THE USE OF NORMOBARIC HYPOXIA AND HYPEROXIA FOR THE ENHANCEMENT OF SEA LEVEL AND/OR ALTITUDE EXERCISE PERFORMANCE

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Doctoral Dissertation
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MEDNARODNA PODIPLOMSKA ŠOLA JOŽEFA STEFANA
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Doctoral Dissertation

UPORABA NORMOBARIČNE HIPOKSIJE IN HIPEROKSIJE ZA IZBOLJŠANJE ŠPORTNE SPOSOBNOSTI NA NIŽINI IN/ALI VIŠINI

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To my three shining stars and Minca, who make my life beautiful.

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Abstract

Adaptation to altitude can enhance performance if the hypoxic dose and the frequency of the exposures are appropriate. While many hypoxic training modalities exist, the protocols utilizing short intermittent hypoxic exposures are gaining popularity. However, the effects of such novel hypoxic protocols on athletic performance are unclear. The aim of this thesis was to investigate the effects of selected hypoxic modalities on performance at simulated altitude and sea level. We undertook three studies that investigated the effect of hypoxic exposure during exercise (***Study I***) and rest (***Study II*** & ***III***) on performance and select hematological and ventilatory responses. Since erythropoietic stimulation is one of the main objectives of hypoxic manipulations the last study tested a novel protocol inducing higher relative changes in O_2 partial pressure by utilizing successive breathing of hyperoxic and hypoxic gas mixture. Given that glycoprotein hormone erythropoietin (EPO) is the chief regulator of endogenous red blood cells production, EPO production was the focal point of ***Study IV***.

Although training in hypoxia has been suggested to improve sea level and altitude performance, most studies have only evaluated its effect on maximal aerobic capacity in either normoxia or hypoxia. In ***Study I*** we evaluated the effect of the intermittent hypoxic training modality (IHT) on both normoxic and hypoxic endurance performance and aerobic capacity. Eighteen healthy male subjects underwent 20 training sessions in either a normoxic (F_IO_2 = 0.209) or hypoxic (F_IO_2 = 0.120) environment. Both the control and IHT group subjects trained at an intensity corresponding to 50% of peak power output attained in normoxia or hypoxia, respectively. Before, during, upon completion and 10 days after the training period, the aerobic capacity ($\dot{V}O_{2peak}$) and endurance performance (80% of $\dot{V}O_{2peak}$) of the subjects were determined under normoxic and hypoxic conditions. Normoxic $\dot{V}O_{2peak}$ increased significantly only in the control group whereas hypoxic $\dot{V}O_{2peak}$ did not improve in either group. The control group exhibited significant improvements in normoxic, but not hypoxic peak power output and endurance performance, whereas the IHT group only exhibited improvements in normoxic endurance performance. The tested IHT modality used in ***Study I*** had no significant effect on altitude and sea level performance compared to the same relative intensity normoxic training.

It has also been suggested, that short intermittent exposures to hypoxia at rest can beneficially effect ventilatory responses and subsequently enhance performance. In ***Study II*** we thus investigated the effects of twenty intermittent hypoxic exposures at rest (IHE) on aerobic capacity and endurance performance in normoxic and hypoxic conditions. Eighteen healthy male subjects were equally assigned to either control or IHE group. Both groups performed a 4-week moderate intensity cycling exercise training. The IHE group additionally performed one hour of IHE prior to the exercise training sessions. The IHE consisted of seven cycles alternating hypoxic and normoxic air breathing, for 5 and

3 minutes duration respectively. During hypoxic exposures the inspired F_IO_2 varied from 0.125 to 0.095. The same testing periods as in ***Study I*** were used to evaluate $\dot{V}O_{2peak}$, and endurance performance in normoxia and hypoxia. Both groups showed similar improvements in normoxic $\dot{V}O_{2peak}$, with no changes in hypoxic condition. Both groups increased endurance performance in normoxia at the post test, whereas only the IHE group preserved this improvement also at the after testing. The IHE group showed higher levels of minute ventilation at post and after testing compared to the control. Although IHE did not improve hypoxic performance and aerobic capacity, it can be beneficial for normoxic performance. In particular, our data show that the addition of IHE to endurance training has the ability to preserve the improved performance in normoxia longer than endurance training alone. The underlying mechanism seems to be related to enhancements of ventilatory responses.

While short hypoxic modalities were shown to have the ability to stimulate ventilatory acclimatization, the minimal dose required is currently unresolved. ***Study III*** investigated the effect of only four short intermittent hypoxic exposures (SIH) on hypoxic performance, ventilatory responses and modulation of muscle and cerebral oxygenation. Nineteen healthy male subjects who participated in the single blind, placebo controlled study were randomly assigned to either the short intermittent hypoxic (SIH; n = 10) or the control (n = 9) group. Each participant underwent four sessions in a climatic chamber (4 $h \cdot day^{-1}$) under either hypoxic (F_IO_2 = 0.120) or a placebo normoxic (F_IO_2 = 0.209) conditions, respectively. Prior to and after the exposures all subjects performed a constant power test to exhaustion (CP) in normobaric hypoxia (F_IO_2 = 0.120) at a work load corresponding to 75 % of the previously determined normoxic $\dot{V}O_{2peak}$. Oxygen saturation and minute ventilation were measured continuously. NIRS was used to monitor cerebral and muscle concentration changes of oxy-hemoglobin, deoxy-hemoglobin and total hemoglobin. Neither group significantly improved the CP performance time. Despite the unchanged CP time, only the SIH group showed significant increases in both minute ventilation (+ 15 %) and oxygen saturation (+ 4 %) during the second test. The deoxy-hemoglobin in the vastus lateralis significantly increased following SIH only. No significant differences were observed between groups and testing periods in cerebral and respiratory muscle oxygenation. Compared to a placebo control, the SIH significantly altered ventilation and blood oxygen saturation during intensive hypoxic exercise. The results of this study indicate that four intermittent hypoxic exposures have the ability to induce some degree of ventilatory acclimatization, but do not improve maximal aerobic performance in hypoxia.

Study IV investigated a novel breathing protocol that could provide an indirect means for performance enhancement, through erythropoiesis stimulation**.** Recent findings suggest that besides acute and chronic tissue hypoxia, as employed by contemporary hypoxic training modalities, relative decrements in tissue oxygenation, using a transition of the breathing mixture from hyperoxic to normoxic, can also augment erythropoietin (EPO) production. This hypothesis, termed the "*Normobaric oxygen paradox*" has already been applied in clinical settings. To further clarify the importance of the relative change in tissue oxygenation on plasma EPO concentration [EPO], and its possible application for enhancing performance through subsequent oxygen flux augmentation, we

evaluated a novel protocol of successive hyperoxic and hypoxic breathing. Eighteen healthy male subjects were assigned to either IHH (n = 10) or the control (n = 8) group. The IHH group breathed pure oxygen (F_IO_2 = 1.00) for 1-hr, followed by a 1-hr period of breathing a hypoxic gas mixture (F_IO_2 = 0.150). The control group breathed air (F_IO_2 = 0.209) for the same duration (2-hrs). Blood samples were taken just before, after 60 minutes, and immediately after the 2-hr exposure period. Thereafter samples were taken at 3, 5, 8, 24, 32 and 48 hrs after the exposure. During the breathing interventions subjects remained in supine position. There were significant increases in absolute [EPO] within groups at 8 and 32-hrs in the control and at 32-hrs only in the IHH group. No significant differences in absolute [EPO] were observed between groups following the intervention. Relative [EPO] levels were significantly lower in the IHH than in the control group, 5 and 8-hrs following exposure. The tested protocol of consecutive hyperoxic-hypoxic gas mixture breathing did not induce [EPO] synthesis stimulation. Moreover the transient attenuation in relative [EPO] in the IHH group, was most likely due to a hyperoxic suppression. Hence, the IHH protocol does not seem to be a promising tool for erythropoiesis stimulation.

Collectively the findings of the second and the third study show that intermittent normobaric hypoxic applications can potentially have beneficial effects on select physiological indices, without affecting performance. Only IHT did not show any benefits, most probably attributable to the relatively low training workload. Both IHE and SIH have shown that ventilatory adaptation can be expected following 20 or only 4 exposures, respectively. Prolonged retention of the enhanced performance following IHE, 10 days following the protocol cessation, indicates the possibilities of IHE application if exercise training cannot be performed. According to the findings of the final study, the use of hyperoxia as means of increasing the relative change in tissue oxygenation cannot be recommended for enhancing EPO endogenous production. Thus, athletic and clinical applications based on the "*Normobaric oxygen paradox*" theory are currently unwarranted. In conclusion, while intermittent hypoxia at rest can potentially have beneficial effects on select physiological indices there is no transfer of these benefits that would result in performance enhancement.

Povzetek

Višinska aklimatizacija lahko izboljša športno sposobnost, če je hipoksična doza in frekvenca izpostavitev zadostna. V zadnjem času, se poleg uveljavljenih daljših protokolov, vse bolj uveljavljajo protokoli višinskega treninga, ki uporabljajo kratke prekinjajoče izpostavitve hipoksiji. Učinki tovrstnih protokolov na športno sposobnost še niso jasni in dokazani. Cilj doktorskega dela je bil ugotoviti učinkovitost izbranih kratkotrajnih hipoksičnih protokolov za izboljšanje športne sposobnosti v hipoksiji in normoksiji. V ta namen smo izvedli tri raziskave, ki so preučevale učinke kratkotrajne izpostavitve hipoksiji med vadbo (***Raziskava I***) in mirovanjem (***Raziskavi II in III***) na športno sposobnost ter izbrane hematološke in ventilatorne kazalce. Poleg direktnih učinkov na sposobnost, smo v zadnji raziskavi (***Raziskava IV***) preučevali spremembe plazemske koncentracije eritropoetina po novem protokolu, ki vključuje zaporedno dihanje hiperoksične in hipoksične mešanice. Povečana koncentracija hormona eritropoetina, ki je glavni regulator eritropoeze v človeškem telesu, lahko poveča količino rdečih krvnih celic in s tem oksiforne kapacitete krvi, ter tako potencialno izboljša prenosa kisika do mišic. To lahko posredno izboljša športno sposobnost, zato je bil odziv eritropoetina na dihalno manipulacijo glavni predmet zadnje raziskave.

Kljub dejstvu, da nekatere raziskave kažejo na uporabnost vzdržlivostne vadbe v hipoksiji, so se do sedaj raziskave osredotočile na raziskovanje učinkov le na višini ali le na nižini. V prvi raziskavi (***Raziskava I***) smo preučevali vpliv vadbe v hipoksiji na maksimalno porabo kisika in vzdržljivostno sposobnost tako v normoksiji kot hipoksiji. Osemnajst zdravih, mladih prostovoljcev je sodelovalo v raziskovalnem protokolu, ki je vključeval 20 enournih vadbenih enot razporejenih preko štirih tednov. Pol preiskovancev (kontrolna skupina) je vadbo opravilo v normoksičnih (F_IO_2 = 0.209), druga polovica (eksperimentalna skupina) pa v hipoksičnih pogojih (F_IO_2 = 0.120). Individualna vadbena obremenitev je predstavljala 50 % maksimalne obremenitve v normoksiji oz. hipoksiji. Testi maksimalne poraba kisika ($\dot{V}O_{2peak}$) in vzdržljivostne sposobnosti (konstantna obremenitev 80% $\dot{V}O_{2peak}$) v normoskiji in hipoksiji so bili opravljeni pred, med, takoj po zaključku in deset dni po zaključenem protokolu. Normoksična $\dot{V}O_{2peak}$ se je povečala le v kontrolni skupini, hipoksična $\dot{V}O_{2peak}$ pa se v obeh skupinah ni statistično značilno spremenila. Značilno povečanje vzdržjivostne sposobnosti v normoksiji je bilo ugotovljeno v obeh skupinah, izboljšanje maksimalne moči pa le pri kontrolni skupini. Uporabljeni protokol vadbe v hipoksičnih pogoji v primerjavi z relativno enako intenzivno vadbo v normoksiji ni učinkovito izboljšal športne sposobnosti ne v hipoksiji in ne v normoksiji.

Poleg vadbe v hipoksiji študije kažejo tudi na možno učinkovitost kratkotrajnih prekinjajočih izpostavitev hipoksiji v mirovanju, za izboljšanje športne sposobnosti kot posledice ventilatorne

adaptatacije na hipoksijo. Cilj druge raziskave (***Raziskava II***) je bil preučiti učinke dvajsetih izpostavitev prekinjajoči hipoksiji v mirovanju na aerobno kapaciteto in vzdržljivostno sposobnost v hipoksiji in normoksiji. Osemnajst zdravih mladih prostovoljcev je bilo naključno izbranih in razporejenih v kontrolno in eksperimentalno skupino. Vsi preiskovanci so opravili štiri tedenski vadbeni protokol (20 vadbenih enot) zmerne intenzivnosti na kolesu (50 % $\dot{V}O_{2peak}$). Eksperimentalna skupina je pred vsako vadbeno enoto izvedla še enourno hipoksično vadbo. Hipoksično vadbo je sestavljalo sedem ciklov, pri katerih se je izmenjevalo dihanje hipoksične mešanice (5 minut) in normoksičnega zraka (3 minute). Koncentracija kisika v vdihanem zraku je bila med hipoksičnim delom med 0.125 in 0.095. Testi maksimalne porabe kisika in vzdržljivostne sposobnosti (konstantna obremenitev 80% $\dot{V}O_{2peak}$) so bili, v normoksiji in hipoksiji, izvedeni v enakih obdobjih kot v prvi raziskavi. Obe skupini sta značilno izboljšali maksimalno porabo kisika v normoksiji. V hipoksiji ni bilo značilnih sprememb. Prav tako sta obe skupini izboljšali vzdržljivostno sposobnost po končanem protokolu, le eksperimentalna skupina pa je to izboljšavo zadržala tudi 10 dni po koncu protokola. Pri eksperimentalni skupini smo, glede na kontrolno, izmerili značilno povečano ventilacijo med naporom, takoj po in deset dni po protokolu. Rezultati kažejo, da je testirani protokol lahko učinkovit za izboljšanje oz ohranjanje športne sposobnosti v normoksiji in ne v hipoksiji. Ventilatorna adaptacija je najverjetnejši mehanizem, ki omogoči podaljšano ohranitev izboljšane sposobnosti.

Kljub dejstvu, da nekatere raziskave kažejo na učinkovitost kratkotrajnih hipoksičnih protokolov za ventilatorno aklimatizacijo, najmanjša doza potrebna za učinke še ni znana. V tretji raziskavi (***Raziskava III***) smo ugotavljali učinke štirih kratkotrajnih izpostavitev hipoksiji na sposobnost premagovanja napora v hipoksiji, ventilatorne parametre in oksigenacijo mišic ter možgan med naporom. Devetnajst mladih, zdravih prostovoljcev, ki so sodelovali v tej enosmerno slepi raziskavi, smo naključno razdelili v eksperimentalno (n = 10) in kontrolno skupino (n = 9). Vsak preiskovanec je v klimatski komori preživel štiri ure na dan, štiri dni zapored. Preiskovanci eksperimentalne skupine so bivali v hipoksiji (F_IO_2 = 0.120), preiskovanci kontrolne skupine pa v normoksiji (F_IO_2 = 0.209). Pred in po protokolu so vsi preiskovanci izvedli vzdržljivostni test v hipoksiji s stalno obremenitvijo (75 % $\dot{V}O_{2peak}$). Med testom smo vseskozi merili kapilarno saturacijo in minutno ventilacijo. Za merjenje sprememb v oksigenaciji možgan in mišic smo uporabili NIRS in stalno nadzorovali relativne spremembe oksi-hemoglobina, deoksi-hemoglobina in celokupnega hemoglobina. Nobena skupina ni značilno izboljšala vzdržljivostne sposobnosti po protokolu. Kljub temu je le eksperimentalna skupina značilno povečala ventilacijo (+ 15 %) in saturacijo (+ 4 %) med naporom. Koncentracija deoksihemoglobina je bila v mišici vastus lateralis ob koncu napora povečana le v eksperimentalni skupini. Drugih značilnih sprememb v oksigenaciji možgan ali respiratornih mišic med naporom ni bilo. Glede na kontrolno skupino je testirani hipoksični protokol značilno vplival na saturacijo in ventilacijo med naporom. Rezultati tretje raziskave kažejo da kratkotrajna štirikratna izpostavitev ponavljajoči hipoksiji lahko privede do delne ventilatorne aklimatizacije, a ne vpliva značilno na sposobnost premagovanja maksimalnega napora v hipoksiji.

Z zadnjo raziskavo doktorskega dela (***Raziskava IV***) smo ugotavljali učinke novega dihalnega protokola, ki bi, z uporabo zaporednega dihanja hiperoksične in hipoksične mešanice, lahko preko

stimulacije eritropoetina (EPO) posredno izboljšal športno sposobnost. Zadnja spoznanja kažejo, da povečano produkcijo EPO lahko poleg akutne in kronične izpostavitve hipoksiji izzovemo tudi z uporabo relativnih sprememb v oksigenaciji z uporabo menjave med dihanjem hiperoksične dihalne mešanice in zraka. Teorija na kateri sloni domneva je poimenovana "*Normobarični kisikov paradoks*" in je že uvedena tudi v klinično prakso. Cilj raziskave je bil potrditi vpliv relativnih sprememb oksigenacije na plazemsko [EPO] koncentracijo in možnost uporabe le teh kot sredstva za izboljšanje športne sposobnosti preko povečanja oksiforne kapacitete krvi. Osemnajst zdravih mladih prostovoljcev smo naključno razdelili v dve skupini: Intermitentno hiperoksično-hipoksično (IHH; n = 10) in kontrolno (n = 8) (placebo). Preiskovanci v IHH skupini so prvo uro protokola dihali čisti kisik (F_IO_2 = 1.00), drugo uro pa hipoksično mešanico (F_IO_2 = 0.150). Preiskovanci v kontrolni skupini so dve uri dihali zrak (F_IO_2 = 0.209). Krvni vzorci so bili odvzeti tik pred dihanjem, po prvih 60 minutah in po koncu dihalnega protokola. Po protokolu so bili dodatni vzorci odvzeti v sledečih urah: 3, 5, 8, 24, 32 in 48 ur po protokolu. Med dihanjem so bili preiskovanci vseskozi v ležečem položaju. V kontrolni skupini je bil [EPO] značilno povečan 8 in 32 ur po protokolu, v skupini IHH pa samo 32 ur po protokolu. Med skupinama v [EPO] ni bilo statistično značilnih razlik pri nobenem vzorčenju. Relativne spremembe [EPO] so bile značilno nižje v IHH glede na kontrolno skupino 5 in 8 ur po protokolu. Testirani protokol zaporednega dihanja hiperoksične in hipoksične mešanice ni povečal endogene produkcije eritropoetina. Glede na rezultate lahko trdimo, da je protokol v prvih osmih urah po zaključku dihanja povzročil malenkostno zmanjšano produkcijo eritropoetina, najverjetneje kot posledica hiperoksične supresije. Testirani protokol ne kaže uporabne vrednosti za stimulacijo produkcije eritropoetina.

Rezultati druge in tretje raziskave kažejo na možne pozitivne učinke prekinjajoče hipoksične vadbe na izbrane fiziološke kazalce, brez značilnega vpliva na športno sposobnost. Edino vadba v hipoksiji ni pokazala nobenih izmerjenih pozitivnih učinkov, kar najverjetneje lahko pripišemo majhni absolutni vadbeni obremenitvi. Tako 20 kot tudi samo 4 izpostavitve prekinjajoči hipoksiji, lahko povzročijo določeno stopnjo ventilatorne aklimatizacije. Podaljšano zadržanje izboljšane sposobnosti premagovanja napora v raziskavi tri kaže na možnost uporabe intermitentne hipoksije tudi kot sredstva za vzdrževanje ventilatornih prilagoditev takrat, kadar vadbe posameznik ne more izvajati. Glede na rezultate zadnje raziskave lahko trdimo, da uporaba hipoksije za povečevanje sprememb v oksigenaciji, z namenom stimulacije produkcije eritropoetina ni priporočljiva. Zaključimo lahko, da so trenutno tako športne kot klinične aplikacije ki slonijo na teoriji "*Normobaričnega kisikovega paradoksa*" preuranjene in nepotrjene. Za konec lahko, kljub dejstvu da različni kratkotrajni hipoksični protokoli lahko izboljšajo določene fiziološke kazalce zaključimo, da je vpliv teh izboljšav na športno sposobnost zanemarljiv.

Abbreviations

2,3-DPG	=	2,3-diphosphoglicerate
[HHb]	=	Deoxygenated hemoglobin concentration
[O_2Hb]	=	Oxygenated hemoglobin concentration
[tHb]	=	Total hemoglobin concentration
BF	=	Body fat
BM	=	Body mass
CBC	=	Complete blood count
$\dot{V}CO_2$	=	Carbon dioxide output
CP	=	Constant power test
CV	=	Coefficient of variation
$\dot{V}_E$	=	Minute ventilation
EPO	=	Erythropoietin
FEV_1	=	Forced expiratory volume in 1 sec
Fer	=	Ferritin
F_IO_2	=	Fraction of inspired oxygen
FVC	=	Forced vital capacity
HCT	=	Hematocrit
Hb	=	Hemoglobin
HIF-1α	=	Hypoxia inducible factor 1 alfa
HR	=	Heart rate
HVR	=	Hypoxic ventilatory response
HYPO	=	Hypoxic
IHE	=	Intermittent hypoxic exposures
IHH	=	Intermittent hyperoxic – hypoxic exposures
IHT	=	Intermittent hypoxic training
LH-TL	=	Live high – Train low
LH-TLH	=	Live high – Train low & high
MCH	=	Mean corpuscular hemoglobin
MCV	=	Mean corpuscular volume
MVV	=	Maximum voluntary ventilation
NORMO	=	Normoxic
N_2	=	Nitrogen

$\dot{V}O_2$	=	Oxygen uptake
$\dot{V}O_{2peak}$	=	Peak oxygen uptake
PEF	=	Peak expiratory flow
PO_2	=	Partial pressure of oxygen
Ret	=	Reticulocytes
RBC	=	Erythrocytes
rHuEPO	=	Recombinant human erythropoietin
RPE	=	Rate of perceived exertion
RPE_{cen}	=	Ratings of centrally perceived exertion
RPE_{leg}	=	Ratings of peripherally perceived exertion
RPE_{dys}	=	Ratings of perceived exertion for dyspnea
SD	=	Standard deviation
SE	=	Standard error
SIH	=	Short intermittent hypoxia
S_pO_2	=	Capillary oxyhemoglobin saturation
SV	=	Stroke volume
SVC	=	Slow vital capacity
T_{lim}	=	Time to exhaustion
T_{sprint}	=	Sprint time
TT	=	Time trial
TRIMP	=	Training impulse
Trf	=	Transferrin
VEGF	=	Vascular endothelial growth factor
V_{max}	=	Maximal speed
W_{compl}	=	Completed workload
W_{max}	=	Peak power output
WL	=	Work load

1 Introduction

1.1 Background

At all times, human organism strives towards a stable internal environment. This process, termed homeostasis, is especially important to constantly provide a milieu, allowing cellular metabolic functions to proceed at optimal efficiency. Alterations of this equilibrium can be provoked by changes of the internal (human body) and external (environmental) conditions. It is dynamically maintained and controlled through a complex network of positive and negative feedback responses. While a human organism tries to counteract these changes, they are crucial for the occurrence of adaptations.

Since O_2 availability is essential for mammalian existence, a decrease in partial O_2 pressure (PO_2) is one of the eminent environmental challenges. For example, it was assumed by the physiologists in the middle of the 20th century, that no human could ever ascend the world's highest mountain (Mount Everest, 8845 m) without the help of supplemental O_2. It was believed, that since PO_2 at the top is reduced to only 25% compared to sea level, this PO_2 would only just be sufficient for rest at that altitude. However, in 1978 two climbers, Habeler and Messner were the first to reach the top without the use of supplemental O_2. A feat considered impossible and never done before showed an example of substantial human adaptation plasticity as well as the ability of a human organism, to beneficially adapt to hypoxic stimuli. As these adaptations were recognized, the ideas of inducing beneficial adaptations for residence and exercise at altitude as well as at sea level were suggested.

Sport science gained interest in hypoxic stimuli in the beginning of the 1960s. At that time, the International Olympic Committee appointed Mexico City (2300 m) as the site for the 19th Olympic Games in 1968. Until then, little was known regarding the effects of hypoxia on athletic performance and possible adaptational benefits of hypoxic exposure. Consequently a number of studies were done during that time to help the athlete's preparation. Since that time, different models of hypoxia application received vast scientific attention. Besides the altitude related effects, the enduring hypothesis about the advantageous effects of different hypoxic protocols on sea level performance was proposed. To date there is general agreement, that some altitude training protocols can be beneficial for performance at altitude and sea level when the hypoxic dose is sufficient. However, the findings regarding the benefits of different hypoxic training modalities incorporating short intermittent exposures remain inconclusive.

1.2 Rationale of the thesis

The main aim of athletes engaged in regular training is improvement of performance. While many different training possibilities exist, hypoxic training is among the most popular additional training tools, employed mainly by endurance professional and recreational athletes worldwide. Although an extensive number of studies has been performed in the last five decades about the different hypoxic training applications on both performance and working ability at sea level and altitude, a number of unresolved problems remain [1]. The aim of the current thesis was to investigate these problems, and ultimately provide athletes and coaches with useful information. Currently, among the most interesting are the issues regarding the effects of shorter hypoxic applications, compared to the longer, more established modalities [2]. In particular, some hypoxic training paradigms have unequivocally been show to have the ability to improve performance at both sea level or at altitude [3]. This does not hold true for the modalities employing hypoxic exposures lasting less than 8 hours per day and three weeks in total [3]. Although the scientific evidence for using hypoxic modalities employing shorter hypoxic exposures is minimal and inconclusive, they continue to be used and advertised in the sport science community and are also promoted in some clinical applications [4, 5]. Therefore, one of the aims of the present thesis was to investigate the effects of the two widely used hypoxic modalities employing short exposures to hypoxia under controlled condition to determine their potential ability to improve performance under both, hypoxic and normoxic conditions.

The time course and the magnitude of deacclimatization i.e. the diminishing of the hypoxic adaptation induced effects, following different hypoxic perturbations are also currently unresolved. Even if it is of major interest when employing protocols to improve subsequent performance or working capacity [6]. For this reason we also investigated the persistence of the possible beneficial effects following hypoxic training protocols. The time course of changes is important for efficient modeling of athletic training and pre competition tapering programs as well as for assessment of occupational hazards when deploying workers to high altitude working areas [7].

In addition the thesis also addressed the issue regarding the magnitude of the hypoxic dose needed to induce beneficial adaptations using hypoxic exposures [8]. Since a hypoxic dose is defined by single and aggregate hypoxic exposure duration and level of hypoxia, the control and comparison of these variables is difficult. The smallest hypoxic dose has not yet been firmly established; especially using normobaric hypoxia that can nowadays be simply utilized at sea level. The shortest possible time required to obtain benefits is motivated, for example, by urgent deployment of rescue or military personnel to high altitude areas, requiring fast acclimatization. Short hypoxic protocols would also benefit athletes providing only minor disturbance of athletic training. With the obvious lack of studies investigating the effects of short intermittent hypoxic exposure on subsequent performance at altitude, the thesis incorporates a study investigating the effect four intermittent exposures on subsequent performance.

We aimed to improve the efficiency of the shorter hypoxic training protocols by testing a novel approach of increasing the relative changes during hypoxic exposures using hyperoxia. In particular, it has been suggested recently, that erythropoietin (EPO) synthesis augmentation can, besides absolute hypoxia, be induced with relative changes in renal tissue O_2 availability [9]. Since it is firmly established that augmented total hemoglobin mass enhances performance [10] and the production of the red blood cells is

primarily controlled by EPO we investigated the changes of EPO plasma levels as opposed to performance. We reasoned that a combination of both hyperoxia and subsequent hypoxia would significantly augment EPO levels and could therefore offer a plausible option for improvement of the efficiency of the currently used protocols. Our assumption was based on the fact, that the relative PO_2 changes in currently used hypoxic protocols are small, and if this relative hypoxia would prove physiologically relative, it would provide an enormous advantage for the hematological effectiveness of hypoxic training modalities. This would not only be applicable to sport via subsequent improvements of O_2 flux capacity and performance, but could also have other application in clinical environment. Namely for treatments of different hematological maladies and illnesses where recombinant EPO is heavily used in therapy [11]. Besides the obvious physiological benefits the economical advantage would also be possible.

1.3 Originality of the thesis

The thesis addreses a number of unresolved issues regarding the use of normobaric hypoxic training modalities, their effects and potential applications. Primarily, the effects of two most commonly used modalities employing short hypoxic exposures, namely IHT and IHE have up to now received little scientific attention. In particular, to date, and to the best of our knowledge, no controlled studies have investigated the effects of these two modalities on both performance in hypoxia and normoxia employing a similar study design. Moreover very few studies have considered the deaclimatization period and incorporated the testing after a certain period following either IHT or IHE. Given the attention and number of studies dealing with the other hypoxic training modalities (LH-TL, LH-TH), the lack of the studies regarding the shorter modalities necessitate further studies. This thesis incorporates two controlled studies investigating the effects of both IHT and IHE on performance in hypoxia and normoxia. Four testing periods were included to monitor the possible changes during, immediately after and ten days after the training protocol cessation. Thus, these were the first studies investigating the effects of IHT and IHE on performance following 10 training sessions, 20 training sessions and the effects of deacclimatization period.

Even more inconclusive are the studies regarding the effects of short preacclimatization IHE modalities for altitude performance enhancement. While few studies have investigated the effects of natural (hypobaric) hypoxic preacclimatization on subsequent performance at altitude, the effects of the protocols employing normobaric hypoxia are still indefinite. While the minimal dose has been suggested to be between 3 to 4 hours repeatedly for at least four days [12], this was not clearly demonstrated before. Therefore, the thesis incorporates a study testing the effects of four intermittent normobaric hypoxic exposures on performance in simulated hypoxia.

The improved O_2 flux from the alveoli to the working muscles is considered to be among the main beneficial outcomes of hypoxic training modalities. However, it seems that this can only be achieved using longer hypoxic exposures. The augmented O_2 flux is enabled by enhanced EPO synthesis and subsequent erythropoiesis stimulation leading to augmentation of hematocrit (HCT) and hemoglobin (Hb). Even though the main physiological mechanism, namely absolute renal tissue hypoxia has been well established, recently other possible mechanisms have also been proposed (i.e. increased relative change in partial O_2

pressure above hypoxic treshold). With the aim of improving the efficiency of protocols employing shorter hypoxic exposures we tested a novel approach of inducing higher relative changes in inspired O_2 pressures and monitored its effect on subsequent EPO *de novo* synthesis. For the first time in the literature, this model (subsequently combining hypoxia and hyperoxia) has been used to investigate its effect on endogenous EPO *de novo* production.

1.4 Thesis organization

The thesis contains eight chapters. The structure follows the thesis aims and research questions. In particular, after the introduction Chapter 2 provides a concise review of the currently used hypoxic training modalities, possible applications, and its purported physiological mechanisms of action. Following the presentation of the aims and hypotheses the subsequent chapters present four independent studies performed with the aim of resolving the research questions and assessing the hypotheses. The included studies have been separately published or submitted for publication consideration to peer-reviewed international journals:

Study I: Normoxic and hypoxic performance following 4 weeks of IHT.

- *Published in Aviation, Space, and Environmental Medicine*

Study II: Effects of IHE on performance in normoxia and hypoxia.

- *Under rewiev in Scandinavian Journal of Medicine & Science in Sports*

Study III: Short intermittent hypoxic exposures augment ventilation during hypoxic exercise.

- *Prepared for submission to Respiratory Physiology & Neurobiology*

Study IV: Acute hyperoxia followed by mild hypoxia does not increase EPO production.

- *Published On-line in European Journal of Applied Physiology*

Study I (Chapter 4), investigated the effects of training in normobaric hypoxia (IHT) as compared to the same relative work load training in normoxia. Study II, investigating the effects of twenty intermittent hypoxic exposures at rest on exercise performance in normobaric hypoxia and normoxia is presented in Chapter 5. Chapter 6 presents a study investigating the potential preacclimatization ability of only four intermittent hypoxic exposures for enhancements of working capacity in normobaric hypoxia (Study III). The last study investigating the effects of subsequent hyperoxic and hypoxic air mixture breathing on EPO *de novo* synthesis augmentation (Study IV) is presented in Chapter 7. The final chapter sums up the findings and conclusions of all the studies performed in general summary and gives a forward perspective about the future use of the tested modalities. Moreover it also includes future research implications and directions for subsequent studies. Appendix includes the list of peer reviewed publications and international conference presentations, arising from this thesis. The pertinent ethics approvals are also attached in the appendix.

2 Oxygen manipulations for enhancement of performance. A review.

2.1 Introduction

At present a vast number of professional and recreational athletes use hypoxic training as means of additional training for boosting their performance [3]. Although deleterious effects of exposure to altitude were primarily recognized, the potential beneficial adaptations to hypoxic perturbation have also been seen and sought after. The issue of inducing beneficial adaptations while, simultaneously avoiding the detrimental consequences, led to the development of several hypoxic training modalities [1]. From traditional altitude training camps, where athletes are exposed to chronic continuous hypoxia, to brief intermittent hypoxic exposures, repeated in series, all aiming to improve performance or working capacity at either altitude or sea level. Although numerous hypoxic training modalities exist, they all relay on efficient manipulation of the hypoxic dose. That, achieving by modifying either the duration of a single exposure, level of hypoxia and the number of aggregate exposures [13].

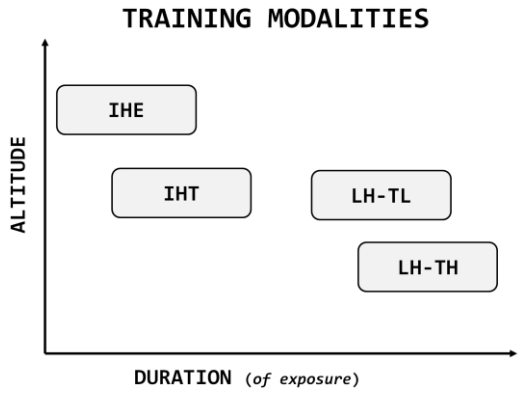

Figure 1.1: *Contemporary hypoxic training modalities.*

While permanent residence at altitude during moderate altitude sojourns (LH – TH) was, and still is a popular form of hypoxic training, the modalities employing shorter intermittent exposures are coming to the forefront of both athletes and researchers interest [14]. For this, there are several reasons. Firstly, while continuous hypoxia can beneficially affect the O_2 flux capacity these benefits can be abated by the negative consequences of chronic hypoxic exposures. In particular, the dehydration, deconditioning, altitude sickness effects, food aversion and especially, in regards to training, the inability to perform high intensity exercises [15]. All these factors can lead to overreaching and overtraining when the sum of training and hypoxic impulse is outsized [15]. Minor

interruption of both, life style and training process is the next obvious advantage of the intermittent protocols. It is without a doubt beneficial, if for the same purported result one needs to dedicate two hours per day as compared to whole day. Particularly, since athletes already perform large amounts of training volume necessitating vast amount of time. This is also in line with the difficulties in logistics, since not everybody has the opportunity to travel to high altitudes. While for longer periods that seems like a viable options when employing shorter exposures the constant travel to and from the terrestrial altitude is not always possible. Especially due to those reasons, the use of simulation of altitude is increasing in popularity, and in sequence the technological possibilities are expanding [12].

The debate about the different physiological effects of hypobaric and normobaric exposures to hypoxia is ongoing and the results of the contemporary studies, do not provide a uniform answer. Some studies show that hypobaric hypoxia leads to lower arterial O_2 saturation and grater hypoxemia during exercise compared to normobaric hypoxia [16]. It has also been shown that serum EPO response is greater when subjects are exposed to terrestrial altitude as compared to simulated altitude [17]. However the same study suggested that levels of responses were comparable when measured in environmental chamber or at natural altitude exposure. Furthermore, similar ventilatory adaptations to intermittent hypoxia have been shown to occur when using either normobaric or hypobaric exposures [5]. Since the technological advancements and convenience of normobaric hypoxic application is improving, all studies within the thesis were performed using normobaric hypoxia application. As discussed below, there are a few ways to simulate hypoxic environment.

A decrease in barometric pressure and thus lowering of PO_2 occurs when a person ascends to higher altitudes (hypobaric hypoxia). However as mentioned, the travel to the mountain region and back is not always very convenient or even possible when employing different hypoxic training modalities. Moreover, since some world regions do not offer an appropriate terrestrial altitude (Australia, Scandinavia), this has led to fast technological development of hypoxic stimulation facilities and enabled contemporary athletes to utilize simulated altitude exposures worldwide. At present, there are a handful of methods, using different technological methodologies that allow for hypoxia simulation [3]. While hypobaric chambers provide the best approximation of natural altitude by making use of both hyperbaria and hypoxia, their use is not widespread. This is mostly due to constructional and technological requirements of assembling pressure resistant buildings. Of late, the use of normobaric simulations of hypoxia using different gas mixture manipulation inducing a decrease in F_IO_2 without a decrease in barometric pressure (normobaric hypoxia) is increasing in popularity. Among the most often used and convenient technologies are O_2 filtration or N_2 dilution [3, 16]. The first method utilizes a specific membrane, reducing the molecular O_2 concentration within the ambient air that is drown from outside to the application space. The generator pumps the O_2 depleted air to either a room, or a custom made tent. The O_2 filtration is most commonly used in small personal portable hypoxicators. Nitrogen dilution is a similar method inducing normobaric hypoxia with the simultaneous induction of ambient air and 100 % N_2 to the targeted environment. Thus by introducing excessive N_2 to ambient air the percentage of O_2 within the gas mixture is reduced to the desired level. This type of application is usually installed to permanent rooms or laboratories. Possible hypoxia

applications are presented in Fig 1.2.

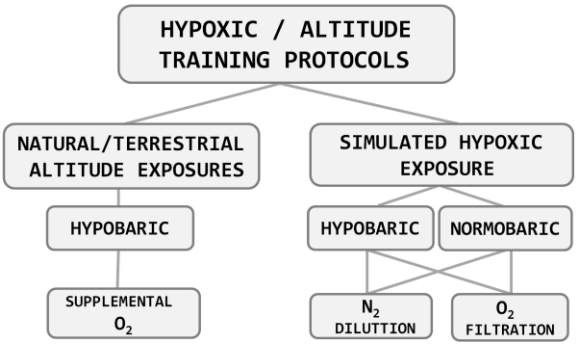

Figure 1.2: *Technological possibilities of hypoxic training application.*

Thus, there are multiple options for applying hypoxic stimuli both, in relation to the selected modality employed and the type of hypoxia used. Even though, for obvious reasons the use of shorter intermittent hypoxic protocols seems to be superior as compared to chronic and longer duration protocols, in regards of time consumption and athlete engagement, the actual effects and the underline mechanism remain indefinite [4, 18]. Due to the lack of controlled studies investigating the effects of shorter hypoxic modalities, especially on performance at altitude and sea level, further studies are warranted [12]. They should determine the magnitude of effects and the necessary hypoxic doses for improvements of performance. The following chapter provides an overview of the main contemporary hypoxic training modalities, and their purported effects.

2.2 Contemporary hypoxic training modalities

2.2.1 Live High – Train High (LH-TH)

The LH-TH, also termed the Classical altitude training, has been a popular modality for the last few decades. The results of the controlled studies investigating LH-TH are not uniform [19]. Incorporation of both sleeping and training at moderate altitudes, usually between 1500 – 3000 m above sea level for three to four weeks is the main characteristic of this modality. Due to the fact that permanent residence at altitude necessitates acclimatization along with adjustment of training volume and intensity, this modality is divided in phases. These include acclimatization phase, aimed at adapting the athletes to reduced PO_2, primary training phase where both the volume and intensity are progressively increased and tapering phase before returning from altitude in which the training load is reduced, to allow the athletes to recover from hypoxic and training induced exhaustion [14]. They are followed by the post altitude phase, where, although still debated, three within phases were suggested, compromising of initial increase in ability for 2-4 days, followed by a decrease and an yet again an increase in performance ability after two to three weeks following exposure. The LH-TH is almost exclusively performed at terrestrial altitudes, where constant altitude residence is conveniently performed as compared to permanent living, training and sleeping within the simulated hypoxic facility. This brings

about another possible beneficial effect of this modality i.e. the "training-camp" effect that has been shown to have a possible although a placebo effect [2]. Compared to other training modalities, LH-TH aims at benefiting from altitude acclimatization related effects and concomitant use of hypoxia during training to increase the level of exercise induced hypoxia within the muscle [20].

The results of the studies investigating the effects of LH-TH are not uniform, but it has been shown that some benefits can be expected. Since, the permanent residence allows for efficient acclimatization the performance or working capacity at altitude has been show to improve [21]. The findings regarding the LH-TH effects on sea level performance are more diverse. Some of the controlled studies have shown favorable effects, namely the increases in $\dot{V}O_{2peak}$ [22] and maximal performance in the incremental test [23]. On the other hand, other studies did not show any favorable effect on aerobic capacity [24] or have even shown a decrease in mean running velocity during the supramaximal test following LH-TH [25]. The acclimatization induced by continuous residence and training at altitude has been shown to result in augmentation of total hemoglobin mass [26] and was also shown to be significantly correlated with concomitant changes in $\dot{V}O_{2peak}$ [22]. Regarding the non-hematological parameters, although no enhancement of muscle tissue oxidative capacity has been observed [27], the buffering capacity was shown to increase significantly, following two weeks of LH-TH at altitudes between 2100 - 2700 m [28].

The main training related drawbacks of LH-TH are the decrements in exercise intensities, due to impaired aerobic capacity at altitude and subsequent lower mechanical and neural training load arising from inability to perform highest intensity exercise [22]. However some studies have nevertheless showed increases in sea level performance following higher intensity training performed during SH-TH [23]. Another setback arises from the possible negative effects of chronic hypoxic exposures namely, the deterioration of muscle function and size, that has been show to occur at higher altitudes [15, 29]. Since LH-TH is performed at natural altitude it does not allow for individual adjustment of exposure altitude. While this, in light of large individual variability observed in response to hypoxic perturbations [30] is an obvious weakness, it can partially be adjusted by repeatedly modifying and regularly monitoring the individual training intensity, to avoid the possible overreaching and subsequent detraining [31].

2.2.2 Live High – Train Low (LH-TL)

Considering the above mentioned detrimental effects of chronic hypoxic exposure, especially in regards to training and conditioning, the possibility of applying hypoxia during the rest while still performing normal sea level training came to light. The LH-TL protocol was introduced in a seminal study by Levine and Stray-Gundersen in the last decade of the previous millennia [22]. They investigated the effects of three modalities either: (i) LH-TL, (ii) LH-TH and LL-TL, namely living and training at sea level. Their findings have shown that, following the tested protocol, the subjects of both LH-TL and LH-TH groups significantly increased the Hb, erythrocyte volume, and $\dot{V}O_{2peak}$. However, only the LH-TL group significantly increased the 5000-m running time. This confirmed their premise of the ability of LH-TL to concomitantly enable sufficient acclimatization to improve O_2

flux while maintaining the sea level training benefits. Therefore the LH-TL is based on simultaneous benefits of acclimatization (↑ total Hb mass, ↑ HCT), with negligible interruption to athletic training and avoidance of possible chronic hypoxia induced detrimental effects.

While the original study was performed at terrestrial altitude, the LH-TL paradigm was soon also applied using different hypoxia stimulation methods, mostly using normobaric hypoxia [32]. This was a logical improvement of modality, due to the inconvenience and stress of constant transportations to and from altitude, when residing at terrestrial altitude. The technological advancements have nowadays enabled the athletes to undergo LH-TL modality at home using small personal hypoxicator devices and sleeping tents. This novelties have subsequently lead to adjustment of the protocol, i.e. applying hypoxia only during sleep while living and training in normal conditions (Sleep high – Train low) [33]. This has lead to the problem of comparing the effects of using different LH-TL hypoxia applications. Moreover, it also opened an issue of the necessary hypoxic dose for assumed benefits when employing the simulated LH-TL or Sleep high-Train low modality [34].

The studies investigating LH-TL at natural altitude have shown that if modality is performed for at least four weeks at altitudes between 2500 to 2800 m, subsequent augmentation of both red cell mass and $\dot{V}O_{2peak}$ can be seen. Thus subsequently leading to improved performance [35]. There is more variability in the findings of studies dealing with simulated LH-TL [33]. Regarding the hematological benefits, the results consistently vary according to both, cumulative and daily hypoxic dose. The studies employing longer daily exposures ranging between 12-17 $h \cdot day^{-1}$ have shown increases in red cell volume [36] and serum transferrin [37]. On the other hand, the studies investigating shorter daily exposures (6-12 $h \cdot day^{-1}$) during LH-TL did not show any modifications of Hemoglobin mass [38] or Reticulocyte count [39]. Even though the main postulated mechanism for performance improvements were reasoned to be the blood related enhancements of O_2 flux capacity [22], a number of other hypoxia inducible fatcors (HIF-1 & HIF-2) driven mechanisms on molecular level have been shown to occur [40]. Namely, the improvements in exercise efficiency related to the augmented muscle cells mitochondrial efficiency, improved acid base cell status regulation, improved muscle cells metabolic efficiency and increased muscle buffering capacity [41]. Moreover improvements in hypoxic ventilatory response [42] and muscle Na-K ATP-ase activity have also been documented [43].

Besides the improvements in altitude performance, the main incentive is to improve performance back at sea level. While the majority of the studies employing natural LH-TL show positive benefit for subsequent sea level performance [35], the results of the simulated LH-TL studies variable findings [33]. Whilst some of the studies showed increases in both $\dot{V}O_{2peak}$ [36] and time trial performance [44], other studies in both elite runners and cyclists did not find any significant improvements in sea level performance following simulated LH-TL [45, 46]. The majority of the discrepancies between the studies finding most probably arise from differences in hypoxic doses [34] it is of outmost importance to ensure the sufficient amount hypoxia is applied to the athlete. According to the current findings, he optimal LH-TL altitude seems to be between 2200 to 2500 meters and between 2500 to 3100 meters for the for hematological and non hematological adaptation respectively [14]. The second determinant, namely the duration of hypoxic exposure, must be at least four weeks when aiming for augmented

erythropoiesis [22], but can be shorter (two to three weeks) when aiming for non hematological benefits [40]. Lastly, the daily dose of hypoxia seems mandatory to exceed 12 h per day. This is especially important for erythropoiesis stimulation, since longer daily exposures seem to be superior to the shorter ones [34]. Therefore, when employing the LH-TL modality, the optimal daily hypoxic exposure duration seems to be between 16 – 22 hours daily and can be reduced when the main aims are non-hematological benefits [14]. Since studies have shown wide interindividual variability to hypoxic training modalities [47], the individual responses have to be taken into account and the programs should be individually customized whenever possible.

2.2.3 Intermittent hypoxic training (IHT)

While the intermittent applications of hypoxia stimuli is used in all but the LH-TH modality the use of intermittent in IHT refers to short periods of hypoxia (minutes to two hours) interspersed between normoxia. It has been reasoned that by applying brief hypoxic exposures similar effects can be expected as with chronic ones, without the detrimental effects of the late. In particular IHT refers to use of both hypoxic in training impulse concomitantly. The premise of the IHT lays in the utilization of the hypoxic stimuli during the training to surplus the normal training load. Since aerobic capacity decays inversely with increasing altitude the same absolute workload presents a higher relative workload when training is performed under hypoxic condition [48]. Thus, inducing specific signaling effects on molecular level in muscular tissue, possibly leading to beneficial changes in muscle cell properties relevant to performance [18]. These adaptations are the fundamental aim of IHT, since the duration of the hypoxic impulse is insufficient to enable the occurrence of hematological beneficial changes in red blood cell number or total hemoglobin mass [14]. The IHT is usually performed for no longer than one to two hours per day. Therefore, when employing IHT the main aim of the training should not be the hematological benefits but improvement of efficiency and working capacity of muscles. Moreover even though the hypoxic exposure duration is shorter, ventilatory benefits, namely higher ventilator levels, have already been shown following IHT [49]. In addition, the fact that IHT only employs short hypoxic periods, presents at least two advantages. Firstly, the avoidance of deleterious effects of chronic high altitude exposures on muscle tissue [29] and secondly it presents only a slight disturbance to the usual athlete daily routine. However, as has already been shown, the amplified training impulse induced by concomitant training load and hypoxia can also swiftly lead to overreaching or overtraining if the total training load is not adjusted accordingly [50].

For convenience, the IHT is habitually performed in simulated hypoxia under either normobaric or hypobaric condition. The simulated altitude used in IHT usually varies between 2500 to 5000 meters, as altitudes $\leq$ 2500 m have little effect and at altitudes $\geq$ 5000 meters, the sufficient exercise training load is hard to achieve for all but the really top acclimatized athletes [12]. Regardless of the mode of hypoxia the results of the studies show a general tendency for improving both, performance and $\dot{V}O_{2peak}$ under hypoxic conditions. On the other hand, the majority of the studies did not find any advantages of IHT training for sea level performance [18]. Table 1.1 summarizes the studies investigating IHE performed in normbaric hypoxia on selected markers of performance. As can be

seen, there are discrepancies between both the study designs and results. Although a tendency of beneficial results when employing greater IHT training duration exist, a study by Bailey [51] showed that twelve 30 minute sessions interspersed between four weeks have the ability to improve normoxic $\dot{V}O_{2peak}$. Similar findings were shown in trained runners employing 30 IHT sessions over a period of 6 weeks [49].

Table 1.1: *Designs and data metrics of the preceding studies investigating normobaric IHT.*

IHT training	Condition / Subjects	Outcome metric	Metric response	Conclusions	Ref.
≈120 min+40 min 5·week^{-1}/6 weeks Normal training	C: F_IO_2= 0.209 H: F_IO_2= 0.145 Trained runners	$VO_{2peakNORMO}$ T_{lim}	C: + 1.2 % H: +5.3 % ↑ C: + 10 % H: +35 % ↑	IHT is superior over normoxic training	[49]
60 min 6·week^{-1}/3 weeks 80% VO_{2peak}	C: F_IO_2= 0.209 H: F_IO_2= 0.12-0.10 Untrained	$VO_{2peakNORMO}$ $VO_{2peakHYPO}$ HVR	C: + 9.3 % ↑ H: +6.7 % ↑ C: - 0.2 % H: +4.8 % C: ≈ H: +54 %	IHT induces hypoxia preacclimatization	[52]
120 min 6·week^{-1}/3 weeks 75 % VO_{2peak}	C: F_IO_2= 0.209 H: F_IO_2= 0.12-0.10 Untrained	$VO_{2peakNORMO}$ $VO_{2peakHYPO}$	C: + 0.5 % H: + 6.4 % C: + 1.9 % H: + 11 % ↑	IHT improves $VO_{2peakHYPO}$ if performed at the same relative WL	[53]
30 min 3·week^{-1}/4 weeks 80% HR_{max}	C: F_IO_2= 0.209 H: F_IO_2= 0.135 Untrained active	$VO_{2peakNORMO}$ $W_{maxNORMO}$	C: + 4 % H: + 13.5 % ↑ C: + 7 % ↑ H: + 4.6 % ↑	IHT improved normoxic aerobic capacity	[51]
30 min 5·week^{-1}/6 weeks 75 % VO_{2peak}	C: F_IO_2= 0.209 H: F_IO_2= 0.12 Untrained	$VO_{2peakNORMO}$ $VO_{2peakHYPO}$ $W_{maxNORMO}$ $W_{maxHYPO}$	C: + 9.5 % ↑ H: + 11.1 % ↑ C: + 3.4 % ↑ H: + 7.2 % ↑ C: + 13.2 % ↑ H: + 11.3 % ↑ C: + 9.5 % ↑ H: + 14.3 % ↑	IHT induced similar performance benefits as C but improved $VO_{2peakNORMO}$ Significantly more	[54]
30 min 3·week^{-1}/8 weeks 75 % VO_{2peak} Single leg	C: F_IO_2= 0.209 H: F_IO_2= 0.135 Untrained	$VO_{2peakNORMO}$ T_{lim}	C: + 13 % ↑ H: +11 % ↑ C: + 400 % H: + 510 %	No functional benefit of IHT	[55]
12.5 min 3·week$^{-1/}$5 weeks High intensity	C: F_IO_2= 0.209 H: F_IO_2= 0.153 Trained swimmers	$VO_{2peakNORMO}$	C: +5.6 % ↑ H: +3.8 % ↑	No additive effect of IHT	[56]
3·30 (20, 10) min 3·week^{-1}/4 weeks 80 &50% VO_{2peak}	C: F_IO_2= 0.209 H: F_IO_2= 0.15 Team sport players	$VO_{2peakNORMO}$ $W_{maxNORMO}$	C: +8 % ↑ H: +7.2 % ↑ C: +17 % ↑ H: +15.5 % ↑	No additive effect of IHT	[57]

C: Control group; H: IHT group; F_IO_2: Fraction of inspired oxygen; $VO_{2peakNORMO}$: maximal aerobic capacity in normoxia; T_{lim}: time to exhaustion; $VO_{2peakHYPO}$: maximal aerobic capacity in hypoxia; HVR: hypoxic ventilatory response; $W_{maxNORMO}$: peak power output in normoxia; $W_{maxHYPO}$: peak power output in hypoxia; + increase; - decrease; ↑ significant ($P < 0.05$).

Regarding the effects of normobaric IHT on performance in hypoxia, two studies have shown the beneficial preacclimatization effect. Namely, the study by Benoit et al. [52] showed that although subsequent performance was similar between groups, the HVR was only significantly increased in the IHT group, thus showing the potentially beneficial ventilatory acclimatization effect. The second study showed benefits of IHT over normoxic training on hypoxic $\dot{V}O_{2peak}$ stipulating, that the training was performed at the same relative work load in both conditions [53]. On the other hand, some studies did not find any significant additional effect of IHT on performance metrics on either normoxic $\dot{V}O_{2peak}$, W_{max} or time to exhaustion in both trained and untrained subjects [55-57]. The only study investigating the IHT effects in both normoxic and hypoxic condition, performed by Geiser et al. [54], did not find any significant differences between IHT and normoxic training, besides a significantly greater increase in normoxic $\dot{V}O_{2peak}$ in the IHT group compared to the control.

As mentioned, the main mechanism responsible for the possible improvements following ITH seems to be the changes on molecular level in muscle tissue. Since the training response is always modulated through integrated signaling pattern induced by mechanical load, metabolic disturbance, neuronal activation and hormonal adaptations, the identifying of specific IHT responses pathways is complex [58]. The transcriptional signaling and subsequent adaptations are during hypoxia governed at the molecular level by HIF-1α related processes that not only master the regulatory genes for erythropoiesis but also glycolysis and pH regulation [59]. The subsequent advantageous effects include greater buffering capacity, lactic acid tolerance and improved muscle efficiency [52]. Expectedly, no hematological benefits have been reported from IHT studies, mostly due to insufficient daily and aggregate hypoxic dose to stimulate erythrocyte production stimulation [56, 60].

2.2.4 Intermittent hypoxic exposures (IHE)

Similarly to the IHT the Intermittent hypoxic exposures (IHE) refer to using brief hypoxic exposures, whereas at rest, as means of improving wellbeing or performance. For purposes of clarity the IHE modality can be divided into two models of application. The first one refers to the use of brief (3 – 6 minutes) of relatively high levels of hypoxia (F_IO_2 = 0.15 – 0.09) interspersed with periods of normoxia of similar duration. This model was introduced and broadly used in the eastern European states for both clinical applications and performance enhancements [5]. Typically, normobaric hypoxia is used for this model since even simulation of altitude using hypobaric chamber does not readily permit the constant changes of PO_2. The second IHE model stands on intermittent applications of "chronic" hypoxic exposures lasting between 30 minutes to 5 hours. This model was commenced for high altitude expeditions preaclimatization protocols, with the aim or reducing the acclimatization time on one hand and improving performance at altitude on the other [61]. Owing to the longer constant exposures, both simulated normobaric and hypobaric hypoxia may be used depending on the technological options. Both models seem to be a promising means of hypoxia application, especially in regard to time consumption and convenience. However, the results regarding their effects on performance and selected physiological variables are less appealing [4, 12].

The accountable physiological mechanism relate to augmentation of respiratory sensitivity, neural

effects, and improved mitochondrial activity [5]. Thus indicating, that the primary adaptational aims are not hematological, but relay on augmented ventilation and substrate utilization [8]. Even if one of the early studies showed that significant blood related benefits in Ret count, Hb and HCT can be expected when employing only nine 90 minutes exposures to hypobaric hypoxia during the period of three weeks [62], subsequent studies did not confirm their findings [63, 64]. Thus, showing that higher hypoxic dose is required to promote the erythropoietic response.

Table 1.2: *Designs and data metrics of the preceding studies investigating short normobaric IHE.*

Subjects	IHE protocol	Outcome metric	Metric response	Conclusions	Ref.
Team sport players	∑ 60 min, 6:4 7·week^{-1}/3 weeks S_pO_2= 90 - 77 %	V_{max}	C: - 0.3 % H: + 1.7 % ↑	Substantial improvement in sprint performance	[65]
		T_{sprint}	C: - 0.5 % H: - 5.1 % ↑		
Active elderly	∑ 20 min, 5:5 10 days S_pO_2= 85 - 86 %	W_{sub}	H: 11.3 % ↑	IHE has beneficial effects on work capacity and hemodynamics	[66]
		BP	H: - 7.9 mmHg ↑		
Multisport athletes	∑ 90 min, 5:5 5·week^{-1}/3 weeks F_IO_2= 0.13-0.10	T_{3000}	H: - 2.3 % ↑ C: - 0.6 %	IHE is likely beneficial for multisport athletes	[67]
Elite rowers	∑ 90 min, 6:4 7·week^{-1}/3 weeks S_pO_2= 90 - 80 %	$W_{mean5000}$	C: ≈ H: + 0.6 %	IHE is unlikely to have a major effect on performance	[64]
		$W_{mean500}$	C: ≈ H: - 2.2 %		
Elite runners	∑ 70 min, 5:5 5·week^{-1}/4 weeks F_IO_2= 0.12-0.10	$VO_{2peakNORMO}$	C: ≈ H: ≈	Not a sufficient stimulus to enhance performance	[63]
		T_{lim}	C: ≈ H: ≈		
Trained runners	∑ 60 min, 6:4 7·week^{-1}/2 weeks F_IO_2= 0.11-0.10	$VO_{2peakNORMO}$	C: - 0.3 % H: + 2,2 %	IHE has no effect on aerobic or anaerobic performance	[68]
		W_{max}	C: - 0.2 % H: + 2.4 %		
Team sport players	∑ 90 min 7·week^{-1}/2 weeks F_IO_2= 0.15-0.10	V_{max}	C: < 1 % H: < 1 %	IHE is not suggested for games preparation	[69]
		T_{sprint}	C: ≈ H: ≈		
Team sport players	∑ 60 min, 6:4 7·week^{-1}/2 weeks F_IO_2= 0.13-0.10	V_{max}	C: ≈ H: ≈	No effect of IHE on altitude impaired performance	[70]
		T_{sprint}	C: ≈ H: ≈		

C: Control group; H: IHE group; ∑: total IHE time; Hypo:Normo exposure; F_IO_2: Fraction of inspired oxygen; V_{max}: maximal speed; T_{sprint}: sprint time; W_{sub}: submaximal work; BP: Blood pressure; T_{3000}: 3000 meters run time; $W_{mean5000}$: mean power during the 5000 m test ; $W_{mean500}$: mean power during the 500 m test; $VO_{2peakNORMO}$: maximal aerobic capacity in normoxia; T_{lim}: time to exhaustion;W_{max}: peak power output; + increase; - decrease; ≈ no change; ↑ significant ($P < 0.05$).

As noted the results regarding the effects of IHE on performance remain elusive. Table 1.2 summarizes the studies investigating normobaric IHE modality employing brief alternations between hypoxic and normoxic exposures. As can be seen only three out of eight studies found significant benefits of IHE on performance metrics. Whilst the studies by Wood et al. [65] and Hamlin et al. [67] showed significant improvements following 21 (60 min) and 15 (90 min) sessions of IHE in sprint time and 3000 meters run time respectively. Interestingly, the study by Shatilo et al. [66] found

benefits in submaximal working capacity after only ten 20 minute sessions of relatively mild IHE (S_pO_2 = 85%). In contrast other controlled studies employing blinded design did not show any benefit for either aerobic [63] or anaerobic [68] performance at sea level and at moderate altitude [70].

Similarly, studies investigating longer continuous IHE model do not show uniform results. Since this IHE model can be applied using either simulated normobaria or hypobaria, the related discrepancies have to be taken into account. In particular, when IHE was applied using hypobaric hypoxia, the majority of the studies showed possible benefits for performance [71, 72] and ventilatory acclimatization [73]. On the contrary, the results of normobaric IHE studies, although few, that are listed in Table 1.3 do not suggest that the use of normobaric IHE seems to be beneficial for performance at altitude. Interestingly, they show that prolonged IHE can have a positive effect on sea level performance. Namely, the study by Burtscher et al. [74] showed that three 2 hour IHE sessions per week (5 weeks) can be beneficial for running performance and exercise economy, although the benefits for trained athletes tend to be dependent on their training phase. Benefits in exercise economy have also been confirmed by Katayama et al. [75]. The three studies investigating effects of normobaric IHE on altitude performance did not find any significant benefits [7, 76, 77].

Table 1.3: *Designs and data metrics of the preceding studies investigating longer normobaric IHE.*

Subjects	IHE protocol	Outcome metric	Metric response	Conclusions	Ref.
Trained athletes	120 min 3·week^{-1}/5 weeks F_IO_2= 0.15-0.10	T_{lim} VO_2	C: + 0.4 min H: + 1 min ↑ C: - 0.3 ml·kg^{-1}·min^{-1} H: - 2.3 ml·kg^{-1}·min^{-1}↑	IHE can be beneficial depending on the training period	[74]
Trained runners	180 min 7·week^{-1}/2 weeks F_IO_2= 0.123	$VO_{2peakNORMO}$ T_{3000}	C: ≈ H: + 0.7 % C: + 0.8 % H: - 1.1 %	IHE can augment running economy and thus enhance performance	[75]
Trained runners	60 min 7·week^{-1}/1 week F_IO_2= 0.123 F_IO_2= 0.155	V_{EHYPO} HVR	C: ≈ H: ≈ C: ≈ H: ↑	No effects of IHE on altitude exercise ventilation.	[76]
Untrained active	180 min 7·week^{-1}/1 week F_IO_2= 0.12	TT_{HYPO} S_pO_{2HYPO}	C: - 12 % H: + 2.7 % C: ≈ H: ≈	Seven IHE exposures does not improve altitude performance	[7]
Trained cyclists	60 min 7·week^{-1}/1 week F_IO_2= 0.125	$W_{meanHYPO}$	H: ≈ compared to C	No positive effect on altitude performance	[77]

C: Control group; H: IHE group; F_IO_2: Fraction of inspired oxygen; T_{lim}: time to exhaustion; VO_2: exercise oxygen consumption; $VO_{2peakNORMO}$: maximal aerobic capacity in normoxia; T_{3000}: 3000 meters run time; V_{EHYPO}: Minute ventilation in hypoxia; HVR: hypoxic ventilatory response; TT_{HYPO}: Hypoxic time trial performance; S_pO_{2HYPO}: Capillary oxyhemoglobin saturation in hypoxia; $W_{meanHYPO}$: mean power in hypoxia; + increase; - decrease; ≈ no change; ↑ significant ($P < 0.05$).

According to the current findings, the efficacy of both continuous and brief IHE is not firmly established. The main source of discrepancies in findings and complex pooling of the IHE studies results is the hypoxic dose, and its modulation [4]. Namely the exposure times, level of hypoxia and

the duration of the interventions, as the main determinants of the hypoxic dose. Thus, the pivotal question regarding the minimal IHE dose for inducing beneficial adaptations remains to be answered. Further strictly controlled studies seem necessary, to clarify the open issues.

2.2.5 Combinations of hypoxic modalities

The previous subchapters reviewed the four basic modalities currently most widely used in elite athletes. As all modalities manipulate the amount and application of the hypoxic dose, it is not surprising that different combinations within the protocols exist. Namely with the rationale of combining the hypoxic training modalities in such a manner, that they allow for utilization of only the parts presumably beneficial. While many possibilities exist, a recent comprehensive review [14] suggested two training modalities modifications. Since LH-TL has been shown to be an efficient modality for improving even elite athletes performance at sea level [13], they use it as a baseline modality. They suggest to alternate the nights spend at hypoxia with the ones in normoxia at a ratio of 5-2 or 6-1. Moreover since beneficial alternations within the muscle on molecular level has been established using IHT, they suggest to substitute two or three training sessions per week at sea level with training in hypoxia. Presumably this combination would be of most benefit for intermittent sport athletes benefiting to both aerobic and anaerobic exercise ability [14]. The combination of LH-TL and IHT has already been shown effective in a recent study by Robertson et al. [78]. They compared the effects of three weeks long protocol of combining LH-TL/IHT versus IHT only. Compared to IHT the LH-TL/IHT combination provokes a substantially improved $\dot{V}O_{2peak}$, total Hb mass and time trial performance. They concluded that the combination of LH-TL/IHT seems to be a promising mixture of hypoxic modalities for physiological capacity enhancements.

Pre-exposures for altitude acclimatization and attenuation of altitude induced reduction of working capacity also often consist of different hypoxic modality combinations. Usually they combine both IHE models with concomitant IHT [12], even though there was reportedly no significant differences between the inclusion or exclusion of IHT in prolonged IHE model [79]. On the other hand, according to a recent review [80], while the effects of IHE with or without IHT may be similar for shorter duration protocols when employing longer protocols an inclusion of IHT may prove beneficial. These benefits can refledt in both, increased performance at altitude as well as in the reduction of altitude related detrimental effects [80].

Collectively, the data on performance effects of different hypoxic training modalities is still inconclusive. While some studies have shown the ability of all modalities to be an effective booster of performance, others have not. A recent meta analysis on the effects of different hypoxic training modalities on sea level performance showed that the LH-TL is currently the most effective model for inducing beneficial changes in performance [2]. With other modalities i.e. LH-TH and IHE can also be beneficial, especially for sub elite athletes and untrained subjects. In conclusion, the studies investigating different hypoxic modalities have unequivocally established that sufficient hypoxic dose can improve maximal aerobic capacity and other performance related physiological mechanisms at sea level and altitude and can thus be advantageous for performance [3, 13, 34].

2.3 Mechanisms effecting performance related to hypoxic stimuli

Human organism is extremely susceptible to changes in O_2 availability and thus initiates responses concomitantly with the onset of the hypoxic exposure. As has been shown in the previous chapter, improvements in performance can occur following different hypoxic training modalities. This section will present the main purported physiological mechanisms underlying the effects of hypoxic modalities on performance. While both aerobic and anaerobic processes contribute significantly to exercise performance, the O_2 delivery is of primary importance for any exercise longer than a few seconds. The main determinants of O_2 delivery to muscle tissue can be seen from in Fig 3. This pathway, also termed the "Oxygen delivery cascade" represents the flow of ambient O_2 from air to cellular - muscle level [81]. It starts with ambient air transfer and is followed by the gas diffusion in the lungs.

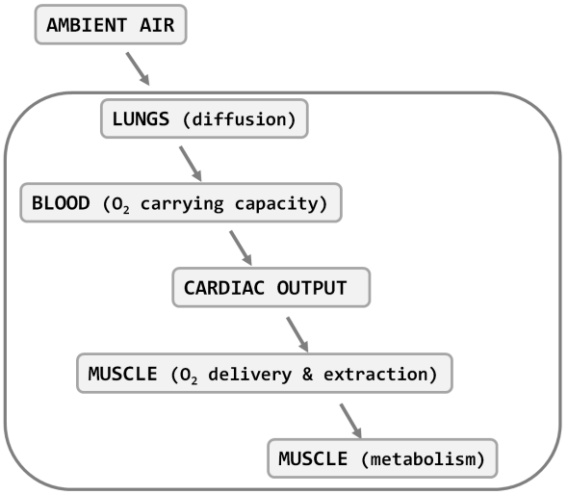

Figure 1.3: *Oxygen pathway from ambient air to muscles.*

Oxygen carrying capacity of the blood, cardiac output and muscle blood flow thereafter determine the supply of O_2 to the muscle cells. On the molecular level within muscle cells, the O_2 extraction by the cells and the metabolic efficiency and capacity finally limit the amount of energy available for muscle contraction and relaxation. In short, all the listed factors, although to a different extent, determine endurance performance in both hypoxia and normoxia. Moreover and even more importantly, each step of the cascade can be altered as a response to hypoxic or exercise training stimuli. The subsequent subchapters will present the hypoxia related physiological mechanisms from acute responses to chronic adaptations. The selected mechanisms of hypoxia induced changes related to performance can be seen in Fig 4.

Modulation of adaptations to hypoxic perturbation can, in terms of timing, be devided in acute (short term) and chronic (long term). The acute responses occurring immediately after the onset of hypoxic exposure are manly controlled through the nervous and endocrine system. Thus inducing prompt up-regulations of pulmonary and cardiovascular systems by, sympathetic efferent activity augmentation, vagal stimulation withdrawal and increased catecholamine activity [81]. This involves activation of stress limiting systems, mobilization of energy and its transportation to the functional

systems. In the long term adaptations those processes are followed by a phase of gene transcription activation that enables the transition from acute to chronic adaptation. After exposures of longer duration there is a successive activation of the regulatory genes consequently leading to enhanced gene coding for proteins involved in transport and utilization of O_2, angiogenesis and hematopoiesis [82].

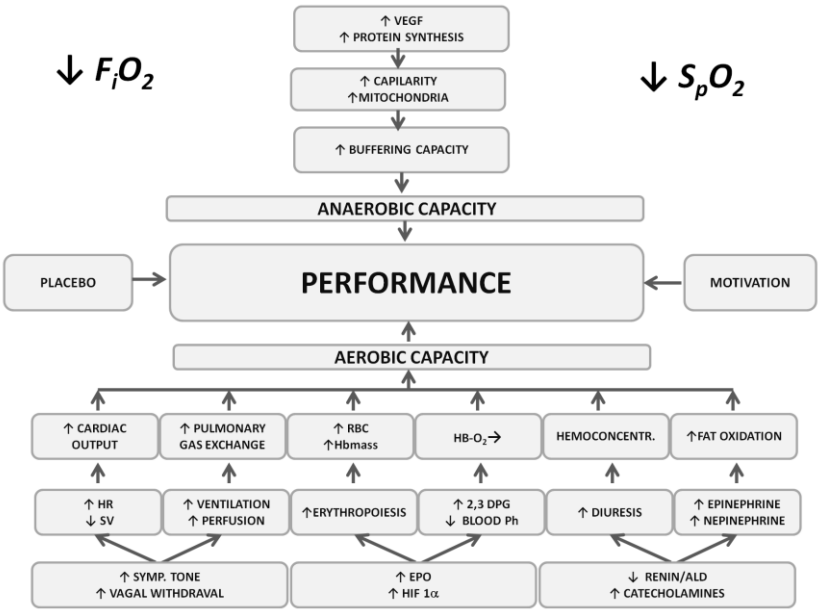

Figure 1.4: *Physiological mechanisms accountable for possible improvements following hypoxic exposures.*

Long term adaptations at the molecular level are chiefly regulated by expression of specific target genes that are upregulated during alternations of O_2 tension. The gene upregulation is governed by corresponding transcription factors that induce gene transactivation through a complex DNA binding and assembling mechanisms [82]. While a wide range of transcriptional factors are suggested to be O_2 regulated the hypoxia inducible factors seem to play a crucial role [83]. In particular the HIF1-α was the first transcription factor shown to be essential for hypoxia induced expression of EPO and other hypoxia related adaptational genes responsible for angiogenesis and metabolic reprograming [83]. While HIF1-α is under normoxic condition subjected to ubiquitination and proteasomal degradation, during tissue PO_2 reduction this process is inhibited and allows for HIF1-α stabilization and transcriptional activity [82].

2.3.1 Ventilatory adaptations

The immediate physiological response that occurs with the onset of hypoxia and reduces the induced hypoxemia is increased ventilation ($\dot{V}_E$) [12]. The initial increase in pulmonary ventilation results from the decreased arterial O_2 saturation sensed by arterial chemoreceptor's (carotid bodies) located primarily in the aorta [84]. In particular, the upregulation of the hypoxic ventilatory response involving augmented sensitivity of the carotid bodies and their afferents activity is the main underlying mechanism of ventilator adaptations to hypoxic exposures [5]. The most abundant acute change in $\dot{V}_E$ occurs with exercise in hypoxia, while the resting $\dot{V}_E$ does not change significantly until the altitude

exceeds 3000 m. This $\dot{V}_E$ increase has been termed the hypoxic ventilatory response (HVR) and has been shown to change during the course of acclimatization [85]. The increased HVR augments CO_2 excretion and thus induces respiratory alkalosis (pH > 7.4). This leads to elevated the excretion of bicarbonate by the kidneys that consequently normalizes pH [12]. Following the initial increase there seems to be a decline in HVR occurring around 20 minutes following the initiation of the hypoxic exposure, also known as the hypoxic ventilatory decline. Even if the changes in $\dot{V}_E$ seem to be among the main acute responses to different hypoxic perturbations, it has also been shown to adapt substantially when employing different hypoxic training modalities [85]. In particular besides longer chronic exposures and LH-TL protocols, increases in hypoxic chemosensitivity and subsequent increases in $\dot{V}_E$ have also been shown following shorter IHE and IHT training modalities [6, 12]. Higher ventilatory levels following adaptation to hypoxia can thus be achieved and is related to subsequently increased S_pO_2 [86]. Even though the increased HVR response seems beneficial for performance at altitude the studies investigating the effects of increased hypoxia induced ventilator chemosensitivity did not find any effect on ventilation at sea level even if the HVR has been significantly augmented [87].

At high ventilatory levels, occurring often during hypoxic exercise, the diffusion capacity of the lungs may pose a limitation to O_2 consumption [88]. In particular the short transit time of the RBC through the pulmonary vessels may not allow for complete blood pressure equalization between the alveolar gas and capillary blood [88]. However following altitude acclimatization this effect is, at least at rest, abated by lowering of diffusion limitations and concomitant decrements in cardiac output [89]. The possible hypoxia induced polycythemia can prolong the diffusion related problems especially during heavy exercise at altitude [89]. The response of the respiratory system therefore acutely provides an increased deliverance of ambient air to the alveoli and under the conditions of sufficient diffusion allows for increased amounts of O_2 to enter the plasma and subsequent binding to hemoglobin. The total hemoglobin mass, and other related hematological parameters, thereafter determine the amount of O_2 transferrable, per unit of blood, from lungs to working muscles.

2.3.2 Hematological modifications

The convective transport of O_2, powered by cardiac output and muscle blood flow, is enabled by the blood. The bloods plasticity and adaptability permits important adjustments of the blood O_2 carrying capacity to transport the available O_2 from lungs, via hart, to the various body organs. While regulatory adaptations of the blood are governed on the hormonal level and are induced over time, notable changes can already be seen immediately following acute hypoxic exposure. Namely, the primary hematological response to hypoxic stimuli is the hemoconcentration [90]. It is induced by the reduction of plasma volume that immediately provides increased relative percentage of the red blood cells (RBC) in the blood. The RBC are the main means of O_2 transport since most of O_2 is binded to the hemoglobin (Hb), the main constituent of RBC, with a negligible amount is diluted in the plasma [91]. Collectively, the amount of Hb and haematocrit are therefore the most important factors affecting the O_2 transportation capacity of the blood. An increase in RBC and associated increase in Hb

therefore provides greater O_2 flux. Thus, by increasing the O_2 carrying capacity of the blood, and augmenting O_2 flux to the muscles, provides advantage for endurance performance [3].

Hematopoiesis is governed by hormone Erythropoietin (EPO) under HIF-1α transcription and signaling activity. EPO is the key role erythropoietic hormone that stimulates erythropoiesis. It is chiefly synthesized by pertibular fibroblasts of the renal cortex as a consequence of a negative feedback loop responses to renal tissue hypoxia [92]. Its concentration can increase significantly in response to hypoxic exposure, due to hypoxic stimuli to the kidneys and liver. Significant increases in serum EPO have been shown after exposures as short as 84 min of simulated altitude corresponding to 4000 m [93]. Marked increase in serum EPO, as high as 353 % from baseline level, have been shown to be induced by acute hypoxic exposure [94]. Collectively these studies have shown that EPO production can be enhanced using hypoxia. However this does not necessarily always lead to subsequent increased production of RBC and elevation of total Hb mass [95]. Although ample evidence exist that sufficiently long chronic exposure to natural altitude provokes increases in EPO and subsequently augments total Hb mass, the responses of EPO to different hypoxic training modalities are not uniform. Even though hematological responses to simulated LH-TL are variable, it seems that only a sufficient hypoxic dose can directly affect the hematopoiesis. Namely, the study by Saunders et al. [96] showed that contrary to their previous findings, simulated LH-TL can enhance total hemoglobin mass if the aggregate sum of hypoxic exposure is 400 hours or more. Their findings are in agreement with the analysis, performed by Levine, regarding the necessary amount of hypoxic dose to induce hematological benefits [34]. Table 1.4 summarizes the findings of selected studies investigating effects of normobaric intermittent hypoxic training modalities on hematological adaptations.

Since sufficient hypoxic dose is a prerequisite for enhanced hematological response, the short exposure durations during IHT usually do not show any beneficial effect. Although some indirect benefits (i.e. Ferritin reduction and Reticulocyte concentration increase), have been shown following IHT [50] neither study employing normobaric hypoxic training did not show significant changes. Only the study by Meeuwseen [97] reported significant improvements in hematocrit (HCT) and Hb following IHT performed in hypobaric chamber. Collectively, the available IHT studies show that hematological benefits cannot be expected following one to two hours of daily exposures [14]. Similarly the effects of both IHE models on hematology are limited. As can be seen from Table 4, neither brief nor prolonged IHE model did not induce any significant changes in neither EPO nor RBC and Reticolucyte count. Thus, showing that IHE exposures performed from a few days to two weeks do not enhance blood O_2 carrying capacity [4].

Table 1.4: *Outcomes of various hypoxic training modalities studies on selected hematological variables.*

Subjects	Modality	Outcome metric	Metric response	Conclusions	Ref.
Multisport athletes	LH-TL Evaluation of different protocols	EPO Ret	C: ≈ H: + 80 % ↑ C: ≈ H: ≈	EPO increase is not necessarily followed by increase in Ret.	[44]
Endurance athletes	LH-TL 10 days F_IO_2= 0.15-0.16	EPO Ret	C: ≈ H: ↑ C: ≈ H: ↑	LH-TL significantly stimulated erythropoiesis	[98]
Ednurance athletes	LH-TL 23 days F_IO_2= 0.17	tHb mass Ret	C: - 1 % H: -1.8 % C: ≈ H: ≈	Red blood cell production is not stimulated	[39]
Elite runners	LH-TL 46 days F_IO_2= 0.17	tHb mass	C: ≈ H: +4.9 %	Extended LH-TL augments total hemoglobin mass	[96]
Elite runners	IHE ∑ 70 min, 5:5 5·week^{-1}/4 weeks F_IO_2= 0.12-0.10	EPO Trf Ret	H: ≈ compared to C H: ≈ compared to C H: ≈ compared to C	No effect on hematological parameters	[63]
Trained runners	IHE 180 min 7·week^{-1}/2 weeks F_IO_2= 0.123	EPO RBC	C: + 5.8 % H:- 6 % C: ≈ H: - 2 %	No significant differences between groups	[75]
Trained swimmers	IHT 12.5 min 3·week$^{-1/}$5 weeks High intensity	HCT Hb	C: ≈ H: ≈ C: ≈ H: ≈	No significant effect of IHT on hematological parameters	[56]
Trained cyclists	IHT 60 min 3·week$^{-1/}$ 6weeks F_IO_2= 0.16	Fer Ret	C: - 42 % ↑ H: - 37 % ↑ C: + 100 % ↑ H: + 100 % ↑	Augmented erythropoiesis following IHT	[50]

C: Control group; H: IHE group; LH-TL: Live high - train low; IHE: Intermittent hypoxic exposures; IHT: Intermittent hypoxic training; EPO: Erythropoietin; Ret: Reticulocytes; tHb mass: Total hemoglobin mass; Trf: Transferrin; RBC: Red lood cells; HCT: Hematocrit; Hb: Hemoglobin; Fer: Ferritin; + increase; - decrease; ≈ no change; ↑significant ($P < 0.05$).

The adjustment of the blood O_2 affinity of is one of the most significant contributors to hypoxic adaptation. It plays an irreplaceable role both in O_2 diffusion within the lungs and even more importantly in the O_2 diffusion from the blood to various organs [99]. The red blood cell concentration, pH, partial pressure of CO_2, 2,3-diphosphoglyceric acid concentration and temperature, are among the most important factors that influence the O_2 affinity [99]. Regarding performance at altitude, there seems to be no agreement on the question of whether the decreased or increased O_2 affinity is more advantageous at different altitudes. Lowering of the blood O_2 affinity, mainly due to an increase in diphosphoglicerate (DPG) following hypoxic training modalities, appears to be advantageous for performance at altitudes up to 5,000 m altitude [100]. Its benefits relate to reductions of the circulatory load required to assure adequate tissue oxygenation due to maximizing the arterial-venous O_2 saturation process. However above 5,000 m altitude the increased blood O_2 affinity is preferable due to the fact the maintenance of high arterial O_2 saturation is a paramount at extreme altitudes [101].

2.3.3 Cardio-vascular adjustments

The responses of the cardiac function are also among the most important changes that occur during hypoxic exposure and help to restore and augment the systemic O_2 transport. The main adjustments of the system are the changes in cardiac output (CO). It has been suggested, that the CO increases during acute hypoxic exposure [102]. On the other hand some data suggest that the CO remains approximately the same [103] or even slightly decreases [104]. The changes are modulated through adjustments of heart rate (HR) and/or stroke volume (SV) as the two determine the CO. The normal hemodynamic response to hypoxic exposure is an increase in HR (tachycardia). It occurs almost immediately after the onset of acute hypoxia and is due to redistribution of blood flow to the brain, kidneys and skeletal muscles which require greater O_2 dependency [82]. The increase is facilitated mainly through sympathetic nervous system activation [105]. Unlike in acute hypoxia during the acclimatization resting HR tends to decrease to normal (sea level) values or even below. The reductions in HR during acclimatization are mainly due to decrease in sympathetic activity on one hand and increases in parasympathetic activity on the other [105]. The SV on the other hand decreases during acute hypoxia and remains decreased during chronic exposure [106].

When combining hypoxic exposure with exercise, initially an increase in CO occurs, providing sufficient amount of O_2 to be transported to exercising muscles and other tissues [81]. During submaximal exercise, this adaptation (↑ CO) allows for similar amount of O_2 to be transported and utilized by the exercising muscles as compared to sea level. The increased CO during exercise is similarly to rest in hypoxia manly mediated by an increase in HR [107], since SV seems to be only marginally affected initially [108]. With prolonged acclimatization CO is reduced for a given power output as a result of both decrease in HR and SV [81]. HR progressively decreases as a result of both, the cardiac responsiveness attenuation to adrenergic stimulation [109] and an increase in vagal tone [105]. The reduction in SV has been shown to recede following two weeks, and although the exact mechanisms are unidentified the well established plasma volume loss during initial acclimatization period might be one of the factors [81].

The muscle and cerebral blood flow also responds actively to hypoxic exposure. With the purported increase in CO the body accommodates the changes in muscle perfusion [110]. It has already been firmly established that the peripheral blood delivery becomes more efficient after altitude exposure [111]. In particular, through a decrease of the ratio between fiber and number of capillaries therefore enabling a greater muscle blood flow, a phenomenon, directly related to the decrease in muscle fiber volume. We could therefore speculate that the blood flow would be increased within the exercising muscle in hypoxia, since it has recently been shown that the quantity of hemoglobin bound O_2 is the crucial factor influencing the muscle blood flow [112]. However, some studies, show different results [108], indicating that the amount of blood flow decreases during exercise at altitude. This is supposed to be the result of an increases in saturation levels and RBC, which allow for a lower blood flow to induce the same O_2 delivery to muscle tissue and also shows that the O_2 delivery to muscles during exercise is strictly regulated and uneffected by prolonged hypoxic exposure [105].

2.3.4 Muscle tissue adaptations

The muscle tissues are among the main end users of the delivered O_2 at exercise. It has been reasoned that the changes on molecular level within the muscle tissue are one of the crucial outcomes of different hypoxic training modalities. This holds especially true for IHT modality, where a concomitant exercise and hypoxic stimuli should result in specific response of muscle tissue. Given that exercise results in muscle relative hypoxia and since hypoxia per se decreases the myoglobin O_2 saturation decreasing intramyocellular O_2 partial pressure this leads to metabolic load surplus [113]. As endurance training solely augments muscle oxidative capacity and enhances muscle capillarity, the addition of hypoxia stimuli to training should result in pronounced response of muscle tissue [114]. The molecular level responses are governed by transcriptional and signaling activity of HIF1-α. This in turn activates a vast number of genes with functional significance for muscle tissue remodelling and adaptation [18]. That is also confirmed by the complex pattern of gene expression following IHT but not normoxic endurance training, advocating for a specific muscle tissue response to training in hypoxia [115]. These responses are the main non-hematological adaptations to different hypoxic training modalities [40]. Among the main muscular tissue related results of hypoxic training adaptations are the enhanced concentration of the glycolytic enzymes, myoglobin and angiogenic factor VEGF [116]. Among the possible beneficial adaptations following hypoxic training is also increased buffering capacity and thus, augmented muscle pH regulation capacity. In the seminal study, Mizuno et al. [28] showed that buffering capacity can be enhance after only two weeks of IHT. These findings were confirmed in subsequent studies of IHT, while not all LH-TL showed augmented buffering capacity of the skeletal muscles following the protocol [40]. Moreover, improved coupling of muscular bioenergetics and mitochondrial function has been shown to enhance mitochondrial efficiency [117].

While these adaptations are obviously beneficial, also some detriemental consequences of chronic hypoxia and exercise have been reported. In particular, muscle mass and perfusion reduction, as well as mitochondrial and oxidative enzymes capacity and activity attenuation, have been shown following chronic hypoxic exposures [108, 118]. Since these unfavorable consequences occur following prolonged chronic hypoxic exposures they can be avoided by selecting appropriate hypoxic training modalities (i.e. shorter intermittent exposures). Even though it has been speculated that hypoxia induces muscle hypotrophy, the evidence from the studies show that if chronic exposure to altitude does not exceed 3500 meters, and the nutrition and activity levels are matched, the hypotrophy of muscle tissue does not occur [90]. This shows that also by employing LH-TH protocols certain negative effects on muscle function can be avoided. Also, the paradoxical lactate values, obtained from different studies acclimatization studies have to be mentioned. In particular, after the acute exposure, the lactate values tend to be higher than at sea level, but after a prolonged stay at altitude, they are paradoxically reduced. This »lactate paradox« appears with lower absolute and relative values, compared to the same relative workload at sea level. The characteristics of this phenomenon have been debated recently, but no final consent has been reached since the studies show variable results [119].

2.3.5 Heterogeneity of the physiological responses to hypoxia

After the onset of altitude exposure, the magnitude and timing of the physiological responses vary among individuals. It has been shown in many studies that some individuals adapt to hypoxic stimulus much faster and more efficiently as compared to others, that may even not be able to acclimatize properly [81]. Some studies [30, 120] on altitude training protocols have even suggested dividing individuals, according to their physiological responses, and named them accordingly ("responders" – "non responders"). Their results have shown a wide inter-individual variability, especially in the hematopoietic response to hypoxic training modalities. The non – responders did not improve endurance performance or $\dot{V}O_{2peak}$, but these differences were not related to the changes in EPO production. They suggested that it is not possible to determine the response of a person to altitude training solely by monitoring erythropoiesis. The possible genetic determinants of this variability have also been investigated [121]. They investigated the correlations between EPO gene markers or eight genes involved in EPO regulation and subsequent variations in post altitude EPO levels but did not find any significant correlation. The problem of differentiation between responders and non-responders to altitude training therefore remains to be answered. Currently there is no efficient prediction method for individual responses to hypoxic stimuli, thus performing the protocols and carefully monitoring the responses seems to be the only reasonable option.

The differences between genders in adaptation capacities following altitude exposure are also an important consideration. The possible differences in erythropoiesis and hematological variables are of most interest, since this has been postulated as one of the key responses to different hypoxic training protocols [19]. The basic prerequisite for hypoxic training to be effective is the normal iron stores. However, due to mechanical haemolysis, sweating, low iron intake and blood loss due to menstruation in female athletes, this is often not the case [122]. Female trained athletes frequently experience low Hb and HCT levels due to the above mentioned chronic depletion of iron stores. The problem can therefore occur if the iron levels are not sufficient to follow the hematopoetic demands. However, it has recently been shown that no differences in responses to altitude training between male and female athletes exist if the iron levels are sufficient [47]. Another consideration regarding the gender differences is the effect of ovulation. The effects of different phases of a menstrual cycle, on altitude acclimatization responses have not yet been thoroughly described. However, according to the latest findings regarding the changes during different phases of menstrual cycle, considerable differences cannot be expected [123]. Even though the differences between males and females in responses to altitude training seem negligible, this area needs further scientific investigation. Thus the monitoring of hormonal and iron status of female athletes before, during and after hypoxic training seems to be warranted.

2.4 Current use of hyperoxia in sport

Hyperoxia refers to excess levels of O_2 and is within a human organism manifested as increased oxygenation of the tissues. It is a result of breathing elevated O_2 levels under either normobaric or

hyperbaric condition. While a reduction in F_IO_2 leads to impairment of performance, on the contrary, when inspired O_2 fraction is increased, endurance performance has been shown to improve [124]. Clearly, the pressure of inspired O_2 influences performance since the O_2 availability is among the main determinants of prolonged muscle work. In that manner, the use of higher F_IO_2 levels during athletic training has been widely utilized. Even though the use of additional O_2 is, for reasons of fairness and equality, not allowed in official competitions, many athletes use it during their training. In particular it has been shown that training in hyperoxia allows for higher workloads, under the same heart rate and rates of perceived exertion [125]. Moreover it has been shown that the duration of the training at a given work load can be prolonged under hyperoxic condition. These findings suggested, that by applying hyperoxia during training, the overloading effect of training, especially in regards to mechanical load could be achieved easier as compared to normoxic training. The mechanism involved in the purported hyperoxic training adaptations, include the reduction of glycogen utilization and subsequent lactate production attenuation [126]. Moreover, hyperoxia also induces decrements in exercise induced epinephrine production and heart rate [127]. Even if the physiological effects have been firmly established, the translation of these to improved performance following hyperoxic training is unclear. This model has also been integrated to the LH-TL hypoxic training modality. Namely, the additional O_2 was used during training at altitude to simulate the training at sea level. It has been shown that significant increases in arterial oxyhemoglobin saturation and subsequent increases in power output can be attained by using supplemental O_2 during the LL-TH training sessions at altitude [128]. Regarding the sports application the use of supplemental O_2 is also widely used for high altitude expeditions and underwater diving.

Breathing O_2 enriched air instantly leads to a significant PO_2 increase within all organs and tissues and initiates several different physiological mechanisms [129]. These adjustments are beneficially used in clinical praxis for treatment of several medical disorders, mostly related to therapeutic use for hematological disorders, traumatic soft tissue injuries and treatments of thermal injuries [130]. Of most interest for the thesis is the utilization of hyperoxia as means for augmenting the efficiency of hypoxic training modalities. Namely it has been suggested recently, that relative decrease in PO_2 induced by hyperoxia and subsequent nomoxia can trigger the negative loop response of EPO production [9]. Thus subsequently, increased EPO levels can lead to enhanced hematopoiesis and augmentation of total Hb mass. This mechanism has already been proposed and used in clinical environment for treatment of anemia [11]. Applying this to sport science, the mechanism would provide a simple means for naturally boosting HCT and total Hb volume thus, enhancing performance by improving the O_2 flux. Altough the findings of Balestra et al. [9] show that the relative change is important, some other studies did not find the same results. In particular, the findings of the study by Kokot et al. [131] showed a significant decline in EPO levels following hypoxia. Furthermore, recent studies by Momeni et al. [132] and Keremidas et al. [133] did not show any significant augmentation or have even shown a transient decrease of EPO concentration following short normobaric hyperoxic exposure, respectively. The discrepancies in findings show, that this intriguing subject needs more scientific attention.

2.5 Drawbacks of oxygen manipulations

Changes in PO_2 lead to significant and immediate responses of human physiological systems. As has been shown in the previous paragraphs, these responses can lead to beneficial adaptations. However, the homeostasis maintaining physiological responses can also lead to harmful and detrimental consequences, especially when the adaptation time is insufficient. In short, while O_2 is essential for human life, it can also act as a toxin, when its levels are either above or below normal for a certain amount of time. Firstly, we will focus on the effects of the decreased PO_2. If an unacclimatized person is acutely exposed to either simulated or natural altitude above a certain threshold, undesired medical disorders can occur. Firstly, the condition termed acute mountain sickness usually manifests. While it is uncommon below 2000 meters above sea level the vast majority of the sea level dwellers experience it when acutely exposed to 3800 meters or above [134]. It is characterized by headache, nausea, fatigue, anorexia and sleep disturbance. The symptoms usually abate within days if enough time is provided for sufficient acclimatization. Although acute mountain sickness is usually self limiting, it can lead to pulmonary and/or cerebral edema. Both strenuous exercise and further ascent can advance this detrimental process rapidly. While the pulmonary edema is induced by multitude factors, the main mechanism seems to be the change in pulmonary hypoxic vasoconstriction leading to raised pulmonary arterial pressure. Thereby inducing stress failures of the vessels and allowing for proteins and certain blood constitutes to enter the interstitial space. On the other hand, the mechanism responsible for the cerebral edema are not yet fully understood, but it seems that it has a vasogenic origin leading to increases in permeability of the blood-brain barrier [134]. High altitude related edemas present lethal medical conditions requiring immediate medical treatment and evacuations to lower altitudes. Even though these conditions must be avoided during utilization of different hypoxic training modalities, one has to realize the possible outcomes of inadequate acclimatization.

In regards to training status, all the above mentioned drawbacks have to be taken into account. Special care should be taken to prevent their onset. In particular the possible consequences of sleep disturbance, anorexia and lassitude do not contribute beneficially to performance capacity [12]. All this disturbances, plus hypoxia per se, can also lead to unfavorable changes within the muscle tissue. These usually occur following chronic exposures to severe hypoxia and among others include the loss of muscle mass with concomitant decreases in muscle fiber size [18]. It has also been shown, that muscle oxidative capacity can be affected by the decrements of respiratory chain and Krebs cycle enzyme activities [135]. Moreover, in mountaineers, a significant increases of lipofucin within the muscle fibers, possibly a consequence of increased oxidative stress, have been shown following expedition [18]. Jointly these data show, that the use of hypoxic training modalities does not only bring about the beneficial effects but can also induce detrimental changes on both central and local level.

Similarly as with the decreased PO_2 its elevated partial pressure can also lead to potentially negative effects. The noxial effects of excessive O_2 are summed in the term oxygen toxicity. It can be manifested in many ways, although two primary outcomes exist: pulmonary and central nervous system toxicity. The pulmonary toxicity is usually the consequence of prolonged hyperoxic breathing

under normobaric condition (> 10 hours). It manifests as tickling and coughs at the beginning and can lead to severe coughing and periods of dyspnea [136]. On the other hand, breathing high O_2 levels under elevated pressure (hyperbaria) can lead to central nervous system toxicity. The early symptoms, of this dangerous medical condition, include visual and audile disturbances and can rapidly advance to severe seizures and subsequent unconsciousness. However when applying normobaric hyperoxic breathing for less than 4 hours daily, no adverse effects can be expected [137].

3 Aims and Hypotheses

The aims of the thesis were the following:

- To test the hypothesis that normobaric hypoxic training can improve performance at both sea level and simulated altitude.[1]
- To examine the effects of intermittent hypoxic exposures at rest on performance.[2]
- To investigate whether normobaric hypoxic protocols employing short exposures can improve oxygen flux through hematological and ventilatory adaptations.[1, 2, 3]
- To investigate whether short intermittent hypoxia can alter performance at altitude.[3]
- To test the hypothesis that combining hypoxic and hyperoxic breathing can augment erythropoietin production.[4]

[1] *Study I*: Normoxic and hypoxic performance following 4 weeks of IHT.

[2] *Study II*: Effects of IHE on performance in normoxia and hypoxia.

[3] *Study III*: Short intermittent hypoxic exposures augment ventilation during hypoxic exercise.

[4] *Study IV*: Acute hyperoxia followed by mild hypoxia does not increase EPO production.

4 Normoxic and hypoxic performance following 4 weeks of IHT

4.1 Introduction

This study investigated the effects of performing endurance training under hypoxic condition on subsequent exercise performance in normoxia and simulated hypoxia. The ability of hypoxic training in improving sea level and altitude aerobic performance depends on whether the hypoxic "dose", or rather the extent and duration of the hypoxic stimulus, is sufficient to initiate physiological changes that would be manifest in improved performance [13]. Several different hypoxic training modalities have been developed over the last few decades [138]. One such training regimen advocates daily training sessions in a hypoxic environment, and has been termed "intermittent hypoxic training" (IHT). It is based on the principle of the establishment of substantial reductions in the partial pressure of O_2 in muscle cells, induced by concomitant hypoxia and exercise. Thus, stimulating specific signaling pathways, mediated mainly by the hypoxia-inducible factor (HIF), and resulting in performance-relevant changes within the muscle [18]. The main allure of the IHT is the potential concomitant benefits of both training and hypoxic acclimatization, whereas the main drawback of this modality is the inability to perform high intensity training [1].

Nevertheless, results regarding the effect of the IHT training regimen on exercise performance remain equivocal. Studies on moderately trained subjects or trained subjects [18] using normobaric hypoxia have reported both improvements in altitude [52, 54], and sea level performance [55, 139], as well as no effect on hypoxic [50, 140] and normoxic performance [87, 141]. Bakkman et al. [142] have even suggested that IHT may be disadvantageous, especially for muscle oxidative function, compared to the same training in normoxia.

Since the main aim of the IHT modality is to enhance sea level performance, very few studies have included the evaluation of both sea level and altitude performance following the hypoxic training protocol. For athletes participating in winter sports, hypoxic training may be valuable in the preparation for summer training camps at altitudes where snow covered terrains are available. Altitude acclimatization conducted prior to attendance at an altitude training camp may improve the quality of the training at altitude. Among the few studies using trained athletes, that have incorporated testing of hypoxic performance following IHT protocol [50, 143], the results are also not consistent. Whereas Terrados et al. [143] observed improvements in total work performed following hypoxic training, others have not [50]. Similarly, very few studies using untrained subjects [52-54] performed tests under both hypoxic and normoxic conditions and controlled the subjects' training. This is especially important since the strict control of training permits elucidation of the separate effects of training and

hypoxia *per se*.

The differences in the hypoxic doses used by the various studies, namely level of simulated altitude, total duration of the daily exposure and number of exposures, make it difficult to compare the results of different studies that have utilized the IHT regimen [1], and may also be the source of some of the discrepancies.

Further studies investigating performance outcomes also in hypoxia are clearly warranted, to elucidate the potential benefits of hypoxic training on altitude performance [12, 18]. Although the results of the studies on both trained and untrained subjects are not very compelling, it seems, that IHT may be used effectively, even by elite athletes, for altitude competition preparation [3]. In addition, the possible beneficial effects of IHT protocol for hypoxic performance could provide the rationale for using it as a preparation tool for soldiers and pilots performing demanding missions at altitude [144]. Since some studies have shown potential benefits and the optimal dose has not yet been identified, we decided to test a specific IHT regimen, that we hypothesized, according to previously tested protocols, would provide benefits.

The aim of the present study was, therefore, to evaluate the effect of a specific IHT regimen in moderately active subjects on sea level and altitude performance and aerobic capacity with a carefully controlled and identical training of all subjects. We hypothesized that as a consequence of training in normobaric hypoxia, improvements in hypoxic performance will be greater than after normobaric normoxic training.

4.2 Methods

4.2.1 Subjects characteristics

Eighteen healthy, young and moderately active males participated in this study. The participants had no previous endurance training history, and were randomly assigned to either the Hypoxic (n=9; age: 20.1 (3.0) yrs; stature: 182.8 (4.3) cm; body mass: 77.4 (8.7) kg; BMI: 23.1 (2.3) $kg \cdot cm^2$; BF: 11.7 (4.1) %) or Control (n=9; age: 22.1 (4.0) yrs; stature: 179.3 (5.0) cm; body mass: 72.9 (9.7) kg; BMI: 22.6 (2.4) $kg \cdot cm^2$; BF: 10.4 (3.0) %) group ($p < 0.05$). Subjects gave their informed consent prior to the study, which was approved by the Institutional ethics committee, and was performed in accordance with the guidelines of the Helsinki Declaration.

4.2.2 Study design

The training protocol (Fig. 4.1) consisted of twenty training sessions on a cycle ergometer over a period of four weeks (5 sessions per week). Hypoxic and normoxic aerobic capacity and endurance performance were evaluated before (Pre), in the middle (Mid), at the end (Post), and ten days after (After) the IHT training program (Fig. 4.1). Subjects were requested not to participate in any physical activity at least two days before, and not to drink caffeinated beverages at least 4 hrs prior to the performance tests. Each test period (Pre, Mid, Post and After) comprised two days of testing, separated

by a rest day. During the test days, two performance tests were conducted on each day: one incremental ($\dot{V}O_{2peak}$) and one constant power (CP) test to exhaustion in either normoxic or hypoxic conditions (Fig. 4.1). The order of the hypoxic and normoxic tests was randomized and counterbalanced. Anthropometric measurements, hematological examinations, and pulmonary function tests were conducted on separate days before the exercise test days (Fig. 4.1).

4.2.3 Endurance exercise training

Each training session comprised a 5-min warm-up at 20 % of normoxic peak power (W_{max}), followed by a 60-min bout of exercise, and a 5-min recovery period. The exercise intensity corresponded to the heart rate (HR) achieved at 50% W_{max}, determined on a previous occasion from tests of maximal aerobic capacity ($\dot{V}O_{2peak}$) in normoxia and hypoxia. Thus, the Control group trained at 50% of their normoxic W_{max}, whereas the Hypoxic group at 50% of their hypoxic W_{max}. The W_{max} was calculated by the equation: $W_{max} = PO_{final}$ (t/60 × 30 W), where PO_{final} refers to the last workload completed, and t is the number of seconds [145]. The Control group performed all their training sessions in normobaric normoxia, at 300 m above sea level (altitude of the Jozef Stefan Institute laboratory). The Hypoxic group performed their training in a climatic chamber (IZR d.o.o., Skofja Loka, Slovenia) at the Jozef Stefan Institute, which maintained the air temperature and humidity at 25 °C and 50 %, respectively. The normobaric hypoxic environment (F_IO_2 = 0.12) within the climatic chamber was maintained with a Vacuum Pressure Swing Adsorption system (b-Cat, The Netherlands) that delivered O_2 depleted air to the chamber. Samples of the chamber air were regularly analyzed for O_2 and carbon dioxide (CO_2) content, and the delivery of the O_2-depleted air was regulated according to the results of the gas analysis. In the event that the O_2 level achieved the pre-set fraction of O_2, delivery of O_2-depleted air was discontinued. In the event that the O_2 level dropped below the pre-set level, or the CO_2 concentration increased by 0.5 %, a large industrial-type fan was activated, drawing normoxic air from the external environment into the chamber. Since there was a constant flow of gas into the chamber, a relief valve prevented any undue pressure fluctuations within the chamber. During the training, all subjects' HR was monitored continuously (Hosand system, Italy), and the external workload was adjusted to maintain the target HR. The HR monitoring software enabled the establishment of an individual training HR interval for each subject. The individual interval was set within ± 4 beats per minute of the targeted HR measured during the first $\dot{V}O_{2peak}$ test, attained in normoxia (Control group) or hypoxia (Hypoxic group), respectively. Whenever the subject's HR was out of the set interval a visual signal prompted the researcher to either increase or decrease the work rate accordingly. During each training session, subjects provided ratings of perceived exertion on a Borg scale (0 - 10), separately for the peripheral (RPE_{leg}) and central (RPE_{cen}) sensations.

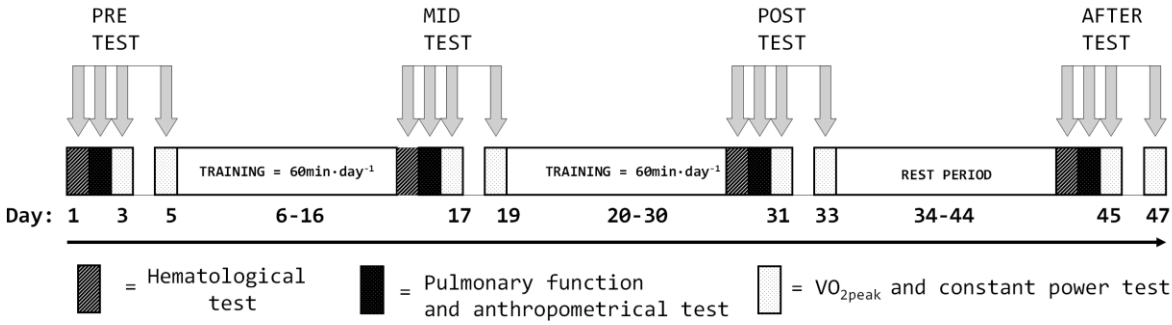

Figure 4.1: *Schematical presentation of the testing and training schedule.*

4.2.4 Anthropometry and pulmonary function testing

Measurements of body mass, body stature and skinfolds were performed in the Pre and Post testing periods only. Percent body fat was calculated from nine skin fold measurements (triceps, subscapular, chest, suprailiac, abdominal, thigh (mid, above, below) & inguinal sites) according to the equation of Jackson and Pollock [146]. The pulmonary function tests were performed at each testing period (Fig. 4.1) and comprised: forced vital capacity (FVC), forced expiratory volume in 1 sec (FEV_1), peak expiratory flow (PEF), slow vital capacity (SVC), and maximum voluntary ventilation (MVV). The tests were performed according to the criteria published by Miller et al. [147]. The pneumotachograph (Cardiovit AT-2plus, Schiller, Baar, Switzerland) was calibrated before each test with a 3-L syringe. Each test was performed three consecutive times and the highest of the three acceptable values was used for the following analysis.

4.2.5 Hematological tests

Blood samples were drawn from the antecubital vein on the morning of the first exercise test day in each of the four testing periods (Fig. 4.1). The subjects were overnight-fasted prior to the procedure. The samples were analyzed by a hematological laboratory (Adria laboratories d.o.o., Ljubljana, Slovenia) using the cythochemical impedance method (Pentra120; Horiba ABX Diagnostics) for hemogram (coefficient of variation: <2 %), and the turbo-bidiametrical method (Hitachi 912; Roche Diagnostics) for transferrin analysis. Blood samples were analyzed for red blood cell count (RBC), hemoglobin (Hb), hematocrit (HCT), transferrin and ferritin.

4.2.6 Exercise testing

All exercise performance tests were performed in the same sea level laboratory (Valdoltra Orthopaedic Hospital, Ankaran, Slovenia), under equivalent environmental conditions. The normoxic (F_IO_2=0.21) and hypoxic (F_IO_2=0.12, corresponding to altitude of 4500 meters) $\dot{V}O_{2peak}$ tests comprised an incremental-load exercise to exhaustion, performed on an electrically braked cycle-ergometer (ERG 900S, Schiller, Baar, Switzerland). The protocol commenced with a 10-min rest period, and a 2-min warm-up at a work rate of 60 W. The tests in hypoxia included a 5-min rest period in normoxia, and a

5-min rest period in hypoxia prior to the 2-min warm-up. Thereafter, the load was increased each minute by 30 W. $\dot{V}O_{2peak}$ was taken as the average of the 60-sec peak $\dot{V}O_2$ values prior to termination of the test. Attainment of $\dot{V}O_{2peak}$ was confirmed on the basis of the following criteria: respiratory exchange ratio >1.1, plateau in $\dot{V}O_2$ during the last 15 sec of the trial, and inability to maintain the cycling cadence at the required level of 60 rpm.

The normoxic and hypoxic constant power tests were performed at an intensity corresponding to 80% of normoxic $\dot{V}O_{2peak}$. The protocol consisted of a 2-min rest period (either in normoxia or hypoxia, depending on the test), a 2-min warm-up at a work rate of 60 W, and then cycling to exhaustion with the pre-determined workload. Time to exhaustion was determined by the number of seconds the subject was able to sustain the pedaling cadence of 60 rpm. During the $\dot{V}O_{2peak}$ tests, the $\dot{V}O_2$ and ventilation ($\dot{V}_E$) were recorded at 10-sec intervals with a metabolic cart (CS-200, Schiller, Baar, Switzerland). Subjects breathed through a low resistance two-way valve (Model 2, 700 T-Shape, Hans Rudolph, Inc., Shawnee, USA). In the normoxic condition they inspired room air, and in the hypoxic condition they inspired a pre-mixed humidified breathing mixture (0.12 O_2, 0.88 N_2) from a 200-L Douglas bag. Flow and volume calibrations of the metabolic cart were conducted with a 3-L plexiglas syringe prior to each test session. The gas analyzers were calibrated with two standard calibration gas mixtures. During the tests we also continuously monitored HR (Vantage NVTM, Polar Electro, Kempele, Finland) and capillary oxyhemoglobin saturation (S_pO_2) using a pulse oxymetry device (Nellcor, BCI 3301, Boulder, USA) with ± 2 units accuracy across the range of 70-100% [148]. If necessary, we pre-warmed the finger used for the measurement of S_pO_2 with warm water.

4.2.7 Data analysis and statistical evaluation

Differences between group means, in the two conditions, over the training period were analyzed with a 3-way ANOVA [group (Control and Hypoxic) × condition (normoxia-hypoxia) × testing period (Pre, Mid, Post, After)]. A Tukey *post-hoc* test was used to compare the specific differences. The significance level was set at 5%. Due to technical problems (malfunction of the gas sensor cell within the metabolic cart) encountered in four of the hypoxic Pre $\dot{V}O_{2peak}$ tests, we used a regression model to predict the missing Pre $\dot{V}O_2$ hypoxic values from the power output - $\dot{V}O_{2peak}$ relationship produced from the normoxic and available hypoxic tests. Due to this same technical problem, the Mid $\dot{V}O_{2peak}$ results are also omitted from the analysis. All statistical analyses were performed using Statistica 5.0 (StatSoft, Inc., Tulsa, USA). All data are presented as means (SD) unless otherwise indicated.

4.3 Results

Exercise training

The average power output, HR and S_pO_2 values during each week for the Hypoxic and Control groups are presented in Table 4.1. The power output of training increased by ~15 Watts in the Control and by ~10 Watts in the Hypoxic group, and was significantly lower in the HYPO group at all times. The HR remained fairly constant (± 3 %) during the training in both groups, although there were some significant fluctuations in both groups at the 2nd, 3rd and 4th training week. However, rates of perceived

exertion were significantly higher in the Hypoxic group compared to the Control group during all training sessions (Table 4.1). For the HYPO group, the values of S_pO_2 during the training remained similar throughout the training period.

Table 4.1: *Average weekly power output (PO_{mean}), heart rate (HR), pheripheral (RPE_{leg}) and central (RPE_{cen}) rate of perceived exertion and capillary oxyhemoglobin saturation (S_pO_2) of the control and hypoxic group during training sessions.*

	CON group				HYPO group			
	1st week	2nd week	3rd week	4th week	1st week	2nd week	3rd week	4th week
PO_{mean} (watts)	152 (18)	158 (17)	159 (16)	167 (21) *	114 (22) #	118 (25) #	122 (23) #	124 (19) # *
HR (beats·min^{-1})	146 (9)	140 (7) *	140 (7) *	144 (8)	151 (8) #	148 (8) #	145 (9) # *	145 (9) *
RPE_{leg}	2.2 (0.8)	2.2 (0.9)	2.3 (1.2)	2.6 (1.4)	5.2 (1.2) #	5.4 (1.1) #	6.2 (2.2) # *	5.5 (1.1) #
RPE_{cen}	1.7 (0.9)	1.8 (0.7)	2.0 (1)	2.0 (1.3)	4.7 (0.9) #	4.8 (0.8) #	5.6 (1.1) # *	4.7 (0.9) #
S_pO_2	/	/	/	/	79 (3)	78 (3)	78 (4)	80 (5)

Values are mean (SD). (*) Significant differences within groups between week averages ($p < 0.05$); (#) differences in weekly averages between groups ($p < 0.05$).

Anthropometrics, pulmonary function and hematology

No significant changes were noted in subjects' anthropometry after the IHT protocol. The values of the pulmonary function tests in both groups ranged between 5.5 (1.2) l. and 5.9 (0.6 l) l. for FVC; 4.6 (0.9) l. and 4.9 (0.5) l. for FEV_1; 5.0 (0.8) l. and 5.9 (1.1) l. for SVC and between 183 (3) l. to 201 (27) l. for MVV. There were no differences between or within groups at any testing period. Similarly, no significant differences in any of the hematological variables were noted between the Control and Hypoxic groups Pre, Mid, Post and After the IHT (Table 4.2).

Table 4.2: *Values of erythrocytes (RBC), hemoglobin (Hb), hematocrit (HCT), transferrin (Trf) and ferritin (Fer) at all testings. No significant differences were shown in both groups ($p < 0.05$).*

	CON group				HYPO group			
	PRE	MID	POST	AFTER	PRE	MID	POST	AFTER
RBC (10^{-12}•L)	5.0 (0.3)	5.0 (0.3)	4.8 (0.2)	5.0 (0.2)	5.0 (0.3)	4.9 (0.3)	5.1 (0.2)	5.0 (0.4)
Hb (g•L^{-1})	150 (10)	151 (13)	141 (12)	145 (12)	142 (9)	142 (10)	142 (10)	146 (10)
HCT (%)	0.45 (0.0)	0.46 (0.03)	0.44 (0.03)	0.45 (0.03)	0.45 (0.03)	0.44 (0.03)	0.45 (0.03)	0.45 (0.03)
Trf (g•L^{-1})	2.6 (0.3)	2.5 (0.3)	2.6 (0.3)	2.6 (0.4)	2.9 (0.4)	2.7 (0.3)	2.7 (0.3)	2.7 (0.3)
Fer (ng•mL^{-1})	91.8 (63)	85.8 (68)	76.9 (44)	68.5 (42.6)	77.2 (26.6)	76.8 (9.1)	86.8 (29.4)	71.8 (21.0)

Values are mean (SD).

Exercise tests

Hypoxic $\dot{V}O_{2peak}$, W_{max} and S_pO_2 values were lower than the normoxic values in all testing periods (Figs. 4.2 & 4.3). The Control group improved their Post and After normoxic $\dot{V}O_{2peak}$ compared to the Pre, but hypoxic $\dot{V}O_{2peak}$ remained unchanged. There were no hypoxic training-induced changes observed in the normoxic and hypoxic $\dot{V}O_{2peak}$ of the Hypoxic group (Fig. 4.2). W_{max} improved After LH-TL only for the Control group in normoxia (Fig. 4.3). No changes were observed in hypoxic W_{max} Pre, Post and After the LH-TL protocol for both groups.

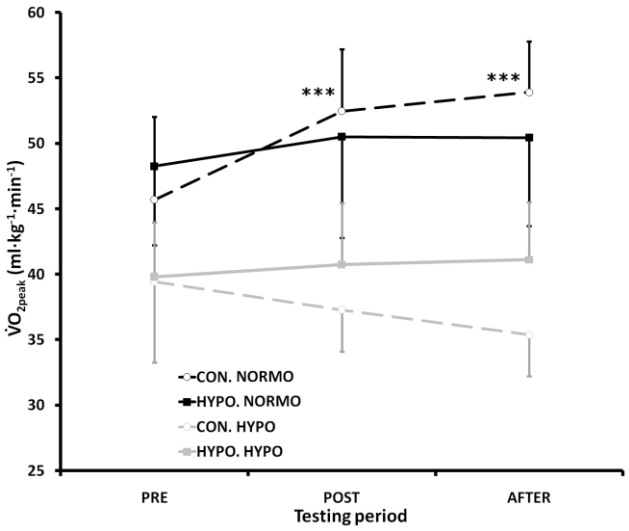

Figure 4.2: *Peak oxygen uptake ($\dot{V}O_{2peak}$) before (PRE), immediately after (POST) and 10 days after the training (AFTER) of both control (CON) and Hypoxic (HYPO) group in normoxic (NORMO) and hypoxic (HYPO) condition. *** denotes a statistically significant difference compared to PRE values at the p < 0.01 level.*

$\dot{V}_E$, HR, and S_pO_2 at normoxic and hypoxic W_{max} are presented in Fig. 4.3. There were no significant differences in either normoxic or hypoxic $\dot{V}_E$ values at W_{max} over the training period, or between groups. The maximal exercise HR in normoxia decreased significantly at the Post and After testing in the Hypoxic group, but not in the Control group. Although S_pO_2 was lower in hypoxia compared to normoxia, no significant differences between groups or Pre, Post and After IHT were observed.

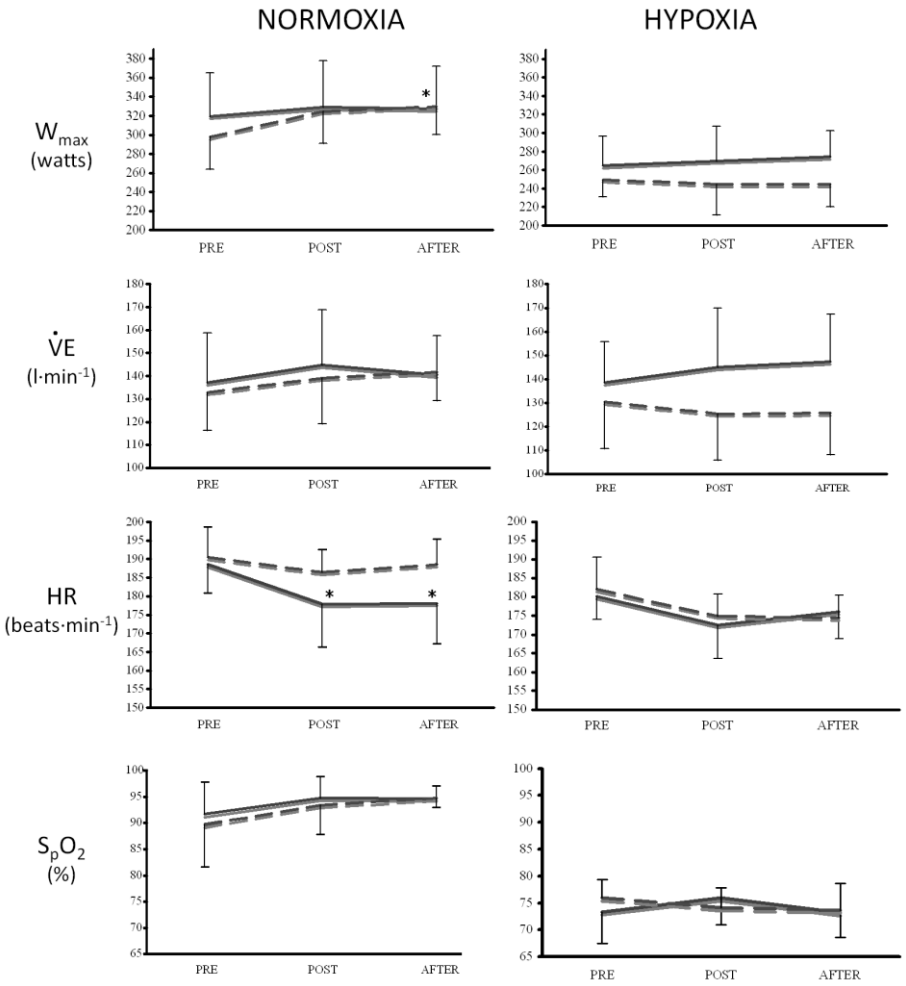

Figure 4.3: *Maximal values of power output (W_{max}), minute ventilation ($\dot{V}_E$), heart rate (HR) and capillary oxyhemoglobin saturation (S_pO_2) during $\dot{V}O_{2peak}$ tests in normoxic (left panel) and hypoxic (right panel) PRE, POST and AFTER the IHT protocol. Full line = HYPO group; Dashed line = CON group. Significant differences from PRE testing are presented as (* $p < 0.05$).*

Mean (SD) endurance performance times are presented in Fig. 4.4. Time to exhaustion at a work rate of 80% normoxic W_{max} was significantly lower in the hypoxic compared to the normoxic condition in both groups during all testing periods (Fig. 4.4). Time to exhaustion improved in both, Control (Post, $p<0.01$) and Hypoxic (Post and After, $p<0.05$) groups, but only under normoxic conditions. No significant differences were observed in the hypoxic condition between groups, or over the testing periods.

The constant power tests in hypoxia were performed at the same absolute power output as in normoxic condition (at 80 % W_{max} Pre Normoxic), therefore at a significantly higher relative workload. The relative power outputs of the hypoxic constant power tests, compared to hypoxic $\dot{V}O_{2peak}$ tests remained constant at all testing periods and were similar in both groups (97 (4) %, 96 (9) %, 95 (12) % and 94 (9) %) for the Hypoxic and (96 (7) %, 97 (10) %, 99 (14) % and 99 (9) %) for the Control group at Pre, Mid, Post and After testing, respectively.

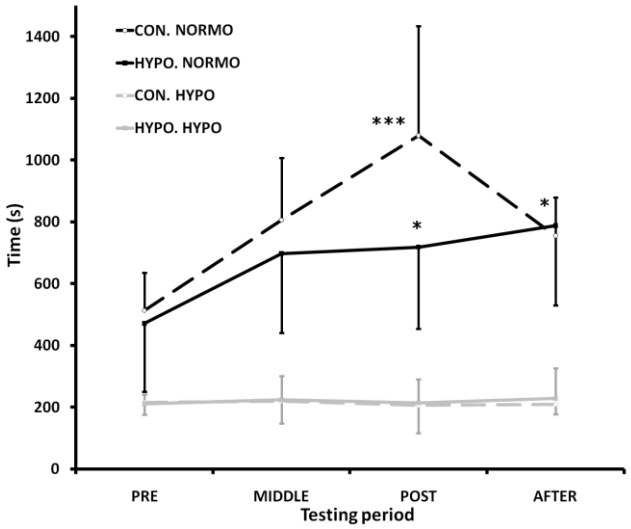

Figure 4.4: *Time to exhaustion in endurance performance PRE, MID, POST and AFTER the LL – TH protocol of both CON and HYPO group in normoxic (NORMO) and hypoxic (HYPO) condition. Statistical significance as compared to PRE is presented (* $p < 0.05$, *** $p < 0.01$).*

4.4 Discussion

Comparison of the results of the Control and Hypoxic groups revealed that IHT did not induce any significant improvements in either normoxic or hypoxic $\dot{V}O_{2peak}$ and endurance performance. In view of the lack of changes in the hematological and ventilatory responses after IHT, it is not surprising that normoxic and hypoxic performance were not enhanced.

Similar to the other studies investigating IHT regimen using untrained subjects [18], we did not find any changes in Hb, Hct, RBC and other hematological parameters. Our training program comprising 20 one-hour sessions over four weeks in normobaric hypoxia at a simulated altitude of 4500 m did not alter the selected hematological variables, most probably due to the insufficient hypoxic dose. Rodriguez et al. [149] and Hendriksen and Meeuwsen [150], using 30 and 60 min longer exposures, respectively, to hypobaric hypoxia (4000-5000 m) showed that 9 to 10 exposures over three weeks induced significant increases in Hct, Hb and RBC concentrations. We can therefore speculate that in the present study both the level of simulated altitude and the duration of the exposure to altitude were insufficient to induce beneficial hematological changes. Moreover we can also hypothesize, that hypobaric hypoxia may be more efficient compared to normobaric hypoxia also in terms of simulated erythropoiesis, as differences in physiological responses to both have already been shown [151].

The improvements reported by Geiser et al. [54] in sea level performance following IHT in untrained subjects, are not confirmed by the IHT protocol used in the present study. The differences in the performance results are most likely due to higher training intensity (80% $\dot{V}O_{2peak}$) and longer protocol duration (6 weeks) employed by Geiser et al.. Both groups in the present study trained at the same relative work intensity, corresponding to 50% of normoxic W_{max} for the Control group, and 50% of hypoxic W_{max} for the Hypoxic group. Thus, the Hypoxic group trained at an absolute work rate that was approximately 18-20 W lower. The improvements in normoxic and hypoxic performance

observed in the Control group are due to training *per se*, whereas the fact that no difference was observed in the normoxic performance of the Hypoxic group probably reflects the lower level of absolute training intensity. Although some studies on untrained subjects [87, 152] have reported no differences in performance following normoxic and hypoxic training conducted at the same absolute work rate, it has been demonstrated that the absolute workload [115] seems to be more important in terms of the acute muscular adaptation.

Studies investigating the effect of hypoxic training on exercise performance have therefore designed the training protocol to include regular exercises conducted at either the same absolute [87, 143], or the same relative [139, 142] work rate. Although the former might be more relevant in terms of maintaining or improving muscle strength, the latter allows the elucidation of the cardiorespiratory responses (strain) to exercise. The main changes reported in muscle tissue following IHT training in trained and untrained subjects include the up-regulation of hypoxia-inducible factor (HIF-1α), increases in vascular endothelial growth factor (VEGF) [135], increased capillary density, resulting in greater muscle buffering capacity and lactate tolerance, improved muscle efficiency and improvements in O_2 transfer [18]. We can speculate that these changes were not induced in our Hypoxic group since we did not observe any significant changes in the performance tests.

Our results are in agreement with the findings of previous studies performed in hypobaric hypoxia on untrained athletes [60, 141]. These studies did not show any improvements in altitude aerobic capacity, and reported similar changes in normoxic aerobic capacity in both experimental and hypoxic training groups, indicating that it was the training *per se* which caused the improvements in sea level performance. However, Katayama et al. [6, 73] reported that 7 hypoxic exposures with or without training lasting 1.5 hr at altitudes greater than 4000 m caused an increase in the S_pO_2 and maximal aerobic capacity. This improvement has been attributed solely to the increased exercise ventilatory response, and not the hematological changes. Since we did not observe any significant changes in the exercise ventilatory response following IHT at 4500 m, this fact could also explain why no significant changes were found in the exercise tests of the hypoxic group occurred compared to the control group (Fig. 4.3). Although the shorter duration of each training session in our study could be a viable explanation, the already mentioned study of Geiser et al. [54] showed that benefits can also be expected using only 30 minute training sessions.

Interestingly, the only significant change in IHT group during the normoxic $\dot{V}O_{2peak}$ tests was a significant decrease of peak HR in the Post and After tests. Although such an effect is usually not expected, it has also been shown to appear in trained athletes, following normoxic endurance training, most likely due to a decrease in the sympathetic drive [153].

This phenomenon has also been associated with overtraining [154]. When incorporating a hypoxic training regimen in an athlete's training schedule, care must be taken that the sum of the basic (normoxic) and hypoxic training does not induce overtraining, as observed by Ventura et al [50]. Overtraining was most likely not a significant contributing factor to the results of the present study, since we did not observe any reductions in ferritin levels, that may be indicative of the initial phase of overtraining-induced reduction in performance following IHT [155].

As noted by Chapman et al. [30], individual variation in the response to acute and chronic exposures to hypoxia also needs to be considered. Analyzing the individual responses of the subjects participating in the present study, we observed variability in subjects' responses to hypoxic training. In particular, Post hypoxic performance improved (6-35%) in only four subjects in the Hypoxic group, compared to their Pre hypoxic performance. However, the difference between the "responders" and "non-responders" in the Hypoxic group could not be attributed to the differences in any of the measured hematological variables.

We also have to address the limitations of our study. Since the aim of the study was to test a specific IHT dose in untrained subjects, our results have to be interpreted in that manner. The absence of a blinded design and a low exercise training intensity are possible limitations of our study. However, it is extremely difficult to perform high intensity training sessions and especially to blind subjects when exercising in severe hypoxia.

Despite some reports [139, 143, 156] of the benefits of the IHT modality to altitude performance, the results of the present study demonstrate no improvement in either altitude or sea level performance. Most likely, the hypoxic dose used was below the critical dose required to result in hypoxia-induced improvements in performance. Since the exercise training was conducted at a simulated altitude of 4500 m, it is unlikely that an insufficient altitude was the cause of the difference. Most probably it was the duration and the frequency of the exposures that would need to be modified to achieve the reported benefits.

5 Effects of IHE at rest on performance in normoxia and hypoxia

5.1 Introduction

Since the training performed in hypoxia did not show significant improvements in either normoxia or hypoxia (Study I) we hypothesized, based on previous reports that, intermittent hypoxic exposures additional to moderate endurance training could provide and advantage over exercise training solely. Moreover, since one of the main drawbacks of the IHT is the necessary decrement of absolute exercise intensity, we wanted to test the efficiency of separate short intermittent hypoxic exposures on performance. We therefore employed similar study design to investigate the effects of 20 intermittent hypoxic exposures on performance and other physiological parameters.

For purposes of performance augmentation, the use of different hypoxic training protocols is gaining popularity. However, since the designed beneficial hypoxic protocols require at least four weeks of daily exposures lasting ≥ 12 h [33] or even ≥ 20 h [13], there is an ongoing pursuit for new training regiments necessitating less time. The application of intermittent exposures (IHE) at relatively high levels of hypoxia during rest (F_IO_2 = 0.13 – 0.9) offers a plausible option. IHE has been previously studied in the eastern European countries in order to treat medical problems (e.g. asthma, hypertension) and to enhance athletic performance [5]. Considering the vast amount of training performed by the endurance athletes, the main advantage of the IHE protocols over longer hypoxic/altitude exposures is the shorter time consuming nature and simplicity of application. Recent technological advancements in production of small portable hypoxic devices made these kind of protocols available to a broad range of users, including athletes [68]. Due to the nature of application (short intervals) the IHE protocols are usually performed by utilizing normobaric hypoxia.

Despite the technological improvements in the area, the effects of IHE on normoxic exercise performance remain ambiguous and controversial. Namely a few studies showed beneficial improvements following IHE under normoxic conditions in sprint performance [157], maximal speed [65] and 3000 m time trial performance [67] in trained athletes. Nevertheless, there are several studies that did not detect any benefits on normoxic performance following IHE [63, 64, 68, 69, 158]. These different results could either be attributed dissimilar durations of the protocols, to various levels of hypoxia used, or to a placebo effect [159].

Besides, there is a lack of studies investigating the effects of IHE on hypoxic performance [158]. Hamlin and colleagues [158] reported no beneficial changes for selected performance measures including 6 × 70 m sprints, maximum speed at 1550 meters altitude, following 13 IHE sessions.

Moreover, there is a paucity of data concerning the time course of physiological changes following

the IHE protocols; one of the crucial problems from the athletes' point of view. Wood and colleagues [65] showed that following IHE the substantial beneficial effects on performance were still present nine days following training cessation. The beneficial effects of continuous intermittent exposures performed for three weeks, three times per week for 90 minutes at 4500 m [160] declined significantly three weeks after the cessation of the protocol.

Therefore, we aimed to investigate the effects of the IHE training protocol on aerobic capacity and performance in normoxia and hypoxia during, post and 10-days after the cessation of the protocol. To eliminate the possible effects of the different training activity (e.g. athletic training) on functional test outcomes, all participants performed the same endurance exercise training. We hypothesized that the addition of IHE to exercise training would provide benefits for performance at both, altitude and sea level compared to exercise training alone due to certain degree of ventilator acclimatization.

5.2 Methods

5.2.1 Subjects characteristics

Eighteen healthy young male subjects were recruited for this study. All subjects were free of lung and heart diseases and were not anaemic. Subjects gave their written consent after being informed about the study's risks involved, and familiarized with the study design and the experimental protocols. The protocol was approved by the National Committee for Medical Ethics at the Ministry of Health (Republic of Slovenia) and conformed to the Declaration of Helsinki. Subjects were instructed to maintain their normal diet and to refrain from alcohol and nicotine during the testing periods. Upon selection, the subjects were assigned to either the control (CON) or the intermittent hypoxic (IHE) training group (Table 5.1).

5.2.2 Study design

The study outline is presented in Fig. 5.1. The experimental protocol consisted of twenty training sessions performed five times per week over a four-week period for both groups. All the participants carried out the tests on four occasions: before (PRE), following 10 training sessions (MID), upon the end of the training (POST), and 10-days after the cessation of training (AFTER). Each testing consisted of pulmonary function assessment, hematological tests and four exercise tests, including both $\dot{V}O_{2peak}$ and constant power test in hypoxic and normoxic condition. Each exercise test was performed under equivalent environmental conditions in the same laboratory at sea (Valdoltra Orthopedics Hospital, Ankaran, Slovenia). The constant power tests were performed in a random and counterbalanced order. On each test day, subjects performed a maximal aerobic capacity test ($\dot{V}O_{2peak}$) in the morning and a constant power test (CP) in the afternoon. All tests were conducted at the same time of the day for each subject to avoid diurnal variations. Moreover, during the exercise cessation period, subjects refrained from any physical training, and followed their normal daily routines.

Table 5.1: *Subject baseline characteristics.*

	CON group (n=9)	**IHE group** (n=9)
Age (yrs)	22.1 (4.1)	22.2 (3.8)
Stature (cm)	179.3 (4.9)	181.1 (7.3)
Body mass (kg)	72.9 (9.7)	74.2 (5.7) *
Body fat (%)	10.4 (2.9)	11.3 (4.7)

Values are mean (SD). * P<0.05 differences between groups.

The training protocol consisted of endurance exercise training performed by both groups while IHE protocol was performed by the IHE group only. At each training session the IHE group initially performed the IHE protocol and after a rest period (15 min) performed the exercise training.

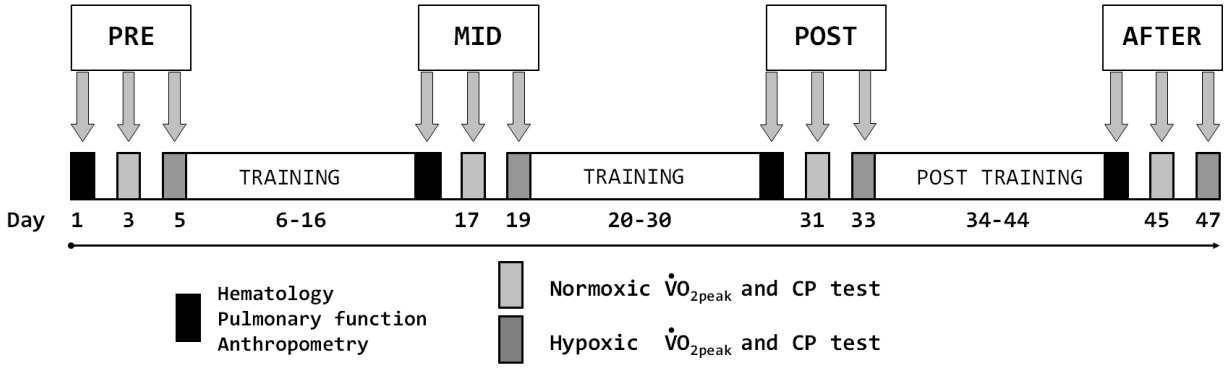

Figure 5.1: *Schematical presentation of the study outline.*

5.2.3 Endurance exercise training

All subjects performed twenty endurance exercise training sessions ($5 \cdot week^{-1}$) on cycle ergometers (Bike forma, Technogym, Cesena, Italy). Each training session consisted of a 5-minute warm up period, 60-minute cycling and a 5-minute cool down period. The intensity of the exercise corresponded to the heart rate (HR) measured at the 50% of normoxic peak power output (W_{max}). The warm up and cool down periods were performed at 60 W. During each training session the subject's heart rate was constantly monitored and recorded with a telemetric system (Hosand TMpro®, Verbania, Italy). The HR monitoring system enabled us to continuously monitor an individual training HR range for each subject. The individual interval was set within ± 4 $beats*min^{-1}$ of the targeted HR measured during the first normoxic $\dot{V}O_{2peak}$ test. If the subject's HR was out of the set interval a visual signal prompted the investigator to either de- or in- crease the work rate accordingly. Rate of perceived exertion (RPE) was also reported during training sessions by all subjects, according to 0-10 Borg scale [161]. The training load was calculated for each individual at each training session using a heart rate based TRIMP score [162].

5.2.4 Intermittent hypoxic exposures at rest

Prior to each exercise training session, performed the IHE protocol and after a rest period (15 min) continued with the exercise training. The IHE protocol comprised of 20 exposure sessions over a four-week period. Each session consisted of a 4-minute resting period in a seating position, followed by seven cycles of hypoxic exposure (5-min hypoxia × 3-min normoxia). The level of hypoxia was based on previous studies [67, 68] and was gradually reduced every five training sessions (Table 5.2). The inspiratory side of the mask was connected to a portable hypoxic gas generator (Everest Summit, Hypoxico, New York, USA), with the universal mask kit and adjustable high altitude adapter. During each session, the $F_IO_2$level of the breathing mixture of each individual was additionally monitored with an oximeter (Hypoxico, New York, USA) that was attached to the O_2 monitor port on the tube connecting the hypoxicator and the mask. Capillary oxyhemoglobin saturation (S_pO_2) was continuously monitored and recorded with a finger oxymetry device (Nellcor, BCI 3301, Boulder, USA). The finger oxymetry device had ± 2 units accuracy across the range of 70-100% with acceptable resilience to motion artefact [148]. During each hypoxic period of IHE the subjects reported their perceived rates of exertion (RPE) according to 0-10 Borg scale.

Table 5.2: *Weekly (5 sessions) average fraction of inspired oxygen (F_IO_2), capillary oxyhemoglobin saturation (S_pO_2), heart rate (HR) and rate of perceived exertion (RPE) of the IHE group during the intermittent hypoxic exposure sessions.*

	IHE SESSIONS			
	1 - 5	6 - 10	11 - 15	16 - 20
F_IO_2 (%)	0.125	0.115	0.105	0.095
S_pO_2 (%)	81 (3)	75 (1) *	73 (1) *	72 (1) * #
HR (beats·min^{-1})	78 (2)	78 (2)	78 (1)	78 (3)
RPE	2.1 (0.3)	2.5 (0.1)	2.9 (0.4) *	2.8 (0.3) *

Values are mean (SD).
* significanly different from wk 1 (P<0.05)
# significantly different from wk 2 (P<0.05)

5.2.5 Anthropometry and pulmonary function tests

Subjects' body mass (BM), stature and body fat (BF) were measured at PRE and POST testing periods. Body fat was estimated from nine skinfold measurements (subscapular, chest, triceps, suprailiac, abdominal, thigh (mid, above, below) & inguinal) according to the standard equation [146]. The pulmonary function was assessed at each testing period (Fig. 5.1). Forced vital capacity (FVC), forced expiratory volume in the first second (FEV_1), slow vital capacity (SVC), peak expiratory flow (PEF) and maximum voluntary ventilation (MVV) were measured using a pneumotachograph (Cardiovit AT-2plus, Schiller, Baar, Switzerland). All tests were performed according to the guidelines published by Miller et al. [147]. The device was calibrated with a 3 liter syringe before each test. Each test was performed three consecutive times and the highest of the three acceptable values obtained was used for the subsequent analysis.

5.2.6 Hematological tests

The blood samples were taken in the morning of the first testing day at each testing period as presented in Fig. 5.1. The subjects were overnight fasted and blood samples were drawn from the antecubital vein. All samples were analysed for red blood cell count (RBC), hemoglobin (Hb), hematocrit (Hct), transferrin and ferritin. For hemogram and transferin analysis the cythochemical impedance method (Pentra120; Horiba ABX Diagnostics, Montpellier, France) and the turbo-bidiametrical method (Hitachi 912; Roche Diagnostics, Basel, Switzerland) were used, respectively. The subjects did not receive any iron supplementation, since the levels of all subject's ferritin were and remained above 30 $ng \cdot ml^{-1}$ throughout the experimental period.

5.2.7 Incremental and constant power tests

Each subject performed two incremental exercise tests to exhaustion ($\dot{V}O_{2peak}$) on an electrically braked cycle-ergometer (ERG 900S, Schiller, Baar, Switzerland). During the normoxic test, subjects inspired ambient air (F_IO_2= 0.21; $\dot{V}O_{2peak}$ NORMO) and during the hypoxic test they inspired a humidified hypoxic gas mixture (F_IO_2= 0.12; $\dot{V}O_{2peak}$ HYPO) from a 200-liter Douglas bag. During all $\dot{V}O_{2peak}$ tests, the subjects breathed through a two-way valve (Model 2, 700 T-Shape, Hans Rudolph, Shawnee, USA), while their oxygen uptake ($\dot{V}O_2$) and ventilation ($\dot{V}_E$) were measured breath by breath using a metabolic cart (CS-200, Schiller, Baar, Switzerland) and subsequently averaged every 10 seconds. The pneumotachograph was calibrated with a 3 liter syringe prior to each test; the gas analyzers were calibrated with two different standard gas mixtures. During the tests, the subjects reported their perceived rate of exertion (RPE) on a Borg scale (0-10), separately for peripheral (RPE_{leg}) and central (RPE_{cen}) sensation of effort.

The testing protocol consisted of 10-min resting period in normoxia or 5-min in normoxia and 5-min in hypoxia, if the test was performed under hypoxic condition. The rest was followed by 2-min warm up at a work rate of 60 Watts. Thereafter, the work rate was increased for 30 Watts each minute until the subjects could no longer sustain the pre-determined cadence. The criteria for the attainment of the $\dot{V}O_{2\ peak}$ values, that was calculated as the highest 60 seconds average, were: pedaling cadence < 60 rpm, and plateau in O_2 consumption.

Additionaly, two constant power (CP) tests were also performed at each testing period breathing either ambient air (CP_{NORMO}; F_IO_2= 0.21) or humidified hypoxic mixture (CP_{HYPO}; F_IO_2= 0.12). After 2 minutes resting period (either in normoxia or hypoxia for NORMO or HYPO test, respectively), the subjects performed 2 minutes warm up (60 watts), followed by cycling to exhaustion on the pre-determined workload. The exercise workload corresponded to 80% of the PRE $\dot{V}O_{2peak}$ NORMO for both tests. The number of seconds the subject was able to maintain the pedalling cadence above 60 rpm determined the final performance. During all tests, HR and S_pO_2 were continuously monitored with a heart rate monitor (Vantage NVTM, Polar Electro Oy, Kempele, Finland) and a pulse oxymeter (Nellcore, BCI 3301, Boulder, USA), respectively.

5.2.8 Data analysis and statistical evaluation

All analyses were performed using Statistica 5.0 (StatSoft, Inc., Tulsa, USA). Differences between groups were defined using a 3-way analysis of variance (ANOVA) (group × condition × testing period). A 4-way ANOVA was employed to analyze the relative submaximal values of incremental and CP exercise tests (group × condition × testing period × relative intensity). A *Post-hoc* test (Tukey HSD) was used for further analysis to identify the specific differences in case of a significant main effect. Due to technical problems encountered on some of the hypoxic Pre $\dot{V}O_{2peak}$ tests (four tests), we used a regression model to predict the missing PRE $\dot{V}O_2$ hypoxic values from the power output - $\dot{V}O_2$ relationship produced from the normoxic and the available hypoxic tests. All data are reported as means (SD) unless indicated otherwise. The alpha level of significance was set a priori at 0.05.

5.3 Results

The mean workload (IHE: 160 (26) Watts; CON: 159 (19) Watts) and HR (IHE: 145 (9) beats·min^{-1}; CON: 143 (8) beats·min^{-1}) during the overall training period show that the absolute workload of both groups was similar. The average training power output increment between the first and the last training session was 6 % and 7 % for the IHE and CON group, respectively. During the training, the work load was regulated for each subject so that the targeted HR range remaind constant. No differences were noted between the groups in TRIMP scores.

Table 5.2 shows the variables measured during the intermittent hypoxic exposures. The average S_pO_2 was 75.5 (5) % during hypoxic exposure bouts and 95 (2) % during normoxic bouts. The values of S_pO_2 during hypoxic exposures decreased throughout the training period, concomitantly with the decreasing F_IO_2 in the inspired breathing mixture (Table 5.2). The ratings of perceived exertion waere significantly higher during the last ten training sessions. No changes were observed in HR between sessions.

There were no changes in the % BF between or within both groups. The CON group had significantly increased BM on the POST test (74.4 kg) compared to the PRE. It has to be noted that the CON group had significantly lower BM compared to the IHE group before the training protocol ($P< 0.05$) (Table 5.1). No significant changes were observed in parameters of pulmonary function test except the peak exspiratory flow (PEF), which was significantly increased at the MID testing in the CON group (Table 5.3). PEF was also significantly higher at the PRE testing in IHE group compared to the CON. No significant differences were found within the groups at different testing periods in any of the measured hematological variables (Table 5.3). Furthermore, there were no significant differences between the groups.

Table 5.3: *Results of pulmonary function and hematological tests conducted before (PRE), in the middle (MID), at the end (POST) and 10 days after (AFTER) the training period, for the control (CON) and experimental (IHE) group.*

	CON group				IHE group			
	PRE	MID	POST	AFTER	PRE	MID	POST	AFTER
FVC (L)	5.6 (0.9)	5.5 (1.2)	5.6 (1.1)	5.5 (1.7)	5.7 (0.8)	5.7 (0.9)	5.8 (0.7)	5.7 (0.9)
FEV_1 (L)	4.9 (0.5)	4.6 (0.9)	4.7 (0.8)	4.7 (1.3)	5 (0.6)	4.9 (0.7)	4.8 (0.6)	4.8 (0.8)
SVC (L)	5.1 (0.8)	5.0 (0.8)	5.6 (1.3)	5.4 (1.6)	5.5 (0.8)	5.3 (0.8)	5.6 (0.8)	5.1 (0.9)
PEF (L)	8.9 (2.2)	10.3 (1.7) *	10.1 (1.5)	9.7 (1.4)	10.9 (1.3) #	10.5 (1.3)	11.1 (1.7)	10.4 (1.6)
MVV ($L \cdot min^{-1}$)	183 (34)	187 (34)	185 (29)	172 (75)	199 (32)	204 (36)	205 (39)	200.9 (36)
RBC ($10^{-12} \cdot L^{-1}$)	5.0 (0.3)	5.1 (0.3)	4.8 (0.2)	5.0 (0.2)	5.1 (0.3)	5.1 (0.3)	5.1 (0.2)	5.2 (0.15)
Hb ($g \cdot L^{-1}$)	15.1 (1.0)	15.5 (0.9)	14.3 (1.2)	14.7 (1.3)	15.1 (0.8)	14.5 (0.9)	15.0 (0.7)	15.1 (0.6)
HCT (%)	45 (3.0)	47 (2.2)	44 (3.0)	46 (4.0)	46 (3.0)	46 (2.0)	46 (3.0)	46 (2.0)
Trf ($g \cdot L^{-1}$)	2.7 (0.3)	2.5 (0.4)	2.7 (0.2)	3.2 (1.5)	2.8 (0.2)	2.6 (0.1)	2.6 (0.1)	2.6 (0.2)
Fer ($ng \cdot mL^{-1}$)	71 (33)	67 (66)	62 (30)	68 (42)	79 (44)	86 (27)	82 (44)	89 (36)

Values are mean (SD); # significant ($P < 0.05$) differences between groups; * significantly ($P < 0.05$) different from PRE test values.
FVC, Forced vital capacity; FEV_1, Forced expiratory volume in 1 second; SVC, Slow vital capacity; PEF, Peak expiratory flow; MVV, Maximum voluntary ventilation; RBC, Erythrocytes; Hb, Hemoglobin; HCT, Hematocrit, Trf, Transferrin; Fer, Ferittin.

Table 5.4: *Peak values of minute ventilation ($\dot{V}_E$), heart rate (HR), power output (W_{max}), capillary oxyhemoglobin saturation S_pO_2 and peripheral (RPE_{leg}) and central (RPE_{cen}) ratings of perceived exertion during normoxic and hypoxic $\dot{V}O_{2peak}$ tests before (PRE), in the middle (MID), at the end (POST) and 10 days after (AFTER) the training period, for the control (CON) and experimental (IHE) group.*

	CON group				IHE group			
	PRE	MID	POST	AFTER	PRE	MIDDLE	POST	AFTER
					$\dot{V}O_{2peak}$ NORMO			
$\dot{V}_{Epeak}$ ($L \cdot min^{-1}$)	132 (18)	140 (19)	139 (21)	141 (13)	135 (30)	143 (30)	147 (28)	153 (30) *
HR_{peak} ($b \cdot min^{-1}$)	190 (8)	187 (4)	186 (6)	188 (7)	189 (8)	181 (7.3)*	186 (7)	187 (8)
W_{max} (Watt)	294 (35)	309 (29)	322 (34) *	327 (31) *	314 (54)	324 (45)	337 (53) *	342 (59) *
S_pO_2 (%)	89 (8)	94 (2)	93 (6)	95 (2)	91 (3)	92 (5)	92 (6)	91 (5)
$RPE_{leg\ peak}$	8 (1.5)	8.7 (1.3)	9.2 (0.8)	9 (1)	9.4 (1.1)	8.2 (1.4)	8.8 (1.3)	9.1 (1.2)
$RPE_{cen\ peak}$	6.7 (1.7)	7 (2)	7.6 (2.3)	7.6 (2.3)	8.7 (1)	6.9 (1.5) *	8 (1.9)	8.4 (1.6)
					$\dot{V}O_{2peak}$ HYPO			
$\dot{V}_{Epeak}$ ($L \cdot min^{-1}$)	130 (20)	121 (23)	125 (20)	125 (17)	140 (16)	137 (19)	142 (23)	145 (26)
HR_{peak} ($b \cdot min^{-1}$)	182 (8)	177 (6)	175 (6)	174 (6)	181 (7)	175 (7)	177 (7)	178 (11)
W_{max} (Watt)	250 (19)	247 (28)	247 (34)	244 (25)	271 (38)	263 (37)	280 (36)	283 (43)
S_pO_2 (%)	76 (6)	73 (4)	74 (5)	74 (5)	73 (6)	74 (5)	73 (5)	74 (6)
$RPE_{leg\ peak}$	7.3 (2.8)	7.4 (2.5)	7.9 (1.5)	8.6 (1)	9.3 (1.1)	8.3 (1.3)	8.5 (1)	8.6 (1.8)
$RPE_{cen\ peak}$	7.1 (2.1)	7.7 (1.6)	7.6 (2.2)	7.9 (3)	8.4 (1.1)	6.9 (2) *	7.3 (2.4)	8.8 (1.2)

Values are mean (SD).
* Significantly ($P < 0.05$) different from PRE test values.

The results of the $\dot{V}O_{2peak}$ tests are presented in Fig. 5.2. No differences were noted between groups in $\dot{V}O_{2peak}$ before the protocol. Both groups significantly increased their $\dot{V}O_{2peak}$ NORMO over the course of training. No changes were observed in the $\dot{V}O_{2peak}$ HYPO in neither group. The peak values

of cardio - respiratory variables during the $\dot{V}O_{2peak}$ tests are presented in Table 5.4. The peak $\dot{V}_E$ significantly increased in the IHE group at the AFTER testing only (Table 5.4). The HR_{peak} decreased significantly only in the IHE at the MID tests compared to the PRE ($p \leq 0.05$). The W_{max} NORMO increased significantly in both groups at POST and AFTER testing periods ($p \leq 0.05$). No changes were observed in the W_{max} HYPO. No significant differences were observed between or within groups in peak S_pO_2 and RPE_{leg} values. The peak RPE_{cen} was significantly lower in IHE group at the MID testing only in both $\dot{V}O_{2peak}$ NORMO and $\dot{V}O_{2peak}$ HYPO.

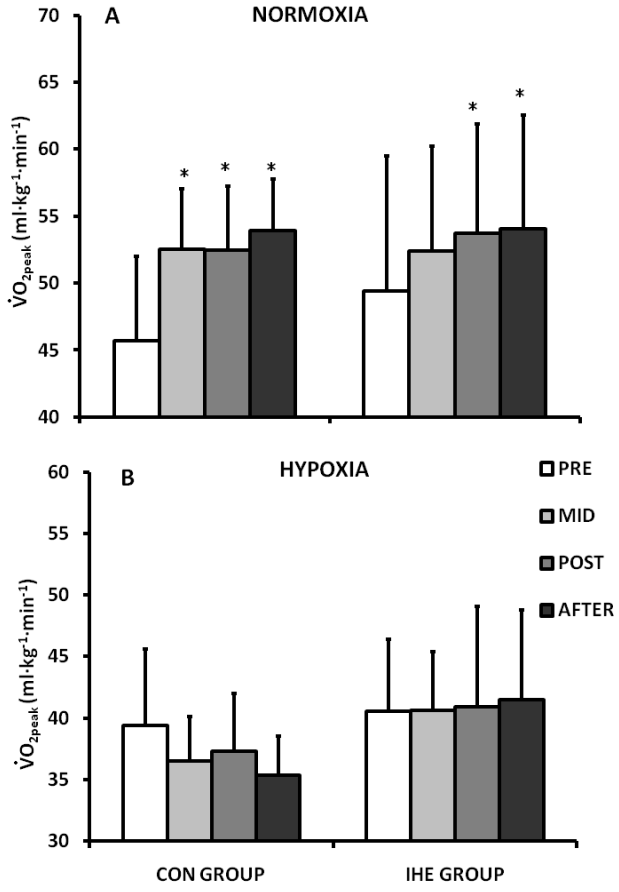

Figure 5.2: *Normoxic (A) and hypoxic (B) peak oxygen uptake ($\dot{V}O_{2peak}$) before (PRE), after 10 training sessions (MID), at the end (POST) and 10 days after the training period (AFTER) for the control (CON) and intermittent hypoxic exposure (IHE) groups. Values are means ± SD. * P<0.05; ** P<0.01 denotes statistically significant from PRE values.*

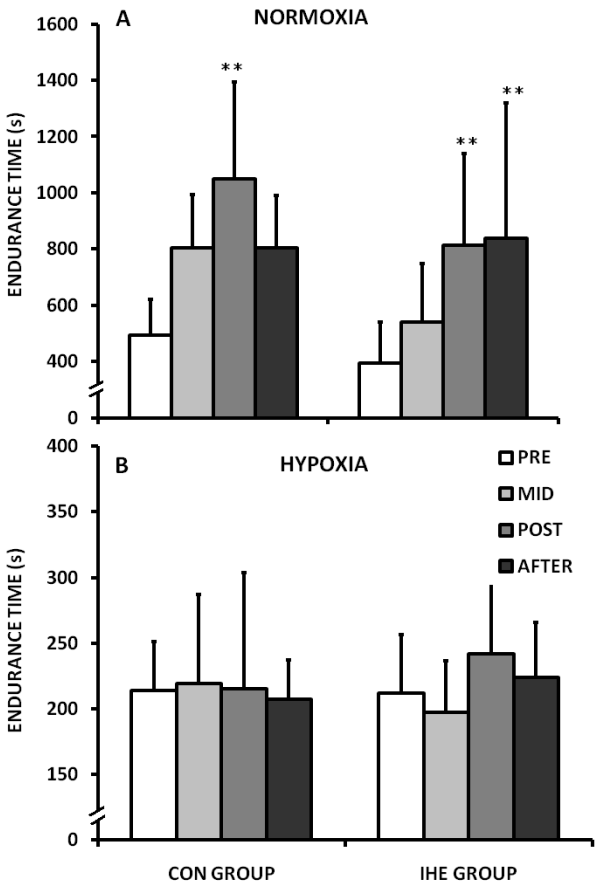

Figure 5.3: *Normoxic (A) and hypoxic (B) endurance times determined with a constant power (CP) testbefore (PRE), after 10 training sessions (MID), at the end (POST) and 10 days after the training period (AFTER) for the control (CON) and intermittent hypoxic exposure (IHE) groups. Values are means ± SD. ** denotes statistically significant differences from PRE values (P<0.01).*

The CP_{HYPO} test results are presented in Fig. 5.2. Before the protocol the groups had comparable endurance performance. CP_{NORMO} was significantly increased from baseline in both groups at the POST. Only the IHE group maintained this improvement at the AFTER test (Fig. 5.3). No changes were noted in CP_{HYPO} in both groups at any testing period (Fig. 5.3). The peak values of $\dot{V}O_{2peak}$, S_pO_2, RPE_{leg} and RPE_{cen} during both CP_{HYPO} and CP_{NORMO} were similar between groups (Table 5.5). Only HR_{peak} was significantly lower during the POST CP_{NORMO} test in the CON group and during the MID CP_{HYPO} test in the IHE group. The analysis of the relative values of $\dot{V}_E$ during the CP_{NORMO} test showed significantly higher values of $\dot{V}_E$ at 60 %, 80 %, and 100 % of relative exercise time at the POST test and 100 % at the AFTER test for the IHE group compared to the CON (Fig. 5.4). Both groups showed increases in $\dot{V}_E$ at 20 % relative exercise time at MID, POST and AFTER testing, whereas there was a significant decrease at the POST testing in $\dot{V}_E$ at 80 % an 100 % exercise time of the CON group (Fig. 5.4). No significant differences in the relative $\dot{V}_E$ values were observed within groups during the CP_{HYPO} tests.

Table 5.5: *Peak values of oxygen uptake ($\dot{V}O_{2peak}$), heart rate (HR), capillary oxyhemoglobin saturation (S_pO_2), peripheral (RPE_{leg}) and central (RPE_{cen}) rate of perceived exertion during the CP_{NORMO} and CP_{HYPO} tests, before (PRE), in the middle (MID), at the end (POST) and 10 days after (AFTER) the training period, for the control (CON) and experimental (IHE) group.*

	CON group				IHE group			
	PRE	MID	POST	AFTER	PRE	MID	POST	AFTER
				CP_{NORMO}				
$\dot{V}O_{2peak}$ (mL·kg^{-1}·min^{-1})	45.3 (6)	48.4 (6)	43.8 (9)	46.1 (6)	49 (6)	53.3 (6)	51.7 (6)	51.2 (8)
HR_{peak} (beats·min^{-1})	190 (6)	187 (8)	182 (5) *	185 (7)	187 (9)	180 (8)	185 (9)	184 (11)
S_pO_2 (%)	93 (2)	94 (2)	94 (1)	94 (1)	94 (4)	94 (4)	94 (2)	93
$RPE_{leg\ peak}$	7.5 (1.4)	-	8.8 (1)	8.1 (1.5)	8.6 (2.1)	7.6 (1.9)	8 (1.6)	8.7 (1.3)
$RPE_{cen\ peak}$	7 (1.7)	-	7.7 (1.8)	7.7 (2.5)	7.7 (2)	6.5 (2.1)	7.9 (2.2)	8.3 (1.7)
				CP_{HYPO}				
$\dot{V}O_{2peak}$ (mL·kg^{-1}·min^{-1})	44.2 (11)	39.1 (4)	36.5 (6)	36.8 (5)	42.8 (5)	43.5 (5)	43.7 (5)	43.1 (4)
HR_{peak} (beats·min^{-1})	181 (8)	178 (5)	175 (8)	176 (7)	179 (9)	170 (9) *	177 (8)	178 (8)
S_pO_2 (%)	72 (6)	72 (4)	73 (6)	72 (4)	72 (5)	75 (6)	73 (5)	72 (4)
$RPE_{leg\ peak}$	6.2 (2.5)	7.4 (2.9)	8.3 (1.7)	8 (1.7)	9.1 (0.8)	7.8 (1.8)	8.6 (1.6)	8.5 (1.6)
$RPE_{cen\ peak}$	7 (2.2)	7.1 (1.8)	9.3 (1)	7.9 (2.2)	8.9 (0.7)	7.8 (1.7)	8.2 (1.6)	8.2 (1.7)

Values are mean (SD).
*significantly ($P < 0.05$) different from PRE test values.

5.4 Discussion

The present study investigated the effects of intermittent hypoxic exposures at rest on $\dot{V}O_{2peak}$ and CP test under normoxia and hypoxia. The results did not show any additive effect of IHE on aerobic capacity and endurance performance in normoxia and hypoxia. However, the novel finding of this study is that the tested IHE protocol may retain beneficial adjustments at sea level, since only the IHE group maintained their improved performance in CP_{NORMO} test 10 days after the end of training intervention.

Improvements in normoxic $\dot{V}O_{2peak}$, following similar or even lower (45 % HR_{peak}) training intensity protocols have already been demonstrated in healthy young adults [163, 164]. Our results have shown similar improvements in $\dot{V}O_{2peak}$ NORMO for both CON and IHE group (Fig. 5.2), thus indicating, that IHE did not provide any additional benefits for sea level aerobic capacity compared to endurance training *per se*.

Similarly, the present study show no additional benefits of IHE on endurance performance compared to the exercise training *per se* at MID and POST training period. This is in line with the results of studies using trained athletes, that showed no additive effects of IHE on normoxic $\dot{V}O_{2peak}$, running economy [63, 68] and 5000 m running performance [64]. On the other hand, the study by Hamlin [67] showed improvements in 3000 meters running time, likely to be beneficial for non elite athletes. The conclusions of Hamlin have also been questioned by Bartsch [4], who attributed the measured effect to the inhomogeneous distribution of performance level and gender between the groups, rather than the IHE effects *per se*.

Although some studies investigating the effects of different intermittent hypoxic protocols on

performance showed possible benefits for altitude performance [72] this was not confirmed by our study. Namely, no significant improvements in hypoxic $\dot{V}O_{2peak}$ or hypoxic CP tests were found in either group during and after the protocol. Our results are in agreement with the findings of Hamlin [158] who tested the effects of a similar IHE protocol on performance at altitude. The tests were performed at a moderate altitude (1550 m), although high enough to significantly impair performance in game specific tests of rugby players. They found that the IHE proocol provided some benefits on submaximal HR and La levels during hypoxic exercise, but induced no changes in the game specific test performance variables.

Even though increases in Hb, Hct and reticulocytes concentrations following short term intermittent hypoxia have been shown in some studies [67, 149], other studies did not display similar benefits [63, 68]. In general it seems that short hypoxic exposures do not provide haematological benefits [95], most probably due to the insufficient hypoxic dose [4]. Although one of the dominant paradigms of improved performance following the majority of altitude training protocol still seems to be arising through accelerated erythropoiesis and subsequent polycythemia, other factors have also been suggested that could provide benefits [40]. The main non haematological benefits include ventilatory adaptations, improved muscle efficiency, greater muscle buffering capacity and improved lactic acid tolerance [40].

Our findings about the de-acclimatization period are in accordance with the findings of Wood [65] who showed that possible benefits of IHE were still present 9 days after the IHE cessation. They have showed improved sprint performance, reduced exercise lactate (La) and HR levels POST and AFTER the IHE, thus indicating that IHE can provide benefits for high intensity running performance. Although these results were promising, they were not confirmed in a subsequent study performed by the same research group [69], that showed no benefits of IHE over similar training and placebo IHE protocol. On the other hand, Katayama et al. [160] showed that three weeks after a continuous intermittent hypoxic exposures protocol (90 min per session/3 times per week/3 weeks @ 4500 m), all benefits were diminished. Therefore, according to our results and Katayama's findings the potential benefits of IHE could be present for 10 days, but no more than 20 days after the cessation of the intervention.

The effect of IHE on ventilatory regulation and sensitivity seems to be the main proposed mechanism underlying the potential benefits observed [165, 166]. Also according to our study, the prolongation of improved performance at sea level can be ascribed to ventilatory adaptation as shown by the increased $\dot{V}_E$ at the POST and AFTER CP_{NORMO} tests (Fig. 5.4). However, this finding is in contrast with the results from another study by Katayama [167]. They showed that continuous IHE did not provide any ventilatory benefits during consequent sea level exercise, whereas in our study we found significant increases in maximal and submaximal $\dot{V}_E$ during CP_{NORMO} test following IHE.

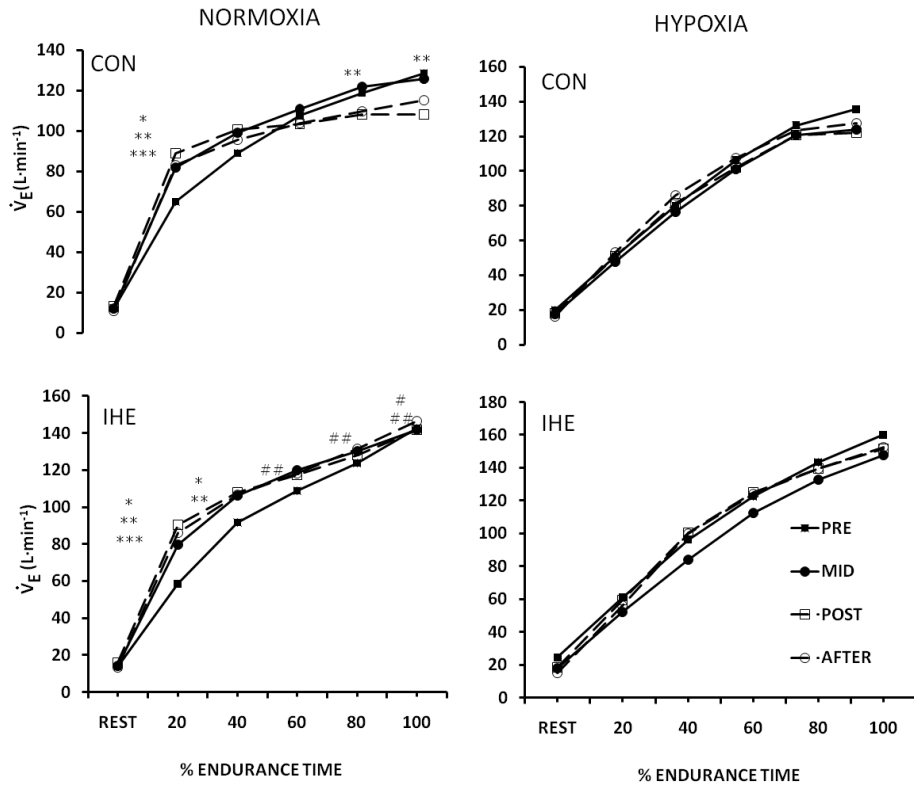

Figure 5.4: *Minute ventilation ($\dot{V}_E$) during the normoxic (CP_{NORMO}) and hypoxic (CP_{HYPO}) constant power test at the same relative endurance time for the control (CON) and intermittent hypoxic exposure (IHE) groups. Values are means ± SD. Statisticaly significant (P<0.05) within groups: (* PRE and MID, ** PRE and POST, *** PRE and AFTER) and between group (## POST, # AFTER) differences.*

The different results can probably be ascribed to the dissimilar hypoxic doses, since both the above-mentioned studies used only seven continuous one-hour exposures performed during one week. In addition, the increase in maximal normoxic exercise ventilation have also been shown following nine 3-5 hour exposures to 4000 meters [62]. This is consistent with the findings of Sheel et al. [168], indicating that longer exposure periods or more severe hypoxia are required to elicit a change in sea level exercise ventilation. However, it seems that there is no clear link between the hypoxic ventilatory response increases and ventilatory response changes during sea level exercise [169]. In particular, Foster et al. [169] did not observe any differences in normoxic and hypoxic exercise ventilatory responses between different IHE protocol durations. It therefore seems that the functional effects of increased chemo sensitivity following IHE on sea level exercise ventilation are not clear [168]. Even though our results showed benefits in sub-maximal and maximal ventilation following IHE, they have to be interpreted as a reflection of combined IHE and exercise training, compared to the IHE alone.

Our results do not show any benefits oh IHE on hypoxic exercise ventilatory responses. However, some studies showed that the increased hypoxic chemo sensitivity following short hypoxic exposures provide increases in ventilation and capillary oxyhemoglobin saturation (S_pO_2) during exercise in hypoxia [6, 86, 170]. Most probably these discrepancies can also be explained by the shorter duration of the hypoxic exposure, since both above mentioned studies used continuous exposures lasting an hour or two.

As can be seen from the results, the levels of S_pO_2 were decreasing concomitantly with the decreases of F_IO_2 in the inspired air (Table 5.2). The F_IO_2 levels used during our IHE protocol are comparable to other studies performed to investigate the effects of IHE on performance and aerobic capacity, although the tested hypoxic stimulus was more intense in the last sessions. In particular, the F_IO_2 in our study was 9.5 % compared to 10 % used by Tadibi et al. [68]. The duration of hypoxic and normoxic bouts between the IHE was shorter (5:3 min) compared to (6:4 min) used in some other studies [65, 68] although the total number of sessions was higher in this one (20 vs. 15 sessions).

The novelty of our study is also a strictly controlled and identical training in both groups. We therefore eliminated the possible differences in the measured outcomes due to the different training programs performed concomitantly with the IHE; a possible occurrence in studies where subjects perform their normal training during the testing protocol [65, 68]. This also allowed us to elucidate the specific changes induced by the training *per se* and the specific effects of IHE.

In conclusion the tested IHE protocol did not improve performance and aerobic capacity in hypoxia or normoxia during and immediately after the protocol. However the prolongation of improved performance (CP_{NORMO}) 10 days after the IHE protocol cessation showed potentially additive effect of IHE over exercise training alone. IHE therefore seems to be a promising protocol for training effects prolongation at sea level and/or as a substitute for exercise training during the periods when physical training cannot be performed.

6 Short intermittent hypoxic exposures augment ventilation during hypoxic exercise

6.1 Introduction

It is well established, that chronic or intermittent hypoxia can enhance endurance performance [4, 171], however, the minimal hypoxic dose, needed to induce beneficial adaptations, is still indefinite. This was the main incentive for the presentstudy (Study III), which investigated the effects of only four intermittent hypoxic exposures on performance, and cerebral and muscle oxygenation during exertion. Since one of the aims of the tesis was to investigate the shortest possible protocols we reasoned, based on the results of Studies I and II and currently available data, that the selected hypoxic dose would be sufficient to augment performence or at least modify physiological responses to hypoxic exercise.

Enhancements in sea level performance, have been noted following daily 20-hr exposures to natural (2000 - 2500 m), or corresponding simulated altitude [13, 33]. Although there is as yet no consensus regarding the underlying mechanisms [172, 173], the increased blood O_2 carrying capacity is among the main mediators of the observed benefits. In contrast, it has been demonstrated that shorter hypoxic protocols may also enhance altitude performance primarily by initiating ventilatory adaptations [72]. Thus, if the aim is improvement in altitude performance or work capacity, the hypoxic dose can be reduced. Hematological benefits (e.g. increases in hemoglobin mass and hematocrit) cannot be anticipated as a result of short ($\leq$ 7 days; $\leq$ 5 hours·day^{-1}) intermittent hypoxic protocols (SIH), due to the insufficient exposure duration and frequency [3] although enhancement of erythropoietin release has been reported [174]. The potential application of SIH induced hypoxic adaptations relates to modern military operations, requiring fast deployments to high altitude areas [12] or in pre-acclimatization protocols before altitude expeditions and competitions [86]. The pivotal physiological mechanism behind improvements in altitude performance following SIH is the increased chemosensitivity to hypoxia [73, 175], leading to increases in ventilation which in turn mediates increased blood O_2 saturation during subsequent hypoxic exercise [6, 86, 87]. It has been shown, that hypoxic exercise ventilatory response is significantly increased after only one exposure to hypoxia (30 min, F_IO_2 = 0.120) with no significant effect of either the duration of hypoxic exposure [176] or activity during the exposure [8, 177]. In particular, it has already been reported that even a few intermittent hypobaric hypoxic exposures (5-7 days, 1-4 h·day^{-1}, at altitudes $\geq$ 4000 m) can improve performance in hypoxia and increase both exercise minute ventilation ($\dot{V}_E$) and blood oxygen saturation (S_pO_2) [72, 80].

Whereas short exposures to altitude (hypobaric hypoxia) have yielded favorable results in terms of improvement in altitude performance [71, 72], there is a lack of data regarding the effects of similar short exposures utilizing normobaric hypoxia on altitude performance [12]. Indeed, the only study that has investigated altitude performance following seven normobaric hypoxic exposures has not observed any improvements in working capacity [7]. These results suggested that the reported differences in responses to normobaric or hypobaric hypoxia [16, 151], although debatable, may play an important role regarding the SIH efficiency. The aim of the present study was to investigate the effects of four intermittent normobaric hypoxic exposures on the exercise ventilatory response and exercise capacity in hypoxia.

We also tested the hypothesis that the SIH induced ventilatory adaptations would have an effect on oxygenation modulation and blood flow distribution in the brain and respiratory and leg muscles. Namley, the findings of Harms et al. [178] suggest that a significant redistribution of O_2 flux occurs during high energy demands of respiratory muscles. In particular, during heavy exercise loads (≥ 80% $\dot{V}O_{2peak}$) the increased work of breathing can effect endurance performance through the redistribution of the blood flow from the exercising limbs to the respiratory muscles [179]. This regional redistribution can also be seen in untrained subjects, with lower absolute minute ventilation levels [179]. Thus, since an enhanced ventilatory response to hypoxia increases exercise ventilation, we hypothesized that the changes in oxygenation pattern and blood flow would be more pronounced following SIH.

6.2 Methods

6.2.1 Subjects characteristics

Active, healthy and non-smoking male subjects were recruited for participation in the study. All applicants, low altitude (~ 300 m) residents, were specifically screened for absence of asthma, hematological and kidney disorders and altitude residence (> 2500 m) within the preceding three months. Nineteen subjects meeting the above criteria were upon selection randomly assigned to either the SIH (n = 10) or the Control (n = 9) group.

Table 6.1: *Subject characteristics.*

	SIH group (n=10)	**CON group (n=9)**
Body height (cm)	180 (3)	179 (3)
Body mass (kg)	74.2 (4.9)	77.4 (11.2)
% body fat	9.5 (4.4)	11.2 (4.6)
Age (years)	22.9 (1.6)	22.3 (3.4)
$\dot{V}O_{2peak}$ (mL·kg^{-1}·min^{-1})	48.8 (7.5)	47.3 (5.1)
W_{max} (W)	322 (43)	330 (40)
$\dot{V}_{Emax}$ (L·min^{-1})	127.7 (16.4)	130.2 (18.1)

Values are mean (SD). VO_{2peak}, W_{max} and V_{Emax} measured in the graded exercise test.
VO_{2peak}, maximal oxygen uptake; W_{max}, peak power output, V_{Emax}, maximal pulmonary ventilation.

There were no significant differences in the observed physical and cardiorespiratory variables between the two groups (Table 1). Prior to participation in the study all subjects signed their written informed consent, completed separate health and training questionnaires and were familiarized with the study protocol, as well as with the risks involved.

6.2.2 Study design

The study was performed as a randomized, single blind, placebo controlled design using one independent factor (SIH v. Control) and two repeated measures; trial (PRE v. POST) and treatment (hypoxia v. normoxia (sham)). The study design consisted of two testing trials and four intermittent exposures in-between. Both groups participated in four daily exposures in a climatic chamber maintained at either normobaric hypoxic (SIH group) or normobaric normoxic (Control group) condition. Exercise tests were performed the day before (PRE) and the day after (POST) the intermittent exposures. The subjects were requested to refrain from strenuous exercise for at least 24 hours before the exercise tests and during the duration of the 6-day protocol. On the PRE testing day each subject performed a graded exercise test ($\dot{V}O_{2peak}$) in the morning and a hypoxic constant power test (CP) in the afternoon, with a minimal 6-hr interval between the tests. Subjects' anthropometric characteristics (height, mass) were measured before the intermittent exposures and percentage body fat was calculated from nine skin folds measurements. POST tests were performed during the same hours of the day as for the PRE tests. The experimental protocol was approved by the National ethics committee and conducted according to the Helsinki declaration guidelines. The outline of the study design is presented in Fig 6.1.

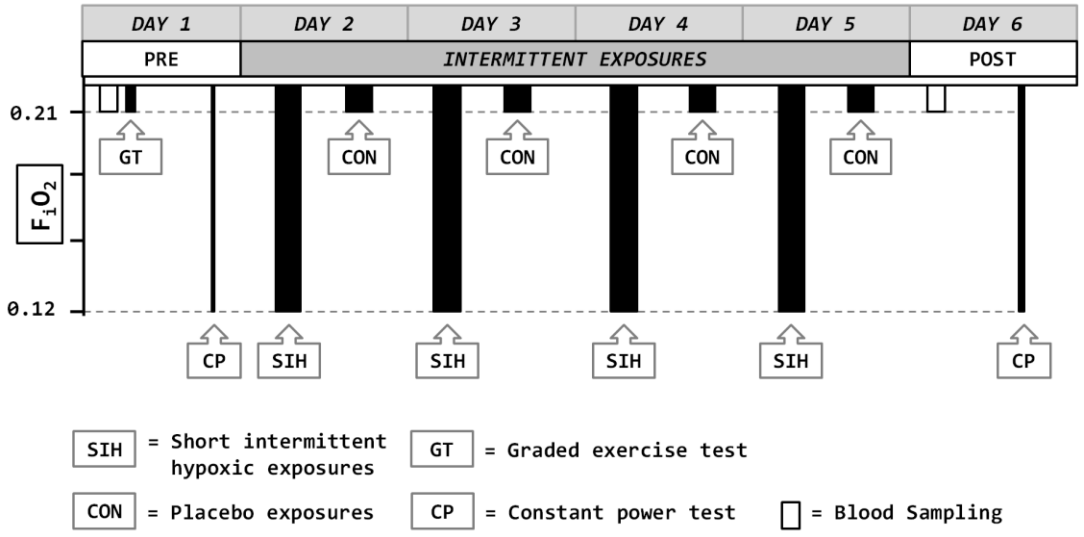

Figure 6.1: *Schematical presentation of the study design.*

6.2.3 Intermittent hypoxic exposures

The intermittent hypoxic protocol was performed on four subsequent days. Each day subjects resided for four hours in an altitude chamber (IZR d.o.o., Skofja Loka, Slovenia) at the Jozef Stefan Institute, under stable environmental conditions (air temperature = 24 °C, humidity = 50 %). For the SIH group,

the normobaric hypoxic environment (F_IO_2 = 0.120 ± 0.05; Equivalent air altitude = 4200 m) within the altitude chamber was maintained using a Vacuum Pressure Swing Adsorption system (b-Cat, Tiel, The Netherlands), that generated and delivered the O_2-depleted air to the chamber. The air samples were continuously analyzed for O_2 and CO_2 levels. The mean CO_2 levels in the altitude room did not exceed 0.05 %. For the Control group the F_IO_2 was kept constant at 0.209. During the exposures heart rate (HR) and capillary oxyhemoglobin concentration (S_pO_2) were recorded at a 2-min intervals using a portable heart rate monitor (Polar S810i, Kempele, Finland) and a portable pulse oxymetry device (Nellcor, BCI 3301, Boulder, USA) with ± 2 units accuracy across the range of 70-100% [148], respectively. The subjects were naïve regarding the exposure and were unable to observe any of the F_IO_2, HR and S_pO_2 readings. Subjects completed a self assessment portion of the Lake Louise Mountain sickness questionnaire (0 - 15), to obtain the individual Lake Louise score (LLS) [180].

6.2.4 Exercise testing

All exercise tests were performed in the same laboratory (Jozef Stefan Institute, Ljubljana, Slovenia), located at 300-m altitude under constant environmental conditions (air temperature = 22 ± 1,5 °C; humidity = 40 ± 7 %; ambient pressure = 978,2 ± 6,4 hPa). On the morning of the PRE testing day the subjects performed a graded exercise test to voluntary exhaustion on electromagnetically controlled cycloergometer Ergo Bike Premium (Daum electronics, Fürth, Germany). Resting and exercise cardiorespiratory responses were measured using a Quark CPET metabolic cart (Cosmed, Rome, Italy). During the test the subjects breathed through an oro-nasal mask (Vmask, Hans Rudolph, Shawnee, USA), connected to a two-way valve. The turbine flowmeter and gas analyzer were calibrated before each test using a 3-L syringe and two different gas mixtures, respectively. The testing protocol commenced with a 5-min resting period, followed by a 3-min warm up at a work rate of 60 W. Thereafter the workload was increased each minute by 30 W. The subjects were required to maintain a cadence of 60·min^{-1} throughout the whole test and were given strong verbal encouragement. The test was terminated when the subject was unable to maintain the assigned cadence. In addition, a plateau in O_2 uptake and a respiratory exchange ratio (RER) > 1.1 were used to confirm the attainment of the maximal O_2 consumption ($\dot{V}O_{2peak}$). $\dot{V}O_{2peak}$ was defined as the highest 60-s average of O_2 uptake ($\dot{V}O_2$) over the course of the test. Peak power output (W_{max}) was calculated using the following equation [145]: $W_{max} = W_{COMPL} + (t/60 \times 30)$. W_{COMPL} corresponds to the last completed workload and *t* corresponds to the number of seconds during final uncompleted workload.

Prior to the CP test, subjects performed a short 5 minute warm-up at a work load ranging from 40 to 90 W, on an electromagnetically-braked cycloergometer model Ergo Bike Premium (Daum electronics, Fürth, Germany). The test comprised a 3-min rest period in normoxia, followed by a 3-min rest period breathing a hypoxic gas mixture. Thereafter the subjects performed a 2-min warm up followed immediately by an increase in work rate to an individualy pre-determined level. The individual absolute work rate was identical to the work load attained at 75 % of $\dot{V}O_{2peak}$ measured during the graded exercise test. Thus, representing the work load corresponding to 100% of hypoxic

$\dot{V}O_{2peak}$, as a 25% decrement from normoxic $\dot{V}O_{2peak}$ was assumed [181]. During the tests, with the exception of the initial warm-up and a 3-min rest period, subjects breathed a pre-humidified hypoxic gas mixture (F_IO_2 = 0.120; F_iN_2 = 0.880) from a 200 L Douglas bag. The subjects breathed through a two way low resistance valve (Model 2, 700 T-Shape, Hans Rudolph, Shawnee USA) and were instructed to maintain their cycling cadence above 60·min^{-1}. Subjects reported their ratings of perceived exertion (RPE) using a modified (0-10) Borg scale [182]. They reported RPE separately for the respiratory (RPE_{dys}) and leg (RPE_{leg}) sensation. Continuous breath-by-breath measurements of $\dot{V}O_2$, carbon dioxide output ($\dot{V}CO_2$) and minute ventilation ($\dot{V}_E$), were performed in the same manner, using the same equipment described for the graded exercise test. Throughout the entire test HR and S_pO_2 were continuously measured using a portable heart rate monitor (Polar S810i, Kempele, Finland) and a portable pulse oxymetry device (Nellcor, BCI 3301, Boulder, USA) with ± 2 units accuracy across the range of 70-100% [148], respectively. Strong verbal encouragement from the experimental personnel was given during the whole test. The failure to maintain a cycling cadence above 60·min^{-1} resulted in termination of the test. The final score of the test was determined as the number of seconds a subject was able to maintain the assigned cycling cadence.

6.2.5 NIRS measurement

Near infrared spectroscopy (NIRS) enables continuous real time tissue oxygenation measurement during rest and exercise [183]. However, only a handful of studies utilized it for monitoring changes following different hypoxic exercise training protocols [184]. Given that NIRS allows for monitoring oxygenation and blood volume during exercise, it can be used for assessing the balance of muscle O_2 demand and supply [179]. The subjects were during all CP tests instrumented with three pairs of NIR probes to continuously monitor light absorption across cerebral and muscle tissue (Oxymon MK III, Artinis Medical systems, Zatten, Netherlands). The cerebral probes were placed over the left frontal cortex region. Leg muscle probes were positioned over the distal belly of the right vastus lateralis muscle, 15 cm above the knee and 5 cm laterally from the tight midline. The probes were also positioned on the right serratus anterior muscle, fixed in the sixth intercostal space on the anterior auxiliary line. The measurements of accessory respiratory muscles oxygenation has been shown to be useful, for assessment of respiratory work, as they play an important role in maintaining ventilation levels when diaphragm (primary respiratory muscle) is fatigued [185]. Elastic bandages were used to preclude any external light source affecting the optodes. Probe positioning and stabilization techniques were performed according to the previously published reports using the same device [179, 186]. The instrument recorded and calculated the micromolar changes in tissue oxy-hemoglobin (Δ[O_2Hb]) and deoxy-hemoglobin (Δ[HHb]) across time. The changes were calculated according to the Beer-Lambert law from two differential path length factors of 4.95 for muscle and 5.93 for cerebral tissue using two NIR lights wavelengths (780 & 850 nm). The sum of Δ[O_2Hb] and Δ[HHb] defined the total hemoglobin (Δ[tHb]) that can be used as an index of regional blood volume changes [187]. All measurements were normalized to reflect the changes from the resting period prior to the initiation of exercise. The data were recorded at 125 Hz and were filtered using Moving Gaussian algorithm prior

to analysis.

6.2.6 Hematological tests

Blood samples were collected from the antecubital vein on the morning of both PRE and POST tests (Fig. 1). Subjects were fasted prior to the procedure. All blood samples were assayed by a clinical biochemical laboratory (AdriaLab, Sinlab Group, Ljubljana, Slovenia). The samples were analyzed for red blood cell count (RBC), hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), using the cythochemical impedance method (Pentra120; Horiba ABX Diagnostics, Montpellier, France) (CV <2%). Transferin and ferritin were analyzed using the immunoturbidimetrical method (Cobas 6000, Roche Diagnostics, Basel, Switzerland) (CV <2,6%).

6.2.7 Data analysis and statistical evaluation

Anthropometrical characteristics, $\dot{V}O_{2peak}$, W_{max}, and $\dot{V}_{E\ max}$ of both groups were compared using Students two tailed *t*-tests. Differences between group means in CP tests, over the experimental period were analyzed using a 2-way ANOVA [group (SIH, Control) × testing period (PRE, POST)]. Three way ANOVA was used to compare the effects of the tested protocol during the CP tests in both groups across both relative (rest, 20, 40, 60, 80, 100 % CP time) and absolute (rest, 1, 2, 3, 4, 5 min) times and across PRE and POST testing [group (SIH, Control) × work load (relative, absolute) × testing period (PRE, POST)]. Where a main effect was observed a Tukey *post-hoc* test was used to compare the specific differences. Criterion level for significance was set *a priori* at $P < 0.05$. The data are presented as means ± SD unless otherwise indicated. All analyses were performed using Statistica 5.0 (StatSoft, Inc., Tulsa, USA).

6.3 Results

Significant difference was observed between groups in S_pO_2 during the SIH (day 1: 82 %, day 2: 83 %, day 3: 83 %, day 4: 84 %) and Control (day 1: 97 %, day 2: 97 %, day 3: 97 %, day 4: 97 %) trials. The average HR levels during the intermittent exposures were comparable for both groups (SIH = 69 ± 3 beats·min^{-1}; CON = 68 ± 4 beats·min^{-1}). The average LLS values were < 0.7 during all exposures in the SIH group and < 0.5 in the CON group. No adverse side effects associated with the hypoxic exposure (nausea, headache, dizziness) were noted. When interviewed at the end of experiment all subjects in both groups perceived their exposure as being hypoxic and not normoxic. As can be seen from Table 6.2 there were no significant differences in hematological parameters between the PRE and POST trials.

Table 6.2: *Selected hematological parameters of both groups before (PRE) and after the experimental protocol. No significant differences were shown ($p < 0.05$).*

	SIH group		CON group	
	PRE	POST	PRE	POST
RBC (10^{-12}·L)	4.9 (0.4)	4.9 (0.3)	4.9 (0.2)	4.8 (0.3)
Hb ($g \cdot L^{-1}$)	150 (9)	151 (9)	148 (10)	147 (9)
HCT (%)	0.44 (0.03)	0.44 (0.02)	0.44 (0.03)	0.43 (0.03)
MCV (fL)	90.1 (2.9)	90 (2.9)	90.2 (4.5)	89.9 (4.2)
MCH (pg)	30.9 (1.1)	30.8 (1.1)	30.4 (1.8)	30.7 (1.7)
MCHC ($g \cdot L^{-1}$)	341.3 (4.7)	340.9 (5.8)	337.8 (4.9)	340.4 (5.4)
Transferrin ($g \cdot L^{-1}$)	1.9 (0.3)	2.1 (0.3)	2.3 (0.5)	2.3 (0.4)
Ferritin ($ng \cdot mL^{-1}$)	129 (60)	117 (53)	126 (65)	124 (59)

Values are mean (SD). Rbc, red blood cells; Hb, hemoglobin; Hct, Hematocrit; MVC, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

No significant differences were observed in time to exhaustion during the hypoxic CP test either between the Control and SIH groups or the PRE and POST trials (SIH [PRE = 295 ± 115 sec, POST = 338 ± 90 sec]; Control [PRE = 350 ± 99 sec, POST = 371 ± 106 sec]). There was a tendency, although non-significant for an increase in the duration of the CP test in both the SIH (14 %) and Control (6 %) group. Analysis of the $\dot{V}O_2$, $\dot{V}CO_2$, HR and RPE_{dys} values, revealed no significant changes between PRE and POST tests in both group. Both, $\dot{V}_E$ and S_pO_2 were significantly higher during the POST test in the SIH group only. The differences occurred at 40, 60 and 80 % of endurance time in $\dot{V}_E$ and at 20, 40, 60 and 100 % of endurance time in S_pO_2 as can be seen in Fig 2. Similarly, there was a significant decrease in RPE_{leg} during the POST test, compared to the PRE in the SIH group only. The RPE_{leg} values (median (range)) decreased significantly during the 40 and 60 % of endurance time in the SIH group (PRE 4 (2-7), 6 (3-8); POST 3 (2-4), 4 (3-7)) and remained similar for the CON group (PRE 4 (2-7), 6 (4-7): POST 4 (2-5), 6 (4-6)), respectively.

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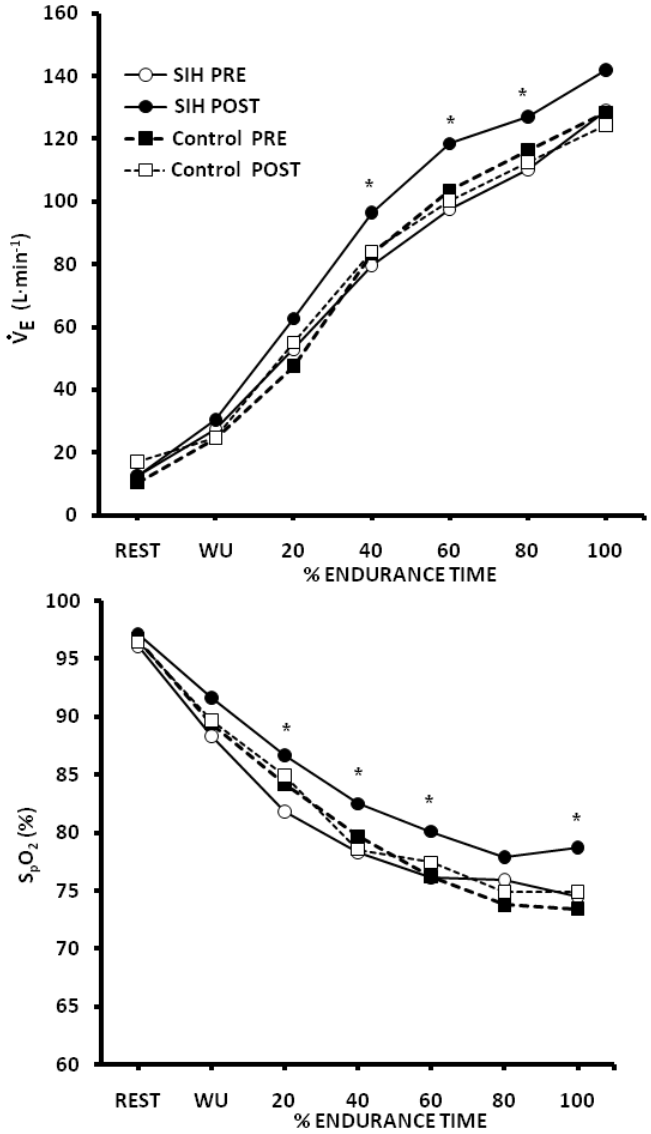

Figure 6.2: *A) Minute ventilation ($\dot{V}_E$), and B) capillary oxyhemoglobin saturation (S_pO_2) during constant power test in hypoxia of both groups before (PRE) and after the experimental protocol (POST). WU: warm up.* indicates significant difference from PRE (P < 0.05). For purpose of clarity the SD's are not shown.*

The only significant difference between groups and trials observed was the significantly higher deoxygenation (↑Δ[HHb]) of the vastus lateralis muscle during the POST compared to the PRE test in SIH group (Fig 6.3). Concomitant with the pronounced, significant deoxygenation (↓Δ[O_2Hb], ↑Δ[HHb]), evident during all tests, was a steady increase in regional blood volume (↑Δ[tHb]). Δ[tHb] was significantly higher during the last part of the test in both group (80 & 100 % endurance time). Significant deoxygenation pattern (↓Δ[O_2Hb], ↑Δ[HHb]) was similar for the serratus anterior muscle, with the exception that there were no significant differences between PRE and POST trials in neither group (Fig 6.4). The serratus anterior regional blood volume was significantly decreased (↓Δ[tHb]) following 20 % of relative CP time and remained fairly constant afterwards, following a similar pattern in both groups during both trials (Fig 6.4). The NIRS oxygenation concentration changes from resting condition in Cerebral frontal cortex are presented in Fig 6.5. The cerebral oxygenation showed a similar trend of significantly decreased oxygenation (↓Δ[O_2Hb], ↑Δ[HHb])

with a tendency, although non-significant, of an increase in regional blood volume (↑Δ [tHb]). There were no significant differences between groups and testing periods in cerebral oxygenation. Both the deoxygenation pattern (↓Δ[O_2Hb], ↑Δ[HHb]) and the regional blood volume changes (Δ[tHb]) in all three regions were not significantly different between neither groups nor testing trials.

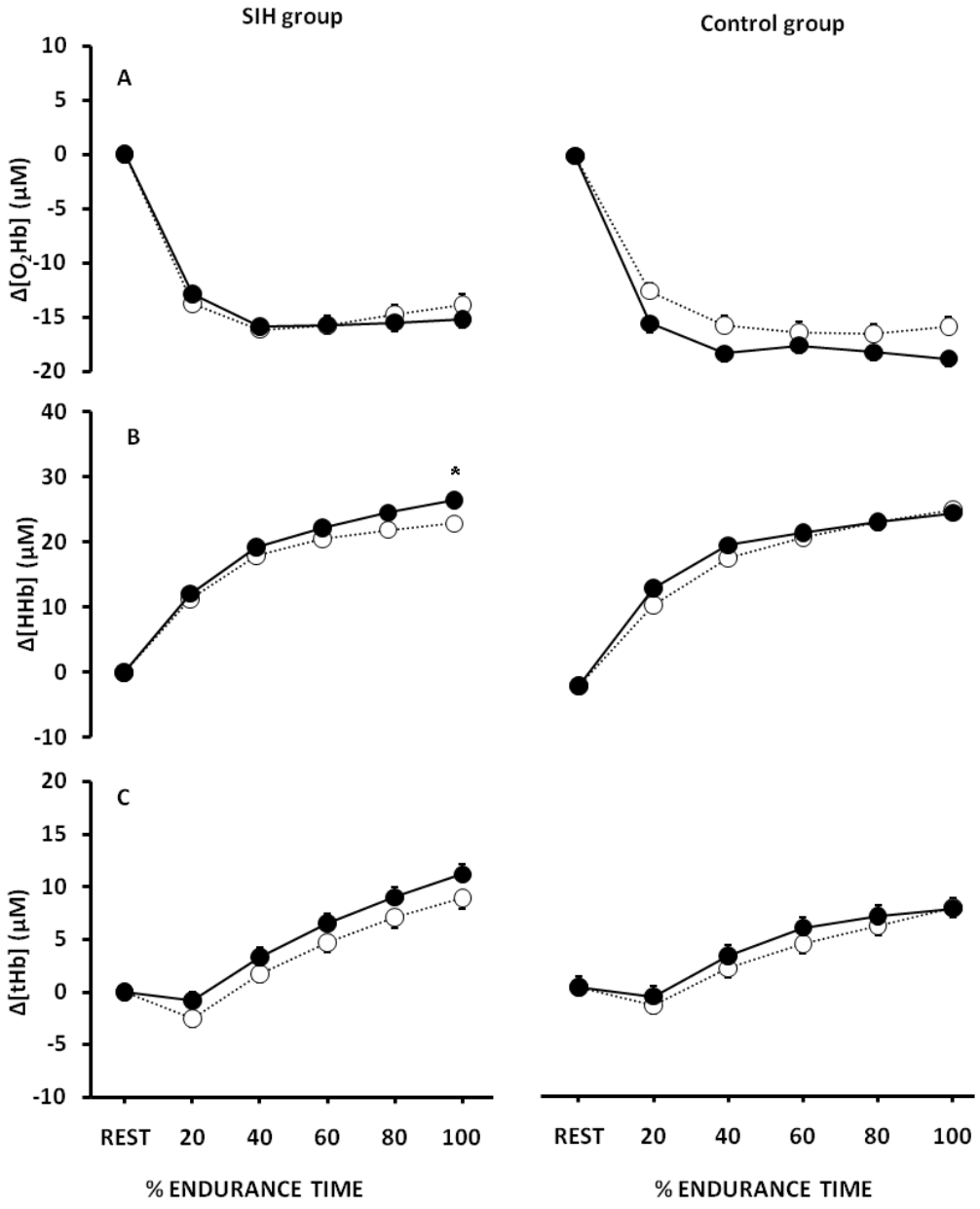

Figure 6.3: *Relative changes from resting values (means ± SE) in the concentration of the oxygenated (A), deoxygenated (B) and total hemoglobin (C) in the vastus lateralis muscle, as measured with NIRS, during the PRE (○) and POST (●) hypoxic constant power tests for the control (CON) and short intermittent hypoxic (SIH) exposure groups. Values are plotted as a function of relative exercise time. * denotes significant difference between PRE and POST values (P < 0.05).*

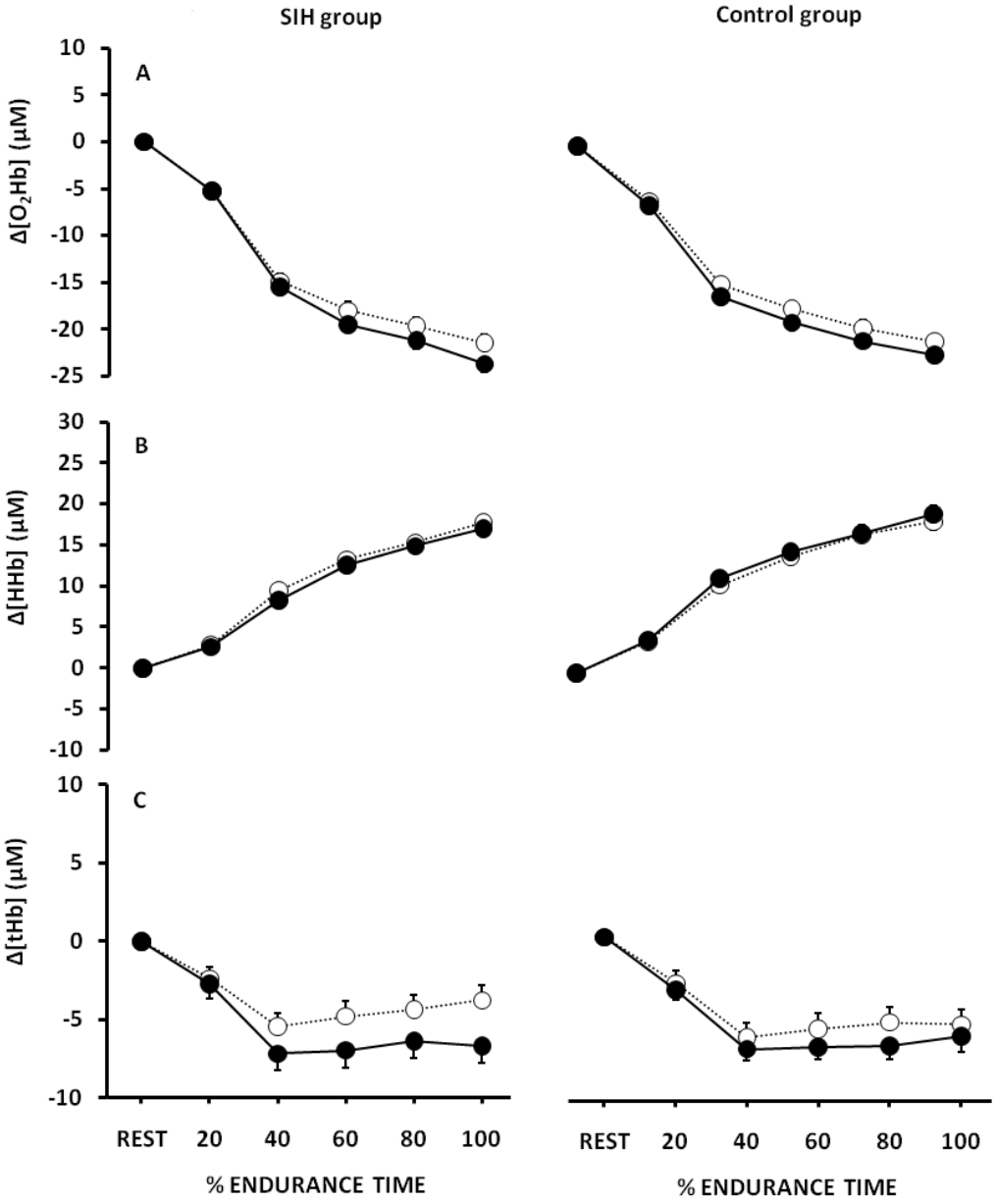

Figure 6.4: *Relative changes from resting values (means ± SE) in the concentration of the oxygenated (A), deoxygenated (B) and total hemoglobin (C) in the serratus anterior muscle, as measured with NIRS, during the PRE (○) and POST (●) hypoxic constant power test for the control (CON) and short intermittent hypoxic (SIH) exposure groups. Values are plotted as a function of relative exercise time.*

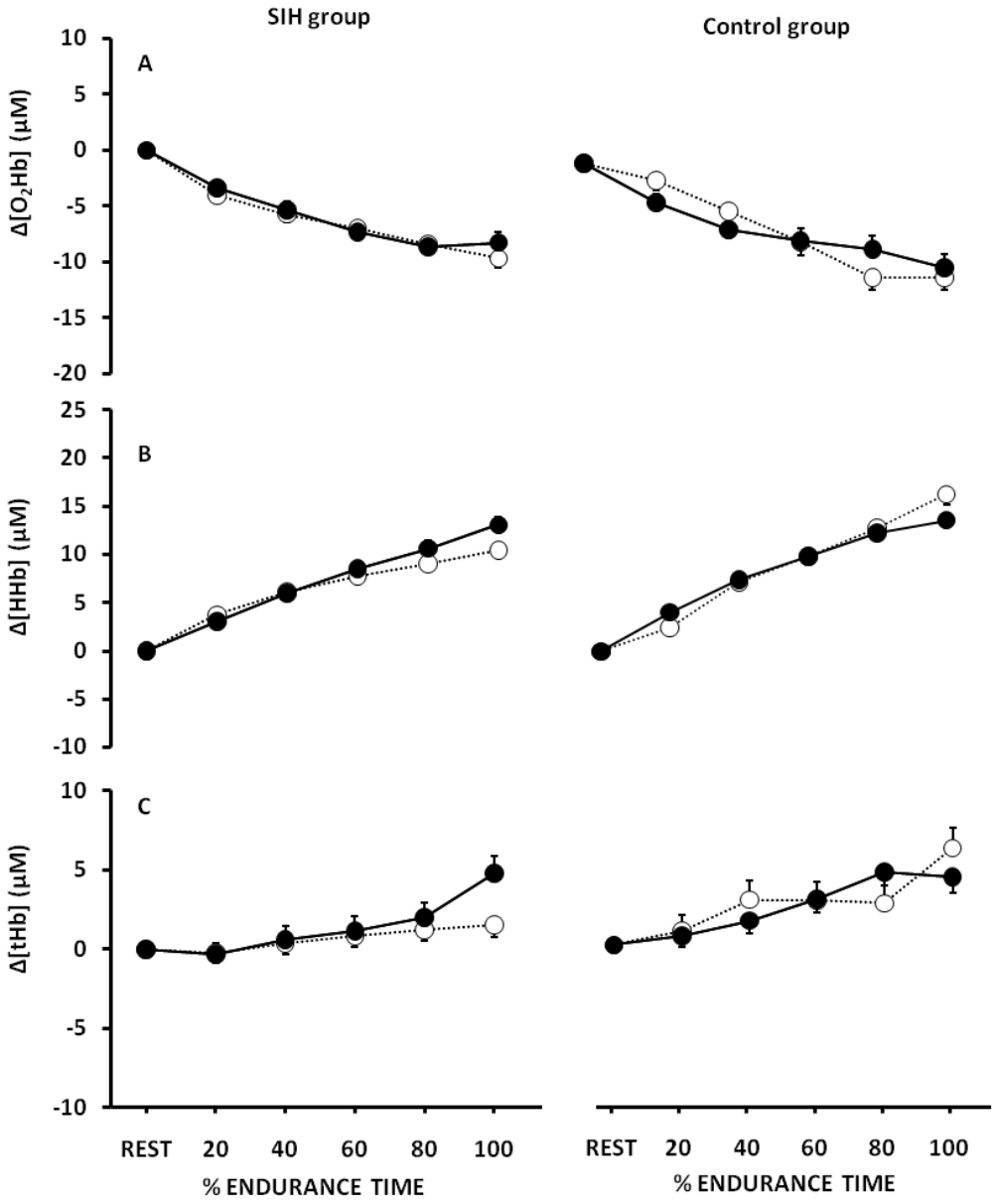

Figure 6.5: *Relative changes from resting values (means ± SE) in the concentration of the oxygenated (A), deoxygenated (B) and total hemoglobin (C) in the left frontal cortex, as measured with NIRS, during the PRE (○) and POST (●) hypoxic constant power test for the control (CON) and short intermittent hypoxic (SIH) exposure groups. Values are plotted as a function of relative exercise time*.

6.4 Discussion

The main finding of the present study is that four intermittent exposures to normobaric hypoxia augment ventilation and blood O_2 saturation levels during hypoxic exercise. These adaptations do not affect either time to exhaustion, or regional blood volume redistribution and oxygenation in the exercising limbs and accessory respiratory muscles during the hypoxic constant power test. The SIH protocol also did not modify cerebral oxygenation during hypoxic exercise.

To date, the majority of studies investigating short intermittent exposures to hypoxia, as a means of

pre-acclimatization to hypoxic environment, have used hypobaric hypoxia [8, 177] and have reported beneficial effects on hypoxic performance [4, 12]. Namely, the studies have shown that improvements in altitude performance can be expected following 15 [71] or even only 7 [72] short intermittent exposures. The augmented ventilatory response to hypoxia as a result of SIH has also been well documented. In particular, the studies by Ricart et al [86], and Katayama et al. [6] demonstrate that, the increases in hypoxic chemosensitivity, ventilation and O2 saturation can be achieved following SIH protocols consisting of twelve 2-hr exposures or seven 1 hr exposures, respectively. This ventilatory adaptations are initiated by the SIH induced increases of the peripheral chemoreceptors sensitivity, and provide the focal underlying mechanism for the enhancement of performance following SIH [168]. The augmented sensitivity has been shown to provide enhanced ventilatory response during both rest [175] and exercise [6] in hypoxia. Furthermore the increased $\dot{V}_E$ induces increases in alveolar and arterial oxygenation levels [168] that subsequently lead to improved working capacity. Collectively, the data on SIH protocols employing hypobaric hypoxia show that hypoxic exercise performance can be enhanced [6, 71, 72, 86].

On the contrary, these findings were not reflected in the results of a study investigating SIH performed in normobaric hypoxia [7]. The study of Beidleman and colleagues [7] is among the few controlled studies directly investigating the effects of short intermittent normobaric pre-acclimatization protocol ($3h \cdot day^{-1}$, 7 days, 3000 – 4500 m simulated altitude). Compared to the present study, they performed seven exposures and combined them with moderate exercise training during the second part of each exposure (2 x 25 min). Similarly to our results, they did not find any benefits for altitude performance, although they performed the tests in hypobaric hypoxia while our tests were performed in normobaric hypoxia. On the other hand they did not find any significant changes in ventilatory and cardiovascular responses or in the ratings of perceived exertion during exercise at altitude. This is contrary to our findings, showing increased ventilation and blood O_2 saturation as a consequence of the SIH protocol. Factors that can be implicated in the observed discrepancies are: timing of the tests (60 h following SIH; 24 h following SIH), environmental conditions (hypobaric v. normobaric), and hypoxic dose duration and frequency (3 hours x 7 days; 4 hours x 4 days). It would appear therefore that normobaric SIH can stimulate the hypoxic ventilatory response immediately following the cessation of the protocol, but this enhancement does not seem to persist for 60 hours. However, since Katayama et al. [188] showed, that the increased hypoxic ventilatory response can persist up to two weeks following the SIH, the time course of the re-setting of the chemosensitivity remains questionable. Accordingly, these findings suggest that in practice similar protocols should be used in such a manner that the subsequent re-exposure occurs rapidly following the SIH protocol.

Regarding the mode of hypoxia (hypobaric v. normobaric) used, one could assume that the normobaric SIH may be less efficient as hypobaric since two similarly designed studies investigating either hypobaric [72] or normobaric SIH [7] showed different results. While they found improvements in both performance and ventilatory responses following hypobaric SIH, neither was seen following normobaric SIH. This inconsistency could be explained by the purported differences in responses to normobaric or hypobaric hypoxia. The skepticism exists as to whether the responses to both are similar

[151]. The Equivalent air altitude model assumes that similar physiological responses should occur as a result of exposure to the same partial O_2 pressure regardless of the [barometric pressure x F_IO_2] combination. However, the gas density related changes in alveolar diffusion and ventilation as well as in pulmonary blood flow distribution may underlie the potential differences [151, 189]. While some studies have shown differences including greater hypoxemia, and lower S_pO_2 levels in subjects exposed to hypobaric as compared to normobaric hypoxia [16], the responses seem to be sufficiently similar for normobaric exposures to provide a faithful surrogate for hypobaric hypoxic exposures [189]. Our findings provide further evidence, that normobaric SIH can induce significant ventilatory adaptations, similar to those observed in the studies employing hypobaric hypoxia [6, 86]. Moreover, a recent study [190], investigated the effects of 48-hrs normobaric hypoxic exposure on normoxic performance and reported increases in normoxic exercise ventilation without a concomitant improvement in performance, similar to the findings of the present study. Thus, showing that normobaric hypoxia could also influence ventilatory parameters during normoxic exercise. Collectively, these finding suggest that similar ventilatory responses can be anticipated following the same SIH dose, regardless of the manner in which hypoxia is established.

Whether SIH induced ventilatory acclimatization effects the oxygenation patterns and blood flow in the exercising limb and respiratory muscles was the second aim of the present study. The redistribution of blood flow during heavy exercise resulting in augmented oxygenation of the respiratory muscles and a reduced oxygenation of the exercising limb muscles, has been demonstrated in healthy subjects [179] and in chronic heart failure patients [191]. We hypothesized that this regional redistribution would also be evident following SIH, as changes in regional oxygenation patterns, due to the increased ventilatilatory levels. Based on the measurements of the oxygenation of the serratus anterior and vastus lateralis muscles, we conclude that no such effect is evident following an SIH protocol.

As suggested by Harms et al. [192] a significant decrease in leg muscle oxygenation enhances the onset of fatigue, mostly due to the higher O_2 flux requirements of the respiratory muscles, at higher ventilation levels. Although they have shown that this phenomenon occurs during heavy load constant power exercise, we did not observe these changes during the hypoxic CP test. Although we considered, that this discrepancies could have been attributed to the differences in training status of the subjects used (highly trained vs. moderately active), the study by Legrand et al. [179] confirmed the existence of the phenomenon also in healthy active subjects. They have essential shown that during last phases of the incremental exercise test, the leg muscle oxygenation decrement was attenuated and related to the accelerated drop in the accessory respiratory muscles oxygenation. This was not observed in the hypoxic constant power test in our study, since the blood volume (Δ[tHb]) of both respiratory and leg muscles did not show any significant cut off point but have decreased or increased steadily. Even if we found significantly higher deoxygenation ($\uparrow\Delta$[HHb]) of the vastus lateralis muscle during the POST testing, this did not significantly effect either Δ[tHb] or $\Delta[O_2Hb]$. Moreover, our data also did not show any significant attenuation of the decrease in $\Delta[O_2Hb]$ of the vastus lateralis and pronounced decrease in Δ[HHb] of the serratus anterior muscle during the last part of the exercise.

Thus, we can say that this phenomenon is not observed during the hypoxic CP test. Our data are also in line with the findings of Kowalchuk et al. [193], who did not observe any changes in leg muscle oxygenation as a result of increased work of breathing during constant power heavy work load exercise. The underlying physiological mechanism for the reported phenomenon has been been reasoned to be the sympathetically mediated vasoconstriction of the exercising leg muscles, that has been shown to occur during high intensity exercise levels [178]. No significant change in the Δ[tHb] of the vastus lateralis during the last part of the CP indirectly demonstrate that most probably, no significant vasoconstriction was present in the legs. The inconsistent results could not have been attributable to the subjects not pushing themselves to the limit since both the rates of perceived exertions (RPE_{dys}; RPE_{leg}) and HR levels were during the last phases of the CP comparable to the ones achieved during the final phases of the graded exercise test.

Although ventilation levels were significantly higher during the POST test in the SIH group, both the respiratory and leg muscle oxygenation was unaffected. Thus, the higher absolute ventilation levels, and a concomitant increase in the work of breathing, did not result in greater deoxygenation of the respiratory muscles, nor in an attenuated regional blood volume. Moreover it also did not the oxygenation pattern in the vastus lateralis. A significant increase in the S_pO_2 during CP confirmed, that higher ventilatory levels improved blood oxygenation however this was not reflected in the muscle oxygenation pattern. Therefore, whilst the ventilatory acclimatization was seen, it did not have any significant effect on neither oxygenation levels nor the modulation of blood volume distribution during the CP test in hypoxia.

The main limitation of the previous studies investigating short intermittent hypoxic protocols is the lack of a placebo control group, since it has been suggested, that a placebo itself may have potent effects on performance and outcomes of different hypoxic training modalities [2]. Therefore, we included a matched controlled group who underwent an identical protocol of exposures, except in normoxic condition, as the SIH group, to eliminate and investigate the possible placebo effect. In the present study, there was no evident placebo effect.

Even though we did not find any differences in the selected hematological parameters, some studies have reported improvements in hemoglobin, red blood cell content and reticulocyte count following SIH [62]. However the study was performed in hypobarichypoxia and incorporated nine exposures lasting between 3 and 5 hours.

Besides chronic hypoxic pre-exposures the SIH protocols thus provide a viable means for inducing pre-acclimatization. The advantages of intermittent over chronic hypoxic exposures are: 1) Better quality of training: due to lower levels of absolute work rate achieved at altitude, the training effect is reduced. 2) Improved appetite: there is evidence that appetite is affected during altitude sojourn [194]. 3) The logistics of normobaric SIH protocol are less complex. Moreover, as has been shown by Katayama at al. [195], the subjects who prior to high altitude exposures undergo a SIH protocol, tend to re-adapt faster although the time between the two hypoxic exposure may be as long as one month. Thus, SIH protocols appear to have a beneficial influence on the process of adaptation in later on hypoxic re-exposures.

In conclusion our results demonstrate that four intermittent normobaric exposures can induce ventilatory acclimatization without affecting hypoxic endurance performance. The SIH protocols may be of practical benefit for inducing ventilatory adaptations prior to high altitude sojourn, when the pre-acclimatization time is limited and the hypoxic exposure commences soon (< 60 hrs) after the SIH protocol.

7 Acute hyperoxia followed by mild hypoxia does not increase EPO production

7.1 Introduction

Since studies (I, II, III) have not showed convincing benefits for performance enhancements following relatively short hypoxic exposures, this lead us to conclusion that the tested protocols and/or hypoxic doses were not efficient. Therefore the intention of the last study (Study IV) was to improve the efficiency of contemporary hypoxic training modalities, by investigating the novel approach for improving O_2 flux capacity and efficiency through augmented erythropoietin sinthesis and subsequent erythropoiesis. Thus, in Study IV we investigated the effects of combining acute hypoxic and hyperoxic exposures on erythropoietin synthesis as the main agent of the hematological benefits following hypoxic training protocols.

Although the debate whether the main benefits following different hypoxic acclimatization protocols arise from hematological [173] or non-hematological [40] changes is still ongoing (see point-counterpoint JAP 2005), it is well established that sufficiently long and strong hypoxic exposure can induce beneficial hematological adaptations [196-198] and subsequent performance enhancements through blood O_2 capacity augmentation [22, 199]. This adaptation is especially important for endurance athletes, providing them with competitive advantage [113]. Its importance is also clearly emphasized with a broad use of illegal recombinant EPO and other simulating EPO agents in elite sport [10, 200]. Enhanced erythropoiesis is also sought after as an important factor when employing pre-acclimatization protocol before altitude expeditions, to subsequently improve performance at altitude and reduce the risk of altitude related medical problems [12].

The key role player in this physiological adaptation is glycosolated hormone Erythropoietin (EPO). EPO is mainly produced by the kidney interstitial fibroblasts [201] in response to reduced O_2 tissue availability [202, 203]. This hypoxia-induced EPO production is well established [174, 197, 198]. Eckardt et al. [93] have also shown that the EPO response has a dose dependent nature, since single exposures to simulated altitudes corresponding to 3000 m and 4000 m above sea level for 5.5 hours caused transient increases in EPO levels equivalent to 1.8 and 3.0-fold, respectively.

While it is well accepted that absolute tissue hypoxia (i.e. decreased PO_2) induces EPO production, recently Balestra et al. [9, 204] reported that EPO production is markedly enhanced following a return from a short-term hyperoxic exposure to normoxia. Namely, they observed an increase in serum EPO levels 24 and 36 hrs after a 2-hr period of breathing 100 % O_2. The transition from normobaric hyperoxia to normoxia induced a similar response as would normally be observed during the transition

from normoxia to hypoxia. Thus, they termed this phenomenon the "normobaric oxygen paradox" (NOP), since it paradoxically appears to be independent of absolute tissue hypoxia [9].

Although intriguing, the existence of the NOP is still questionable. In particular, subsequent studies either did not find augmented EPO production following an acute normobaric hyperoxic exposure [132, 205], or have even shown a suppressive effect on EPO production [133]. This is in line with the results of previous studies showing the suppression of erythropoietic activity following normobaric hyperoxia in humans [131, 206].

To further elucidate the importance of relative changes in oxygenation on EPO production and to evaluate the NOP theory, the present study investigated the effect of consecutive hyperoxic and hypoxic breathing on EPO production. Based on the assumption that the EPO synthesis is a function of the relative change in, rather than the absolute level of PO_2 [207], we hypothesized that a greater drop in PO_2 (ΔPO_2) would provide a stimulus for EPO synthesis. We therefore monitored plasma EPO levels following a relative change in PO_2 from hyperoxia (PO_2 = 950 hPa) to hypoxia (PO_2 = 140 hPa), reasoning that a ΔPO_2 = 810 hPa should provide a similar or even greater plasma EPO concentration than that observed by Balestra and colleagues, who exposed their subjects to a drop in PO_2 of 765 hPa.

7.2 Methods

7.2.1 Subjects characteristics

Eighteen active healthy male subjects participated in the study. All subjects, were local residents (altitude = 300 m above sea level), and were not exposed to altitudes above 2000 meters within five weeks preceding the experiment. Exclusion criteria were: a history of anemia or bone marrow dysfunctions [208], renal disease [209] and habitual or occasional smoking [210]. Subjects were given a detailed verbal presentation of the protocol and signed their informed consent to participate in the study. They were then randomly assigned to either the control (CON, n = 8), or intermittent hyperoxic/hypoxic (IHH, n = 10) group (Table 1). The subjects were specifically instructed to refrain from any heavy physical activity two days before and throughout the experimental period. The study protocol was approved by the National Committee for Medical Ethics of the Republic of Slovenia, and was conducted according to the guidelines of the Helsinki Declaration.

7.2.2 Study design

The outline of this single-blind, placebo controlled and randomized study protocol is presented in Fig. 7.1. The protocol consisted of two hour exposure period and the pre-exposure, mid-exposure and post-exposure samplings. The post-exposure sampling period lasted two days. Moreover, to ensure similar fitness level between groups, all subjects performed an incremental exercise test to exhaustion on a cycle-ergometer (Daum electronics, Fürth, Germany) to determine the peak O_2 consumption ($\dot{V}O_{2peak}$)

on a separate day, prior to the exposures.

The $\dot{V}O_{2peak}$ testing protocol comprised a 5-min resting period, followed by a 2-min warm-up at 60 W. Thereafter, the workload was increased each minute (25 W· min^{-1}) until exhaustion. Inability to maintain the pedaling cadence above 60 rpm, plateau in the O_2 consumption ($\dot{V}O_2$) and/or a respiratory exchange ratio > 1.1 were the criteria used, to confirm the attainment of the $\dot{V}O_{2peak}$ (calculated as the highest $\dot{V}O_2$ averaged over 60 s during the test). $\dot{V}O_2$ was measured breath-by-breath with a Quark CPET metabolic cart (Cosmed, Rome, Italy).

7.2.3 Breathing protocol

Both groups (CON and IHH) followed exactly the same time schedule on exposure days. They were instructed to eat a standardized breakfast consisting of bread, honey and juice, at least 1.5 hrs before their arrival. Each subject reported to the laboratory during the morning hours (7:30 – 8:00 AM). After being instrumented, subjects were placed in supine position on a gurney, and remained in the same position for the entire 120-min exposure period. The laboratory environmental conditions [temperature = 21.9 ± 0.8 ˚C; relative humidity = 42 ± 1 % and pressure = 978 ± 6.5 hPa] were maintained stable throughout the experiments. Following a 15-min resting period, subjects breathed the assigned normobaric gas mixture via an oronasal mask (7920 AL, Hans Rudolph Inc., Shawnee, USA) with a low resistance two-way valve (2700 NRBV, Hans Rudolph Inc., Shawnee, USA) from a 200-L Douglas bag. Hyperoxic and hypoxic breathing mixtures were decompressed from cylinders, humidified and accumulated in the Douglas bag. Two Douglas bags were used for the IHH group, one bag for each breathing mixture (i.e. hyperoxic and hypoxic). The normoxic breathing mixture (air) was also humidified and collected in the Douglas bag. The CON group breathed air (PO_2= 197 hPa) for 120 minutes, while the IHH group breathed hyperoxic gas mixture (PO_2= 950 hPa) for 60 minutes followed by a 60-min period of breathing the hypoxic gas mixture (PO_2= 140 hPa). Capillary oxyhemoglobin saturation (S_pO_2) (Nellcor, BCI 3301, Boulder, USA) and heart rate (HR) (Polar S810i, Kempele, Finland) were continuously measured during the protocol. Subjects also reported their ratings of perceived exertion for dyspnea (RPE_{dys}) on a modified Borg scale (scale 0-10).

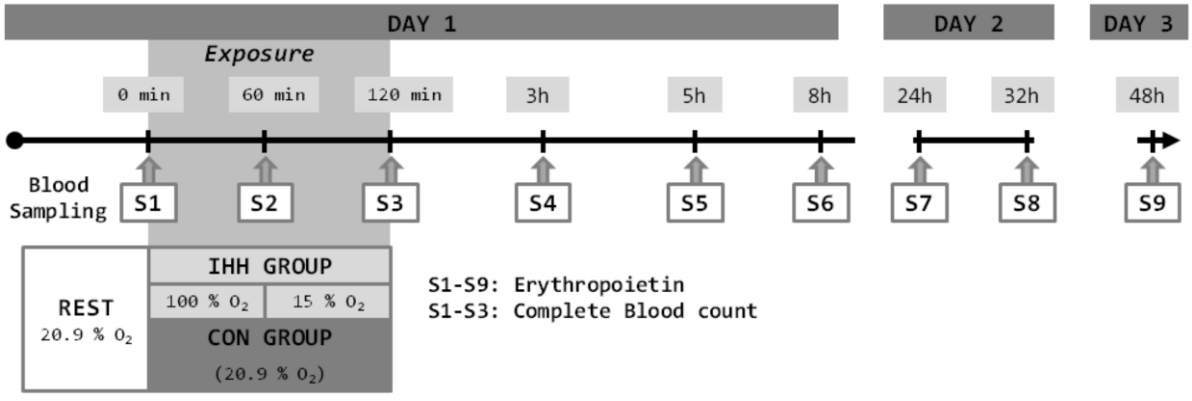

Figure 7.1: *Schematical presentation of the study outline.*

7.2.4 Blood sampling and analysis

Blood samples were taken just before (S1), after 60 minutes of exposure (S2) and immediately after the 120-min exposure period (S3). Thereafter, the samples were taken at 3, 5, 8, 24, 32 and 48 hours after the exposure (S4 – S9). All samples were drawn from the antecubital vein of the subjects' left arm. During each visit and prior to obtaining the sample, subjects continuously rested in the supine position for at least 15 minutes. The total quantity of blood taken during the whole protocol did not exceed 30 mL. Blood samples were immediately stored in BD Vacutainers (K2E, Becton Dickinson, New Jersey, USA) and centrifuged (10 min at 3000 rpm). The obtained plasma was instantly frozen to -80 °C and stored for further analysis. The [EPO] was determined in 100 μL of plasma using sandwich enzyme-linked immunoassay (Quantikine IVD EPO ELISA, R&D Systems, Minneapolis, USA). The method used in the study has been validated before [211]. The quantification of the optical density was performed on a microplate reader Quant (Bio-Tek instruments, Winooski, USA) set at 450 nm and corrected at 600 nm. All samples were assayed in duplicate, whereas only one microplate was used for each subject's samples in order to avoid the possible variability between the plates. All techniques were performed according to the manufacturer's instructions. The sensitivity of the analysis was 0.6 $mU \cdot mL^{-1}$, whereas the estimated coefficient of variation (CV) was 2.9 %.

At first three samplings (S1-S3) additional blood samples (500 μL) were taken to determine the complete blood count (CBC). The samples were analyzed using an automated laser-based hematology analyzer ADVIA 120 (Siemens, München, Germany). The resulting CBC included red blood cells (RBC), hemoglobin concentration (Hb) and hematocrit (Hct) with the estimated CV of 0.9 %, 0.7 % and 1.3 %, respectively.

7.2.5 Data analysis and statistical evaluation

All data are presented as mean ± SD, unless otherwise indicated. Anthropometric data and $\dot{V}O_{2peak}$ were compared using a Students two-tailed t-test for unequal sample sizes. The [EPO] data were analyzed for normal distribution using a modified Kolmogorov-Smirnov test. One-way ANOVA (time) was performed to analyze within group changes in [EPO] and CBC. Differences between groups in [EPO] were analyzed using a two-way ANOVA for repeated measures (group x time)**.** The same analysis was performed to analyze the changes in HR, S_pO_2 and RPE_{dys} during the exposure. When a main effect was observed, a Tukey *post hoc* significance test was employed to identify the specific differences between means. Pearson's correlation analysis was calculated for selected parameters. All analyses were performed using Statistica 5.0 software (StatSoft, Inc., Tulsa, USA). The alpha level of significance was set *a priori* at 0.05.

7.3 Results

Subjects' characteristics are presented in Table 7.1. There were no significant differences between groups in anthropometric characteristics, $\dot{V}O_{2peak}$ or in any of the hematological variables.

Table 7.1: *Baseline subject characteristics.*

	CON group (n=10)	**IHH group** (n=10)
Age (yrs)	25.3 (1.6)	23.3 (2.1)
Stature (cm)	180.2 (7.2)	180.7 (7.1)
Body mass (kg)	74.1 (7.3)	76.8 (13)
BMI (kg/m^2)	22.9 (2.9)	23.4 (3.2)
$\dot{V}O_{2peak}$ (mL/min)	3942 (340)	3572 (540)
W_{max} (watt)	333 (25)	314 (40)
Hb (g/L)	150.9 (6.8)	152.5 (7.5)
HCT (%)	44 (3)	45 (2)

Values are means (SD).

The S_pO_2 of the IHH group significantly decreased immediately following the switch to hypoxic mixture and remained consistently decreased throughout the hypoxia exposure (Fig. 7.2). The HR was not different between groups during the first 60 min of the exposure (Fig. 7.2). During the second 60 min of the exposure, HR was significantly higher in the IHH group breathing a hypoxic gas mixture than in the CON group that continued breathing air. We did not observe any differences between groups in RPE_{dys} during the exposure. No significant changes, from baseline values, were observed in any of the groups after 60 min (S2) or immediately following the 120-min exposure (S3) for any of the measured hematological variables.

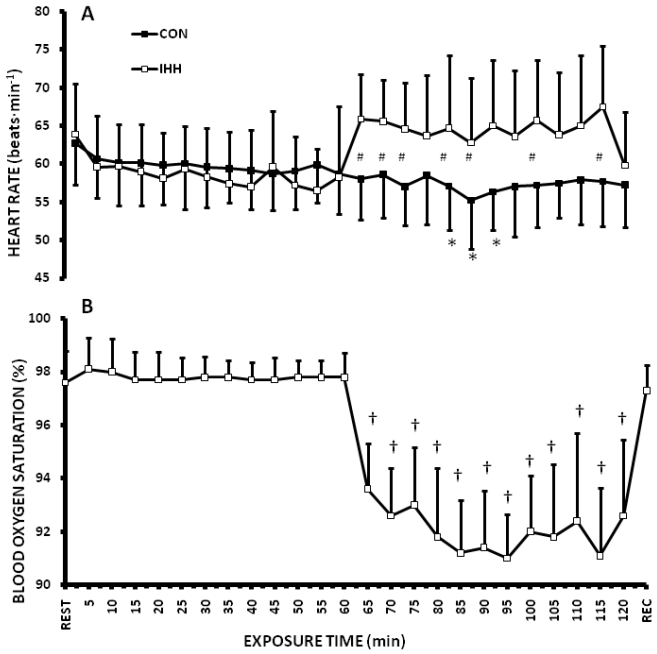

Figure 7.2: *Average values of Heart rate (A) and Capillary oxyhemoglobin saturation (B) during the exposure for the IHH and CON groups (* P < 0.05; signifficant differences from REST, # P < 0.05; signifficant differences between groups, † P< 0.05; significant differences from hyperoxic breathing (first 60 minutes)).*

No significant changes in hematological variables were observed in any of the groups after 60 min (S2) or immediately following the 120-min exposure (S3) compared to baseline (Table 7.2).

There were significant differences in absolute [EPO] within groups at different time periods (Fig. 7.3). Specifically, [EPO] was significantly increased at 8 and 32 hrs after the exposure in the CON group and at 32 hrs in the IHH group. However, no significant differences were noted between groups. Peak [EPO] values were observed 8 and 32 hours following the exposure for the CON and IHH group, respectively (Fig. 7.3). The CON group showed a fluctuation of [EPO] with nadir values measured in the mornings 24 and 48 hrs following the exposure and zenith values in the evening 8 and 32 hrs following the exposure.

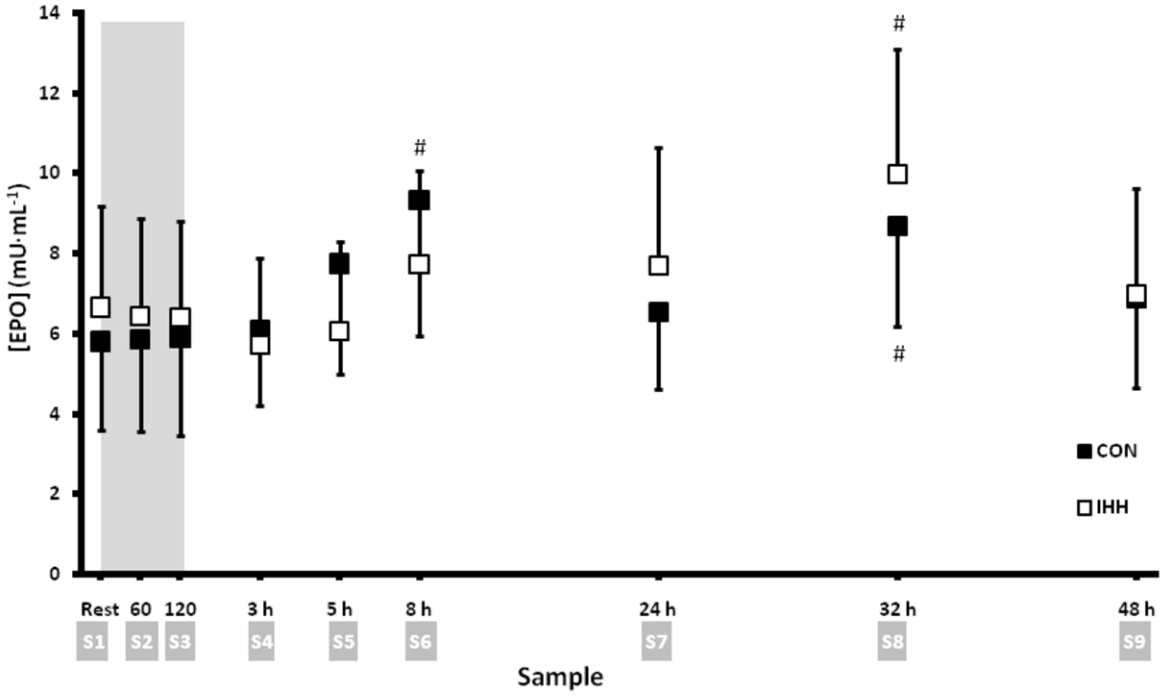

Figure 7.3: *Plasma EPO concentrations before, during and after the exposure in both groups (# P < 0.05; significant differences from REST). Values are mean ± SD.*

Fig. 7.4 presents the relative changes in [EPO], which were significantly different between groups 5 and 8 hrs following the exposure. [EPO] levels increased steadily from the first 8 hours following the exposure in the CON group. In the IHH group, following an initial, albeit non-significant, decrement (3 and 5 hrs), [EPO] began to increase 8 hrs following the exposure, and resumed a similar pattern thereafter, as observed in the CON group. A substantial individual variability in [EPO] response was observed in both groups. The individual changes in absolute [EPO] levels during all testing periods ranged from 35 – 225 % for the CON subjects and from 33 – 165 % for the subjects in the IHH group. These different responses of [EPO] were not related to either $\dot{V}O_{2peak}$ or baseline Hb and Hct values, with correlation coefficients (r) of 0.22, 0.13, and 0.24, respectively.

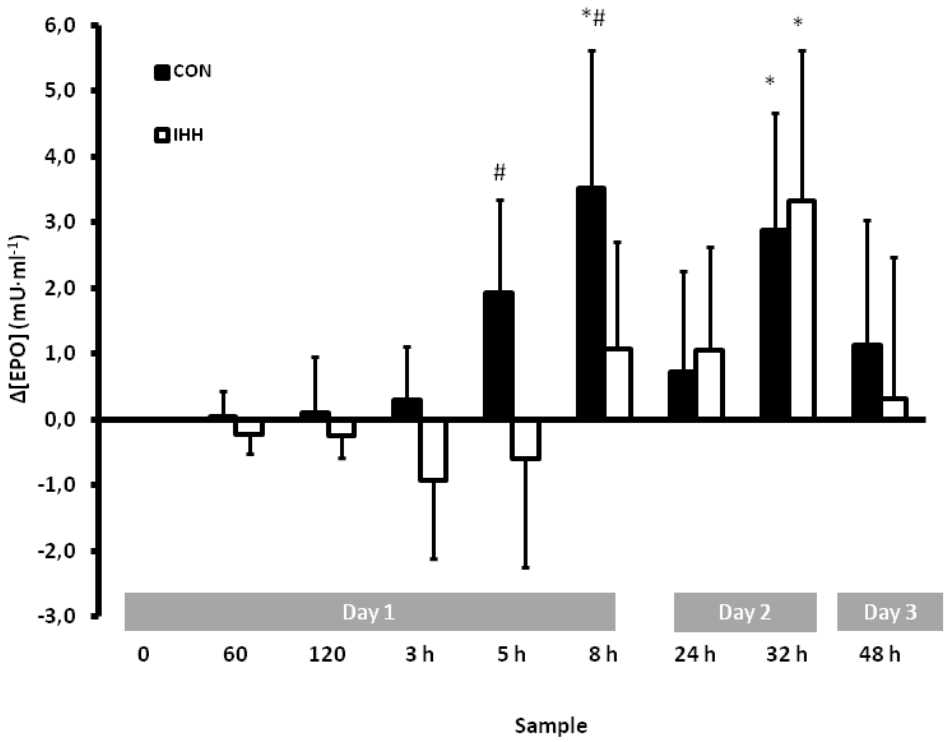

Figure 7.4: *Changes in plasma EPO concentration before, during and after the exposure in both groups. (* P < 0.01; significant differences within groups from REST; # P< 0.01; significant differences between groups). Values are mean ± SD.*

Table 7.2: *Changes in erythrocytes (RBC), Hemoglobin (Hb), Hematocrit (HCT), Reticulocytes (Ret), Mean cell volume (MCV) and Mean cell hemoglobin (MCH) before, during and after the exposure for both groups.*

	CON			IHH		
	Rest	60	120	Rest	60	120
RBC ($10^{12} \cdot L^{-1}$)	5.01 (0.2)	4.98 (0.2)	5.04 (0.2)	5 (0.3)	4.49 (0.3)	5.08 (0.3)
Hb ($g \cdot L^{-1}$)	150 (7)	150 (7)	151 (8)	153 (7)	150 (7)	154 (7)
HCT (%)	0.44 (0.03)	0.44 (0.03)	0.44 (0.03)	0.45 (0.02)	0.44 (0.02)	0.45 (0.02)
Ret (%)	0.83 (0.35)	0.78 (0.25)	0.76 (0.31)	1.07 (0.37)	1.03 (0.35)	1.07 (0.37)
MCV (*f*L)	87.5 (2.3)	87.2 (2.3)	86.9 (2.4)	88.8 (2.7)	89.1 (4.3)	88.2 (2.6)
MCH (*p*g)	30.1 (0.6)	30.1 (0.5)	30 (0.7)	30.6 (0.9)	30.7 (0.9)	30.5 (0.9)

Values are means (SD).

7.4 Discussion

We measured [EPO] levels following successive normobaric hyperoxic and hypoxic gas mixture breathing. Our findings demonstrate that, contrary to the suggestions of Balestra et al. (2004, 2006), relative hypoxia induced by a combination of short-term hyperoxia followed by hypoxic gas breathing does not augment EPO *de novo* production. Compared to the placebo, the IHH protocol induced a transient reduction in [EPO] within the initial 8 hours following the IHH protocol. Our results thus present further evidence against the existence of NOP, and concur with the findings of Keramidas et

al. [133], McGuire et al. [205] and Momeni et al. [132]. The results of the current study show that a combination of short-term hyperoxic and hypoxic breathing does not augment the [EPO] *de novo* synthesis, thus, our findings do not support the existence of the "normobaric oxygen paradox". The validity of our observation is fortified by the study design, incorporating a matched control group undergoing a single-blind placebo protocol.

Absolute renal tissue hypoxia i.e. decreased renal tissue O_2 partial pressure (PO_2) has been well documented as the main trigger of EPO de novo synthesis in humans [197, 212, 213]. Increased [EPO] intensifies the red bone marrow progenitor cells activity in red blood-cell production and can, subsequently lead to augmented hematocrit and total hemoglobin mass [22, 47, 199]. While short periods (90 - 120 min) of hypoxic exposure have been shown to augment endogenous EPO production [149, 174] a distinct dose-related response has also been observed. In particular, Eckardt et al. [93] observed a dose-dependent response in EPO release with ΔPO_2 stimuli of 62 hPa and 80 hPa, resulting from exposures to simulated altitudes, corresponding to 3000 and 4000 m, respectively.

Considering the purported importance of the relative oxygenation changes [9, 204], it is surprising that ΔPO_2 = 810 hPa, as experienced by the subjects in the IHH group did not yield an increase in EPO levels. Especially, since the simulated altitude (~ 3000 m) was above of 2500 m, a speculated altitude threshold for a sustained, short term augmentation of EPO production [1, 197]. While the latter could be explained by the relatively short hypoxic exposure period (60 min), the effects of the relative PO_2 on EPO are more intriguing. Assuming that the relative decrease in PO_2 can enhance EPO production, as proposed by Balestra et al. [9, 204], one would expect to find augmentation of EPO production following immediate transition from hyperoxia to normoxia. On the contrary, the Δ[EPO] response of the IHH group, compared to that of the CON group, demonstrated a transient suppression of EPO production during the first eight hours following the exposure. Our data obviously negate the ability of the relative changes in PO_2 induced by breathing pure O_2 to enhance EPO production. On the other hand, our data support the already reported suppressive effect of the normobaric hyperoxia on EPO release [131, 206]. This hyperoxic suppressive effect on EPO production was recently confirmed by Keramidas et al. [133], demonstrating that two hours of O_2 breathing leads to a transient attenuation of plasma EPO levels during the initial eight hours following the exposure.

Considering the purported importance of the relative oxygenation changes, it is surprising that ΔPO_2 = 810 hPa, as experienced by the subjects in the IHH group did not yield an increase in EPO levels. Especially, since the simulated altitude (~ 3000 m) was above the suggested altitude threshold of 2500 m for augmentation of EPO release [197]. While the latter could be explained by the relatively short hypoxic exposure period (60 min), the effects of the relative PO_2 on EPO are more intriguing. Assuming that the relative decrease in PO_2 can enhance EPO production, as proposed by Balestra et al. [9, 204], one would expect to find augmentation of EPO production following immediate transition from hyperoxia to normoxia. On the contrary, the Δ[EPO] response of the IHH group, compared to that of the CON group, demonstrated a transient suppression of EPO production during the first eight hours following the exposure. Although this obviously negates the importance of relative changes in PO_2 above the hypoxic threshold on EPO production, it further confirms the already reported

suppressive effect of the normobaric hyperoxia on EPO release [131, 206]. This hyperoxic suppressive effect on EPO production was recently confirmed by [133], demonstrating that two hours of O_2 breathing leads to a transient attenuation of plasma EPO levels during the initial eight hours following the exposure.

The NOP theory [9, 204] suggests that in addition to absolute renal tissue hypoxia, relative changes in PO_2 from hyperoxia to normoxia may also induce EPO de novo synthesis. The importance of relative decrements in PO_2 on EPO synthesis is also supported by the findings of Hofso et al. [207]. They found increased plasma EPO in healthy subjects following a deep saturation dive and speculated that the increased EPO concentration was induced by the transition from a hyperoxic to a normoxic breathing mixture. The mechanism proposed to account for this phenomenon includes changes in the concentration of different glutathione forms (GSSG & GSH) within the cells, subsequently effecting the production and deactivation of HIF-1α the key role factor in O_2 sensing mechanism [214]. In particular, both GSH and GSSG play an important role in HIF-1αactivation and deactivation; therefore the maintenance of glutathione equilibrium is essential for HIF-1α stabilization [215]. Since both glutathione forms are strongly connected to naturalization of O_2 free radicals within cells, that increase substantially under hyperoxic conditions [216], they reasoned that the increased GSH cellular concentration following hyperoxia can induce higher levels of HIF-1α activation post hyperoxia. Accordingly if this situation would be followed immediately by a hypoxic stimulus there would be an even further increase of GSH, since hypoxic condition stimulates GSSG reduction to GSH [217]. This increased GSH concentration would therefore further inhibit Von Hippel Lindau factor protein binding to HIF-1α and allow activation and transcriptional activity of HIF-1α and subsequent EPO production enhancement. Despite the plausible mechanism proposed for the "Normobaric oxygen paradox", the evidence in support of this theory remains, at best, equivocal.

Our results, demonstrating substantial subject variability in the nature and magnitude of the EPO response, concur with the findings of previous studies [30, 197, 218]. In particular, within the first 5 hours (S5) following the protocol, seven subjects in the CON and only two subjects in the IHH group exhibited higher values in EPO compared to that observed prior to the exposure (S1). The range of subject variability was similar in both groups, and was not related to either differences in $\dot{V}O_{2peak}$ or in any of the baseline hematological variables. Individual variability in EPO synthesis is likely dependent on the factors regulating the EPO responses [219]. In particular, the transcriptional and post-transcriptional mechanisms involving hypoxia-inducible factors activation, governing EPO gene expressions, may play a leading role in the individual responses [197].

Although some significant changes were observed within groups, these are probably attributable to the natural circadian EPO fluctuations [220]. The studies investigating the existence of circadian fluctuations of [EPO] have provided inconsistent findings and no final conclusion can be made from available data [221]. Some studies have shown a distinct daily variation of EPO concentration in healthy male subjects [220] while others have shown no such variation [222].The results of our study support the notion that significant changes in EPO level do in fact occure during the course of the day. In particular, zenith and nadir values of [EPO] were noted during the evening (S6 and S8) and morning

hours (S7 and S9), respectively. Although factors like heavy exercise [223] or blood-volume distribution [224] have been shown to effect EPO levels, these are unlikely to account for the observed variation seen in the present study, since the subjects refrained from any form of physical exercise during the course of the study and all blood samples were taken following a resting period in supine position lasting at least 15 minutes.

Even though the ΔPO_2 stimulus as a consequence of the IHH protocol did not induce increases in EPO production, the potential of augmenting EPO synthesis using changes in relative PO_2 remains intriguing. The aim of inducing significant erythropoiesis within a short time period is certainly motivated by clinical and field applications. In particular, the improved efficiency of the hypoxic protocols would prove beneficial for endurance athletes, providing them with competitive advantage [113]. In clinical settings, normobaric O_2 breathing is already being promoted in the treatment of anemia [11], and as an adjunct therapy for cancer patients [225]. Although commendable, our results, and the findings of other studies investigating NOP, suggest that the use of NOP for both athletic and clinical application is not warranted. Thus, any recommendations regarding normobaric O_2 breathing as a new training or clinical treatment strategy are currently premature.

8 General summary and conclusions

8.1 General summary

The present thesis investigated the effects of different O_2 manipulations at rest and during exercise on performance in normoxia and hypoxia. In particular, the main focus has been in reducing the time required to initiate beneficial altitude acclimatization. The last study tested a novel hyperoxic - hypoxic protocol that could indirectly augment performance. The conclusions from this thesis may be beneficial to athletes for improving their sea level and altitude performance, for occupational issues related to high altitude work, and finally to a vast amount of recreational athletes who are active in high altitude areas. The main findings of the present thesis can be summarized as follows:

- Moderate intensity hypoxic training is not efficient in improving performance.
- Intermittent hypoxic exposures combined with exercise training do not augment performance.
- Four intermittent hypoxic exposures lasting four hours do not improve performance in hypoxia while augmented ventilation and blood oxygenation saturation can be expected during exertion.
- In all three tested training protocols the hypoxic dose was insufficient and/or inefficient for a robust and substantial improvement in performance.
- Short consecutive breathing of hyperoxic and hypoxic mixture does not enhance EPO synthesis.
- Manipulations of relative changes in PO_2 above hypoxic threshold are currently unwarranted for stimulating EPO production and require further investigation.

Although our findings do not confirm our initial hypothesis, the obtained results give an important amount of information. They show, that when using normobaric hypoxic manipulations in moderately trained, healthy subjects the hypoxic dose should most probably exceed the ones tested in studies I, II and III, if one wishes to improve performance in both normoxia and hypoxia. Furthermore, due to the large individual heterogeneity of responses to a hypoxic stimulus, also shown in the present studies, it is important to continuously monitor the individual responses before, during, and after the utilization

of different hypoxic training modalities.

The results of Study I showed, contrary to the studies showing improvements following ITH in both normoxia [49, 51] and hypoxia [52, 53], that the hypoxic dose employed i.e. (twenty 60 min sessions) was insufficient to induce hypoxia related beneficial effects. Apparently, the same relative workload in hypoxia and normoxia did not induce specific changes during IHT. While the unchanged aerobic capacity and performance in the IHT group could be due to a relatively low training intensity, the control group results, on the other hand, show that in active subjects improvements can be achieved. Our findings concur with a recent suggestion [18] that although specific changes on muscles on molecular level can be expected following hypoxic exercise training, the effect on performance is unlikely to be beneficial. Similarly, the effects of intermittent hypoxic exposures on performance have been questionable [4]. Study II showed that twenty 1-h intermittent hypoxic sessions do not enhance performance. While we expected that the combined exercise training and IHE would prove superior over endurance training only in regards to performance, our results did not confirm our assumptions. However, the prolonged improved performance experienced in the IHE group 10 days following the experimental protocol showed that IHE may have beneficial effects. Augmentation of ventilation during exercise seems to be the prominent response, since improvements in ventilatory responses have previously been shown to occur following short intermittent hypoxic bouts [8, 12]. While, according to our results, the IHE does not seem to enhance performance *per se*, the related adaptational changes can prolong the beneficial adaptations of aerobic training. This finding could indicate another possible application of IHE, for retention of training induced benefits when exercise training cannot be performed (i.e. travel, injury). More importantly, our data show, that the effects of different hypoxic training modalities, do not always occur immediately following the protocol. Although the findings regarding the post hypoxic training de-acclimatization are not uniform, our findings show that also 10 days following the IHE, certain benefits for performance still exist.

Study III added new insight to the current body of knowledge regarding short intermittent hypoxic exposures. In particular, our findings show that, although the performance time was not improved, four consecutive daily exposures to normobaric hypoxia can alter ventilatory responses and blood O_2 saturation during hypoxic exercise. Although our hypothesis of improved performance was not confirmed, our findings show that a certain ventilatory adaptation occurs that could prove beneficial during maximal performance. While this may seem surprising, especially in terms of the studies investigating similar hypoxic doses in normobaric hypoxia [7, 76, 77], similar studies employing hypobaric hypoxia have shown similar results [6, 72]. Collectively our findings underpin the ability of normobaric hypoxic exposures in inducing significant ventilatory acclimatization effects. In practical terms, since the utilization of normobaric hypoxia is nowadays logistically and technologically simple, its use can be promoted as means of pre-acclimatization even if the available time period for acclimatization is short.

Ventilatory adaptations following different hypoxic modalities have thus been confirmed by our results. On the other hand, neither of the tested hypoxic training modalities did induce a significant augmentation of hematological parameters. Since the hematopoiesis is chiefly governed by EPO, we

investigated the effects of a novel breathing protocol on its production and subsequent plasma concentration changes. While hypoxia *per se* is an established enhancer of EPO production [92], the necessary dose and the importance of the relative changes in tissue oxygenation are not yet firmly established. Balestra et al. [9, 204] proposed that EPO production can also be stimulated by tissue oxygenation changes above hypoxic threshold using hyperoxic breathing. Study IV investigated the effects of an additional increase in ΔPO_2 on EPO production by employing consecutive hyperoxic and hypoxic breathing. Our findings do not confirm the effect of relative hypoxia on subsequent EPO synthesis. In particular, our evidence does not support the »Normobaric oxygen paradox« hypothesis, since an increased ΔPO_2 achieved by consecutive acute hyperoxic and hypoxic breathing did not augment EPO synthesis. On the contrary, the tested protocol induced a transient attenuation of plasma EPO levels for 5 hours following the exposure, confirming the previously reported hyperoxia related plasma EPO concentration suppression [131]. Thus, the use of increased changes in tissue O_2 partial pressure above hypoxic threshold does currently not seem to be a promising tool to boost EPO production and subsequently augment O_2 flux and performance.

8.2 (De) Limitations

We used healthy male volunteers as subjects in the studies. Therefore our conclusions should be considered applicable to this population only. The main incentive for the use of healthy active males instead of elite athletes came from the notion that this population has a wider adaptational potential also referred to as »adaptation ceiling«. Compared to the highly trained athletes, untrained active subjects have more room for improvements since it has already been shown that the magnitude of the training effect (hypoxic & exercise training) is inversely associated with the baseline performance levels [226]. This allowed us to directly examine the effects of hypoxia per se, since the training in both Study I and II was of same duration and relative intensity. The larger adaptational potential of non-athletes could however on the other hand mask the small changes that would occur as a result of hypoxia. With this limitation in mind, our results have to be interpreted and applied to normal healthy male population and not elite athletes or females, whose responses may differ.

The statistical power of the analytical methods used in the studies was calculated in advance using previous similar study designs. With the use of means and standard errors of populations from previous studies using similar methodology we calculated that at least eight subjects per intervention was required. In particular, to ensure sufficiently high power for statistical analysis and to reduce the chances of Type II error we performed all studies with at least ten subjects per group or intervention. The ANOVA analysis models were employed when repeated measures between and within groups were performed, as this strict statistical analysis limits the possible Type I error.

In the studies we used two exercise tests that are broadly used in sport and medicine science. Although graded exercise test for measuring $\dot{V}O_{2peak}$, is used extensively for investigating different training effects, it has been suggested to be to coarse to detect the possible subtle changes following adaptation to hypoxic protocols [18]. While this could be a possible problem when dealing with elite athletes it seems of minor importance when investigating moderately trained subjects. The $\dot{V}O_{2peak}$ has

also been shown to be well correlated with performance in endurance events [227] although other factor have to be taken into account (i.e. exercise economy). Even though, many types of exercise testings can be used to monitor athletic performance the use of constant power tests to exhaustion is widespread and adequately reliable [228].

The absolute intensity of the exercise training in both studies I and II was intentionally low, since we wanted to compare the effects of performing training in either hypoxia or normoxia at the same relative work load. If a higher intensity had been chosen it would prove hard or unfeasible to perform the training in hypoxia. The main reason for inclusion of training in study II was to ensure the same level of subject's activity during the study.

A strict control of both experimental and control groups in our studies, regarding the training and daily activity during the course of the protocols, made our obtained results more reliable as compared to many uncontrolled studies previously used to examine the effects of different hypoxic training modalities. Furthermore, the blinded design used in studies III and IV eliminated the possible placebo or a nocebo effect that has already been shown to possibly occur when employing specific training regimens in hypoxia [2].

To reduce the error of [EPO] measurement we assayed each sample in duplicate and used the same assay plate for every sample of the same subject to avoid the variability between plates [229]. Moreover the coefficients of variation were also calculated for both within and between runs and were within the acceptable ranges for these types of measurements. Since EPO responses have shown a large individual variability [30, 221], this could also limit robustness of our findings regarding effects of intermittent hypoxic and hyperoxic breathing on EPO synthesis. However, in view of the fact that we used a total of eighteen subjects and according to the power calculations we believe, that our results reasonably reflect the physiological responses.

Normobaric hypoxia (and hyperoxia) was used in all studies. Hypoxic breathing mixtures during the training studies were generated by either nitrogen dilution (Study I & III) or oxygen filtration (Study II). Due to the fact that differences in responses to either hypobaric or normobaric hypoxia have been reported [16, 151], this has to be taken into account when interpreting the finding of this thesis.

8.3 Conclusions & Perspective

The present thesis provides new insights into the effects of intermittent hypoxic training modalities on performance and related metrics. In summary, the efficiency of hypoxia application during moderate exercise training was not confirmed and can therefore not be recommended for purposes of either sea level or altitude performance enhancement. Secondly, while IHE did not show immediate effects following the protocol, the prolongation of enhanced normoxic performance and ventilatory responses demonstrates its potential beneficial effect. Therefore, further studies investigating the different IHE protocols on performance enhancement are warranted. Collectively, in both tested protocols (IHT & IHE) hypoxic doses were insufficient to result in acute performance enhancements. Further studies should focus on higher hypoxic doses to determine the threshold limit for performance enhancement.

Thus, further studies are warranted to define the optimal dose. Our findings showed that even with the utilization of only four intermittent hypoxic exposures lasting four hours each, some degree of acclimatization is achieved. These data therefore show, that the hypoxic dose used when employing normobaric hypoxic exposures must most probably exceed the ones used in our studies if one wishes to improve performance in either normoxia or hypoxia. Moreover, since only performance metrics may be to coarse to detect the possible subtle changes, induced by short hypoxic stimuli, the changes in muscle properties, central and peripheral oxygenation modulation and ventilatory modifications should be the focus of the forthcoming studies.

Regarding the novel protocol for augmenting EPO de novo production, our findings do not provide any support for the utilization of changes in PO_2 above hypoxic threshold. Although intriguing, we did not confirm the hypothesis underlying the reported »Normobaric oxygen paradox« theory. Thus, its utilization does not seem promising for subsequent use as performance enhancement tool. The use of hyperoxia in clinical applications, already implemented for anemia and cancer patients' treatment, is according to our findings premature and unwarranted. Further studies investigating the importance of relative changes in oxygenation on consequent EPO production should focus on the possible dose response relationship. In conclusion, since the possible effects of short acute and intermittent normobaric hypoxic exposures, not only on performance, but also on selected physiological indexes, are not clearly established further studies on this issue are also needed before a final conclusion can be derived.

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Appendix

Publications araising from the thesis

Peer reviewed publications

Debevec T., Amon M., Keramidas M. E., Kounalakis S. N., Pišot R. & Mekjavić I. B. (2010). ***Normoxic and hypoxic performance following 4 weeks of normobaric hypoxic training***. Aviation, Space, and Environmental Medicine. **81(4)**. 387 - 393.

Debevec T., Keramidas M. E., Norman B., Gustafsson T., Ola E. & Mekjavić I. B. (2011). ***Acute short term hyperoxia followed by mild hypoxia does not increase EPO production: Resolving the "Normobaric oxygen paradox"***. European Journal of Applied Physiology. ***Accepted paper***

International conference presentations

Debevec T., Amon M., Keramidas M. E., Kounalakis S. N. & Mekjavić I. B. (2009). ***The use of live low - train high protocol for the enhancement of endurance performace and aerobic capacity***. 16th International Hypoxia Symposium. Lake Louise, Canada.

Debevec T., Amon M., Keramidas M. E., Kounalakis S. N. & Mekjavić I. B. (2009). ***Hematological responses to two different intermittent hypoxic training regimens***. 14th Annual Congress of the European College of Sport Science. Oslo, Norway.

Debevec T., Keramidas M. E., Amon M., Kounalakis S. N. & Mekjavić I. B. (2009). ***Evaluation of training protocols for the improvement of altitude and sea level performance***. 4th International Symposium High Altitude Influence on Human Performance. Bohinjska Bela, Slovenia.

Debevec T., Keramidas M. E., Norman B., Gustafsson T., Eiken O. & Mekjavić I. B. (2011). ***No evidence for the normobaric oxygen paradox***. 58th Annual Meeting of the American College of Sports Medicine. Denver, USA.

Debevec T. & Mekjavić I. B. (2011). ***Can four normobaric hypoxic exposures enhance performance in hypoxia?*** 14th Annual Congress of the European College of Sport Science. Liverpool, UK.

Ethics approval

All studies in the present thesis were performed in accordance with the procedures approved by the National Ethics Committee of the Republic of Slovenia. Reference numbers (75/05/03, 65/06/04, 108/08/09). The experimental procedures were conducted according to the guidelines of the Helsinki Declaration.

Republika Slovenija
KOMISIJA ZA MEDICINSKO ETIKO
pri Ministrstvu za zdravstvo

Prof. dr. Igor B. Mekjavič
Odsek za avtomatizacijo, biokibernetiko in robotiko,
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Jamova 39, 1000 Ljubljana

Šiev.: 75/05/03
Datum: 20. 05. 2003

Spoštovani gospod Mekjavič,

Komisiji za medicinsko etiko ste 5. 5. 2003 poslali vlogo za oceno načrta študije z naslovom:

"Višinski trening."

Komisija je na današnji seji ocenila, da je raziskava z etične strani sprejemljiva, in Vam s tem izdaja svoje soglasje.

S spoštovanjem in lepimi pozdravi,

V imenu komisije za medicinsko etiko:
prof. dr. Jože Trontelj

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KOMISIJA REPUBLIKE SLOVENIJE ZA MEDICINSKO ETIKO

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Štev.: 65/06/04
Datum: 22. 06. 2003

Spoštovani g. prof. Mekjavić, spoštovana gospa Golja,

Komisiji za medicinsko etiko sta 24. 5. 2004 poslala prošnjo za oceno etičnih vidikov študije z naslovom:

"Razvoj nove metode višinske aklimatizacije."

Komisija je na današnji seji menila, da je raziskava z etične strani sprejemljiva.

S spoštovanjem in lepimi pozdravi,

V imenu komisije za medicinsko etiko:
prof. dr. Jože Trontelj

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KOMISIJA REPUBLIKE SLOVENIJE ZA MEDICINSKO ETIKO

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Štev.: 108/08/09
Datum: 17. 10. 2009

Spoštovani gospod prof. Mekjavic,

Komisiji za medicinsko etiko (KME) ste 17. 8. 2009[1] poslali prošnjo za oceno načrta raziskave z naslovom:

"Srčnožilne in metabolne adaptacije na hipoksijo." "Continuous exposure to hypoxia: cardiorespiratory and metabolic adaptations."

KME je ocenila, da je raziskava etično sprejemljiva, in Vam izdaja svoje soglasje.

S spoštovanjem in lepimi pozdravi,

prof. dr. Jože Trontelj
predsednik Komisije za medicinsko etiko

[1] Opravičujemo se za pozen odgovor.

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Declaration of authorship

DECLARATION

I the undersigned Tadej Debevec declare herewith that:

- The submitted Doctoral Dissertation has been prepared by myself based on my own research and using the sources listed, under the supervision of
  supervisor prof. Igor B. Mekjavic
  co-supervisor Stylianos Kounalakis
  co-supervisor Blaž Jereb

- This Dissertation has not been submitted either in whole or in part for obtaining any other degree at another university or faculty.

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