum of 40 arm strokes. Prior to and post the interventions, oxygen partial pressure and saturation as well as pH, base excess and blood lactate concentration were noted. The main findings to this study revealed the following:

- Peak and mean power in hyperoxic recovery recorded significantly higher results as opposed to normoxia, particularly throughout the third, fourth, and fifth intervals.
- No evident changes were detected in blood lactate, pH or base excess throughout both groups.

Therefore, Sperlich et al. (2011) conclude that peak and mean power are improved as a result of hyperoxia in recovery among elite swimmers.

The studies aforementioned mainly reveal that hyperoxic recovery is conducive to athletic performance and yields positive, desirable outcomes.

2.3 LHTH/LHTL training

Schmitt, Millet, Robach, Nicolet, Brugniaux, Fouillot, and Richalet (2006) tested the impact of the live high train low intervention among 40 elite athletes on aerobic performance and on the economy of work. The study's period was 13-18 consecutive days, and the athletes were split into two equal groups: 20 athletes trained and slept at 1200 meters altitude whereas the other 20 athletes trained at 1200 meters altitude and slept in hypoxic rooms (2500 meters) for 5-6 nights and in hypoxic rooms (3000-3500 meters) for 8-12 nights. Tests were conducted pre- and post- one day and post- 15 days of the camp. The results were as follows: From pre- to post- one day, the VO_{2max} increased by 7.8% in the hypoxic group and by 3.3% in the normal group. From pre- to post- one day, the peak power output increased by 4.1% in the hypoxic group and by 1.9% in the normal group. After 15 days, the VO_{2max} returned to pre-conditions in both groups. After 15 days, the peak power output increased by 8.3% in the hypoxic group and by 3.8% in the normal group. After 15 days, the VO_2 and power at respiratory compensation point increased by 9.5% and by 11.2% respectively for the hypoxic group and by 3.2% and by 3.3% respectively for the normal group. Schmitt et al. (2006) conclude that athletes exposed to hypoxic conditions showed greater increase in peak power output than athletes exposed to normal conditions for the same increase in VO_{2max} .

Also, the efficiency of the live high train low is evident through the higher VO_2 and power at RCP 15 days later.

Robertson, Saunders, Pyne, Aughey, Anson, and Gore (2010) “quantify the reproducibility of responses to the LHTL altitude exposure” through sampling 16 runners to complete two three-week blocks of simulated LHTL for 14 hours per day at 3000 meters or near sea level altitude at 600m. Changes in 4.5 km time trial performance were recorded as well as physiological changes, including $\text{VO}_{2\text{max}}$, running economy, and hemoglobin mass. The results yielded the following conclusions: Reproducible mean improvements $\text{VO}_{2\text{max}}$ and hemoglobin mass can be induced due to 3 weeks of LHTL altitude exposure. Changes in time trials appear to be more variable than the criteria mentioned in the first point. Robertson et al. (2010) conclude that enhanced physiological capacities alongside factors, such as fitness, fatigue, and motivation, positively contribute to competitive performance.

Moreover, Robertson et al. (2010) reports “the changes in hemoglobin mass, performance tests and competitive performance of athletes after exposure to simulated and real altitude trainings.” The participants of this study were nine swimmers that completed up to four 2-week blocks of combined living and training at moderate natural altitude of 1350 meters and simulated LHTL altitude exposure (2600 meters and 600 meters). The competition performance was compared with that of nine similarly swimmers that were not exposed to altitude training. The study reveals that each 2-week altitude block produces on average a 0.9% increase in Hb_{mass} , a 0.9% increase in 4-mM lactate threshold velocity, and a 1.2% enhancement in 2000m time trial test. Competition times remained close to those athletes that were not exposed to altitude training. Therefore, the study concludes that altitude training and exposure programs deliver “modest changes in physiology” but do not necessarily improve competition times (Robertson et al., 2010).

Siebenmann et al. (2012) study the effect of LHTL training strategy. This study was conducted at Centre National de Ski Nordique in Prémaman, France. The studied sample was 16 athletes, of which six were exposed to normobaric normoxia and 10 were exposed to normobaric hypoxia. Moreover, none of the participants have been exposed to hypoxia within the past month before the study. In addition, none of the athletes actually knew of the conditions they were going to face to ensure the elimination of any placebo effect. The total duration of the study was eight

weeks, of which the first two weeks served as a lead in point, where athletes were asked to stay for a minimum of 16 hours inside their rooms (whether normoxia or hypoxia), and baseline testing was performed. The 16-hour curfew carried on for the next four weeks, then finally, in the last 2 weeks of the study, participants were relieved from their rooms. The purpose of this was to monitor how long would the effects of hypoxia last.

The tests conducted on the participants were performed at the hospital La Vallée (Le Sentier, Switzerland). The end results of the study were as follows:

- The study failed to reproduce physiological changes due to hypoxia in the participants in contrast with previous studies.
- Hb_{mass} was not affected as no significant difference was present between the placebo group, and the hypoxic group and the performances of athletes in a 26-km time trial remained similar to pre-study level.
- VO_2 max was not affected by the LHTL strategy. Exercise economy was not affected. The erythropoietic response to LHTL is varies between participants, even those exposed to the same conditions.
- The reduction in plasma volume, which may occur due to the confinement in hypoxic rooms, counteracts any erythropoietic response.
- LHTL does not have a significant advantage over conventional endurance training methods, which, surprisingly contradicts many studies.

The study performed by Robach et al. (2012) tests the following hypotheses:

1. The live high train high intervention improves the $V_{O_2\ max}$.
2. The improvement mentioned above is due to hypoxia induced increase in total Hb_{mass} .
3. The improvement mentioned above is not due to “improved maximal oxidative capacity.”

Robach et al. (2012) sampled 16 endurance athletes, six of which were exposed to normoxia and ten were exposed to normobaric hypoxic with a 3000-meter equivalency for more than 16 hours per day for a period of four weeks. The study concludes that the LHTH intervention did not increase the VO_2 max regardless of the air conditions the athletes were exposed to. The Hb_{mass} increased slightly by 4.6% in 5 out of the 10 athletes exposed to hypoxia, but this was not accompanied with

an increase in the VO_2 max. Thus, Robach et al. (2012) claim that the LHTH intervention has no positive affect on VO_2 max in endurance athletes.

Humberstone-Gough, Saunders, Bonetti, Stephens, Bullock, Anson, and Gore (2013) compare two hypoxic interventions, LHTL and the acute (60-90 min) intermittent hypoxic exposure, to determine the direct effects “on the running and blood characteristics of elite triathletes.”

The criteria examined include “total hemoglobin mass (Hb_{mass}), maximal oxygen consumption, velocity at VO_{2max} , time to exhaustion, running economy, maximal blood lactate concentration ($[La]$) and 3 mM $[La]$ running speed.” Seventeen male and seven female Australian National Team triathletes took part of this experiment and were distributed to groups for 17 days. The three groups are (1) LHTL group (total of 240h of hypoxia), (2) IHE group (total of 10.2 h of hypoxia), and (3) a placebo group. After pre- and post- comparison tests between the three groups, the study concluded that there is no evidence that supports the IHE training method for triathletes (Humberstone-Gough et al., 2013).

Pugliese, Serpiello, Millet, and La Torre (2014) counteract the claims that hypoxia benefits are offset by the inability of athletes to actually maintain high training intensities in such conditions, serving as a counterexample for successful LHTH intervention in two Olympic athletes. The athletes underwent a three-week LHTH camp with 2090 meters and training intensity greater than 91% of race pace. “The training diaries were collected, and sea level performance was recorded before, during, and after the intervention,” yielding positive results (Pugliese et al., 2014).

Moreover, Rodríguez et al. (2015) examine the effects of this altitude training on performance, VO_2 and Hb_{mass} for each of the 4 preparatory in-season training methods for 54 elite swimmers. Three training interventions were included as follows:

- “Living and training at moderate altitude for three and four weeks (Hi-Hi3, Hi-Hi)”
- “Living high and training high and low (Hi-HiLo, 4 weeks)”
- “Living and training at sea level (SL) (Lo-Lo, 4 weeks)”

Measures were conducted initially, directly after finishing, and weekly after returning to sea level for a duration of four weeks. Rodríguez et al. (2015) conclude that a well-designed 3-4-

week program may impair performance immediately after the finish but definitely improves performance after a recovery period at sea level.

In a more recent study, Girard, Millet, Morin, and Brocherie (2017) examined the LHTL and the LHTH methods. Girard et al. (2017) examined twenty field hockey players for 14 days, residing under normobaric hypoxia and training in normoxia or under normobaric hypoxia with a protocol of six sessions, constituted of sprints, passive recovery, and active recovery. Running pattern was assessed prior to and immediately after the intervention. Results revealed the following:

- Running kinematics and spring-mass parameters did not exhibit clear changes.
- No clear changes were also evident in the condition interaction for any parameters as time progressed.
- Heart rate decreased post the intervention.

Girard et al. (2017), thus, conclude that changes in the players' mechanical pattern post the Live High-Train Low and Live High-Train High hypoxic training are not evident.

2.4 Studies on Protocols of Altitude training

Levine and Stray-Gundersen (1997) evaluate the LHTL intervention among 39 elite runners, participating in a two-week lead-phase and followed by a four-week supervised training at sea-level. Participant groups include the following:

- High-Low Group, living at a 2500-meter altitude and training at a 1250-meter altitude
- High-High Group, living and training at a 2500-meter altitude
- Low-Low Group, living and training at a 150-meter altitude

The criteria examined for the three include “maximal oxygen uptake, anaerobic capacity, maximal steady state, running economy, velocity at VO_{2max} , and blood compartment volumes.” Moreover, a 5000-meter time trial was conducted.

The findings revealed that altitude groups had a 5% increase in VO_{2max} and a 9% increase in red cell mass volume. Time trial test, velocity at VO_{2max} , and maximal steady state was evidently improved in the High-Low Group. Therefore, Levine and Stray-Gundersen (1997) conclude that LHTL, over a period of four weeks, enhances performance at sea-level due to the altitude

acclimatization. Furthermore, the maintenance of sea level training velocities accounts for the increase in velocity at VO_{2max} and maximal steady state (Levine & Stray-Gundersen, 1997).

Rusko, Tikkanem, and Peltonem (2004) analyzed the impact of High-High (HiHi) and Hi-Low (Hi-Lo) training strategies. In their analysis, the authors note essential reasons that indicate why High-High fails to yield positive outcomes in particular cases, (1) a low altitude might be insufficient to produce acclimatization effects among athletes in short training periods, (2) the presence of “insufficient training stimuli for the neuromuscular and cardiovascular systems,” (3) an increased risk of stress and infections due to “overtraining symptoms,” and (4) an “influence training intensity and physiological responses during altitude training.” Therefore, Rusko, Tikkanem, and Peltonem (2004) propose normoxia training, HiLo, to counteract the implications of extended hypoxia. They also conclude that HiHi and HiLo may yield positive acclimatization effects, especially in increasing the oxygen transport capacity of blood in case prerequisites, such as a minimum dose of 12 hours per day of hypoxia exposure for at least 3 weeks at an altitude of 2100-2500 meters, are met. Moreover, further studies are required to understand the effects of hypoxic training and the effects of severe hypoxia despite the impact of endurance performance (Rusko, Tikkanem, & Peltonem, 2004).

Bonetti and Hopkins (2009) meta-analytically reviewed “the effects of the adaption to six protocols of natural or artificial hypoxia on performance and related physiological measures in sub-elite and elite athletes.” The six protocols included the following:

1. Live-high train-high (LHTH)
2. Live-high train-low (LHTL)
3. Artificial LHTL with daily exposure to long (8-18) continuous
4. Brief (1.5-5 hours) continuous periods of hypoxia
5. Brief (<1.5 hours) intermittent periods of hypoxia
6. Artificial live-low train-high (LLTH)

Results revealed the following:

In sub-elite athletes, the substantial enhancement of maximal endurance power output was “very likely with artificial brief intermittent LHTL, likely with LHTL, and possible with artificial long continuous LHTL, but unclear with LHTH, artificial brief continuous LHTL, and LLTH.” On

the other hand, among elite athletes, “enhancement was possible with natural LHTL, but unclear with the other protocols.”

Moreover, a VO_{2max} increase proved to be very likely with LHTL in sub elite athletes. However, in elite athletes, a reduction was possible with LHTL. Bonetti and Hopkins (2009) conclude “that natural LHTL is the best protocol for enhancing endurance performance in elite and sub-elite athletes, and other artificial protocols are effective in sub-elite athletes.” Nonetheless, if modifications were introduced to the protocol, further alterations are possible.

Saugy et al. (2016) detected “the changes in physiological and performance parameters after Live High-Train Low (LHTL) altitude camp in normobaric (NH) or hypobaric hypoxia (HH)” to reproduce the actual training practices of endurance athletes. Athletes attended two LHTL 18-day camps, trained at an altitude of 1100 meters to 1200 meters, and lived at an altitude of 2250 meters under normobaric and hypobaric hypoxia conditions. Measurements and oxygen and oxygen saturation (SpO_2) were also recorded for both groups. PiO_2 and training loads were collected day-to-day, and blood samples as well as VO_{2max} were measured pre- and one day post-LHTL. A three-kilometer running test was conducted near sea-level pre- and post- training camps at one, seven, and 21 days. Results indicated an increase VO_{2max} . Moreover, performance enhancement remained similar in NH and HH conditions (Saugy et al. 2016).

2.5 Hyperoxia and Hypoxia

The majority of research studies have examined either hyperoxia or hypoxia separately. However, a few studies have examined them together as presented in this section.

Susta, Dudnik, and Glazachev (2015) use intermittent hypoxia-hyperoxia interventions along with light training schedules to help athletes overcome overtraining syndrome to return to regular performance. Susta et al. (2015) sampled 34 track and field athletes with 15 having the syndrome. These 15 were part of a condition program composed of repeated exposure to hyperoxia with oxygen at 30% and to hypoxia with oxygen at 10%. For a total duration of four weeks, interventions were conducted 90 to 120 minutes post a low intensity 45- to 60-minute exercise session of six to eight cycles, three times a week. The 19 remaining athletes were within the control group, continuing normal training sessions. “Exercise capacity, analysis of heart rate variability and hematological parameters were measured before and after the intervention” (susta et al., 2015).

Exercise capacity improved from 170.8 ± 44.8 W to 191.0 ± 26.9 W. “Heart rate variability revealed an improved sympatho-parasympathetic index - low frequency/high frequency ratio with 1.45 ± 1.71 after the intervention compared to 8.01 ± 7.51 initially.” Hematological parameters remained unchanged. Susta et al. (2015) conclude that a hyperoxia-hypoxia intervention coupled with light training schedules can treat overtraining syndrome recovery in a short period of time.

In a different study, Susta and Glazachev (2015) examine the effects of intermittent hypoxia-hyperoxia interventions on intermittent exercise of a 21-year-old football player over a period of a 3-week daily program. With hypoxic air of 11% oxygen and hyperoxic air of 30% oxygen, the exercise protocol incorporated a minute at 85% of maximum workload followed by a minute at 50% of maximum workload. Post-intervention, the athlete was able to complete 30 stages of the exercise as opposed to 14 stages initially, the heartrate was at 169 beats/minute as opposed to 182 beats/minute initially, and blood lactate was at 5.8 mmol/L as opposed to 7.3 mmol/L initially. Thus, Susta and Glazachev (2015) conclude that intermittent hypoxia-hyperoxia interventions improve cardiovascular efficiency and lactate removal.

Zinner, Hauser, Born, Wehrin, Holmberg, and Sperlich et al. (2015) “examined the effects of breathing oxygen at different partial pressures during exercise recovery on the performance of ten well-trained male endurance athletes with a special focus on upper body muscles and the oxygenation of the m, triceps brachii at sea-level, and an 1800-meter simulated altitude.” Four trial tests were conducted as follows:

1. Trail One was performed under normoxia.
2. Trail Two was performed under hypoxia (H_o , $F_iO_2 = 0.165$).
3. Trail Three was performed under normoxia and hyperoxia with hyperoxia as a recovery phase (HOX , $F_iO_2 = 1$).
4. Trial Four was performed under hypoxia and hyperoxia with hyperoxia as a recovery phase (HOX , $F_iO_2 = 1$).

Each trial involved three 3-minute sessions on a double-poling ergometer with 3-minute intervals of recovery.

After analyzing their results, Zinner et al. (2015) conclude that that hyperoxia with either normoxic or hypoxic training conditions can aid in recovery intervals.

Bayer et al. (2019) assess the effect “of adding intermittent hypoxic-hyperoxic training to a multimodal training intervention on the mobility and perceived health” of old individuals. Thirty-four participants between the ages of 64 and 92 years participated in this five to seven-week test, categorized by the following:

- A multimodal training constituted of “strength, endurance, balance, reaction, flexibility, coordination, and cognitive exercises.”
- Hypoxic intervention consisted of breathing 10-14% oxygen for four to seven minutes.
- Hyperoxic intervention consisted of breathing 30%-40% oxygen for two to four minutes. The placebo treatment group breathed ambient air.

Results did not reveal a difference between hypoxic-hyperoxic intervention and normoxia, accentuating that “adding intermittent hypoxic-hyperoxic training to a multimodal training intervention does not improve perceived health and mobility” (Bayer et al., 2019).

In conclusion, Chapter Two has presented most of the literature on hypoxic and hyperoxic training, highlighting the results of the research studies conducted yet noting the disparity, when present, among these findings and lead us to the problem of this study:

The previous studies had usually investigated the effects of hypoxia and hyperoxia training separately. Scarce number of scientists had tried to combine the two techniques to achieve optimal results. The effects of Intermittent Hypoxia-Hyperoxia Training (IHHT) has been the main focus of a number of studies. Nonetheless, extensive literature on IHHT is yet to be developed.

The only similar experiment was conducted on 32 cardiac patients suffering from comorbidities. The patients were divided as follows: A group that benefited from a five-week program of IHHT and another that benefited from an eight-week exercise program with hypoxia. The results of the experiment revealed that both programs provided the same results in patients; both groups reached the same cardiorespiratory fitness. This signifies that IHHT is a safe alternative for cardiac patients who cannot undergo physical activity (Dudnik, Zagaynaya, Glazachev, & Susta, 2018).

A similar study in ischemic heart disease patients demonstrated that IHHT, after the patients were subjected to fifteen treatments during a three-week period, allowed for a noticeable increase in exercise tolerance and for a decrease of angina attacks as well as gave patients a more positive outlook on their quality of life. Once again, this study proved that IHHT is an effective option for patients suffering from heart conditions. One can thus clearly conclude that past experiences demonstrated that IHHT is a suitable option for patients suffering from diseases that, in normal circumstances, prevent them from practicing any physical activity. Previous findings have also shown that IHHT has beneficial effects on people who have not previously suffered any cardiac issues.

One study examined if hyperoxia has any effect on performance, training stimulus, and recovery. Findings from this study corroborated previous literature, revealing that individuals had an ameliorated performance and longer time to exhaustion when the fraction of inspired oxygen was at 0.3 or more. Moreover, when hyperoxia was used during recovery, the performance of the individual was better during the subsequent exercises (Malette, Stewart, & Cheung, 2017).

CHAPTER THREE

3. PROBLEM AND AIM

3.1. Problem

To the best of the author's knowledge, there has not been any studies and/or experiments conducted that combined hypoxic high intensity interval training with exposure to hyperoxia during recovery. Moreover, all previous studies either studied hypoxia or hyperoxia separately or combined them but without the use of HIIT. Therefore, the aim of this dissertation is to combine, for the first time, HIIT with hypoxia, followed by hyperoxia at rest in healthy able bodied subjects.

3.2. Objective and Hypotheses

Based on the aforementioned, the proposed doctoral dissertation aims at determining the impact of two distinct environments on the athlete's training program. These environments incorporate hypoxia during high-intensity interval training and hyperoxia during recovery. The dissertation also aims at determining the effects of these environments on blood oxygen transport parameters on athlete's performance measured by aerobic capacity. The main hypotheses of the research are set as follows.:

- H₁: A combination of intermittent high intensity hypoxic training and recovery in hyperoxia significantly improve the aerobic capacity than when recovering in normoxia.
- H₂: A combination of intermittent high intensity hypoxic training and recovery in hyperoxia significantly improve the oxygen transport parameters than when recovering in normoxia.

CHAPTER FOUR

4. METHODOLOGY

4.1 Approach and Design

The study follows a quantitative approach to the collection of data. According to Roni, Merga, and Morris (2020), a quantitative approach is most suitable when the data to be collected is numerical in nature. Therefore, quantitative methods require that comparable data across participants is retrieved, and the type of analysis necessitates detecting patterns among the given data in order to attain findings, interpret the results, and derive conclusions.

In addition, the study adopts an experimental design, given that the study aims at establishing a cause-effect relation among a group of variables. The independent variable is manipulated to determine the effects on the dependent variables. Also, participants are assigned to groups in a random manner.

4.2 Sampling Method

A priori power analysis in free G*Power software was used in order to calculate the minimal sample size for desired minimal power. The minimal acceptable power was set at 0.80. The group of Z tests and ANOVA within-between effects were chosen. For the p set at $p < 0.05$, the minimal total sample with expected moderate effect size and for two repeated measurements in three groups was calculated to be 42 total subjects (14 per group). A moderate effect size $f = 0.25$ was chosen. According to Cardinale and Ekblom (2018), the estimated hyperoxia training effects on VO_{2max} and performance were 0.15 to 1.0, respectively, signifying small and large effects. As it was inconclusive, a moderate effect was chosen. Therefore, the sample is comprised of 49 (15% more than required in case of a dropout) able-bodied male students, aged 18 to 20, mainly the students that are studying at sports Academy School and Antonine University in Beirut.

Participants were randomly assigned to one of the following groups (randomization was performed by “lottery” method where the papers with hidden names of the subjects were taken from a bowl and assigned on 1st, 2nd and 3rd group consequently):

1. HIIT Hypo group: High intensity interval training group with recovery in normoxia, N=16
2. HIIT Hypo+ Hyper group: High intensity interval training group with recovery in hyperoxia, N=16
3. CTR: Control group, performing everyday activities, N=16

Participants trained three times a week at *Performance First Club* facilities for a period of four weeks, adhering to their designated training protocol which will be explained later in more details.

4.3 Procedure

4.3.1 Initial and Final Testing

The experiment had two series of aerobic performance testing as well as laboratory blood testing, separated by four micro cycles of training (four weeks). The first series of testing is conducted initially before the intervention and, finally, ten days after the last micro cycle.

Testing consisted of a blood tests to determine the erythrocyte count, hemoglobin, hematocrit, reticulocyte, MCHC (mean corpuscular hemoglobin concentration), MCH (mean corpuscular hemoglobin), and MCV (mean corpuscular volume) parameters. In addition, the aerobic capacity testing is noted by means of all-out test with incremental intensity using treadmill and metabolic analyzer (*Fitmate PRO*, COSMED, Italy).

Before the treadmill procedure, spirometry is conducted in order to exclude the subjects with possible pulmonary obstruction. None of the subjects demonstrated any possible pulmonary obstruction, meaning FEV1 to FVK ratios obtained by spirometry were well above 80% and not indicative of any COPD. The analyzed variables are comprised of parameters at maximal exertion

(VO_{2max} , maximal speed reached, HR_{max}) and parameters at anaerobic threshold (ANT) determined by v- slope method (VO_2 at ANT, speed at ANT, and HR at ANT) expressed as absolute values as well as relative values (percentage of maximum).

In sum, in order to attend to the purpose of the study, the following parameters, within two categories, were measured prior to and post the intervention among the three aforementioned groups.

(1) Category One: Treadmill Parameters, including

- a. Parameters at maximal exertion
 - i. Oxygen uptake (VO_{2Max} measured in $ml/kg \cdot min^{-1}$)
 - ii. Maximal speed reach (km/h)
 - iii. Heart rate (HR_{Max} measured in bpm)
- b. Parameters at anaerobic threshold
 - i. Oxygen uptake (VO_{2ANT} measured in $ml/kg \cdot min^{-1}$)
 - ii. Speed at anaerobic threshold (km/h)
 - iii. Heart rate (HR_{ANT} measured in bpm)

(2) Category Two: Hematological Parameters, including

- a. Erythrocyte count (measured in cells/mcl)
- b. Hemoglobin (Hb measured in g/dl)
- c. Hematocrit (HCT)
- d. Reticulocyte (%)
- e. MCHC (measured in g/dl)
- f. MCH (measured in pg)
- g. MCV (measured in fl)

Measurements for each variable were recorded at the beginning of the study, prior to the intervention, and ten days post the end of the intervention. The figure below represents recorded instances of the treadmill parameters throughout the study.



Figures 1a, b, c, d. Recorded Instances of Treadmill Parameters

The figure below represents the participants' participation in retrieving data for the treadmill parameters.

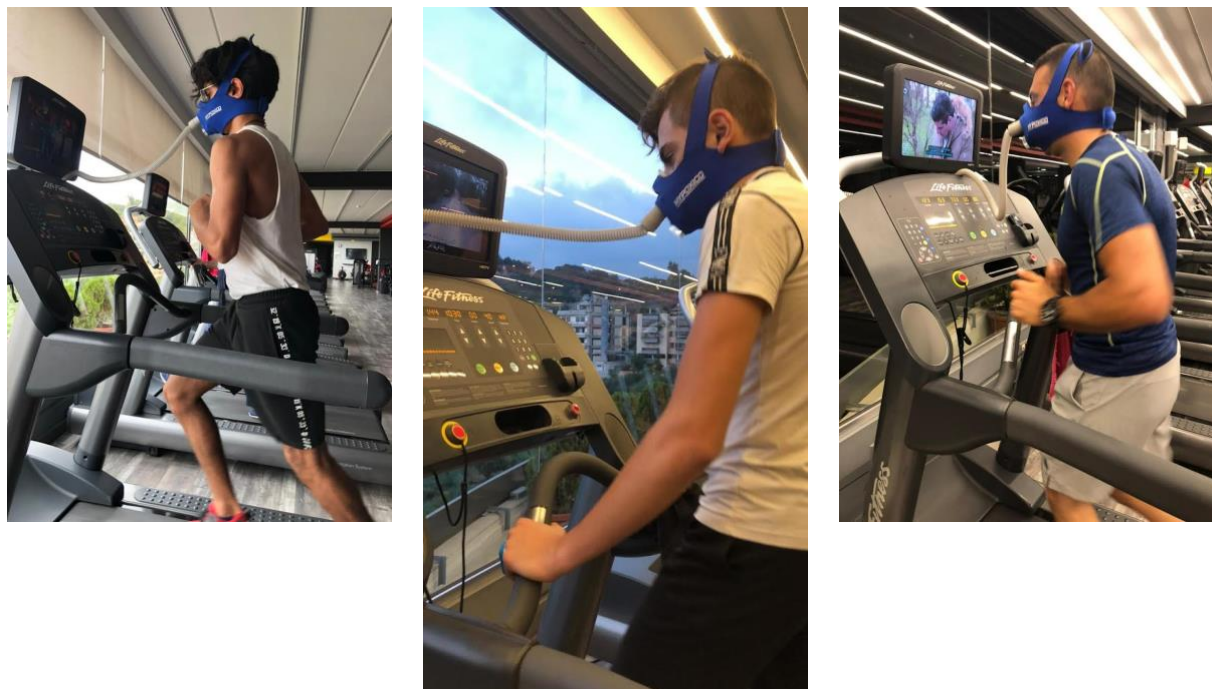


Figure 2. Participants' participation in retrieving treadmill parameters

4.3.2. Equipment and Protocols

As previously mentioned, participants were randomly assigned to one of the following groups. The equipment and protocols of each group were as follows;

4.3.2.1 HIIT- High intensity interval training group, normoxia recovery:

The training protocol in hypoxia was performed for four weeks with *Everest 2 Summit generator* (Hypoxico, USA). This generator is also called hypoxicator and it is portable and quiet. It is the top of the line in hypoxic, altitude simulation technology. This is the ideal system for home or personal-use for any or all of the 3 training methods. Each micro cycle consisted of one week with three training sessions. Each of the four weeks, the intensity of the HIIT and continuous training is increased by 5%. HIIT hypoxia exercise protocol consists of six bouts of one minute at 80 to 95% of HR_{max} , each week at higher

rate, respectively, followed by 2 minutes at 60% of HR_{max} . The recovery protocol for experimental group HIIT is 15 minutes breathing ambient air at rest.



Figure 3. Hypoxico generator (commercial photo from official Hypoxico website)

4.3.2.2 HIIT+O₂- High intensity interval training group, hyperoxia recovery:

Similar to HIIT, the HIIT+O₂ group follows the same high intensity interval procedure in hypoxia. However, the recovery protocol differs. Particularly, the recovery protocol for experimental group HIIT+O₂ was 15 minutes at $FiO_2=0.40$. According to Cardinale and Ekblom (2018), 0.21. to 0.6 are safe ranges. Nowadays oxygen concentrators are also portable and easy to use. The equipment used was DeVilbiss, USA.

4.3.2.3 CTR- Control group, performing everyday activities: The protocol for control group incorporates four weeks of continuing the everyday activities with no training involved and testing within the same timeframe as experimental groups.

The blood samples were take two days prior to the beginning of the study as well as ten days after the end of the intervention in similar conditions and at the same time in the morning and analyzed by a licensed biochemistry laboratory *Laboratoire d'analyse medical* in Beirut.

4.4 Data Analysis Procedures

Given that the study adopts a quantitative approach to the collection of data, data analysis has been conducted via the statistical software, SPSS. Data was uploaded at two instances, prior to the start of the intervention and 10 days post the completion of the intervention. The analyzed variables included parameters at maximal exertion, which are VO_{2max} , maximal speed reached, HR_{max} , and parameters at anaerobic threshold (ANT) determined by v- slope method (VO_2 at ANT, speed at ANT, and HR at ANT) as well as hematological parameters, including Hb, HCT, Erythrocyte, MCV, MCHC, MCH, and Reticulocyte. The statistical test adopted was factorial ANOVA.

4.5 Ethical Considerations

All ethical considerations have been taken into account in this study. Participation was voluntary, and participants were informed about the purpose and procedure of the study. They provided written consent prior to taking part in the study. They were also informed that they are able to withdraw their participation. Moreover, they were given access to the results and were able to ask the researcher any questions or share their concerns if and when needed. Ethics committee of the Faculty of Kinesiology, University of Zagreb approved the ethical part of this thesis on their meeting on 26th March 2019.

CHAPTER FIVE

5.RESULTS

Chapter Five presents the findings of this doctoral dissertation, including descriptive statistics of the anthropometric measurements for the participants of this study. The data was analyzed via statistical software to yield descriptive and inferential statistics.

5.1 Participant Groups and their Characteristics

Forty-nine participants aged 18 to 20 years were randomly assigned to three groups with the following characteristics pertaining to the type of protocol adhered to. The descriptive characteristics related to height and weight are summarized in the table below.

Table 1 Descriptive data by group

	Hyper-Hypo	Hypo	Control
Height (centimeters)	175.94 ± 5.76	175.38 ± 5.95	179.69 ± 6.65
Weight (kilograms)	71.13 ± 15.56	70.38 ± 12.71	73.62 ± 8.28

5.2 Parameters at Maximal Exertion

This section provides a detailed overview of the descriptive and inferential statistics of the parameters at maximal exertion, which include VO_{2Max} (ml/kg·min⁻¹), Maximal Speed Reach (km/h), and HR_{Max} (bpm).

To begin with, the table below displays the descriptive statistics of the parameters at maximal exertion prior to and post intervention.

Table 2 Descriptive Statistics of Parameters at Maximal Exertion

Parameters at maximal exertion	Group	Prior to Intervention		Post Intervention	
		Mean	Stand Error of Mean	Mean	Stand Error of Mean
VO _{2Max} (ml/kg·min ⁻¹)	Hypo+Hyper	49.34	1.24	50.79	1.18
	Hypo	46.89	1.24	47.56	1.18
	Control Group	48.04	1.30	46.71	1.03
Maximal Speed Reach (km/h)	Hypo+Hyper	14.81	0.41	15.47	0.36
	Hypo	13.66	0.41	13.86	0.36
	Control Group	13.88	0.40	13.12	0.35
HR _{Max} (bpm)	Hypo+Hyper	201.12	1.34	200.69	1.06
	Hypo	200.75	1.34	202.94	1.61
	Control Group	199.94	1.30	199.12	1.03

The changes in mean of the parameters at maximal exertion are evident yet minimal as shown in Table 2. The mean of VO_{2Max} increased in both Hypo+Hyper and Hypo groups with an increase of 1.5 and 0.9, respectively, but decreased in the control group from 48 to 46.7 ml/kg·min⁻¹. The mean of maximal speed reach increased the most in the Hypo+Hyper group with an increase of 0.7 km/h, followed by an increase of 0.2 km/h in the Hypo group, but a decrease in the control group by 0.8 km/h. As for heart rate, a slight decrease occurred in the Hypo+Hyper and the control group with 0.4 and 0.8, respectively; whereas an increase of 2.1bpm in the hypo group was observed. The figures below are a line-graph representation of the abovementioned results.

While VO_{2Max} increased most apparently in the Hypo+Hyper group and less apparently in the Hypo group, it decreased in the control group as Figure 3 shows.

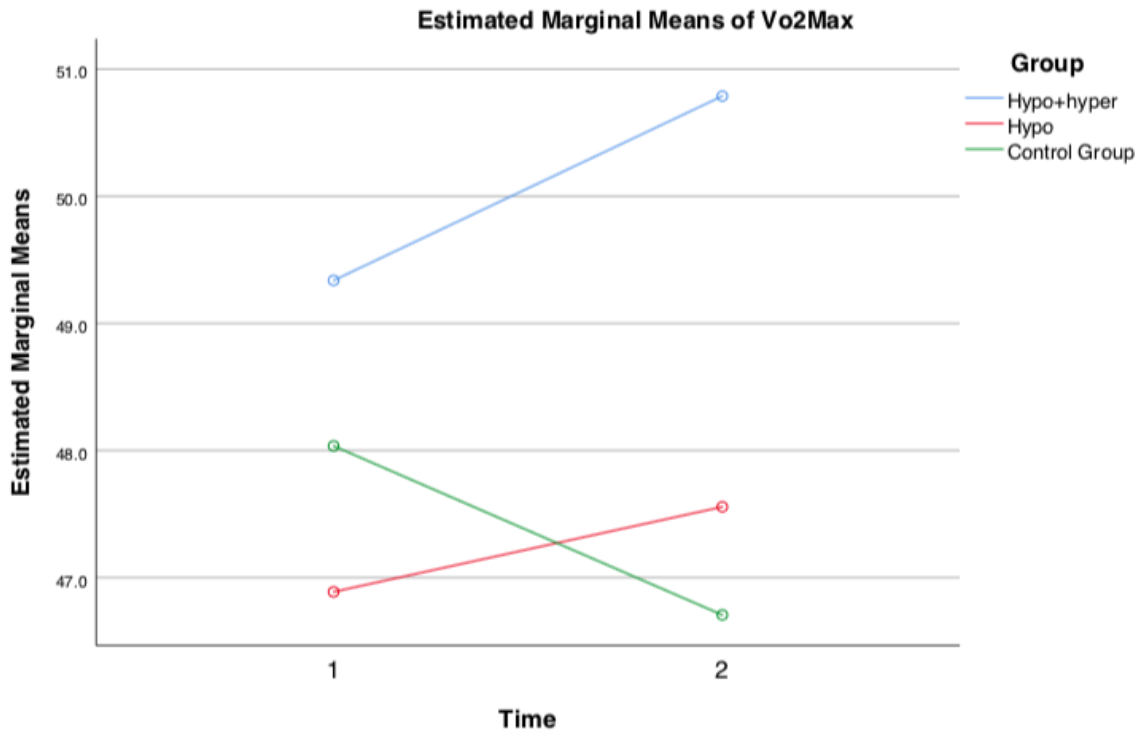


Figure 3
 Estimated Marginal Means of VO2Max (ml/kg/min) before-1 and after-2 intervention at Maximal Exertion

Figure 4 also reveals more significant increase in MAXSpeed in Hypo+Hyper group as opposed to the Hypo, and, once again, a decrease in the control group as it pertains to the maximum speed reach at maximal exertion.

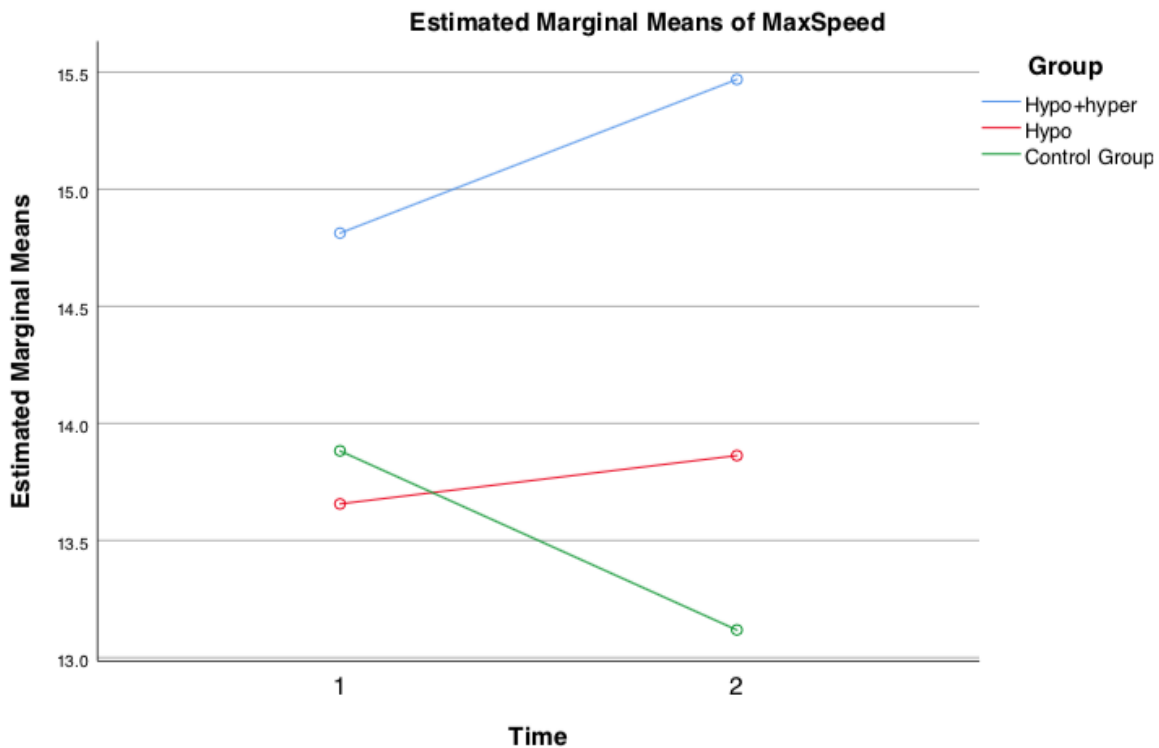


Figure 4
 Estimated Marginal Means of MaxSpeed (km/h) before-1 and after-2 intervention at Maximal Exertion

Unlike VO_{2Max} and MaxSpeed, Figure 5 pertains to the heart rate at maximal exertion. It seems that it shows a more significant increase in the Hypo+Hyper group and a smaller one in Hypo group and decrease in control group. Still, these changes are within several heartbeats so are not considered relevant. Nonetheless, those probably occurred because the first two groups managed to reach their real HRmax after the intervention and because of reaching higher intensities.

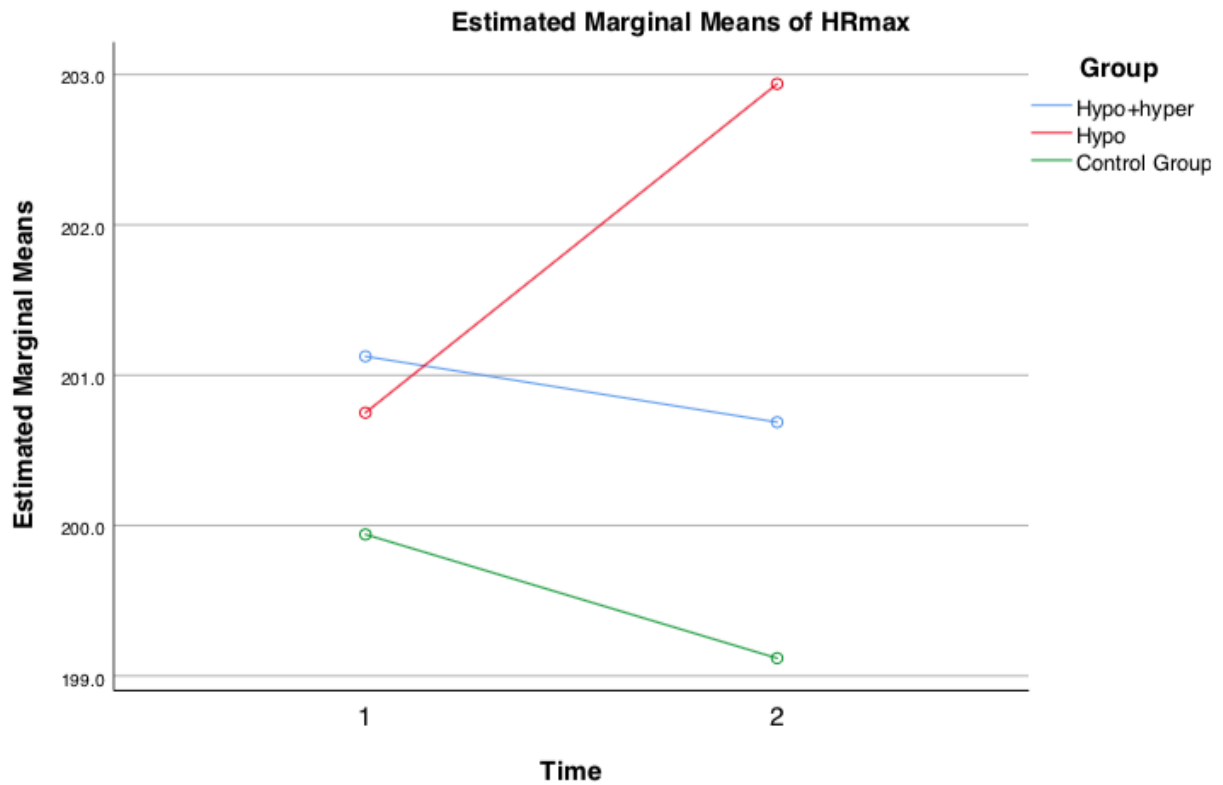


Figure 5
Estimated Marginal Means of HRmax (bpm) before-1 and after-2 intervention at Maximal Exertion

Following the, the table below summarizes the mean different (prior to and post intervention) of parameters at maximal exertion, namely VO_{2MAX} , maximum speed, and HR_{max} .

Table 3. Mean Difference of Parameters at Maximal Exertion for Entire Sample

Parameters at Maximal Exertion			
Measure	Mean Difference (pre-post)	Std. Error	P value
VO_{2MAX} (ml/kg·min ⁻¹)	0.26	0.1	0.105
MaxSpeed (km/h)	0.10	0.8	0.723
HR_{max} (bpm)	0.31	0.5	0.541

*Significant at 0.05

The significance level is marked at 0.05. According to the results presented in the table above, the changes in parameters at maximal exertion are insignificant for the whole sample.

Moreover, based on a pairwise comparison, the table below depicts the results of the parameters at maximal exertion. The table provides the mean difference, the standard error, the significance, as well as the lower and upper bound according to the groups, which was the main aim of the study.

Table 4. Pairwise Comparison of Parameters at Maximal Exertion by groups

Parameters at Maximal Exertion								
Measure	Time	Group I	Group J	Mean Difference (I-J)	Std. Error	P	Lower Bound	Upper Bound
VO ₂ MAX (ml/kg·min ⁻¹)	Pre-Intervention	Hypo+Hyper	Hypo	2.45	1.75	0.503	1.89	6.79
			Control	1.30	1.72	1.000	2.98	5.58
		Hypo	Hypo+Hyper	2.45	1.75	0.503	6.79	1.89
			Control	1.15	1.72	1.000	5.43	3.13
			Control	Hypo+Hyper	1.30	1.72	1.000	5.58
	Post-intervention	Hypo+Hyper	Hypo	3.23	1.67	0.176	0.91	7.37
			Control	4.08	1.64	0.050*	0.00	8.16
		Hypo	Hypo+Hyper	3.23	1.67	0.176	7.37	0.91
			Control	0.85	1.64	1.000	3.23	4.93

Max Speed (km/h)	Control	Hypo+Hyper	4.08	1.67	0.050*	8.16	0.00	
		Hypo	0.85	1.64	1.000	4.93	3.23	
	Pre- Interventi on	Hypo+ Hyper	Hypo	1.16	0.59	0.2	0.30	2.61
		Control	Control	0.93	0.58	0.3	0.50	2.36
	Control	Hypo	Hypo+Hyper	1.16	0.59	0.2	0.261	0.30
		Control	Control	0.23	0.58	1	1.66	1.20
	Control	Hypo+Hyper	Hypo+Hyper	0.93	0.58	0.3	2.36	0.50
		Hypo	Hypo	0.23	0.58	1	1.21	1.66
	Post- interventi on	Hypo+ Hyper	Hypo	1.61	0.51	0.01*	0.34	2.88
		Control	Control	2.35	0.50	0.00*	1.10	3.60
	Control	Hypo	Hypo+Hyper	1.61	0.51	0.01*	2.88	0.34
		Control	Control	0.75	0.50	0.4	0.51	2.00
Control	Hypo+Hyper	Hypo+Hyper	2.35	0.50	0.00*	3.60	1.10	
	Hypo	Hypo	0.75	0.50	0.4	2.00	0.50	
HR_{max} (bpm)	Pre- Interventi on	Hypo+ Hyper	Hypo	0.38	1.90	1.000	4.35	5.10
		Control	Control	1.18	1.87	1.000	3.47	5.84
	Control	Hypo	Hypo+Hyper	0.38	1.90	1.000	5.10	4.35
		Control	Control	0.81	1.87	1.000	3.84	5.46
	Control	Hypo+Hyper	Hypo+Hyper	1.18	1.87	1.000	5.84	3.47
		Hypo	Hypo	0.81	1.90	1.00	5.46	3.84
	Post- interventi on	Hypo+ Hyper	Hypo	2.25	1.87	0.421	5.98	1.48
		Control	Control	1.57	1.	0.881	2.10	5.24
	Control	Hypo	Hypo+Hyper	2.25	1.50	0.421	1.48	5.98
		Control	Control	3.82	1.48	0.039*	0.15	7.49
	Control	Hypo+Hyper	Hypo+Hyper	1.57	1.48	0.881	5.24	2.10
		Hypo	Hypo	3.82	1.48	0.039*	7.49	0.15

*Significant at 0.05

Based on the table 4 above, significant results are apparent post-intervention in VO_{2MAX} , maximum speed, and HR_{max} , particular as it relates to the following groups:

- For VO_{2MAX} , significant differences are visible between the Hypo+Hyper group and the control group but are insignificant between the Hypo+Hyper group and the Hypo group and between the Hypo group and the control group.
- For maximum speed, similarly, significant differences are visible between the Hypo+Hyper group and the control group, significant results are also visible between the Hypo+Hyper group and the Hypo group, but are insignificant between the Hypo group and the control group.
- For HR_{max} , the results are surprisingly significant between the Hypo group and the control group but are insignificant between the Hypo+Hyper group and the Hypo group and between the Hypo+Hyper group and the control group

As such, at maximal exertion, the results reveal that the intervention impacts the Hypo+Hyper group when it comes to VO_{2MAX} and maximum speed. It also impacts the Hypo group when it comes to HR_{max} which was not expected to happen as result of training.

5.3 Parameters at Anaerobic Threshold

This section provides a detailed overview of the descriptive and inferential statistics of the parameters at anaerobic threshold. VO_{2ANT} ($ml/kg \cdot min^{-1}$), ANT Speed, and HR_{ANT} (bpm) are recorded. To begin with, the table below displays the descriptive statistics of the parameters at anaerobic threshold prior to and post intervention.

Table 5. Descriptive Statistics of Parameters at Anaerobic Threshold

Parameters at Anaerobic Threshold	Group	Prior to Intervention	Post Intervention
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		Mean	Std Err	Mean	Std Err
VO _{2ANT} (ml/kg·min ⁻¹)	Hypo+Hyper	37.02	1.02	37.47	0.81
	Hypo	35.18	1.02	35.59	0.83
	Control Group	35.63	0.99	34.61	0.78
Speed at ANT (km/h)	Hypo+Hyper	10.94	0.32	11.13	0.29
	Hypo	10.44	0.32	10.56	0.29
	Control Group	10.82	0.31	10.32	0.28
HR _{ANT} (bpm)	Hypo+Hyper	175.31	2.72	175.56	2.62
	Hypo	172.06	2.82	174.00	2.60
	Control Group	168.94	2.73	170.88	2.52

Table 5 also depicts a minimal change in the different parameters at anaerobic threshold. The change at anaerobic threshold is less evident than that at maximal exertion across all parameters. VO_{2ANT} increased by 0.5 ml/kg·min⁻¹ in the Hypo+Hyper group as well as the Hypo group but decreased by 1.1 in the control group. The speed at anaerobic threshold increased by 0.2 km/h in both Hypo+Hyper group and Hypo group and, again, decreased by 0.5 km/h in the control group. Finally, the heart rate at anaerobic threshold increased by 0.3bpm in the Hypo+Hyper group, by 1.8bpm in the Hypo group, and by 1bpm in the control group. The figures below are a line-graph representation of the abovementioned results.

Figure 6 shows an increase in the Hypo+Hyper group as well as the Hypo group but a decreased in the control group as it pertains to VO₂ at anaerobic threshold.

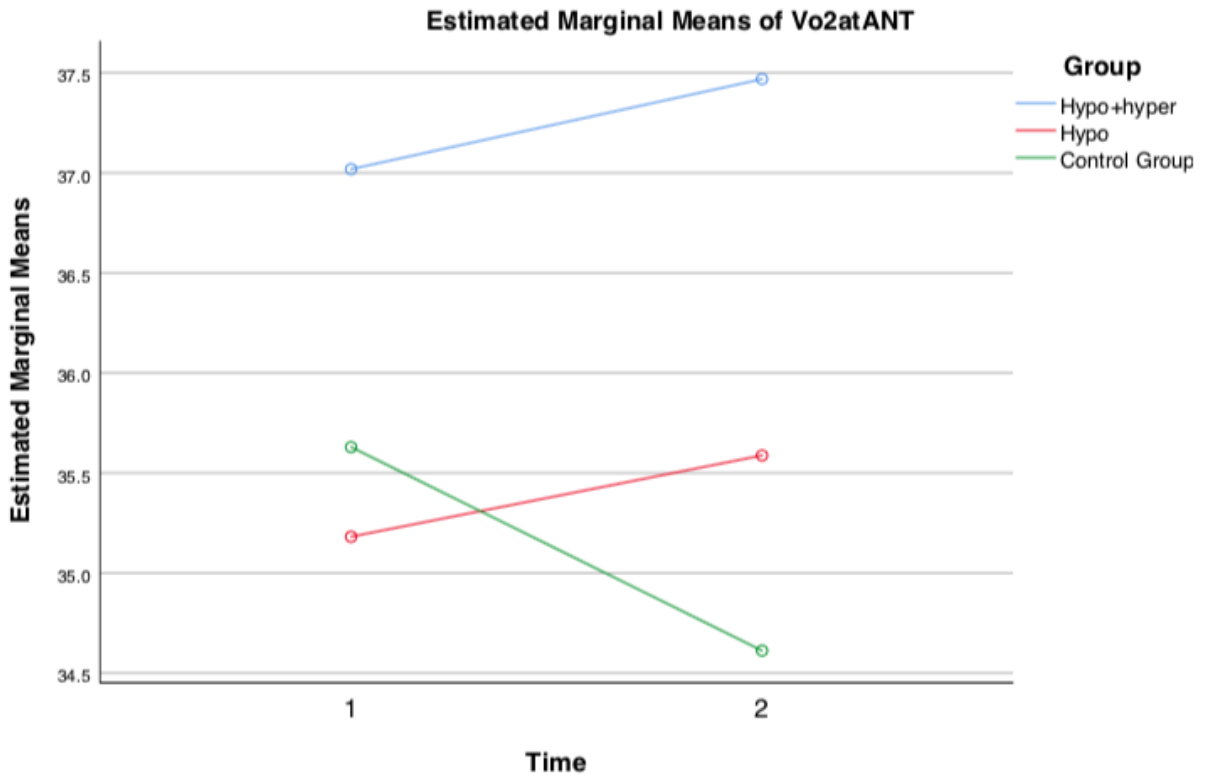


Figure 6
Estimated Marginal Means of VO_2 (ml/kg/min) before-1 and after-2 intervention at Anaerobic Threshold

Figure 7 shows a more significant decrease in the control group through a sharper slope than the increase in both, Hypo+Hyper group as well as the Hypo group, as it pertains to speed at anaerobic threshold.

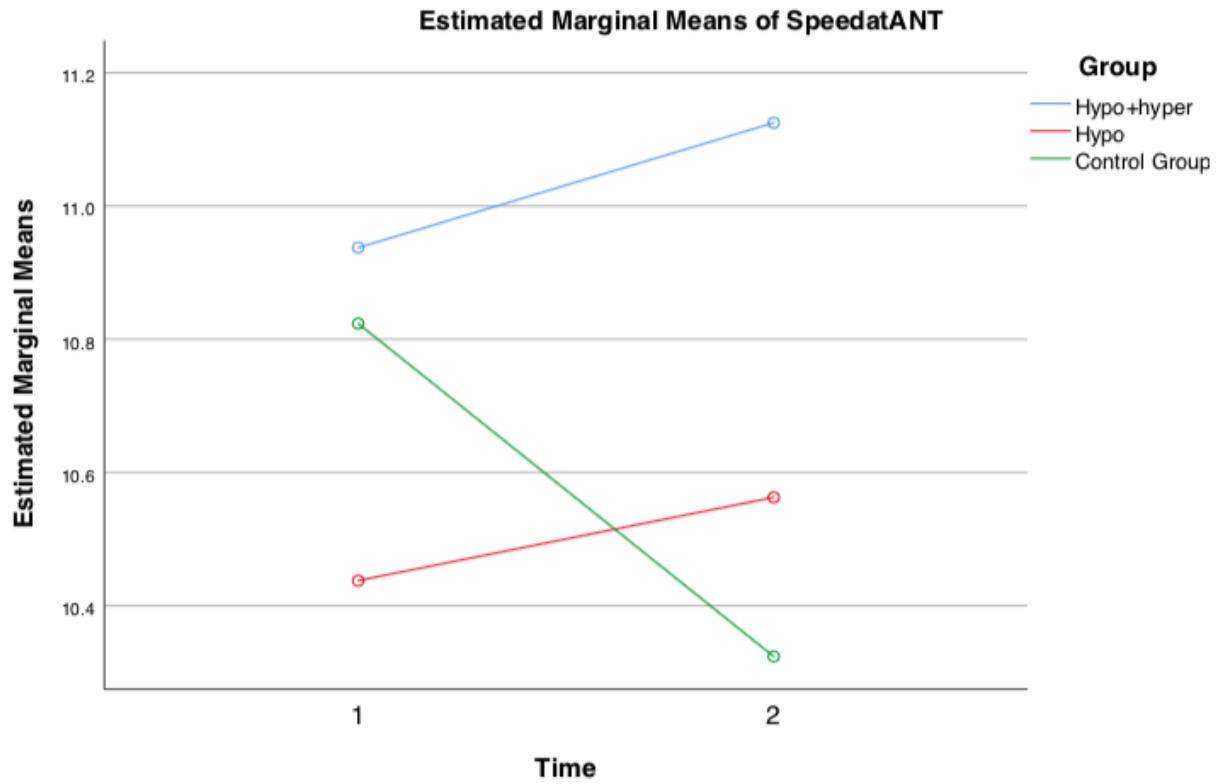


Figure 7
 Estimated Marginal Means of Speed (km/h) before-1 and after-2 intervention at Anaerobic Threshold

Figure 8 shows an increase in all three groups as it pertains to heart rate at anaerobic threshold; however, the increase was more apparent in the Hypo+Hyper group as well as the Hypo group.

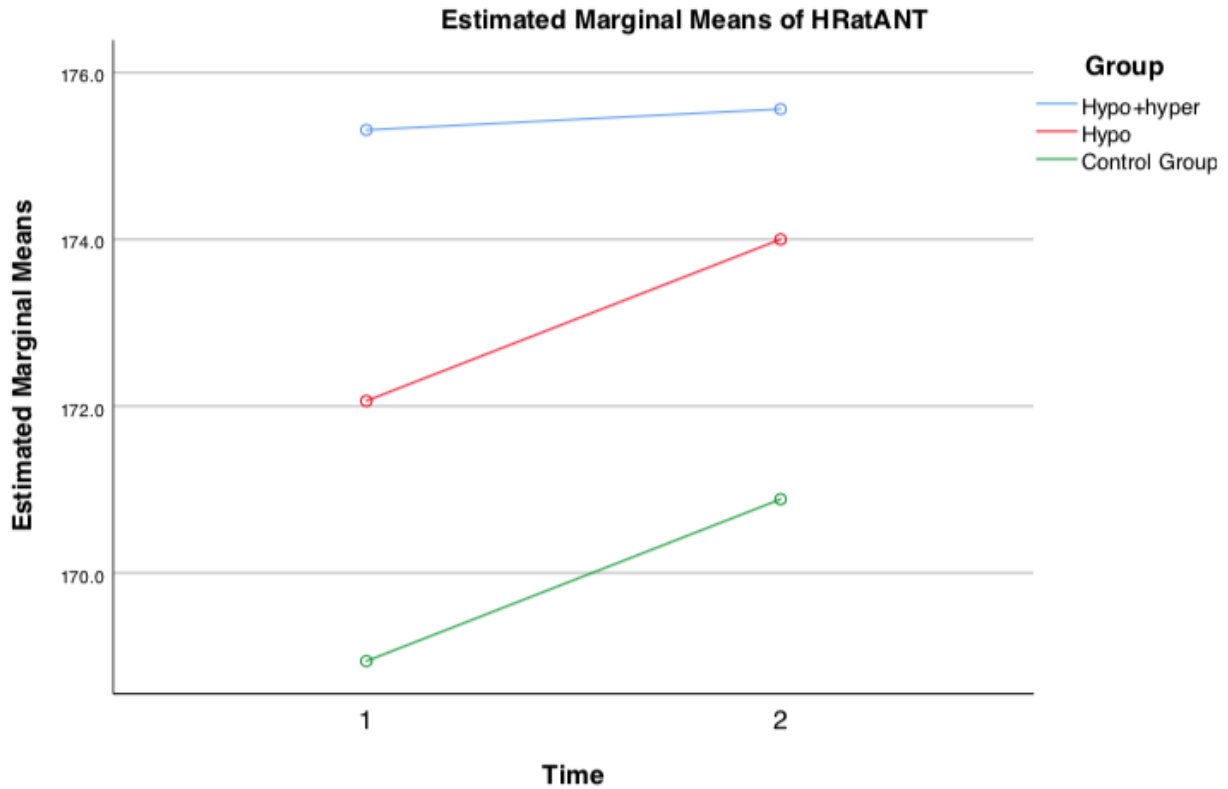


Figure 8
Estimated Marginal Means of HR (bpm) before-1 and after-2 intervention at Anaerobic Threshold

The table below summarizes the mean different (prior to and post intervention) of parameters at anaerobic threshold, namely VO_{2ANT} , speed at ANT, and HR_{ANT} .

Table 6. Mean Difference of Parameters at Anaerobic Threshold for Entire Sample

Parameters at Anaerobic Threshold			
Measure	Mean Difference (pre-post)	Std. Error	P value
VO_{2ANT} (ml/kg·min ⁻¹)	0.05	0.4	0.942
Speed at ANT (km/h)	0.06	0.1	0.530
HR_{ANT} (bpm)	1.4	0.5	0.011*

*Significant at 0.05

According to the table above, the results are insignificant when it comes to VO_{2ANT} and speed at ANT but reveal unexpected significance for HR_{ANT} . Then the results were analyzed by groups.

Table 7. Pairwise Comparison of Parameters at Anaerobic Threshold by groups

Parameters at Anaerobic Threshold									
Time	Group I	Group J	Mean Diff (I-J)	Std Err	P	Lower Bound	Upper Bound		
VO_{2ANT} (ml/kg·min ⁻¹)	Pre-Intervention	Hypo+Hyper	Hypo	1.84	1.45	0.630	1.75	5.43	
			Control	1.39	1.42	1.000	2.15	4.93	
	Post-intervention	Hypo	Hypo+Hyper	1.84	1.45	0.630	5.43	1.75	
			Control	0.45	1.42	1.000	3.99	3.10	
		Control	Hypo+Hyper	1.39	1.42	1.000	4.93	2.15	
			Hypo	0.45	1.42	1.000	3.10	3.99	
	Speed at ANT (km/h)	Pre-Intervention	Hypo+Hyper	Hypo	0.50	0.45	0.820	1.62	1.62
				Control	0.11	0.44	1.000	1.00	1.22
		Post-intervention	Hypo	Hypo+Hyper	0.50	0.44	0.820	1.62	0.62
				Control	0.39	0.44	1.000	1.49	0.72
Control			Hypo+Hyper	0.50	0.44	0.820	1.62	0.62	
			Hypo	0.39	0.44	1.000	1.49	0.72	

HR_{ANT} (bpm)	Control	Hypo+Hyper	0.11	0.40	1.000	1.22	0.99	
		Hypo	0.39	0.40	1.000	0.72	1.49	
	Hypo+Hyper	Hypo	0.56	0.40	0.507	0.44	1.56	
		Control	0.80	0.40	0.148	0.18	1.79	
	Post-intervention	Hypo	Hypo+Hyper	0.56	0.40	0.507	1.56	0.44
		Control	0.24	0.40	1.000	0.75	1.23	
	Control	Hypo+Hyper	0.80	0.40	0.148	1.79	0.18	
		Hypo	0.24	0.40	1.000	1.23	0.75	
	Pre-Intervention	Hypo+Hyper	Hypo	3.25	3.99	1.000	6.67	13.17
		Control	6.37	3.93	0.336	3.40	16.14	
	Hypo	Hypo+Hyper	3.25	3.99	1.000	13.17	6.67	
		Control	3.12	3.93	1.000	6.65	12.89	
	Control	Hypo+Hyper	6.37	3.93	0.336	16.14	3.40	
		Hypo	3.12	3.93	1.000	12.89	6.65	
	Hypo+Hyper	Hypo	1.56	3.68	1.000	7.59	10.71	
		Control	4.68	3.63	0.611	4.34	13.70	
	Post-intervent.	Hypo	Hypo+Hyper	1.56	3.68	1.000	10.71	7.59
		Control	3.12	3.63	1.000	5.90	12.13	
Control	Hypo+Hyper	4.68	3.63	0.611	13.70	4.34		
	Hypo	3.12	3.63	1.000	12.13	5.90		

**Significant at 0.05*

As we can see, there were not so many significant changes noted at anaerobic threshold apart from the oxygen uptake between Hypo+Hyper and Control group.

In sum, regarding the first hypotheses it seems that the above results indicate some moderate changes in group parameters post-intervention, especially in the Hypo+Hyper group and the Hypo group in comparison to Controls. These changes are significant in the following cases;

At Maximal exertion:

- VO_{2MAX} in Hypo+Hyper group increased as opposed to control group (p value 0.05)
- MaxSpeed in Hypo+Hyper group increased as opposed to Hypo group (p value 0.01)
- MaxSpeed in Hypo+Hyper group increased as opposed to control group (p value 0.00)
- HR_{MAX} in Hypo group increased as opposed to control group (p value 0.04)

At Anaerobic threshold:

- VO_{2ANT} : in Hypo+Hyper group it increased as opposed to the control group (p value level 0.04)

4.4 Hematological Parameters

This section presents the hematological parameters, including Hb, Hct, erythrocyte count, MCV, MCHC, MC and reticulocyte . The table 8 below displays the results of the hematological parameters pre-intervention and ten days after the intervention among the three groups, Hypo+Hyper, Hypo, and Control.

Table 8. Descriptive Statistics of Hematological Parameters Prior to and Post Intervention

Parameter	Group	Prior to Intervention		Post Intervention	
		Mean	Std. Err.	Mean	Std. Err
Hb (g/dl)	Hypo+Hyper	14.51	0.20	15.69	0.19
	Hypo	15.04	0.20	15.12	0.19
	Control Group	14.99	0.20	14.92	0.18
HCT (%)	Hypo+Hyper	43.51	0.59	44.03	0.64
	Hypo	45.1	0.58	45	0.64
	Control Group	46.29	0.56	45.84	0.63
Erythrocyte (cells/mcl)	Hypo+Hyper	5.16	0.07	5.19	0.06
	Hypo	5.16	0.07	5.17	0.06
	Control Group	5.21	0.06	5.22	0.6
MCV (fl)	Hypo+Hyper	85.56	1.36	85.44	1.36
	Hypo	84.13	1.36	84.00	1.36
	Control Group	87.00	1.32	87.82	1.32
MCHC (g/dl)	Hypo+Hyper	33.04	0.18	33.28	0.17
	Hypo	33.13	0.18	33.31	0.17
	Control Group	33.48	0.18	33.35	0.17
MCH (pg)	Hypo+Hyper	29.33	0.25	29.40	0.28
	Hypo	29.34	0.25	29.54	0.28
	Control Group	29.84	0.25	29.61	0.27
Reticulocyte (%)	Hypo+Hyper	1.10	0.06	1.12	0.05
	Hypo	1.10	0.06	1.08	0.05
	Control Group	1.12	0.06	1.05	0.05

As depicted in table 8 above, the results reveal the following among the different hematological parameters (the significance tests of this changes will be presented later n text):

- (1) Hb increases the most in the Hypo+Hyper group from 14.5 g/dl to 15.7 g/dl, followed by a slight 0.1 g/dl increase in the hypo group. In the control group, Hb remains constant.
- (2) While HCT increases by 0.5% in the Hypo+Hyper group, it decreased by 0.1% in the Hypo group and by 0.4% in the control group.
- (3) Erythrocyte count remains constant among the different groups, prior to and post intervention.
- (4) Slight changes occur in MCV with a 0.2 fl decrease in the Hypo+Hyper group, consistency in the Hypo group, and a 0.8 fl increase in the control group.
- (5) As for MCHC, slight changes are also visible from 33 to 33.3 g/dl in the Hypo+Hyper group, from 33.2 to 33.3 g/dl in the Hypo group, and from 33.5 to 33.3 g/dl in the control group.
- (6) Similarly, slight changes occur in MCH from 29.3 to 29.4 pg in the Hypo+Hyper group, from 29.4 to 29.5 pg in the Hypo group, and from 29.8 to 29.6 pg in the control group.
- (7) As for Reticulocyte, no significant changes are evident among the three groups prior to and post the intervention.

The following line-graphs are representation of each hematological parameter's means, prior to and post the intervention.

Figure 9 shows a spike in Hb in Hypo+Hyper group, followed by a slight increase in Hb in the Hypo group and a decrease in the control group.

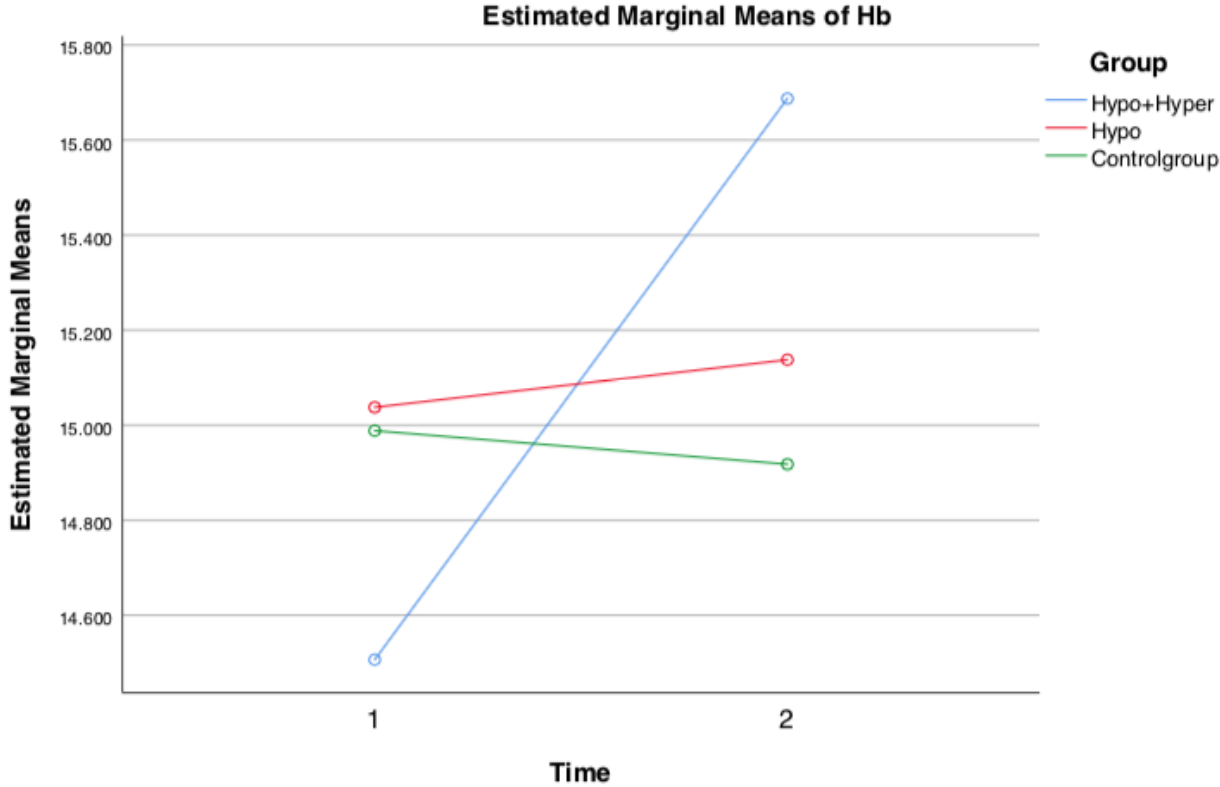


Figure 9
Estimated Marginal Means of Hb (g/dl) before-1 and after-2 intervention

Figure 10 shows a slight increase of HCT in the control group and the Hypo group as opposed to a slight increase Of HCT in the Hypo+Hyper group.

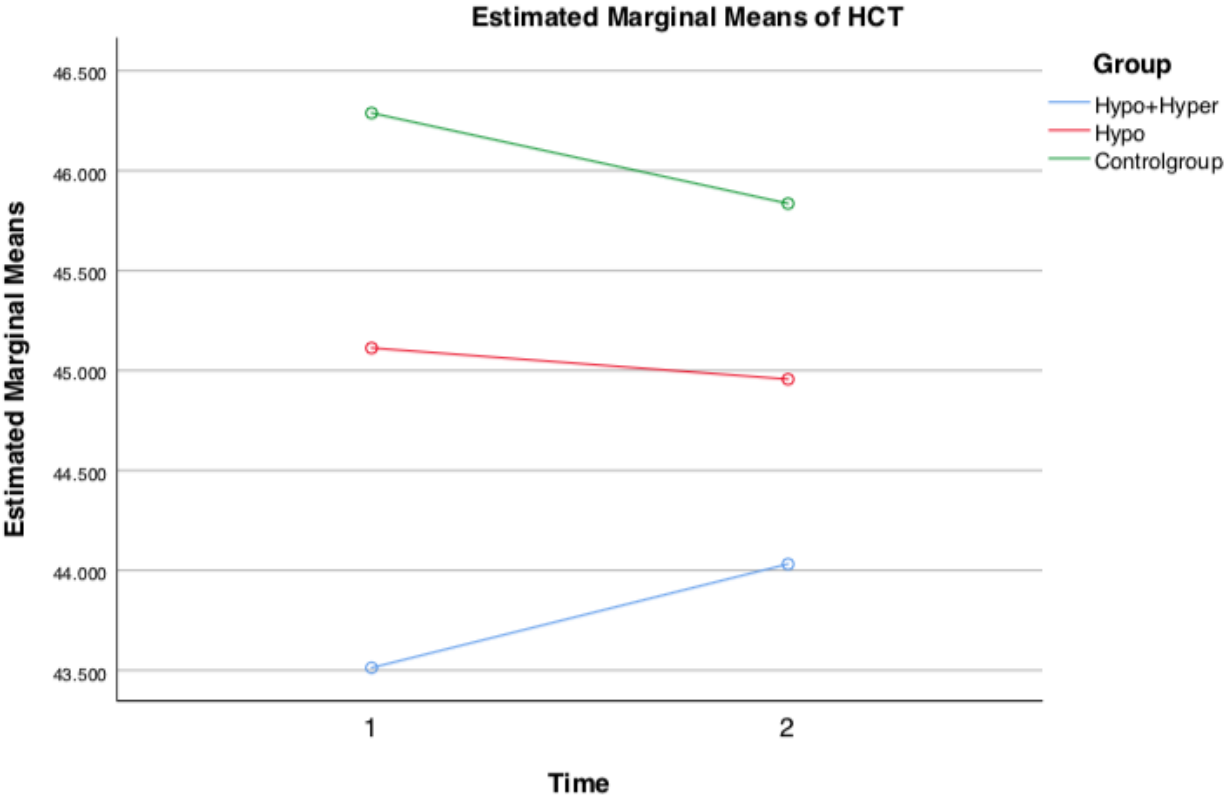


Figure 10
Estimated Marginal Means of HCT before-1 and after-2 intervention

Figure 12 shows an increase of ERT among all three groups, the most apparent remains the control group, followed by the Hypo+Hyper group, then the Hypo group.

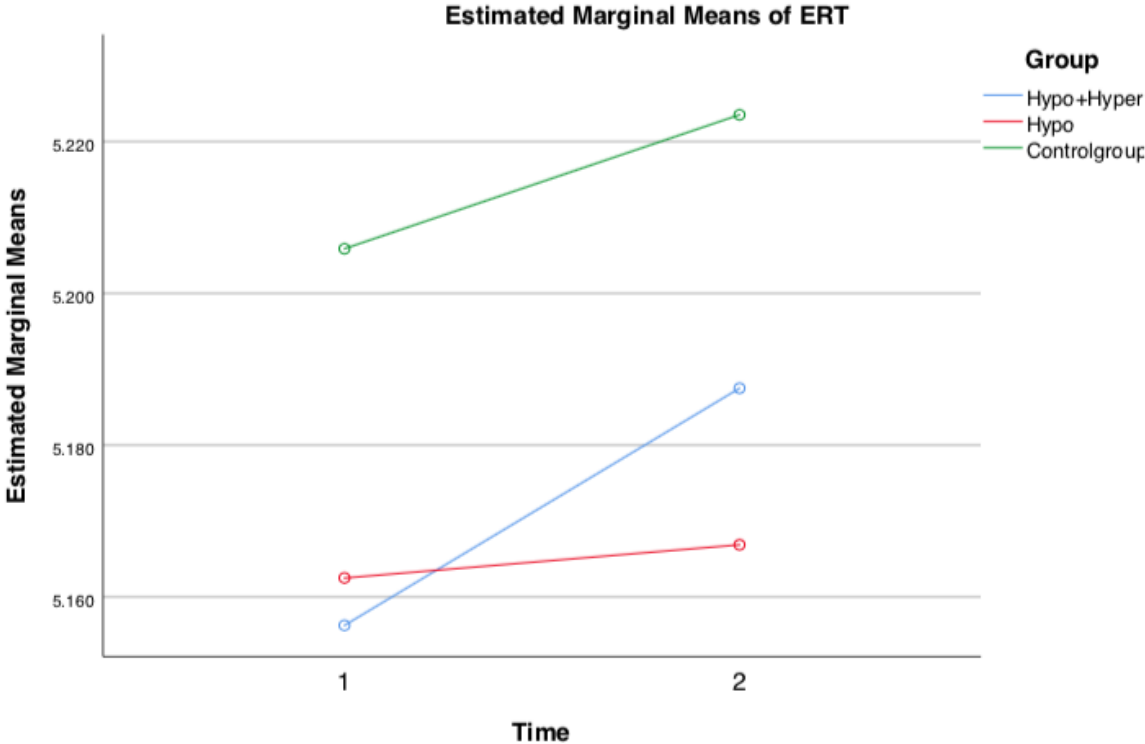


Figure 11
Estimated Marginal Means of ERT before-1 and after-2 intervention

None of the changes in Figure 11 were significant.

Figure 12 reveals an increase of MCV in the control group and a slight decrease of MCV in both, the Hypo+Hyper group and the Hypo group.

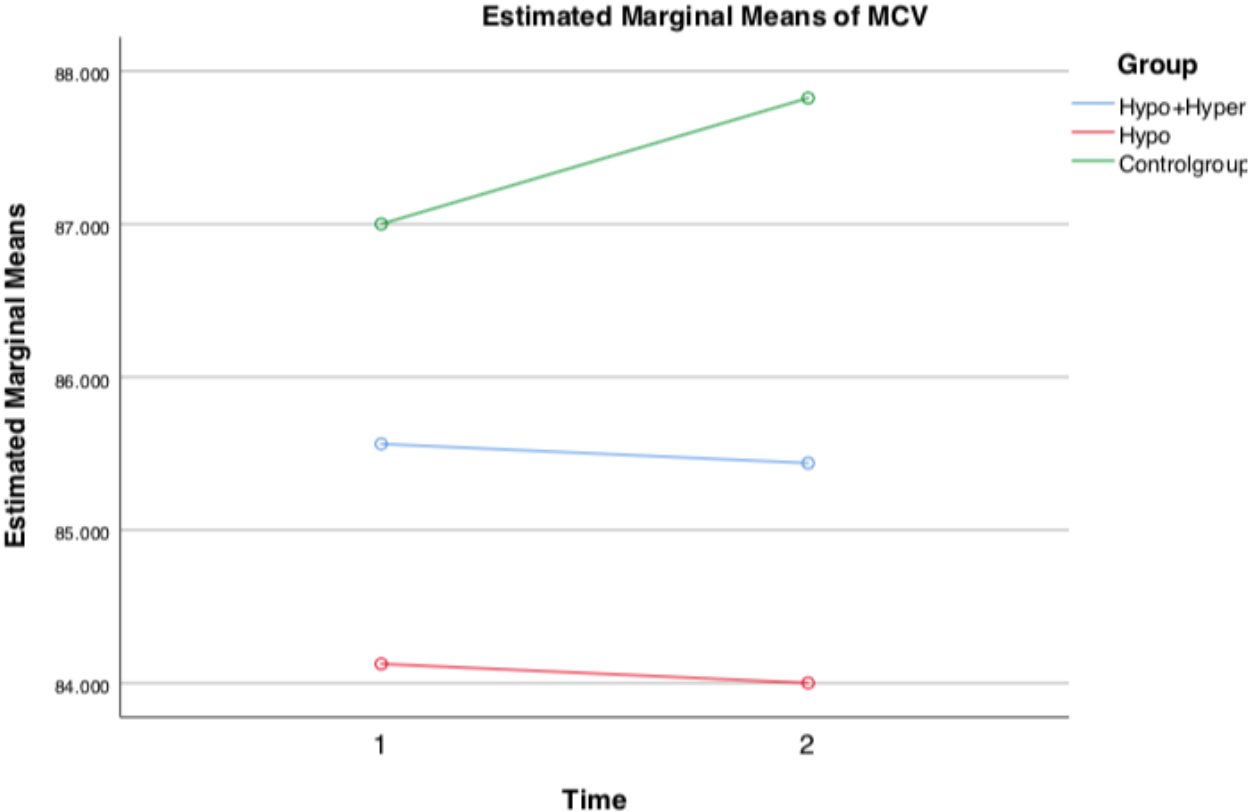


Figure 12
Estimated Marginal Means of MCV before-1 and after-2 intervention

Even though it seems that there is an increase in MCV that increase is not significant in Control group.

Figure 13 shows an apparent increase of MCHC in the Hypo+Hyper group as well as the Hypo group. However, a decrease of MCHC in the control group is evident.

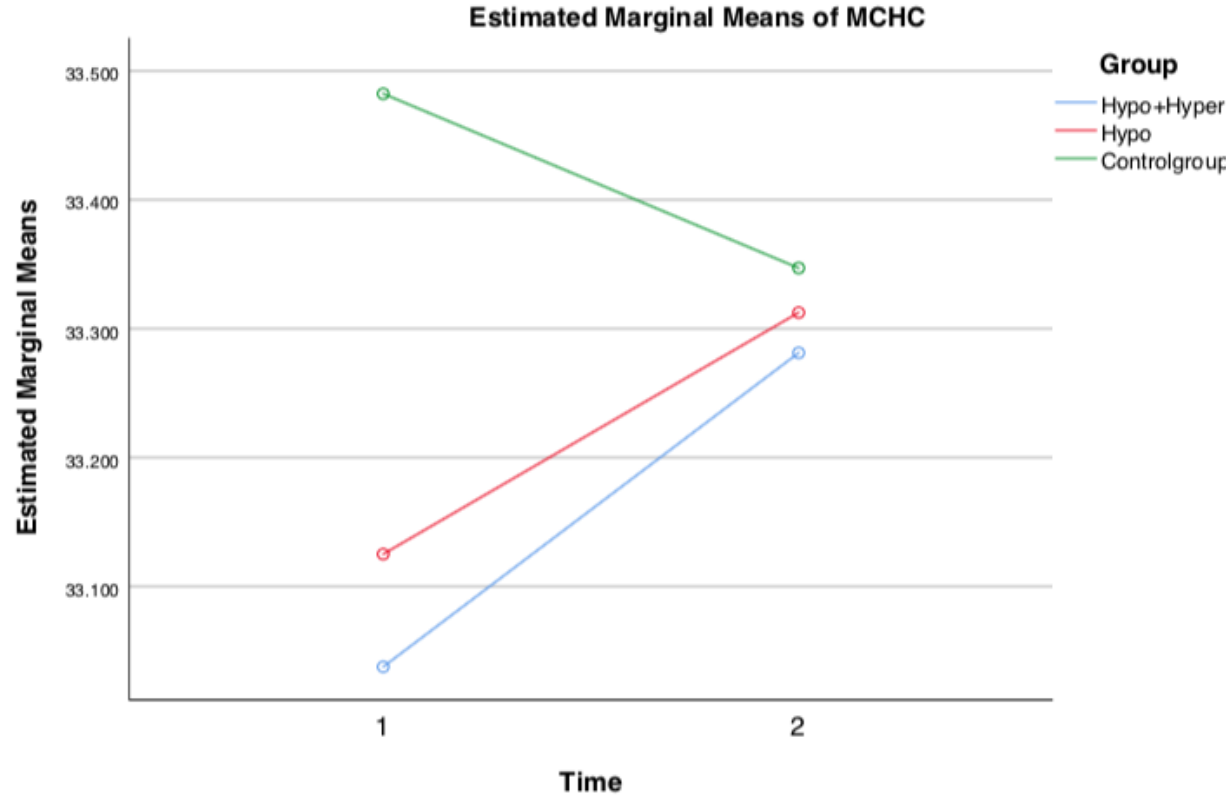


Figure 13
Estimated Marginal Means of MCHC before-1 and after-2 intervention

Similarly to Figure 13, Figure 14 shows an increase of MCH in the Hypo+Hyper group as well as the Hypo group but a decrease of MCH in the control group.

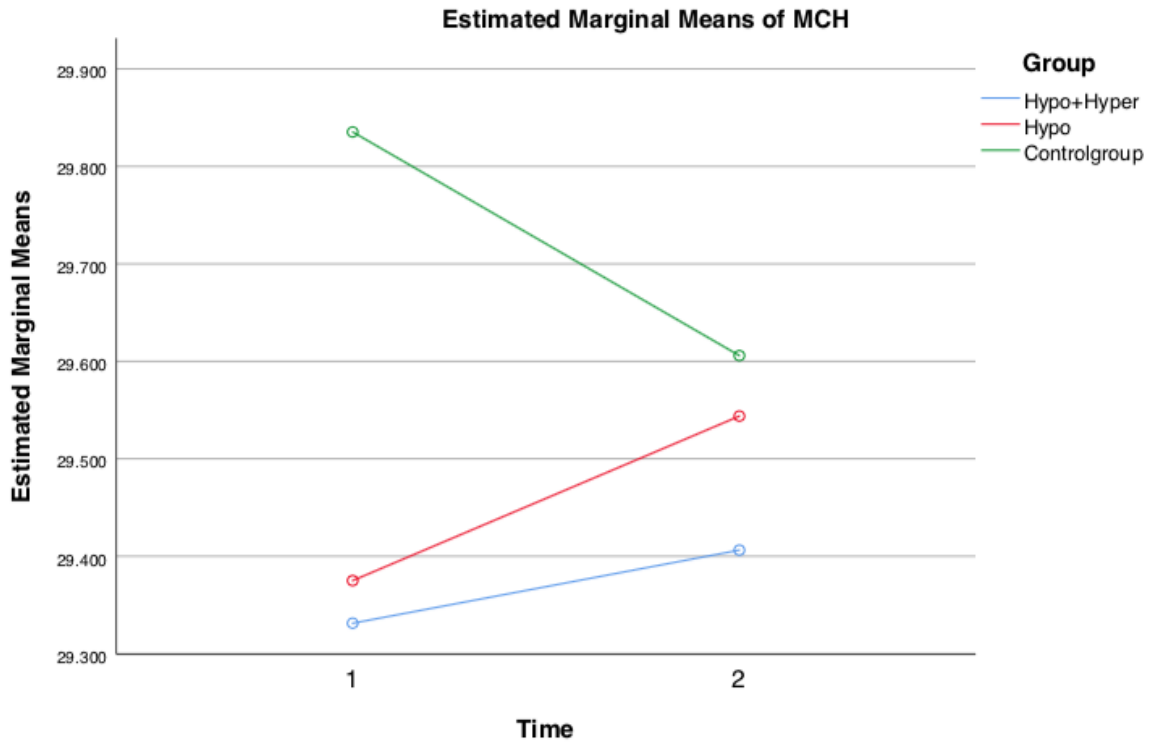


Figure 14
Estimated Marginal Means of MCH before-1 and after-2 intervention

Figure 15 shows a sharp decrease of reticulocyte in the control group, followed by a decrease in the Hypo group, but an increase in the Hypo+Hyper group.

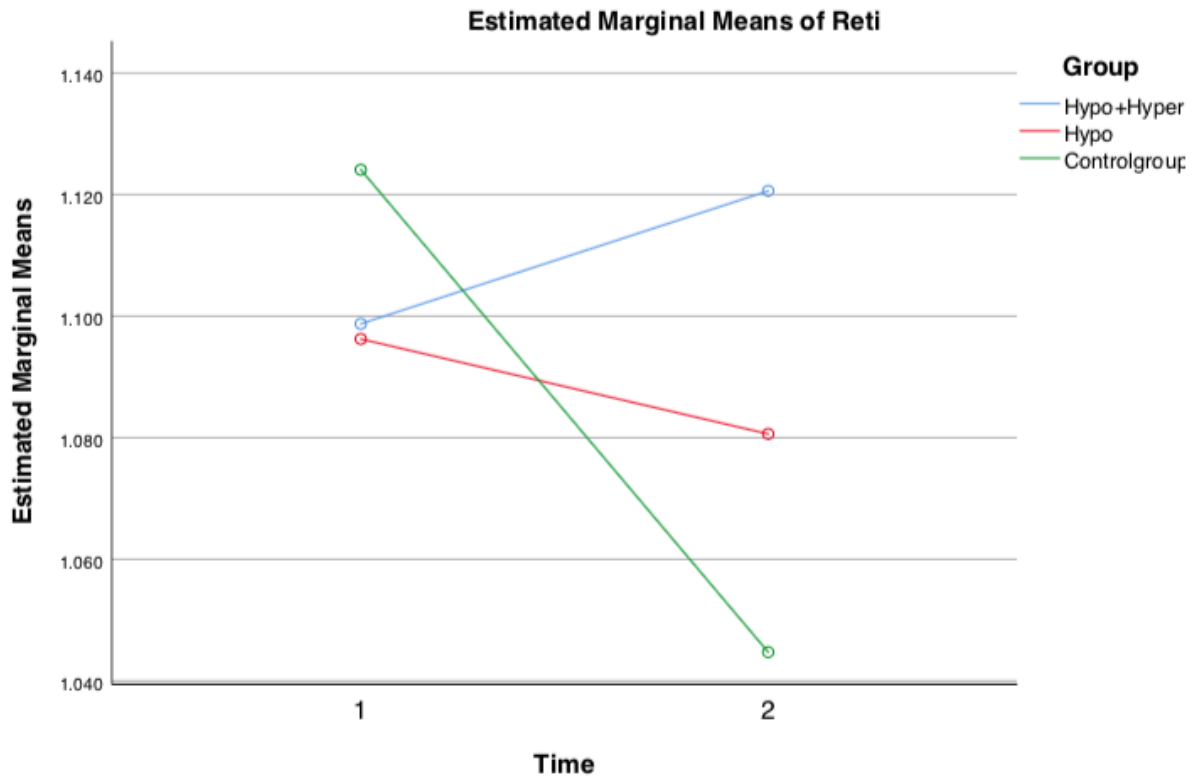


Figure 15
Estimated Marginal Means of Reticulocyte before-1 and after-2 intervention

As such, the results show minor changes in the variables related to the hematological parameters. Through a pairwise comparison, the table 9 below presents data about each of hematological parameters before and after the intervention, comparing the three different groups.

Table 9. Pairwise comparison of hematological parameters pre- and post-intervention

Measure	Time	Group I	Group J	Mean Difference (I-J)	Std. Error	P value	Lower Bound	Upper Bound
Hb (g/dl)	Pre-Intervention	Hypo+Hyper	Hypo	0.53	0.28	0.203	1.24	0.17
			Control	0.48	0.28	0.275	1.18	0.21
		Hypo	Hypo+Hyper	0.53	0.28	0.203	0.17	1.24
			Control	0.05	0.28	1.000	0.65	0.74
		Control	Hypo+Hyper	0.48	0.28	0.275	0.21	1.18
			Hypo	0.05	0.28	1.000	0.74	0.65
	Post-intervention	Hypo+Hyper	Hypo	0.55	0.26	0.127	0.11	1.21
			Control	0.77	0.26	0.014*	0.13	1.42
		Hypo	Hypo+Hyper	0.55	0.26	0.127	1.21	0.11
			Control	0.22	0.26	1.000	0.43	0.87
		Control	Hypo+Hyper	0.77	0.26	0.014*	1.42	0.13
			Hypo	0.22	0.26	1.000	0.87	0.43
HCT (%)	Pre-Intervention	Hypo+Hyper	Hypo	1.60	0.82	0.171	3.64	0.44
			Control	2.78	0.81	0.004*	4.78	0.77
		Hypo	Hypo+Hyper	1.600	0.82	0.171	0.44	3.64
			Control	1.18	0.81	0.457	3.18	0.83
		Control	Hypo+Hyper	2.78	0.81	0.004*	0.77	4.78
			Hypo	1.18	0.81	0.457	0.83	3.18
	Post-intervention	Hypo+Hyper	Hypo	0.93	0.91	0.946	3.12	1.34
			Control	1.80	0.99	0.151	4.03	0.43
		Hypo	Hypo+Hyper	0.93	0.91	0.946	1.34	3.19
			Control	0.88	0.90	0.998	3.11	1.35
		Control	Hypo+Hyper	1.80	0.90	0.151	0.43	4.03
			Hypo	0.88	0.90	0.998	1.35	3.11
Erythrocyte (cells/mcl)	Pre-Intervention	Hypo+Hyper	Hypo	0.01	0.09	1.000	0.24	0.23
			Control	0.05	0.09	1.000	0.28	0.18

MCV (fl)	Hypo	Hypo+Hyper	0.01	0.09	1.000	0.23	0.24	
		Control	0.04	0.09	1.000	0.27	0.19	
	Control	Hypo+Hyper	0.05	0.09	1.000	0.18	0.28	
		Hypo	0.04	0.09	1.000	0.19	0.27	
	Post-intervention	Hypo+Hyper	Hypo	0.02	0.09	1.000	0.20	0.24
		Control	0.04	0.09	1.000	0.25	0.18	
	Hypo	Hypo+Hyper	0.02	0.09	1.00	0.24	0.20	
		Control	0.06	0.09	1.000	0.28	0.16	
	Control	Hypo+Hyper	0.04	0.09	1.000	0.18	0.25	
		Hypo	0.06	0.09	1.000	0.16	0.28	
	Pre-Intervention	Hypo+Hyper	Hypo	1.44	1.93	1.000	3.36	6.23
		Control	1.44	1.90	1.000	6.16	3.29	
	Hypo	Hypo+Hyper	1.44	1.93	1.000	6.23	3.36	
		Control	2.86	1.90	0.412	7.60	1.85	
	Control	Hypo+Hyper	1.44	1.90	1.000	3.29	6.16	
		Hypo	2.86	1.90	0.412	1.85	7.60	
	Post-intervention	Hypo+Hyper	Hypo	1.44	1.92	1.000	3.34	6.21
		Control	2.39	1.89	0.641	7.09	2.32	
	Hypo	Hypo+Hyper	1.44	1.92	1.000	6.21	3.34	
		Control	3.82	1.89	0.148	8.53	0.88	
	Control	Hypo+Hyper	2.39	1.89	0.641	2.32	7.09	
		Hypo	3.82	1.89	0.148	0.88	8.53	
	Pre-Intervention	Hypo+Hyper	Hypo	0.09	0.3	1.000	0.73	0.55
		Control	0.45	0.3	0.261	1.08	0.19	
Hypo	Hypo+Hyper	0.09	0.26	1.000	0.55	0.73		
	Control	0.36	0.25	0.501	0.19	0.28		
Control	Hypo+Hyper	0.45	0.26	0.261	0.19	1.08		
	Hypo	0.36	0.25	0.501	0.28	0.99		
Post-intervention	Hypo+Hyper	Hypo	0.03	0.25	1.000	0.64	0.54	
	Control	0.7	0.24	1.000	0.67	0.54		
Hypo	Hypo+Hyper	0.03	0.25	1.000	0.58	0.64		
	Control	0.04	0.24	1.000	0.64	0.57		

MCH (pg)	Control	Hypo+Hyper	0.07	0.24	1.000	0.54	0.67	
		Hypo	0.03	0.24	1.000	0.57	0.64	
	Hypo+Hyper	Hypo	0.04	0.36	1.000	0.94	0.85	
		Control	0.50	0.35	0.482	1.38	0.37	
	Pre-Intervention	Hypo	Hypo+Hyper	0.04	0.36	1.000	0.85	0.94
			Control	0.46	0.35	0.598	1.34	0.42
	Control	Hypo+Hyper	0.50	0.35	0.482	0.37	1.38	
		Hypo	0.46	0.35	0.598	0.42	1.34	
	Hypo+Hyper	Hypo	Hypo	0.14	0.39	1.000	1.11	0.84
			Control	0.20	0.39	1.000	1.12	0.76
	Post-intervention	Hypo	Hypo+Hyper	0.14	0.39	1.000	0.84	1.11
			Control	0.06	0.39	1.000	1.02	0.90
	Control	Hypo+Hyper	0.20	0.39	1.000	0.76	1.16	
		Hypo	0.06	0.39	1.000	0.90	1.02	
Reticulocyte (%)	Hypo+Hyper	Hypo	0.00	0.09	1.000	0.22	0.22	
		Control	0.02	0.09	1.000	0.24	0.19	
	Pre-Intervention	Hypo	Hypo+Hyper	0.00	0.09	1.000	0.22	0.22
			Control	0.03	0.09	1.000	0.25	0.19
	Control	Hypo+Hyper	0.03	0.09	1.000	0.19	0.24	
		Hypo	0.03	0.09	1.000	0.19	0.25	
	Hypo+Hyper	Hypo	Hypo	0.04	0.7	1.000	0.14	0.22
			Control	0.08	0.07	0.913	0.11	0.26
	Post-intervention	Hypo	Hypo+Hyper	0.04	0.07	1.000	0.22	0.14
			Control	0.04	0.07	1.000	0.15	0.22
Control	Hypo+Hyper	0.08	0.07	0.913	0.26	0.11		
	Hypo	0.04	0.07	1.000	0.22	0.15		

*Significant at 0.05

Results in table 9 regarding the hematological parameters before and after the intervention are of significance when it comes to two parameters, Hb and HCT (unfortunately in HCT parameter the difference was already present pre-intervention so it will not be discussed later) when Hypo+Hyper group is compared to controls. Accordingly, it is important to note that the difference

for Hb, the Hypo+Hyper group as opposed to the control group has a significance level of 0.01 post the intervention.

In the following chapter the results will be discussed and the conclusions drawn on the body of existing literature to corroborate, support, and/or refute the findings. Chapter Six also explains whether the posed hypotheses have been accepted or rejected and under which conditions.

CHAPTER SIX

6. DISCUSSION

Chapter Six discusses the most prominent findings of this doctoral dissertation as well as rejects/accepts the posed hypotheses. As aforementioned, this study aimed at determining the impact of two distinct environments on the athlete's training program. These environments incorporate hypoxia during high-intensity interval training and hyperoxia during recovery. The dissertation also aims at determining the effects of these environments on blood oxygen transport parameters on athlete's performance measured by aerobic capacity. So, this discussion section will address three distinctive parts, each addressing one of the intervention protocols. The training of one of the groups was only the HIIT training in hypoxia which also yielded some interesting results so that will be discussed firstly:

6.1.1 Only hypoxia effects

Even though according to Sinex and Chapman (2015), "Altitude training, both in natural/terrestrial and artificial conditions, has been established as an effective means to improve oxygen transport, RBC volume, and VO₂max, given sufficiently high "doses of elevation and exposure duration" (p. 330). So, we can say that the hypoxic training seems to be a proven method to promote endurance among athletes and is recommended by sport experts and coaches due to its potential benefits. Several studies have previously corroborated the benefits of training in only hypoxia.

Unfortunately, in this study we did not confirm the above-mentioned effects, as the Hypo only group gained some benefits in aerobic endurance but those were not significant so will not be discussed. The reason for that may be that the experiment was too short to obtain the significant VO₂max gains in only Hypo group. Nevertheless, that just stresses the importance of adding hyperoxia recovery if the training period is not adequately long. Similarly, the findings of Hahn

and Core (2001) suggested that it is improbable for the adaptation to hypoxia to improve sea level VO_{2max} to a large extent.

Most of the experiments which confirmed the positive effects of hypoxia had longer exposure to hypoxia stimuli and training than our Hypo subjects. For instance, Feriche et al. (2017) concluded that hypoxia increases the accumulation of metabolite, which, in turn, promotes strength and muscle gains and Hendriksen and Meeuwsen (2003) revealed that the “maximal power output, the anaerobic mean power and the anaerobic peak power” significantly increase in hypoxic training, proving “that intermittent hypobaric training can improve the anaerobic energy supplying system and the aerobic system” (Hendriksen & Meeuwsen, 2003). Hamlin et al.’s (2017) results indicated an improvement in high-intensity intermittent running performance post-hypoxic intervention. Hamlin et al. (2017) also establish that hypoxic intervention improves high-intensity running performance in team-sport athletes, encouraging coaches to use hypoxic training methods to improve athletic performance. So, in this study the above mentioned adaptations might have happened in case the stimuli were longer than 4 weeks.

Hypoxia training should have had improved oxygen repletion, and aerobic performance as repeated exercise in hypoxia enhances the formation of cell mitochondria and myoglobin content according to Hoppeler et al. (2013). An increase in mitochondrial and capillary densities as well as the positive effects on VO_{2max} on maximal power output and on lean body mass were achieved through 30 hypoxic training sessions in that study but the volume of the sessions was almost twice than the volume in our study which may have contributed to their significant results.

Similarly, to no improvement in aerobic endurance parameters in only Hypo group, this experiment did not cause significant increase in hemoglobin or hematocrit values when only hypoxia was applied. It was also not surprising, as this was previously corroborated by Ryan et al. (2014), whose findings revealed that after one day of exposure to hypoxia, Hb_{mass} at high altitude remained the same when compared to sea level. After seven days of exposure to hypoxia, Hb_{mass} increased only by 3.765% at 5260 meters when compared to sea level. After 16 days of exposure to hypoxia, Hb_{mass} increased by 7.666% at 5260 meters when compared to sea level. So, these research findings further justify the possibility of attaining minimal or insignificant changes in Hb_{mass} in hypoxia but

with higher impact on aerobic capacity. Similarly, Saunder et al. (2013) conclude that altitude training increases VO_{2max} of more than half the magnitude of the increase in Hb_{mass} , recommending hypoxic training for endurance athletes. The results of this dissertation also reach similar conclusions as hemoglobin change was insignificant in the Hypo group.

The hypobaric hypoxia, like going to the high altitudes might have yielded different results than these obtained in normobaric hypoxia training. According to Hahn and Core (2001), if a red cell volume and hemoglobin mass were elevated, enhancement in aerobic endurance might be possible. As such, based on Hahn and Core (2001), from a physiological/hematological perspective, the increase of Hb as well as HCT indicate a potential enhancement of overall performance. The group of Mclean et al. (2013) conducted a study on two pre-season camps at an altitude of 2100 meters, one for 19 days and the other for 18 days. Results showed an increase of 4% in Hb_{mass} . but the main difference from our study was the type of hypoxia.

Moreover, natural altitude hypoxia protocols yielded positive performance results among sub-elite as well as elite athletes when adopting a LHTL protocol. Despite these findings, Bonettina and Hopkins (2009) accentuate that various studies did not demonstrate an improved endurance exercise performance as a result of only hypoxic exposure and training; nonetheless, when protocols are used appropriately, improvement in sea-level endurance exercises is noted. Consequently, Sinex and Champan (2015) speculate, “Balancing the positive adaptations that result from training in hypoxia while minimizing effects that can lead to detraining or maladaptation is key to obtaining the greatest benefit from hypoxic training” (p. 326). Additionally, Bonetti and Hopkins (2009) have proved that artificial altitude protocols along with continuous or intermittent exposure while training low have improved endurance among sub-athletes.

The benefits of the intermittent training as proposed in this study is that it prevents the negative effects of long-term hypoxia stays like the influence on body mass. It is well-known that during their sojourns to high altitude, individuals lose fat-free mass. The factors that contribute to this decrement are “hypoxia-induced anorexia, an elevated metabolic rate, and increased physical activity levels, which lead to a negative energy balance.”

Some evidence suggests that “muscle protein synthesis is resistant to external anabolic stimuli” during severe hypoxia exposure. The basic conditions do not contribute to the impact that severe exposure has on muscle protein synthesis. However, acclimatization causes the myofibrillar protein turnover to double and leads to “a net catabolic state” if the energy balance is not achieved. The upregulation in intramuscular proteolysis and dysregulation of anabolic signaling can mediate the catabolic response to hypoxia (Pasiakos et al., 2017). When performing only training in hypoxia without the long term stay and without the real acclimatization in hypoxia some of those effects can be avoided.

In our study the training that subjects performed was an endurance type training. In case of the strength training the results would probably be different. The resistance training under hypoxic condition yields also potential benefits, including muscular strength, hypertrophy, and muscle power. Hence, the field promises innovative methods that foster strength and ensure muscle gains. The increase in the accumulation of metabolite, as a result of hypoxia, is the main mechanism leading to strength and muscle gains (Feriche et al., 2017). Nonetheless, despite the prominent research, the impact of altitude conditions on muscle power by resistance training is yet to be investigated. Future studies need to take several factors, such as nutrition, hydration, and the training load throughout terrestrial altitudes, into consideration as they examine trained athletes’ performances and before they propose novel methods for hypertrophy (Feriche et al., 2017).

The hypoxic method incorporates training at high altitudes in training camps or in a simulated hypoxic environment. However, hypoxia is not always well tolerated by all participants and may have its own risks. It has other apparent short and long-term effects, such shortness of breath, headache, dizziness, and chest pain, among others. In most tissues of the body, the response to hypoxia is vasodilation while, in the central nervous system, multiple oxygen sensors are deployed during acute hypoxia allowing neurons to adapt to the response. The long-term effect could be death immediately after the deprivation or due to the side effect of hypoxia, such as a stroke or other cardiovascular episodes.

Recent techniques and methods are pushing coaches to experiment hypoxic effects on various types of trainings, but further practical studies are needed. With time, hypoxic training developed

into multiple protocols, which include the Live High-Train High (LHTH), Live High-Train Low (LHTL), Intermittent Hypoxic Exposure (IHE), Intermittent Hypoxic Training (IHT), and Repeated Spring Training in Hypoxia (RSH). Research has also revealed that an ideal altitude at which hypoxic training is conducted is between 2200 meters and 2500 meters, exposure to hypoxic altitudes should last four weeks for twelve hours a day, and results can be noted as of 18 days post-exposure.

6.1.2. Hypoxia-Hyperoxia effects

The hyperoxic method, opposite to the hypoxic one, involves training with an exposure to air that contains a high oxygen percentage. New studies, including present study, present a value to the body of knowledge and would be considered as one step further in establishing a consensus about hypoxic training and about the optimal levels and circumstances in which hypoxic training would yield desirable outcomes that can be used to the benefit of the athletes' physiology and performance.

The most evident changes that were observed in our study happened within the treadmill and aerobic endurance parameters and are pertaining to the hyper-hypo group as the significant increase in VO₂max was noted when Hypo-Hyper group was compared to the controls ($p < 0.05$). The change in maximal speed reached was also significantly larger in Hyper-Hypo group as opposed to both Hypo ($p < 0.01$), as well as opposed to control group ($p < 0.001$).

Actually, the main improvement we hoped to obtain in Hyper -Hypo group was that improvement in aerobic capacity as that is the factor that could contribute to performance in real everyday conditions and during sport events. Also, the VO₂max is a good predictor of cardiovascular risk, which is a widely known fact, so this this type of training may also be useful and important for general population, not only athletes.

Even though the significant VO₂max increase was observed in Hypo-Hyper group it cannot be concluded with certainty which mechanism underly that change as the adaptations might have been achieved at the cellular level. Was it the increase in mitochondrial density as well as

mitochondrial enzyme activity; increase of oxygen delivery at transport level meaning the heart adaptations or/and red blood cells adaptations; buffering capacity and the least probable changes at respiratory membrane levels e.g. changes in alveolar diffusion. Out of those possible adaptations the parameters that were under monitoring in this study were red blood cell related parameters.

As the aerobic adaptations occur also on mitochondrial levels the role of citrate synthase might be mentioned regarding the obtained results in hyperoxia group. This enzyme is usually considered to be a marker of good functioning mitochondria and has its role in aerobic energy pathways. The mechanisms that have caused that adaptation might be related to the mechanisms explained in the study of Perry, Talanian, Heigenhauser, and Spriet (2007) who examined whether hyperoxia “improves skeletal muscle oxidative capacity, maximal oxygen consumption, and endurance performance than training in normoxic conditions” on nine recreationally active individuals, randomly assigned to train for six weeks in hyperoxic conditions, 60% oxygen, or normoxic conditions. The training program consisted of three sessions per week each with 10x4 minutes at 90% VO_2 max. After at least six weeks of detraining, the experiment was repeated for six weeks with the other breathing condition. The criteria to be tested were: Training power outputs, VO_2 max, time to exhaustion, citrate Synthase, β – HAD (hydroxyacyl-coenzyme), a dehydrogenase, and mitochondrial aspartate aminotransferase.

The enhancement in power outputs for hyperoxic training (8% higher than normoxia) was proven in Perry et al. study (2007) while both hyperoxia and normoxia produced similar improvements in maximal oxygen consumption (11-12%) and the time to exhaustion was similar as well in both conditions. The citrate synthase was 30% higher for hyperoxia and 32% for normoxia group. The β -HAD was 23% for hyperoxia and 21% for normoxia. Finally, the m-AsAT was 21% for hyperoxia while it was 26% for normoxia. Even though the enzyme activity changed the hyperoxic training did not produce significant enhancements in the aerobic capacity of skeletal muscles, VO_2 max, and exercise performance.

The supply of oxygen to muscle tissues contributes to the control of the VO_2 for high intensity exercises for steps above ventilatory threshold (MacDonald et al. 1997). Interestingly a few studies had accentuated the possible benefits of recovering in hyperoxia and some authors

suggest the “catch” might be in the recovery processes. For example, Pupis et al. (2013) proposed that hyperoxic air accelerates recovery time after a karate or judo match. Also, according to Yokoi et al. (2014), normobaric hyperoxic treatments serve a better recovery for muscles than normal conditions (Yokoi et al., 2014). Kay et al.’s (2008) study shows that a 100% oxygen supply for a 4-minute recovery period after 30-second maximal cycling exercise improves absolute power output.

So for now we mostly speculate that hyperoxic recovery seems efficient when it comes to exercise performance and according to Mallette et al. (2017), but also according to our results, hyperoxic recovery is still an underdeveloped topic in the literature, and further studies are needed to accurately determine its efficacy and its mechanism. They noted that “due to the large variability in the time between subsequent bouts of exercise, more research needs to be conducted to determine the optimal length of time of hyperoxic gas delivery” (p.2).

Control group effects

As expected there were no significant changes observed in a control group. Even though in Figure 16 it seems that the reticulocyte count dropped a lot the actual change is small and is not significant. The seen effect is mostly visible because of the small-scale steps of the y axis.

At the end we can say that sport experts and coaches continuously examine ways to improve athletes’ performance through legal means and away from potential substance abuse. Moreover, the pressure exerted on athletes to perform to the best of their abilities requires implementing training strategies that do not pose physiological and/or psychological implications on the long run. Some of the suggested strategies that allow athletes to better perform and achieve desirable results have been investigated in the literature, chief among these strategies are hypoxic training and hypertoxic training and, sometimes, a combination of both. The previous studies had examined hypoxia and hyperoxia separately. However, some scientists have tried to mix the two techniques to achieve optimal results. The effects of Intermittent Hypoxia-Hyperoxia Training (IHHT) had been the focus of a smaller number of studies. Hence, this doctoral dissertation aimed

at determining the impact of hypoxia during high-intensity interval training and hyperoxia during recovery on the training program and the effects of these environments on blood oxygen transport parameters on performance and aerobic capacity and its unique design is the most prominent specificity of this study.

CHAPTER SEVEN

7. CONCLUSION

7.1 General Conclusion

To the best of the author's knowledge, this study is unique in its design. It assesses the effects of combining hypoxia, hyperoxia, and HIIT with regards to blood oxygen transport and aerobic performance in healthy individuals, providing much-needed information sport experts, coaches, and athletes on devised training programs and their efficiency. It also contributes to the body of knowledge about hypoxia and hyperoxia training and recovery. The study posed two hypotheses:

Hypothesis 1: A combination of intermittent high intensity hypoxic training and recovery in hyperoxia significantly improves the aerobic capacity than when recovering in normoxia.

Hypothesis 2: A combination of intermittent high intensity hypoxic training and recovery in hyperoxia significantly improves the oxygen transport parameters than when recovering in normoxia.

So at the end we can say that the proposed hypotheses which speculated that the best effects might be obtained in Hypoxia+Hyperoxia group should be accepted partially, and under the following circumstances:

- Hypothesis 1 is accepted when it comes to the treadmill parameters pertaining to VO_{2MAX} , Maximal Speed, and VO_{2ANT}
- Hypothesis 2 is accepted when it comes to the hematological parameters pertaining to hemoglobin

All other proposed variables, including HR_{max} , VO_2 at ANT, speed at ANT, and HR at ANT as well as Erythrocyte, MCV, MCHC, MCH, and Reticulocyte have yielded no significant results.

Positive results are shown as it pertains to the Hypo+Hyper group. At maximal exertion, for the Hypo+Hyper group, VO_{2MAX} and maximal speed are enhanced. At anaerobic threshold, for the Hypo+Hyper group, VO_{2ANT} is enhanced. As for hematological parameters, the significant results pertain to two parameters, Hb and HCT, within the Hypo+Hyper group as opposed to the control group at 0.01 significance.

Based on the results, the most optimal training method, among the three proposed (HIIT, HIIT+O₂, and CTR) is that of high intensity interval training group in hypoxia with hyperoxia recovery (HIIT+O₂). With the improvement of parameters pertaining to aerobic capacity and oxygen transport, the above results reveal that the implemented Hyper-Hypo protocol has yielded positive results. Training in the high intensity interval protocol in hypoxia was beneficial but only with the added hyperoxia recovery. The hyper-hypo group, training in hypoxia and recovering in hyperoxia, has witnessed the most significant increase among VO_{2MAX} , Maximal Speed, and VO_{2ANT} . A combination of intermittent high intensity hypoxic training and recovery in hyperoxia significantly improves the aerobic capacity and some of the the oxygen transport parameters than when recovering in normoxia and therefore athletes would benefit the most from that type of training design, especially if their training period in limited to few weeks.

7.2 Limitations

The limitations to this study include the lack of oxygen saturation data during the experiment. Also, maybe some of the other parameters would turn to be significant on a larger sample and in different sample. As such, findings may not be generalized to a low fitness population, may not be applicable to females given the physiological distinctions between males and females, and may not be applicable to those of an older age group.

7.3. Scientific Contribution

As opposed to the previous conducted studies which mostly studied either HIIT or training in hypoxia or hyperoxia separately, this research tried to answer the question whether the combination of hypoxic high-intensity interval training followed by hyperoxic recovery exposure provides additional positive effects of hyperoxia on aerobic endurance and on the oxygen transport

system. The study contributes to the knowledge about the effects of combining hypoxia, hyperoxia, and HIIT with regards to blood oxygen transport and aerobic performance in healthy individuals even though most of these effects were studied previously but separately. Therefore, this study, given that it yields positive results, is a step forward in the training process of many athletes, especially in endurance disciplines.

7.4 Suggestions for Further Studies

To begin with, given that this study is one of a kind with exactly this design, further research should be conducted to corroborate the findings yielded. Moreover, this doctoral dissertation only measures blood oxygen transport parameters on athlete's performance and aerobic capacity. Further studies might work on measuring other physiological parameters like direct oxygen saturation and on incorporating diversified samples (different sport background, different age or sex) in order to ensure optimal strategies that enhance performance among athletes.

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