

INDEPENDENT AND COMBINED EFFECTS
OF HEAT AND HYPOXIC ACCLIMATION ON
EXERCISE PERFORMANCE IN HUMANS
WITH PARTICULAR REFERENCE TO
CROSS-ADAPTATION
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Doctoral Dissertation
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POSAMEZNI IN SKUPNI UČINKI AKLIMATIZACIJE
NA HIPOKSIJO IN VROČINO NA VADBENO
ZMOGLJIVOST PRI ČLOVEKU S POUDARKOM NA
Doktorska disertacija

Supervisor: Prof. Igor B. Mekjavić

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Abstract

The present thesis assesses the theory of cross-adaptation, whereby the adaptation to one stressor (i.e. exercise, heat, hypoxia) could benefit performance during exposure to another stressor. We investigated the independent and combined effects of training, heat and hypoxic acclimation on selected cardiorespiratory, thermoregulatory, hematological and cellular responses during exercise in normoxic (NOR), hypoxic (HYP) and hot (HE) ambient conditions.

Participants conducted 10-d adaptations to I: training, II: hypoxia, III: heat, and IV: combined heat and hypoxic acclimation. Before and after each 10-d adaptation period, participants completed three cycle ergometer exercise tests in NOR, HYP and HE conditions. The first 30-min phase of each test comprised steady-state exercise at 40% NOR $\dot{V}O_{2\text{peak}}$ followed by incremental exercise to exhaustion. The acclimation protocols were:

- I. Participants with higher and lower ($\dot{V}O_{2\text{max}}$: 57.9 ± 6.2 and 41.7 ± 5.0 mL $\cdot$ kg $^{-1}$ $\cdot$ min $^{-1}$, respectively) maximal aerobic power conducted daily 60min exercise at 50% NOR $\dot{V}O_{2\text{peak}}$.
- II. Participants were confined to a normobaric hypoxic environment (partial pressure of oxygen in the inspired air, $P_{iO_2} = 87 \pm 3$ mmHg) and completed daily exercise in hypoxia at a heart rate corresponding to 50% $\dot{V}O_{2\text{peak}}$.
- III. Participants conducted daily 90-min controlled-hyperthermia (target rectal temperature, $T_{\text{re}} = 38.5^\circ\text{C}$) exercise on a cycle ergometer.
- IV. Participants were confined to a hypoxic environment (II) and conducted daily 90-min controlled-hyperthermia exercise in a hot environment (III).
- V. Following the NOR maximal exercise test conducted before and after heat acclimation (III) we immersed participants in water maintained at 28°C for 60 min.

Results of the above acclimation procedures were as follows:

- I. $\dot{V}O_{2\text{max}}$ increased by $+9.2 \pm 8.5\%$ and $+10.2 \pm 15.4\%$ in the untrained group in NOR and HE, respectively. Increases in $\dot{V}O_{2\text{peak}}$ were correlated with baseline values in NOR ($r=-0.6$) and HYP ($r=-0.5$). Peak sweat rate increased in both groups in HE, and was activated at a lower rectal temperature (T_{re}) in the trained group.
- II. $\dot{V}O_{2\text{max}}$ increased by 10.7% and 7.9% in NOR and HE, respectively, whereas no differences were found in HYP. Increases in $\dot{V}O_{2\text{max}}$ did not translate into increases in $\dot{V}O_{2\text{peak}}$ in either NOR or HE.
- III. $\dot{V}O_{2\text{max}}$ and maximal cardiac output (CO_{max}) were not affected. $\dot{V}O_{2\text{peak}}$ was increased in NOR and HE by 41 ± 21 and 26 ± 22 W, respectively, but not in HYP. GME was higher whereas resting T_{re} and sweating thresholds were lower across environmental conditions. The gain of the sweating response decreased in HYP.
- IV. $\dot{V}O_{2\text{max}}$ and the anaerobic threshold were not modified in any of the environments. $\dot{V}O_{2\text{peak}}$ increased by $6.3 \pm 3.4\%$ in NOR and $4.0 \pm 4.9\%$ in HE, respectively. Forehead sweating (magnitude and gain) was augmented in HE. Time to exhaustion in hot hypoxic conditions increased by $12.9 \pm 10.6\%$.
- V. Baseline pre-immersion T_{re} was similar pre- and post-heat acclimation, as was T_{re} cooling rate, the vasomotor response and shivering.

A training effect was observed in the untrained participants (I). Hypoxic acclimation combined with moderate-intensity exercise training improved $\dot{V}O_{2\max}$ in NOR and HE, but did not affect exercise performance or thermoregulatory responses in any of the environmental conditions (II). Our data do not support a beneficial cross-over effect of heat acclimation on $\dot{V}O_{2\max}$ in NOR or HYP conditions (III). A 10-day combined heat and hypoxic acclimation conferred minor benefits in aerobic performance in NOR or HE conditions (IV). Heat production and heat loss responses in a high heat loss environment remained unaltered following heat acclimation (V). The results of the present thesis do not confirm the theory of cross-adaptation in humans.

Povzetek

Ovrednotili smo teorijo križne adaptacije, na podlagi katere naj bi pri človeku adaptacija na enega ali več dejavnikov (npr. vadba, vročina, hipoksija) izboljšala vadbena zmogljivost v drugih okoljskih pogojih. Ovrednotili smo posamezni in skupni vpliv vadbe (treninga), višinske (hipoksične) in vročinske aklimatizacije na kardiorespiratorne, termoregulacijske, hematološke in izvenske odzive med vadbo v normoksičnem (NOR), hipoksičnem (HYP) in vročem okolju (HE).

Preiskovanci so opravili 10-dnevno adaptacijo na: I: vadbo, II: hipoksija, III: vročina, in IV: kombinirano adaptacijo na vročino in hipoksijo. Pred in po vsaki 10-dnevni adaptaciji so opravili tri vadbene teste na cikloergometru s katerimi smo določili maksimalno porabo kisika ($\dot{V}O_{2max}$), maksimalno opravljeno delo (W_{peak}), ter termoregulacijske in kardiorespiratorne odzive v NOR, HYP in HE okolju. V prvi 30-minutni fazi testa so preiskovanci opravili vadbo pri obremenitvi enaki 40% NOR W_{peak} , kateri je v drugi fazi sledila vadba s stopnjevano obremenitvijo do onemoglosti. Aklimatizacijski postopki v omenjenih študijah so bili sledeči:

- I. Preiskovanci z večjo ali manjšo aerobno sposobnostjo ($\dot{V}O_{2max}$: 57.9 ± 6.2 vs 41.7 ± 5.0 mL · kg⁻¹ · min⁻¹) so dnevno vadili na cikloergometru (60 min pri obremenitvi 50% W_{peak}).
- II. Preiskovanci so živeli v normobaričnem hipoksičnem okolju (delni tlak vdihanega kisika, $P_iO_2 = 87 \pm 3$ mmHg) in dnevno izvajali 60-minutno vadbo na cikloergometru (50% HYP W_{peak}).
- III. Med dnevno 90-minutno cikloergometrijo pri temperaturi 35°C smo z ustrezno uravnano obremenitev vzdrževali rektalno temperaturo (T_{re}) pri 38.5°C.
- IV. Preiskovanci so živeli v normobaričnem hipoksičnem (II) okolju in dnevno opravili 90-minutno cikloergometrijo v vročem (III) okolju.
- V. Po končanem NOR testu v študiji III smo pred in po aklimatizaciji preiskovance do vratu potopili v kad z vodo pri 28°C za 60 min. Ovrednotili smo učinek vročinske aklimatizacije na termoregulacijske odzive med ohlajanjem telesnega jedra.

Rezultati zgoraj opisanih adaptacij (I: vadba, II: vadba+hipoksija, III: vadba+vročina, IV: vadba+hipoksija+vročina) so sledeči:

$\dot{V}O_{2max}$ se je povečal pri preiskovancih z manjšo aerobno sposobnost za $+9.2 \pm 8.5\%$ pri NOR in za $+10.2 \pm 15.4\%$ pri HE. Značilno korelacijo smo opazili med W_{peak} in bazalne vrednosti $\dot{V}O_{2max}$ pri NOR ($r=-0.6$) in HYP ($r=-0.5$). Pri obeh skupinah se je znojenje povečalo pri HE. Znojenje se je aktiviralo pri nižji T_{re} pri preiskovancih z večjo aerobno sposobnostjo (I). $\dot{V}O_{2max}$ se je povečal za 10.7% pri NOR in za 7.9% pri HE. (II). $\dot{V}O_{2max}$ in maksimalni iztisni volumen srca (CO_{max}) se nista spremenila. W_{peak} se je povečal pri NOR za 41 ± 21 W in pri HE za 26 ± 22 W, vendar ne pri HYP. Učinkovitost se je izboljšala. Bazalna temperatura telesnega jedra (T_{re}) je bila nižja, kakor tudi pragovi T_{re} za znojenje. Odziv znojenja se je znižal pri HYP (III). W_{peak} se je povečal za $6.3 \pm 3.4\%$ pri NOR in za $4.0 \pm 4.9\%$ pri HE. Znojenje (količina in senzitivnost) se je povečalo pri HE. Pri stopnjevani obremenitvi se je čas do onemoglosti v vročem hipoksičnem okolju povečal za $12.9 \pm 10.6\%$ (IV). Bazalna vrednost T_{re} je bila enaka pred in po aklimatizaciji, kakor tudi hitrost

ohlajanja T_{re} . Vazomotorični odziv in odziv drgetanja sta bila nespremenjena po aklimatizaciji (V).

Učinek treninga je bil značilen pri preiskovancih z nižjo aerobno sposobnostjo (I). Normobarična hipoksična aklimatizacija je izboljšala $\dot{V}O_{2max}$ pri NOR in HE, in ni vplivala na vadbeno sposobnost in termoregulacijo pri NOR, HYP in HE (II). Vročinska aklimatizacija ni vplivala na vadbene odzive pri NOR in HE (III). Deset dnevna kombinirana aklimatizacija na vročino in hipoksijo je zanemarljivo izboljšala aerobno sposobnost pri NOR in HE (IV). Aklimatizacija na vročino ne vpliva na odziv vazomotorike in drgetanja med ohlajanjem telesnaga jedra (študija V).

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Contents

Contents

Acknowledgments	vii
Abstract.....	ix
Povzetek	Error! Bookmark not defined.
Contents	xiii
List of Figures.....	xix
List of Tables	xxi
Abbreviations.....	xxiii
Introduction.....	1
1.1. Thesis Structure	1
1.2. The time-course of adaptation.....	2
1.3. Heat Acclimation.....	2
1.4. Hypoxic Acclimation	4
1.5. A combined stressor approach, the training effect and cross-adaptation between heat and cold	6
1.6. Aims and Hypotheses	8
2 Aerobic gains of a 10-day training regimen are dependent on initial fitness level.....	9
2.1. Methods	10
2.1.1. Participants	10
2.1.2. Study Design	11
2.1.3. Exercise Training	11
2.1.4. Exercise Testing	12
2.1.5. Steady-State and Incremental Exercise	13
2.1.6. Hematological Results	14

2.1.7. Data Analysis	14
2.2. Results.....	15
2.2.1. Exercise Training.....	15
2.2.2. Aerobic Capacity and Cardiovascular Responses.....	15
2.2.3. Submaximal Exercise	18
2.2.4. Thermoregulation	21
2.2.5. Hematological Results and Vascular Volumes	24
2.3. Discussion.....	28
2.3.1. Aerobic Capacity	28
2.3.2. Thermoregulation	29
2.3.3. Hematology.....	30
3 Exercise cardiorespiratory and thermoregulatory responses in normoxic, hypoxic, and hot environment following 10-day continuous hypoxic exposure	33
3.1. Methods.....	35
3.1.1. Participants	35
3.1.2. Study Design.....	35
3.1.3. Hypoxic Acclimation.....	36
3.1.4. Blood sampling and biochemical analysis	37
3.1.5. Exercise Testing.....	37
3.1.6. Steady State and Incremental Exercise to Exhaustion	38
3.1.7. Data Analysis	39
3.2. Results	40
3.2.1. Hypoxic Acclimation.....	40
3.2.2. Body composition and Energy Intake.....	40
3.2.3. Steady-State Exercise.....	40
3.2.4. Hematological Data.....	46
3.2.5. Cardiorespiratory responses to Incremental Exercise to Exhaustion.....	48
3.3. Discussion	51
3.3.1. Acute responses.....	51
3.3.2. Consequences of hypoxic acclimation.....	52
4 Heat acclimation does not affect maximal aerobic power in normoxic or hypoxic conditions.....	57

4.1. Methods	58
4.1.1. Participants	58
4.1.2. Study Outline	59
4.1.3. Heat Acclimation	59
4.1.4. Blood sampling and biochemical analysis	60
4.1.5. Exercise Testing	60
4.1.6. Steady-State and Incremental Exercise	61
4.1.7. Data Analysis	62
4.2. Results	63
4.2.1. Heat Acclimation	63
4.2.2. Steady-State Exercise	63
4.2.3. Incremental Exercise to Exhaustion	69
4.2.4. Hematological Data	71
4.3. Discussion	73
4.3.1. Heat Acclimation	73
4.3.1. Cardiorespiratory responses	73
4.3.2. Thermoregulatory Responses	74
4.3.3. Hematological Data	75
5 No ergogenic effects of a 10-day combined heat and hypoxic acclimation on aerobic performance in normoxic thermoneutral or hot conditions .77	
5.1. Methods	78
5.1.1. Participants	78
5.1.2. Study design	79
5.1.3. Hypoxic Acclimation	79
5.1.4. Heat Acclimation	80
5.1.5. Exercise Testing	80
5.1.6. Steady-State and Incremental Exercise	81
5.1.7. Time to Exhaustion	82
5.1.8. Hematological Data	82
5.1.9. Data Analysis	83
5.2. Results	84
5.2.1. Heat acclimation	84

5.2.2. Hematological Data	84
5.2.3. Submaximal Exercise	87
5.2.4. Exercise Performance.....	88
5.2.5. Thermoregulatory data	89
5.3. Discussion	95
5.3.1. Exercise Performance.....	95
5.3.2. Thermoregulatory data	96
5.3.3. Hematological data	97
6 Autonomic temperature regulation in high heat loss environments following heat acclimation: no evidence of cross adaptation	99
6.1. Methods.....	100
6.1.1. Participants	100
6.1.2. Experimental Design.....	100
6.1.3. Heat Acclimation Protocol (HeA).....	100
6.1.4. Autonomic Thermoregulation	101
6.1.5. Data Analysis	101
6.2. Results.....	103
6.2.1. Heat acclimation	103
6.2.2. Autonomic Thermoregulation Assessment	103
6.3. Discussion	108
6.3.1. Core temperature threshold for shivering	108
6.3.2. Core temperature response and peripheral perfusion.....	108
6.3.3. Immersion response.....	109
6.3.4. Neurophysiological perspective	109
6.3.5. Set-point or interthreshold zone.....	109
6.3.6. Exercise fatigue.....	110
6.3.7. Limitations.....	110
7 Combined effects of hypoxia and heat: importance of hypoxic dose...	111
8 Conclusions	113
References.....	115
Bibliography	131
Publications Related to the Thesis.....	131

Journal Articles.....	131
Conference Papers.....	131
Biography	133
Appendix A	135
Appendix B.....	139

List of Figures

Figure 2.1: (A) Maximal aerobic power ($\text{mL} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$) and (B) peak power output ($\text{W} \cdot \text{kg}^{-1}$) percentage changes from the pre-training trials in all environmental conditions. * $p < 0.05$ versus pre-training within environmental conditions.	16
Figure 2.2: Pre- and post-training individual data for absolute T_{re} sweating thresholds in normoxia (closed circles), hypoxia (open circles), and heat (triangles) in the trained (A) and the untrained (B) individuals.	24
Figure 3.1: Outline of the experimental protocol.	36
Figure 3.2: Temporal response of the mean forehead sweating rate ($\dot{m}_{\text{sw}}$, $\text{g} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$) during exercise in the normoxic (NOR), hypoxic (HYP), and heat (HE) trials before (A) and.....	45
Figure 3.3: Mean changes in the forearm-fingertip skin temperature gradient ($\Delta T_{\text{f-f}}$, $^{\circ}\text{C}$) as a function of the relative change in rectal temperature (ΔT_{re} , $^{\circ}\text{C}$) during the normoxic, hypoxic and heat trials before and after HyA ($n=12$) * $p < 0.05$. SD bars are omitted for the purpose of clarity.....	45
Figure 3.4: Pre- and post-acclimation mean and individual data for extracellular (A) Heat shock protein 72 (HSP72), (B) Heat shock protein 90 (HSP90), (C) Hypoxia-inducible factor 1a (HIF-1a), and (D) Erythropoietin (EPO). * $P < 0.05$	47
Figure 3.5: Pre- and post-acclimation individual data for (A) maximal aerobic power ($\text{VO}_{2\text{max}}$, $\text{mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) and (B) Peak Power Output (W_{peak} , Watts) in normoxia (closed circles), hypoxia (open circles) and in the heat (triangles).	49
Figure 4.1: Cardiorespiratory and performance variables as percentage changes from pre-acclimation. Values are means $\pm$ SD. $\text{VO}_{2\text{max}}$ maximal aerobic power, GME gross mechanical efficiency, VT ventilatory threshold, CO_{max} maximal cardiac output, W_{peak} peak power output. * $P < 0.05$, # $P = 0.07$ vs. pre-HeA within environmental condition.	66
Figure 4.2: Mean changes in the rate of forehead sweating plotted as a function of changes in T_{re} , in (A) normoxic, (B) hypoxic and (C) heat trials both before (●) and after (○) heat acclimation protocol.	67
Figure 4.3: Pre- and post-acclimation individual data for performance variables in NOR (●), HYP (○) and HE (Δ). (A) Peak Power Output (W_{peak}), and (B) maximal aerobic power ($\text{VO}_{2\text{max}}$). The dashed line represents the line of equality.	71
Figure 5.1: Individual and average (bold) percentage changes in plasma EPO concentration before (Pre), during (days 1 and 10), and after (D+1) the 10-day combined acclimation protocol ($n=8$).	87

Figure 5.2: Pre- (horizontal axis) and post- (vertical axis) acclimation individual data for (A) maximal aerobic power and (B) peak power output in normoxia (closed circles), hypoxia (open circles), and heat (triangles).....	91
Figure 5.3: Pre- (horizontal axis) and post- (vertical axis) acclimation individual data for (A) absolute sweating thresholds and (B) peak sweat rate in normoxia (closed circles), hypoxia (open circles), and heat (triangles).....	94
Figure 6.1: Relative changes in rectal temperature (ΔT_{re}) during the first 45 minutes of immersion before and after heat acclimation. Values are means $\pm$ SD.....	103
Figure 6.2: Changes in the forearm-fingertip temperature difference as a function of the relative decrease in rectal temperature for the pre- and post- heat acclimation immersions. *denotes values that are significantly different ($p < 0.05$) from 1 st min values pre-heat acclimation. # denotes values that are significantly different ($p < 0.05$) from 1 st min values post-heat acclimation.	104
Figure 6.3: Change in the cardiorespiratory responses (oxygen uptake, minute ventilation, HR) as a function of time for the immersions pre- and post-heat acclimation. *denotes values significantly different from 10 th min value pre-heat acclimation #denotes values significantly different from 10 th min value both pre- and post-heat acclimation.	105
Figure 6.4: Average relative changes in the shivering and the sweating response (threshold, gain, maximal capacity) as a function of rectal temperature pre- and post-heat acclimation. Standard deviations omitted for clarity. Solid and dashed lines represent values pre- and post-heat acclimation, respectively. Gray and black circles represent the sweating and shivering responses, respectively.	106

List of Tables

Table 2.1: Participants' baseline characteristics. Values are means $\pm$ SD. Abbreviations: BSA, body surface area; $\dot{V}O_{2max}$, maximal aerobic power, W_{peak} peak power output. *Significant difference from trained group.....	11
Table 2.2: Maximal cardiorespiratory responses and peak power output during the incremental exercise to exhaustion pre- and post-training for the trained and untrained groups in normoxic, hypoxic and heat trials (n = 10).....	17
Table 2.3: Cardiorespiratory responses and RPE during constant-work rate cycling before and after heat acclimation for the normoxic, hypoxic and heat trials for the trained (n = 10) and the untrained group (n=10).....	19
Table 2.4: Thermoregulatory responses at rest and during the constant-work rate cycling before and pre- and post-training for the trained (n = 10) and untrained (n = 10) groups in normoxic, hypoxic and heat trials.	21
Table 2.5: Resting hematological variables pre-, on days 2 and 6 and post-training for the trained (n=10) and the untrained individuals (n=10).....	26
Table 3.1: Average daily (acclimation days 1 to 10) energy (kcal $\cdot$ day ⁻¹), fluid (mL $\cdot$ day ⁻¹) and macronutrient (g $\cdot$ day ⁻¹) intakes during the HyA protocol.....	40
Table 3.2: Cardiorespiratory responses and RPE in the last ten minutes of the constant-work rate cycling before and after HyA for the normoxic, the hypoxic and the trial in the heat. (n = 12).....	42
Table 3.3: Thermoregulatory responses at rest and during the last ten minutes of the constant-work rate cycling before and after HyA for the normoxic, the hypoxic and the heat trial (n = 12).	43
Table 3.4: The measured hematological variables pre and post the 10-day HyA.	46
Table 3.5: Maximal cardiorespiratory responses and peak work rate during the incremental exercise to exhaustion before and after HyA for the normoxic, hypoxic and heat trials (n = 12).....	48
Table 4.1: Average daily exercise responses during HeA (n=12).....	64
Table 4.2: Cardiorespiratory responses and RPE in the last 20 of the 30 minutes of constant-work rate cycling before and after heat acclimation for the normoxic, the hypoxic and the heat trial (n = 12).....	65
Table 4.3: Thermoregulatory responses at rest and during the last 20 of the 30 minutes of the constant-work rate cycling before and after heat acclimation for the normoxic, the hypoxic and the heat trials (n = 12).	68

Table 4.4: Maximal cardiorespiratory responses and peak power output (mean $\pm$ SD) during the incremental exercise to exhaustion before and after HeA for the normoxic, the hypoxic and the heat trial (n = 12).	69
Table 4.5: Resting hematological variables pre- and post-heat acclimation (n=12).....	72
Table 5.1: Average daily exercise responses during heat acclimation.	85
Table 5-2 Cardiorespiratory responses and RPE in the last 20 of the 30 minutes of constant-work rate cycling before and after acclimation for the normoxic, the hypoxic and the heat trials. Heart rate, stroke volume and cardiac output values reflect the last minute of submaximal exercise (n = 8).....	87
Table 5.3: Maximal cardiorespiratory responses and peak power output during the incremental exercise to exhaustion pre- and post-acclimation in normoxic, hypoxic and heat trials. (n = 8)	90
Table 5.4: Thermoregulatory responses at rest and during the constant-work rate cycling before and after HA for the normoxic, the hypoxic and the heat trials (n = 8).	92
Table 6.1: Selected average thermoregulatory responses calculated for the first 45 minutes of immersion before and after heat acclimation.	106

Abbreviations

CO	...	Cardiac Output
CO ₂	...	Carbon Dioxide
DEXA	...	Dual X-ray absorptiometry
ELISA	...	Enzyme-linked immunosorbent assay
eNOS	...	Endothelial nitric oxide synthase
EPO	...	Erythropoietin
F _i O ₂	...	Fraction of inspired oxygen
GLUT1	...	Glucose Transporter 1
GME	...	Gross mechanical efficiency
HE	...	Heat trial
HeA	...	Heat Acclimation
Hbmass	...	Hemoglobin mass
HIF-1 α	...	Hypoxia-inducible factor 1 α
HR	...	Heart rate
HSP	...	Heat shock protein
HYP	...	Hypoxic trial
iNOS	...	Inducible nitric oxide synthase
$\dot{m}_{sw}$	...	Minute mass flow of secreted sweat
NOR	...	Normoxic trial
P _{ET} CO ₂	...	Partial pressure of end-tidal carbon dioxide
P _i O ₂	...	Partial pressure of oxygen in the inspired air
PV	...	Plasma volume
RH	...	Relative humidity
RPE	...	Rating of perceived exertion
SD	...	Standard deviation
S _p O ₂	...	Capillary oxyhemoglobin saturation
SV	...	Stroke volume
T _a	...	Ambient temperature
T _c	...	Core temperature
T _{re}	...	Rectal temperature
T _{sk}	...	Weighted mean skin temperature
V _E	...	Minute ventilation
$\dot{V}CO_2$	...	Carbon dioxide production
$\dot{V}O_2$	...	Oxygen uptake
$\dot{V}O_{2max}$	...	Maximal aerobic power

W_{peak}	...	Peak power output
$\Delta T_{\text{f-f}}$	...	Forearm-fingertip skin temperature difference

Chapter 1

Introduction

1.1. Thesis Structure

The thesis outlines a series of studies conducted to assess different aspects of the theory of cross-adaptation.

The Introduction (Chapter 1) presents a short review of the nature of adaptations induced by medium-term heat and hypoxic acclimation protocols. The ergogenic potential of training in thermoneutral normoxic conditions or combining heat and hypoxic acclimation is also discussed. This chapter also introduces the theory, promoted by observations at the cellular level, that adaptation to one stressor may cause a concomitant adaptation to another stressor, or a cross-over of adaptation.

Study I ([Chapter 2](#)) examines the effect of a moderate-intensity exercise training protocol on aerobic performance in trained and untrained individuals, and to what degree such an adaptation to exercise training influences the performance in hot or hypoxic environments. Common to most acclimation studies is the incorporation of an exercise regimen during the acclimation procedure. In such studies exercise also represents a stressor, but is seldom examined separately. This chapter provides an assessment of the effect of training in participants of varying fitness levels on their performance in hot or hypoxic conditions.

Study II ([Chapter 3](#)) addresses the effect of normobaric hypoxic acclimation on exercise cardiorespiratory and thermoregulatory responses in normoxic, hypoxic or hot conditions in healthy males.

Study III ([Chapter 4](#)) addresses the effect of a controlled-hyperthermia heat-acclimation protocol on exercise cardiorespiratory and thermoregulatory responses in normoxic, hypoxic or hot conditions in trained males.

Study IV ([Chapter 5](#)) addresses the effect of a combined heat and hypoxic acclimation protocol on thermoregulation and aerobic performance. Normobaric hypoxic training is being used by athletes to augment their performance in hypoxic and normoxic environments, as outlined in Chapter 3. The fact that many competitions are also conducted in hot climates requires acclimation also to heat, as outlined in Chapter 4. This chapter examines the effect of combining acclimation to heat and hypoxia. Specifically, whether it is synergistic, or whether the concomitant adaptation to the two stressors cancels any potential benefits of each.

Study V ([Chapter 6](#)) addresses the effect of a controlled-hyperthermia heat-acclimation protocol on autonomic thermoregulatory responses in a high-heat loss environment (i.e. during water immersion). While Chapters 3 to 5 examined the adaptation to one stressor on performance during exposure to another, this chapter examined the effect of thermoregulatory adaptation to heat on the thermoregulatory responses during exposure

to a high heat loss environment. Specifically, whether the heat adaptation-induced modification of the characteristics of the sweating response (i.e. increase in the gain of the sweating response, and shift in the core threshold temperature for sweating), would also affect the characteristics of the shivering response (i.e. gain in the shivering response, and the core threshold temperature for onset of shivering).

[Chapter 7](#) provides a critical commentary on the importance of the magnitude of the hypoxic dose applied in acclimation protocols.

The findings of the present thesis are summarized in [Chapter 8](#).

A review of the theoretical background to the studies outlined above is provided in the following sections.

1.2. The Time-Course of Adaptation

Morphological, chemical, functional, and genetic changes that reduce physiological strain when exposed to stress represent forms of adaptation. By being highly-adaptive, humans manage to maintain their internal environment relatively constant even when endogenous/external stressors are imposed. By stressor, a specified treatment or circumstance to which animals are exposed and which induces adaptations is meant (Selye, 1950). The time course of adaptation may be described using a combination of curvilinear growth and decay curves from which six different characteristics may be identified (Adolph, 1956; N. A. Taylor, 2014):

1. A stimulus must exceed a critical threshold before adaptation can be induced.
2. While acutely applied stimuli evoke immediate responses, a latency always occurs before physiological adaptations become evident.
3. For each effector organ affected by the stimulus, there is a physiological and possibly a genetically determined maximum, beyond which there is considerable adaptation resistance.
4. The speed of tissue, organ or systemic adaptations varies broadly within and among individuals.
5. Optimal adaptation results when the cumulative adaptation impulse is maximized, whilst simultaneously ensuring that the overall physiological strain remains within tolerance limits.
6. Once the repeated application of the adaptation stimulus ends, there is a gradual reversal (decay) of the morphological and functional adaptations.

The mechanisms and time-course of adaptation to hypoxia and heat combined with a training-related metabolic stress will be the focus of the following sections. Given that most of the work in this field has been conducted in laboratory conditions, the term “acclimation” will be used followingly to describe experimentally induced adaptation protocols.

1.3. Heat Acclimation

Heat acclimation (HeA) develops upon repeated exposure to environmental conditions that are sufficiently stressful to elevate core and skin temperature (Regan, Macfarlane, & Taylor, 1996) and induce profound sweating (Hessemer, Zeh, & Bruck, 1986). Exercise is used as means to potentiate the increases in core temperature (T_c) whereas high ambient temperatures (T_a) contribute mostly to the increases in skin temperature. Specifically, a T_c as high as 38.5 °C stands as a prerequisite for HeA (Fox, Goldsmith, Kidd, & Lewis, 1963) with further increases not demonstrating an additional effect (Gibson, Mee, et al., 2015).

For a given physical task, the positive phenotypic response to HeA elicits lower core and skin temperatures, augmented sweating and skin blood flow, improved fluid balance, increased cardiovascular stability, enhanced skeletal muscle metabolism, a lower whole-body metabolic rate and an increased acquired thermal tolerance (for detailed reviews on the topic see (Periard, Racinais, & Sawka, 2015; Sawka, Leon, Montain, & Sonna, 2011; N. A. Taylor, 2014). Considering the kinetics of HeA, most studies employ medium-term protocols (7-14 days) even though signs of thermoregulatory, cardiovascular and hormonal adaptation have been observed after as little as 5 days (Garrett, Creasy, Rehrer, Patterson, & Cotter, 2012; Neal, Corbett, Massey, & Tipton, 2016). Horowitz (1998) has proposed a conceptual model characterized by a distinct biphasic pattern. In the first transient phase, a decreased organ output-to-autonomic signal ratio indicates that an augmented efferent activity is necessary to override the impaired peripheral responsiveness and produces adequate effector output. The second stable phase is manifested by an enhanced effector organ-to-autonomic signal ratio as both central and peripheral adaptations improve physiological efficiency.

HeA has been repeatedly shown to mitigate the heat-induced decrements in aerobic performance (Keiser et al., 2015; Lorenzo, Halliwill, Sawka, & Minson, 2010; Racinais, Periard, Karlsen, & Nybo, 2015). Whether this ergogenic potential can be transferred to thermoneutral normoxic conditions remains unresolved. Given that aerobic performance is determined by maximal aerobic power ($\dot{V}O_{2max}$), exercise economy and anaerobic threshold (Bassett & Howley, 2000), an ergogenic effect should be translated into an improvement in any of these indices. Among many studies that have reported increases in $\dot{V}O_{2max}$ in normoxia following HeA (Lorenzo et al., 2010; Sawka, Young, Cadarette, Levine, & Pandolf, 1985; Shvartz et al., 1977), only one recruited well-trained participants (Lorenzo et al., 2010). Multiple studies have failed to demonstrate a similar increase in $\dot{V}O_{2max}$ in participants of a high fitness level (Karlsen et al., 2015; Keiser et al., 2015; Rendell et al., 2017). Given that exercise training is included in HeA protocols, a “training effect” has been implied to mediate these improvements, especially in the cases that exercise economy gains are observed in “one-arm” designs (i.e. no control group such as in the study by Sawka, Pandolf, Avellini, and Shapiro (1983)). Nevertheless, a reduced rate of metabolism might result from the Q_{10} effect; a HeA-induced reduction of the body temperature drives a lower metabolic rate. These findings are not universal with recent studies demonstrating no changes in GME during submaximal exercise following HeA (Karlsen et al., 2015). Moreover, HSP90 stabilizes steroid hormone receptors in the kidney (Bohen & Yamamoto, 1993) and thus supports an aldosterone-driven plasma volume (PV) expansion. Despite the fact that PV expansion is a consistent finding among HeA studies (Karlsen et al., 2015; Keiser et al., 2015; Lorenzo et al., 2010; Rendell et al., 2017), the ergogenic potential of such adaptation remains questionable. In normoxia, the end-diastolic volume of the heart will be enlarged via the Frank-Starling mechanism and facilitate convective oxygen delivery (Lorenzo et al., 2010) which however may be counteracted by the excessive hemodilution (Coyle, Hopper, & Coggan, 1990; Keiser et al., 2015). The preload of the heart is also enhanced by an increased ventricular compliance (Horowitz, Parnes, & Hasin, 1993) while cardiac function appears to be further supported by an increased myocardial efficiency, i.e. a lower metabolic cost of pumping (Horowitz, Shimoni, Parnes, Gotsman, & Hasin, 1986). Considering the anaerobic threshold, a reduced carbohydrate metabolism stands as the main mediator of an increased power at the lactate threshold during exercise in thermoneutral conditions (Young, Sawka, Levine, Cadarette, & Pandolf, 1985).

In hypoxia, aerobic capacity cannot be restored towards baseline values even after hypoxic acclimation (HyA) owing to a persistent reduction in cardiac output (CO) (Saltin, Grover, Blomqvist, Hartley, & R.L., 1968). Even though artificially-induced PV expansion enhances blood flow in the exercising muscles in hypoxia, again the resultant hemodilution

seems to offset the improved oxygen extraction (Calbet et al., 2004). Albeit PV facilitates CO during submaximal exercise (Calbet et al., 2004), whether aerobic capacity remains unaltered (Calbet et al., 2004) or not (Robach et al., 2000) is still under debate. Despite reports of increased cycling time-trial performance in acute hypoxia (Lee, Miller, James, & Thake, 2016; A. C. White et al., 2016), $\dot{V}O_{2\max}$ remained unaffected after HeA in the only study to measure maximal cardiorespiratory responses to-date (A. C. White et al., 2016). From a mechanistic standpoint, PV expansion could indirectly benefit hypoxic aerobic performance in multiple other ways; for instance, a bradycardia-mediated increase in erythrocyte alveolar transit time together with a temperature-related leftward shift in the oxyhemoglobin dissociation curve improve arterial oxygen content while the hypervolemia-enhanced lactate removal delays the heart rate (HR) onset of blood lactate accumulation (Heled et al., 2012). For longer sojourns at altitude, HSPs can protect humans against pulmonary hypertension and altitude-related illnesses (HAPE, HACE) by participating in the production of nitric oxide synthase and maintaining epithelial barrier integrity, respectively. The activation of shared cellular stress response pathways between heat and hypoxia including hypoxia-inducible factor-1 (HIF-1) and heat-shock proteins (HSP), though promising, has not been clearly shown, particularly in humans.

HeA programmes traditionally request individuals to perform fixed-work rate exercise in hot conditions for multiple (~10) days. Subsequently, the thermal load is externally quantified in terms of T_a and humidity. Inevitably, as adaptation progresses, indices of cardiovascular (HR) and thermal strain (core and skin temperature) are reduced over the course of acclimation for the prescribed constant exercise intensity. Another experimental approach that takes into consideration the internal heat load rather than the external strain is the isothermic/controlled-hyperthermia HeA protocol. The variable being monitored here is T_c rather than exercise workload. For reasons of practicality, HR could be monitored instead of T_c . The adaptation impulse is sustained over the duration of the protocol as humans are forced to exercise at an aggregate higher workload during each successive exercise session. Another strength of isothermic models is that adaptation appears to be induced at the end of each session with no exercise cost. Surprisingly, not many studies have adopted the isothermic HeA protocol (Fox et al., 1963; Gibson, Mee, et al., 2015; Patterson, Stocks, & Taylor, 2004; Regan et al., 1996; Rendell et al., 2017).

1.4. Hypoxic Acclimation

Hypoxic acclimation protocols involve sojourns at natural (hypobaric hypoxia) or simulated (normobaric hypoxia) high-altitude. Since the seminal study by Levine and Stray-Gundersen (1997), hypoxic confinements were mostly interspersed with exercise training sessions held in normoxic conditions so that the training intensity is maintained in elite athletes. Despite the fact that increases in the exercise economy (Gore et al., 2001) or anaerobic capacity (Clark et al., 2004) have been considered as contributing factors, the ergogenic effect of HyA is mainly linked to an augmented red cell volume (Levine & Stray-Gundersen, 2005). In acute hypoxia, the greatest challenge that humans face is a desaturation-driven reduction in arterial oxygen content. As a compensatory mechanism, the hemoconcentration is the consequence of a renin-aldosterone axis-mediated (Siebenmann, Cathomen, et al., 2015) decline in PV that develops over the first hours/days (Sawka, Convertino, Eichner, Schnieder, & Young, 2000). During the course of HyA, an expansion of total hemoglobin mass (Hbmass) progressively contributes to the hemoconcentration. Additionally, the hypoxic ventilatory drive restores saturation towards baseline values. These adaptations result in $\dot{V}O_{2\max}$ and endurance performance gains in hypoxic conditions that seem to peak in two weeks (Schuler, Thomsen, Gassmann, &

Lundby, 2007). Similar to HeA, this ergogenic potential is thought to transfer to normoxic thermoneutral conditions. Numerous studies have observed increases in $\dot{V}O_{2\max}$ after HyA protocols (Brugniaux et al., 2006; Wehrlin, Zuest, Hallen, & Marti, 2006) with some of them failing to translate the hematological/aerobic gains into enhanced aerobic performance (Bonne, Lundby, et al., 2014; Robertson et al., 2010). In order for a hypoxic dose to be of sufficient intensity, certain conditions regarding the stimulus and the duration have been suggested (see [Chapter 7](#)); a daily exposure that is higher than 12 h at a minimum altitude of 2,000 m for at least 3 weeks (Rusko, Tikkanen, & Peltonen, 2004). Past studies that scheduled a shorter daily exposure (Ashenden, Gore, Dobson, & Hahn, 1999) or employed a lower simulated altitude (Pottgiesser, Ahlgrim, Ruthardt, Dickhuth, & Schumacher, 2009) failed to observe increases in Hbmass, the main determinant of $\dot{V}O_{2\max}$ in normoxic conditions. Other models that quantify the cumulative hypoxic dose-response have been recently proposed: 1% in either red cell volume or Hbmass per 100 h of hypoxia. Given that the typical error of Hbmass measurement is $\sim 2.2\%$ (Gore, Hopkins, & Burge, 2005), then at least a total of 300 h of hypoxic exposure has been claimed (Clark et al., 2009) to be needed before changes in Hbmass could be detected with confidence. Recent findings even support an erythropoietic effect with as much as 230 h of live high-train low at a (simulated) altitude of 2250 m (Hauser et al., 2017). An attempt has been made to include the employed altitude as a parameter in the calculation of the total hypoxic dose (Garvican-Lewis, Sharpe, & Gore, 2016). The product of total hypoxic exposure (intermittent or continuous) and the hypoxic stimulus expressed in hours and kilometers of ascent, respectively, represents the accumulated hypoxic dose. The typical error of 2.2% in Hbmass measurement will be surpassed by doses higher than 500 km · h. Continuous normobaric hypoxic acclimation *per se*, without concomitant exercise training, does not seem to alter normoxic $\dot{V}O_{2\max}$ and respective peak power output (W_{peak}) (Kounalakis, Keramidas, Eiken, Jaki Mekjavic, & Mekjavic, 2013). This may be due to the confinement protocol restricting the daily physical activity levels of the participants. Indeed, a recent study that investigated the kinetics of Hbmass during such a 28-day confinement in untrained males – incorporating sessions of physical exercise – reported increases in Hbmass in all participants by day 12 (Siebenmann, Cathomen, et al., 2015). Caution should be taken when applying these findings on an individual basis. The individual variation to altitude training has been attributed to several factors such as baseline fitness level (Hauser et al., 2018; Robach & Lundby, 2012) and iron availability, the sustained erythropoietin (EPO) response at altitude, the degree of desaturation that trained humans experience and the training intensity they are able to maintain while training at altitude (Chapman, Stray-Gundersen, & Levine, 1998).

Acute hypoxia has been repeatedly shown to alter the human sweating response but not T_{c} responses (Greenleaf, Greenleaf, Card, & Saltin, 1969; Kacin, Golja, Eiken, Tipton, & Mekjavic, 2007; Kounalakis, Eiken, & Mekjavic, 2012). The mechanism claimed to mediate an increment in the sweating response is the hypoxia-imposed increase in the relative intensity for participants exercising at the same absolute workload. This possibility is further supported by previous studies that utilized muscle ischemia as a means to increase relative intensity for the same absolute workload (Kacin et al., 2005). Central command and muscle metaboreflex-related sympathoactivation reflected in the augmented cardiorespiratory responses might exacerbate sweating during hypoxic exercise. These results are not universal, though, as other studies have shown no change (Coombs, Cramer, Ravanelli, Imbeault, & Jay, 2017) or even an impaired (Kolka, Stephenson, Rock, & Gonzalez, 1987) sweating response during exercise in hypoxic conditions. The former studies reasoned that a lower perception of overall body exertion together with an increased muscle blood flow that attenuated the muscle metaboreflex acted to abate the forehead sweating response in hypoxia following HyA. This may seem a bit paradoxical given that

hypovolemia, one of the main adaptations observed in HyA protocols, impairs evaporative heat loss (Fortney, Nadel, Wenger, & Bove, 1981). Adding to the complexity, the training component of HyA might enhance the magnitude and gain of the sweating response (Nadel, Pandolf, Roberts, & Stolwijk, 1974; Roberts, Wenger, Stolwijk, & Nadel, 1977) and shift the T_c threshold for onset of sweating and vasodilatation toward lower T_c levels (Roberts et al., 1977). During maximal aerobic exercise, the heat-induced decrement in aerobic performance can presumably be attributed to “an impaired arterial oxygen delivery to the exercising muscles as a consequence of impaired cardiac filling when skin temperature and skin blood flow become high and central venous pressure declines” (Nybo, Rasmussen, & Sawka, 2014). As HyA is able to modulate both arterial oxygen delivery and thermoregulatory function, the potential of applying such protocols for individuals that are scheduled to perform in the heat is worth exploring. At a cellular level, this research endeavor is further necessitated by the observations that HIF-1 α – the mediator of the HyA response – might elicit systemic acclimation responses that would facilitate exercise in the heat. Additionally to the erythropietic effect, an increased carotid body sensitivity to oxygen could augment respiratory heat loss, whereas HIF-1 could support maximal skin blood flow via the induction of the vascular endothelium growth factor (VEGF). Whether these promising interactions observed between heat and hypoxia at a cellular level can indeed transfer to the systemic level warrants investigation.

1.5. A Combined Stressor Approach, the Training Effect and Cross-Adaptation Between Heat and Cold

HyA has been shown to enhance $\dot{V}O_{2max}$ in hypoxic (Schuler et al., 2007) or normoxic thermoneutral (Levine & Stray-Gundersen, 1997) conditions. These improvements are possibly mediated by augmented red cell volume (Levine & Stray-Gundersen, 2005) that serves to increase arterial oxygen content. Similarly, HeA has also gained in popularity among athletes as a potential ergogenic aid in aerobic performance in hot conditions. There is a disagreement however, over whether such a protocol increases aerobic performance in normoxic thermoneutral conditions (Lorenzo et al., 2010; Rendell et al., 2017) or not (Karlsen et al., 2015; Keiser et al., 2015). Potential HeA-induced ergogenic mechanisms include PV expansion (Lorenzo et al., 2010), an increase in exercise economy (Sawka et al., 1983) and/or power output at the lactate threshold (Lorenzo et al., 2010; Rendell et al., 2017). Thus, the separate physiological mechanisms associated with HeA and HyA might act synergistically to influence aerobic performance in thermoneutral or hot conditions. The potential improvements that can be derived from such a combined HyA and HeA are associated with either predominantly hematological and cardiorespiratory modifications in the case of the former, and thermoregulatory modifications in the case of the latter. Both heat and hypoxic stimuli to be applied in a combined acclimation protocol should provide the individuals with a sufficient adaptation impulse. The lack of such stimuli may have resulted in insufficient hypoxic (Rendell et al., 2017) or heat (McCleave et al., 2018) doses. Thus, a continuous hypoxic confinement daily interspersed by controlled-hyperthermia training sessions in the heat is recommended to explore an ergogenic combination of heat and hypoxic acclimation.

Both HeA and HyA acclimation protocols commonly include a series of exercise sessions. In the former case, exercise is utilized as a means to facilitate rapid increases in T_c which are known to chiefly modulate heat adaptation (Regan et al., 1996). In the latter case, exercise sessions are included to prevent a deconditioning/detraining effect (Keramidas, Kolegard, Mekjavic, & Eiken, 2016). However, the inclusion of exercise sessions into

acclimation protocols renders it difficult to distinguish the independent effect of exercise training from that of the environmental stressor on the subsequent physiological responses. Control groups have been repeatedly recruited to showcase the so-called “training effect” which, to-date, and in regards to HeA and HyA protocols, remains poorly defined. In previous HeA/HyA studies (Brugniaux et al., 2006; Karlsen et al., 2015; Keiser et al., 2015; Sawka et al., 1983; Wehrlin et al., 2006), the control groups undergoing training/living at normoxic thermoneutral conditions did not improve any of the aerobic performance measures. As such, any positive results observed in the experimental groups should have resulted from the interactive effect between training and the environmental stressor. Otherwise, the inefficiency of the acclimation protocol is attributed to the high fitness level of the participants (Karlsen et al., 2015; Siebenmann et al., 2012).

HeA has been repeatedly shown to lower resting and exercise T_{c} and augment the sweating response in thermoneutral and/or hot conditions (Nadel et al., 1974; Patterson et al., 2004; Roberts et al., 1977); a lower threshold T_{c} as well as increased gain and maximal capacity have been commonly observed. However, a lower sweating threshold T_{c} would narrow the inter-threshold zone unless the shivering threshold T_{c} is simultaneously lowered. In turn, a narrower inter-threshold zone would result in a less economical temperature regulation with more water or energy expenditure despite a higher regulatory efficiency. To date, there is only one study to demonstrate that short-term (5-day) HeA resulted in a concurrent decrease in the shivering and sweating thresholds (Hessemer et al., 1986). Thus, the interthreshold zone remained unaffected with the authors concluding that “[...]there must be some cross-adaptation between heat and cold”.

1.6. Aims and Hypotheses

The overall aim of the present thesis was to examine the independent and combined effects of heat and hypoxic acclimation on aerobic performance, thermoregulation and selected hematological responses in healthy aerobically trained males under normoxic, hypoxic and hot conditions. The experimental protocol was approved by the National Committee for Medical Ethics at the Ministry of Health (Republic of Slovenia, no. 0120-494/2018/9) and conformed to the Declaration of Helsinki guidelines (Appendix A). This general purpose was evaluated in four separate studies, and each of them had a specific aim and hypothesis listed below:

Study I. This study assessed the aerobic performance and thermoregulatory responses during exercise in thermoneutral, hot, and hypoxic conditions following a 10-day continuous hypoxic acclimation protocol.

We hypothesized that hypoxic acclimation would improve performance when exercising in normoxic, normobaric hypoxic, and hot conditions. We also hypothesized that the thermoregulatory responses (core threshold temperature for the onset of sweating and the gain of the sweating response) would be enhanced when exercising in heat and in hypoxia following the acclimation protocol.

Study II. This study mapped the cardiorespiratory and thermoregulatory responses during exercise in thermoneutral hypoxic and hot conditions following a 10-day controlled-hyperthermia heat-acclimation protocol.

We hypothesized that heat acclimation would enhance maximal aerobic power in thermoneutral normoxic, hypoxic and hot conditions. Secondly, we hypothesized that peak power output as a surrogate index of exercise capacity would be higher in all environmental conditions post-acclimation.

Study III. This study investigated aerobic performance and thermoregulatory adaptations in normoxic, hot and hypoxic environments following a 10-day combined heat and hypoxic acclimation protocol in young males.

We tested the hypothesis that the combined-stressor acclimation protocol would enhance aerobic performance and facilitate thermoregulatory function in normoxic and hot conditions.

Study IV. This study investigated the interactive effects of a 10-day moderate-intensity training protocol and baseline fitness level on aerobic performance in normoxic, hypoxic and hot conditions.

We hypothesized that aerobic performance as well as thermoregulatory responses would be facilitated only in the untrained group exercising in normoxic conditions after the application of the training protocol.

Study V. This study investigated the influence of heat acclimation achieved with the exercise controlled-hyperthermia protocol, on the autonomic thermoregulatory responses activated to regulate body temperature in a high heat loss environment.

The null hypothesis was that the T_{c} response to a high heat loss environment will remain unaffected by the controlled-hyperthermia heat-acclimation protocol.

Chapter 2

Aerobic Gains of a 10-Day Training Regimen are Dependent on Initial Fitness Level

Exercise creates a competition between the requirement for oxygen delivery by the exercising muscle and need to dissipate heat from the skin. Exercise training reduces this competition by rendering the sweating response more efficient, thus improving not only aerobic performance, but also exercise thermoregulation. Training in a hot environment will augment the exercise-induced improvements in temperature regulation leading to an improvement in aerobic performance in hot environments. Such adaptation to a hot environment is defined as heat acclimation (HeA). In contrast, adaptation to the hypoxia of high altitude does not require the inclusion of exercise. Nevertheless, the most efficient HeA and HyA protocols include exercise during exposure to the respective environments. In the former case, exercise is utilized as a means to facilitate rapid increases in core temperature (T_c) which serve as the key stimulus for the heat adaptation (Regan et al., 1996). In the latter case, exercise sessions are included to prevent a deconditioning/detraining effect (Keramidas, Kolegard, et al., 2016). The inclusion of exercise sessions into acclimation protocols renders it difficult to distinguish the effect of the adaptation of physiological systems to the environmental stressor under investigation from that of the exercise training. Control groups are not always included in acclimation studies, thus the separation of the training effect from the acclimation effect remains unclear.

Common to acclimation studies is that the efficiency of acclimation protocols is evaluated on the basis of physical performance. Specifically, aerobic exercise performance is determined by $\dot{V}O_{2max}$, GME and the lactate/anaerobic threshold (Bassett & Howley, 2000). In addition to improving performance in the environment to which individuals acclimated (i.e. heat or hypoxia), it soon became apparent that acclimation to heat or hypoxia also improves physical performance in thermoneutral normoxic conditions. The ergogenic contribution of HeA to exercise in thermoneutral conditions was established by Lorenzo et al. (2010), who reported benefits in $\dot{V}O_{2max}$, power at the lactate threshold and time-trial performance in elite cyclists. Subsequent studies failed to verify these improvements in the determinants of aerobic performance (Karlsen et al., 2015; Keiser et al., 2015). Interestingly, studies including only an experimental group have reported a higher GME after HeA incorporating exercise training (Sawka et al., 1983), but not after only a training protocol of a similar duration (Sawka et al., 1983, Chapter 4). Currently, HyA is widely regarded as an ergogenic aid significantly contributing to aerobic

performance. Levine and Stray-Gundersen (1997) reported higher values for 5-km time trial performance, $\dot{V}O_{2max}$, ventilatory threshold and velocity at $\dot{V}O_{2max}$, but not exercise economy in trained runners after a live-high train-low protocol. An augmented $\dot{V}O_{2max}$, an unaltered mechanical efficiency (Lundby et al., 2007), and an increased anaerobic/lactate threshold (Brugniaux et al., 2006; Clark et al., 2004) have been confirmed following HyA, though these are not universal findings (Gore et al., 2001; Siebenmann et al., 2012). $\dot{V}O_{2max}$, W_{peak} or exercise economy in normoxic or hypoxic conditions were not influenced following a 10-day hypoxic confinement that included no exercise sessions (Kounalakis et al., 2013). In the above studies, the control groups undergoing training/living at normoxic thermoneutral conditions did not improve any of the aerobic performance measures. As such, any positive results observed in the experimental groups should have resulted from the interactive effect between training and the environmental stressor. Otherwise, the inefficiency of the acclimation protocol is attributed to the high fitness level of the participants (Karlsen et al., 2015; Siebenmann et al., 2012). Should the training effect during acclimation to heat or hypoxia be a function of the initial pre-acclimation aerobic fitness level, then it can be reasoned that the benefit of an acclimation protocol may have a greater benefit to less-trained compared to well-trained individuals. For the purpose of comparing the efficiency of acclimation regimens in either heat or hypoxia, it would therefore seem imperative to assess the interaction of training and acclimation.

Short-term (e.g. 10-day) training protocols applied in untrained individuals are believed to potentiate heat dissipation responses owing to the training-induced repeated elevations of T_c (Avellini, Shapiro, Fortney, Wenger, & Pandolf, 1982; Hessemer et al., 1986). Augmented heat dissipation might be of peripheral or central origin and respectively expressed as a lower sweating threshold (Nadel et al., 1974) or a higher gain of the sweating response (Roberts et al., 1977). Consequently, decrements in exercise HR, rectal temperature (T_{re}) and skin temperature are observed whereas maximal skin wettedness can also be enhanced after longer protocols (Ravanelli, Coombs, Imbeault, & Jay, 2018). Surprisingly, no study to-date has examined whether these phenotypic adaptations in an untrained population are translated into a lower decrement in aerobic performance in the heat, compared to thermoneutral conditions. Furthermore, there seems to be a negative correlation between the initial fitness level and the decrement in aerobic performance in hypoxia (Martin & O’Kroy, 1993) explained by the excessive reduction in S_pO_2 in the trained individuals (Mollard, Woorons, Letournel, Cornolo, et al., 2007).

2.1. Methods

2.1.1. Participants

Twenty healthy young male volunteers [age (mean $\pm$ SD) = 23.5 $\pm$ 2.6 years] were recruited to participate in the study. Inclusion criteria included males between 18 and 30 years of age, non-smokers and medication free. All participants were near-sea level residents and had not been exposed to altitudes >1500 m or temperatures >30 °C for at least 1 month prior to the start of the study which was held in November and December. None had a history of any cardiorespiratory or hematological diseases. Participants were instructed to abstain from caffeine and alcohol consumption during the course of the entire study. They were extensively informed regarding the study protocol as well as the potential risks involved. During the pre-screening procedure the participants completed a graded exercise test on a cycle ergometer to establish their normoxic $\dot{V}O_{2max}$ and W_{peak} . Aerobic fitness was defined based on $\dot{V}O_{2max}$ values. A $\dot{V}O_{2max}$ lower than 45 mL $\cdot$ kg⁻¹ $\cdot$ min⁻¹ or higher than 55 mL $\cdot$ kg⁻¹ $\cdot$ min⁻¹ was deemed as a prerequisite for the participation in the

lower (untrained) or higher fitness group (trained), respectively in agreement with the values reported in previous studies (Jay, Bain, Deren, Sacheli, & Cramer, 2011; Montero & Lundby, 2017). To further ensure that $\dot{V}O_{2\max}$ reflected the actual participants' cardiorespiratory fitness level, untrained participants were also required not to participate in any organized sports. Cycling and walking for commuting purposes were allowed. Accordingly, trained participants were performing endurance-type activities (running, cycling, swimming) multiple times per week. Participants were informed that the aim of the study was to investigate the effect of a 10-day training protocol on aerobic performance in young males. However, they were blinded to the fact that the data will be analyzed in groups according to their fitness level. Participant baseline characteristics are presented in Table 2.1. For the participants that were included in the study, the measured normoxic W_{peak} was used to determine 1) the individual steady-state workloads during the pre- and post-HA tests (40% of the W_{peak} attained during the preliminary test) and 2) the training intensity to be used during the 10-day training protocol. Prior to the onset of the experiments, informed oral and written consents were obtained from all participants.

Table 2.1: Participants' baseline characteristics. Values are means $\pm$ SD. Abbreviations: BSA, body surface area; $\dot{V}O_{2\max}$, maximal aerobic power, W_{peak} peak power output. *Significant difference from trained group.

Group	Trained (n = 10)	Untrained (n = 10)
Age (years)	23 $\pm$ 2	25 $\pm$ 3
Height (cm)	180 $\pm$ 5	179 $\pm$ 3
Mass (kg)	74 $\pm$ 3	85 $\pm$ 14*
BSA (m ²)	1.96 $\pm$ 0.08	2.05 $\pm$ 0.17
Body Fat (%)	9.2 $\pm$ 2.3	16.3 $\pm$ 4.9*
$\dot{V}O_{2\max}$ (mL $\cdot$ kg ⁻¹ $\cdot$ min ⁻¹)	58 $\pm$ 6	42 $\pm$ 5*
W_{peak} (W)	364 $\pm$ 35	309 $\pm$ 46*
W_{peak} (W $\cdot$ kg ⁻¹)	4.9 $\pm$ 0.5	3.6 $\pm$ 0.4*

2.1.2. Study Design

The study consisted of three parts: pre-training exercise testing, a 10-day exercise training program, followed by the post-training exercise testing. During exercise testing, the participants completed the same maximal exercise performance test in three different environmental conditions (thermoneutral normoxic, thermoneutral hypoxic, and hot normoxic) on separate but consecutive days. The order of the exercise tests was randomized and counterbalanced between participants whereas the order of the tests within participants was the same pre- and post-training. All tests were performed at the same time of the day for a given participant (± 1 hour). Exercise training sessions were held during morning hours (9:00-12:00). Participants were given a 24-h rest prior to and following the 10-day training period.

2.1.3. Exercise Training

All participants underwent daily 60-min supervised cycling sessions over 10 days. Training intensity was similar in relative terms for all individuals, set at 50 % of the W_{peak}

calculated from the individual W_{peak} obtained during the preliminary graded normoxic exercise test. Participants were only informed of the time remaining to the end of the session and were allowed to drink ad libitum during each training session. Exercise HR and SpO₂ were assessed using a finger pulse oximeter (Wristox 3100 Nonin, Plymouth, Minnesota, USA) at 5-min intervals. Ratings of perceived exertion (RPE; 6-20) were also recorded at 5-min intervals. T_a was maintained at 24 °C. The exercise room was well ventilated, ensuring normoxic and normocapnic conditions during training. The participants performed all training sessions at the same time of the day. No other exercise training was allowed during the study. The sessions were supervised by at least two researchers in order to record the training data and to ensure that all participants maintained the desired workloads at all times.

2.1.4. Exercise Testing

All the pre- and post-training trials were conducted in a laboratory situated at 300 m above sea level (Ljubljana, Slovenia). The trials were conducted on a cycle ergometer (Daum, Electronic, Furth, Germany) and comprised two stages; a 30-min steady-state exercise immediately followed by an incremental exercise to exhaustion. During exercise, each participant pedalled at a preferred cadence (between 60 and 90 rpm), which they maintained via visual and verbal feedback throughout the trial. Before (Pre) and after (Post) the 10-day training protocol, participants conducted three trials on three consecutive days. In the normal temperature and normoxic condition (NOR), participants breathed room air (pre: partial pressure of oxygen in the inspired air (P_{iO_2}) = 143.7 ± 0.8 mmHg, post: P_{iO_2} = 143.4 ± 0.7 mmHg) and exercised in thermoneutral conditions (pre: T_a = 23.2 ± 0.7 °C and relative humidity (RH) = $47.2 \pm 2.2\%$, post: T_a = 23.2 ± 0.5 °C and RH = $46.6 \pm 5.9\%$). In the hypoxic condition (HYP), they inspired a hypoxic gas mixture (pre: P_{iO_2} = 92.2 ± 1.5 mmHg, post: P_{iO_2} = 93.2 ± 1.2 mmHg) and exercised in thermoneutral conditions (pre: T_a = 22.8 ± 0.5 °C and RH = $51.2 \pm 1.2\%$, post: T_a = 22.5 ± 0.6 °C and RH = $51.5 \pm 1.3\%$). In the hot condition (HE), the participants inspired room air (pre: P_{iO_2} = 142.6 ± 1.7 mmHg, post: P_{iO_2} = 142.7 ± 1.8 mmHg), but exercised in a hot environment (pre: T_a = 34.1 ± 0.9 °C, RH = $48.1 \pm 4.2\%$, post: T_a = 34.1 ± 1.1 °C and RH = $49.8 \pm 3.0\%$). As stated earlier, the order of the exercise trials was randomized and counterbalanced. A resting day was scheduled immediately before and after the training protocol to minimize the contribution of fatigue during the exercise tests. Body composition was assessed on days 1 and 10 of the training protocol by taking measurements of several skinfolds (chest, thigh, triceps, subscapular, suprailiac, abdominal and axillary) and using the equation of Jackson and Pollock (1978) for the calculation of body fat percentage.

A metabolic cart (Quark CPET, Cosmed, Rome, Italy) was used to obtain the breath-by-breath respiratory responses during the exercise trials. Metabolic energy expenditure was calculated during submaximal exercise using the following equation:

$$M (W) = \dot{V}O_2 \frac{\left(\frac{RER-0.7}{0.3}\right)e_c + \left(\frac{1-RER}{0.3}\right)e_f}{60}$$

where RER respiratory exchange ratio, $\dot{V}O_2$ oxygen uptake ($\text{mL} \cdot \text{min}^{-1}$), and e_c and e_f are the caloric equivalents per liter of oxygen for the oxidation of carbohydrates (21.13 kJ) and fats (19.62 kJ), respectively. Metabolic heat production (H_{prod}) was estimated by subtracting the rate of mechanical work from metabolic energy expenditure. The anaerobic threshold was defined as the $\dot{V}O_2$ that corresponded to the deflection point of the partial pressure of end-tidal carbon dioxide (P_{ETCO_2}) confirmed by the nadir in the respiratory equivalent for carbon dioxide (CO_2) when plotted as functions of the workload during the incremental test (Binder et al., 2008). Before each trial, the O_2 and CO_2 analyzers were

calibrated using two different gas mixtures, and the pneumotachograph was calibrated with a 3-L syringe, in accordance with the manufacturer's recommendation.

HR was derived from electrocardiographic recordings with electrodes in a precordial position. CO was determined using electrical impedance cardiography (Physioflow Q-Link, Manatec Biomedical, Paris, France). The method measures changes in thoracic impedance during cardiac ejection to estimate stroke volume (SV). Six electrodes were placed at the base of the neck and on the xiphoid process. Electrical impedance cardiography has been validated against the direct Fick method during maximal incremental exercise in healthy participants (Siebenmann, Rasmussen, et al., 2015). S_pO_2 was recorded using an ear pulse oximeter (Wristox₂ 3150 Nonin, Plymouth, Minnesota, USA). RPE was obtained using a Borg scale (6-20). During all trials the participants wore only shorts and athletic shoes. The body mass was measured before and after each exercise testing on a weighing scale (± 0.05 kg) (TPT 5N, Libela ELSI, Celje, Slovenia).

T_{re} was measured with a thermistor probe (MSR 145, Henggart, Switzerland) inserted 10 cm beyond the anal sphincter. Skin temperatures were measured at four sites (chest, arm, thigh and calf) using thermistors (MSR 147WD, Henggart, Switzerland) attached to the skin with a single piece of adhesive tape. The assessment of calf, thigh, chest and arm skin temperature enabled the calculation of weighted mean skin temperature (T_{sk}) (Ramanathan, 1964). The ventral fingertip and forearm temperatures were also measured. The forearm-fingertip temperature difference (ΔT_{f-f}) has been validated as an index of vasomotor tone during steady-state exercise (Keramidas, Geladas, Mekjavic, & Kounalakis, 2013). Minute mass flow of secreted sweat ($\dot{m}_{sw}$, $mg \cdot cm^{-2} \cdot min^{-1}$) was measured with a ventilated capsule placed on the forehead. Forced evaporation of sweat under the capsule (surface area $4.8 cm^2$) was achieved by a constant air flow ($1.0 L \cdot min^{-1}$) through the capsule. Minute mass flow of secreted sweat was estimated each minute from the difference between the temperature and the humidity of inflowing and outflowing air. Air temperature was measured with thermistors (LM35, National Semiconductor Corp., Santa Clara, CA, USA) and the RH with capacitance hygrometers (Valvo air humidity sensor, Valvo-Philips GmbH, Hamburg, Germany).

2.1.5. Steady-State and Incremental Exercise

Following a 2-min rest period, participants commenced pedalling on the cycle ergometer. During the first 2 minutes of the exercise, the load was set at 90 W (warm-up). Thereafter, they cycled for 30 min at a steady-state work rate equivalent to 40% of their NOR W_{peak} attained during the preliminary testing. The absolute and not the relative external workload was the same in all three exercise trials (the same power output was employed for a specific participant across environmental conditions). Our experimental design permitted that each participant served as their own control to eliminate variation in other factors that are shown to affect T_c and the sweating response such as body surface area, body mass and adiposity (Cramer & Jay, 2014). Immediately following the 30-min steady-state exercise protocol, participants performed an incremental-load exercise to exhaustion. The initial work rate was set at 100W (2 min), thereafter increasing by 20W every minute until volitional exhaustion (inability to maintain the cycling cadence above 60 rpm). During each trial, S_pO_2 and sweat rate data were recorded at minute intervals whereas HR, CO and SV were measured every 10 s. RPE values were recorded at 5-min and 2-min intervals during the steady-state and the incremental-load exercise, respectively. Attainment of $\dot{V}O_{2max}$, defined as the highest $\dot{V}O_2$ averaged over 30 s, was confirmed when participants met at least three of the following criteria, listed in order of priority: (a) severe fatigue or exhaustion leading to inability to maintain exercise at a given work rate (cycling cadence lower than 60 rpm), (b) a plateau in $\dot{V}O_2$, as indicated by the breath-by-breath values,

despite an increase in power output, (c) a subjective RPE near maximal (Borg scale rating greater than 17) and (d) respiratory quotient greater than 1.10. W_{peak} was calculated according to the following equation:

$$W_{\text{peak}} = \text{work rate of last stage completed} + [(\text{work rate increment}) * (\text{time into current stage}/60)]$$

2.1.6. Hematological Results

Initial resting vascular volumes (pre-training) were calculated from body surface area by the equations of Sawka, Young, Pandolf, Dennis, and Valeri (1992) and post-training volumes were calculated by correcting that initial values for the percent change in PV (Strauss, Davis, Rosenbaum, & Rossmeisl, 1951) as well as total blood and erythrocyte volume (Dill & Costill, 1974).

2.1.7. Data Analysis

Statistical analyses were performed using Statistica 5.0 (StatSoft, Tulsa, OK). Hemodynamic variables (CO, HR and SV) collected during steady-state exercise were analyzed for the last 5 minutes of the steady-state exercise, unless stated otherwise. The dependent thermoregulatory variables of Hprod, sweating response (gain, threshold, maximal capacity) as well as all temperature data were analyzed only for the 30 min of submaximal exercise using a mixed three-way ANOVA with the non-repeated factor of group (two levels: trained and untrained) and the repeated factors of environmental condition (three levels: NOR, HYP and HE) and time (two levels: pre and post). Peak values of cardiorespiratory and hemodynamic variables as well as several exercise performance variables (mechanical efficiency, anaerobic threshold, W_{peak}) were analyzed using again a similar mixed three-way ANOVA. Hematological variables were analyzed using a mixed two-way ANOVA with the non-repeated factor of group (two levels: trained and untrained) and the repeated factor of time (four levels: pre, day 2, day 6 and post). When ANOVA revealed a significant F-ratio for interaction and/or main effect, pairwise comparisons were performed with the Tukey honestly significant difference post-hoc test. In the event that the statistical analysis revealed a non-normal distribution of the data, a non-parametric test was performed. Such was the case for SpO_2 , $\Delta\text{Tf-f}$, plasma EPO concentration and heat-shock proteins (HSP72 and HSP90) which were analyzed using the Friedman non-parametric test. Planned comparisons were carried out using Kruskal-Wallis or Wilcoxon tests to locate specific differences. Baseline sweating has an approximate slope of zero, which changes in abrupt monotonic fashion in response to heat stress. We used segmented regression to identify the breakpoint in the forehead sweating response (msw)-Tre curve. The gain of the localized forehead sweating response was determined as the slope of the linear portion of the relationship between forehead sweating response and Tre using least square linear regression. The reported values are averages of the individual thresholds and slopes. Linear regressions were also used to determine the Pearson's product-moment correlation coefficients (r) between initial W_{peak} and $\text{VO}_{2\text{max}}$ and their respective percent post-training changes or the percentage changes in maximal cardiac output (CO_{max}). Correlation size was interpreted using the correlation classification of Hopkins, Marshall, Batterham, and Hanin (2009): trivial ($r < 0.1$), small ($0.1 < r < 0.3$), moderate ($0.3 < r < 0.5$), large ($0.5 < r < 0.7$), very large ($0.7 < r < 0.9$), nearly perfect ($r > 0.9$) and perfect ($r = 1.0$). Data are presented as mean $\pm$ SD unless indicated otherwise. The alpha level of significance was set *a priori* at 0.05.

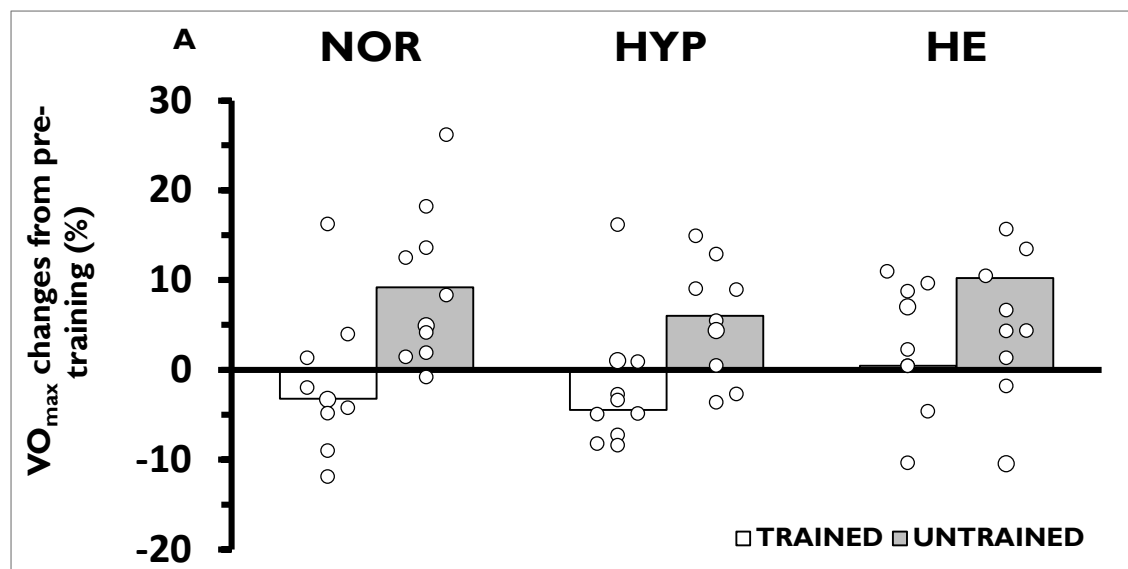
2.2. Results

2.2.1. Exercise Training

Trained individuals were exercising at a higher workload during the training sessions as supported by a main effect of fitness level ($p < 0.01$) on the training load. The two groups of participants experienced a similar relative training stimulus throughout the 10-d period ($p = 0.94$). Participants across groups demonstrated lower HR from day 8 onwards ($p < 0.05$) compared to day 1. The cardiovascular strain induced by training cannot be differentiated between groups (interaction effect between fitness level and time, $p = 0.08$). Untrained individuals tended to have a higher body mass across time ($p = 0.062$). Body fat percentage was lower in the trained group across time ($p < 0.001$) with both groups displaying a lower body fat percentage post-training (trained: 9.2 ± 2.3 vs $8.4 \pm 2.0\%$, $p < 0.01$, untrained: 16.3 ± 4.9 vs $14.8 \pm 4.9\%$, $p < 0.001$).

2.2.2. Aerobic Capacity and Cardiovascular Responses

Figure 2.1A presents the relative changes (%) in $\dot{V}O_{2\max}$ after the 10-d training period in both groups. In the untrained group, $\dot{V}O_{2\max}$ increased in NOR by $+9.2 \pm 8.5\%$ ($+3.4 \pm 2.7 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $p = 0.024$) whereas the respective increases in HYP and HYP were $+10.2 \pm 15.4\%$ ($+3.2 \pm 4.6 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $p = 0.037$) and $+6.0 \pm 5.9\%$ ($+2.1 \pm 1.9 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $p = 0.45$). $\dot{V}O_{2\max}$ in the trained group did not benefit from the training protocol in any environmental condition. More specifically, the magnitude of the changes was $-3.2 \pm 4.7\%$ ($-2.0 \pm 3.0 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $p = 0.53$), $-4.5 \pm 3.5\%$ ($-2.1 \pm 1.6 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $p = 0.68$), $-0.5 \pm 8.2\%$ ($-0.1 \pm 4.4 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $p = 1.0$) in NOR, HYP and HE, respectively. A “very large” correlation ($r = -0.86$, $p < 0.001$) was noted between the initial fitness level and $\Delta\dot{V}O_{2\max}$ in NOR. Weaker negative correlations are reported between initial fitness level and $\Delta\dot{V}O_{2\max}$ in HYP ($r = -0.53$, “large”, $p = 0.016$) and HE ($r = -0.43$, “moderate”, $p = 0.067$).



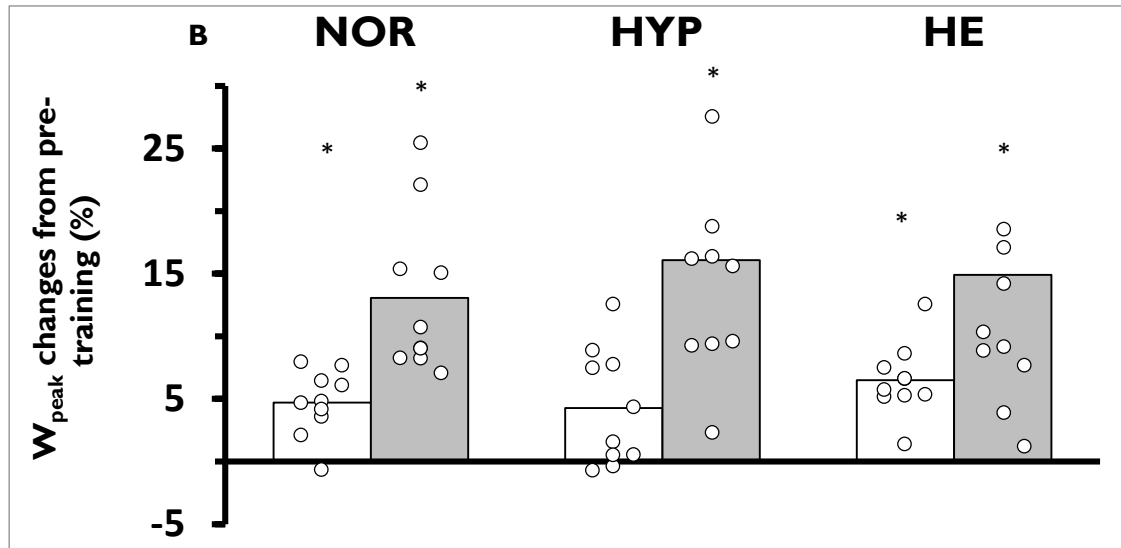


Figure 2.1: (A) Maximal aerobic power ($\text{mL} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$) and (B) peak power output ($\text{W} \cdot \text{kg}^{-1}$) percentage changes from the pre-training trials in all environmental conditions. * $p < 0.05$ versus pre-training within environmental conditions.

Figure 2.1B presents relative changes (%) in W_{peak} (normalized to body mass) after the 10-d training period in both groups. W_{peak} increased by $13.1 \pm 6.4\%$ ($p < 0.001$), $16.1 \pm 9.7\%$ ($p < 0.001$), $14.9 \pm 16.0\%$ ($p < 0.001$) in NOR, HYP and HE for the untrained group. Trained participants did not benefit to the same degree with mean increases reaching $4.7 \pm 2.6\%$ ($p < 0.001$), $4.3 \pm 4.6\%$ ($p = 0.019$) and $6.5 \pm 2.9\%$ ($p < 0.001$) in the respective environments. When W_{peak} was expressed in absolute terms, untrained participants improved their W_{peak} by $12.5 \pm 6.3\%$ ($35 \pm 15 \text{ W}$, $p < 0.001$), $15.2 \pm 9.4\%$ ($36 \pm 19 \text{ W}$, $p < 0.001$) and $13.8 \pm 15.4\%$ ($34 \pm 28 \text{ W}$, $p < 0.001$) whereas the trained group performed also better in NOR and HE with increases reaching $4.1 \pm 3.3\%$ ($15 \pm 11 \text{ W}$, $p = 0.014$) and $6.5 \pm 3.2\%$ ($21 \pm 10 \text{ W}$, $p = 0.002$). W_{peak} did not improve for trained individuals in HYP with the mean change being $4.3 \pm 4.7\%$ ($13 \pm 14 \text{ W}$, $p = 0.18$). Again, there were “large” correlations between baseline W_{peak} , expressed in W , and ΔW_{peak} in NOR ($r = -0.58$, $p = 0.010$) and HYP ($r = -0.52$, $p = 0.018$). ΔW_{peak} in HE was not correlated with baseline W_{peak} ($r = -0.36$, $p = 0.13$).

Table 2.2: Maximal cardiorespiratory responses and peak power output during the incremental exercise to exhaustion pre- and post-training for the trained and untrained groups in normoxic, hypoxic and heat trials (n = 10).

TRAINED	Pre-training		Post-training			
	NOR	HYP	HE	NOR	HYP	HE
$\dot{V}O_{2\max}$ (mL · kg ⁻¹ · min ⁻¹)	58.3 ± 6.3 ^{†#}	47.8 ± 3.8 [#]	52.9 ± 4.9	56.3 ± 5.5 ^{†#}	45.7 ± 4.6 [#]	53.1 ± 5.8
$\dot{V}O_{2\max}$ (L · min ⁻¹)	4.44 ± 0.53 ^{†#}	3.62 ± 0.31 [#]	4.02 ± 0.32	4.26 ± 0.43 [†]	3.46 ± 0.34 [#]	4.04 ± 0.38
W_{peak} (W)	359 ± 44 ^{†#}	302 ± 31 [#]	331 ± 38	373 ± 47 ^{*#†}	314 ± 34 [#]	352 ± 38 [*]
W_{peak} (W kg ⁻¹)	4.71 ± 0.52 ^{†#}	3.98 ± 0.43 [#]	4.36 ± 0.50	4.93 ± 0.58 ^{*#†}	4.16 ± 0.54 ^{*#}	4.64 ± 0.56 [*]
$CO_{\max}$ (L · min ⁻¹)	23.6 ± 3.8	23.2 ± 2.8	24.5 ± 3.2	25.2 ± 4.3	23.4 ± 3.4	25.0 ± 3.0
HR_{peak} (bpm)	192 ± 6	186 ± 9 [#]	194 ± 5	188 ± 7 [†]	180 ± 9 [#]	190 ± 7
SV_{peak} (mL)	123 ± 21	125 ± 17	127 ± 18	134 ± 23	131 ± 23	131 ± 18
AT (mL · kg ⁻¹ · min ⁻¹)	58.3 ± 6.3	47.8 ± 3.8	52.9 ± 4.9	56.3 ± 5.5 [†]	45.7 ± 4.6 [#]	53.1 ± 5.8
$V_{E\text{ peak}}$ (L · min ⁻¹)	186 ± 19	194 ± 10 [#]	178 ± 15	186 ± 15	182 ± 13	185 ± 16
UNTRAINED						
$\dot{V}O_{2\max}$ (mL · kg ⁻¹ · min ⁻¹)	41.5 ± 6.0 ^{††}	35.8 ± 4.9 ^{†#}	40.0 ± 7.0 [‡]	44.9 ± 4.1 ^{*††}	38.0 ± 5.2 [#]	43.2 ± 4.0 [*]
$\dot{V}O_{2\max}$ (mL · kg ⁻¹ · min ⁻¹)	3.50 ± 0.53 ^{††}	3.02 ± 0.27 [#]	3.36 ± 0.48	3.77 ± 0.42 ^{*†}	3.17 ± 0.23 [#]	3.62 ± 0.41 [*]
W_{peak} (W)	293 ± 45 ^{†#}	250 ± 33 [#]	277 ± 48	328 ± 41 ^{*†#}	286 ± 23 ^{*#}	311 ± 39 [*]
W_{peak} (W kg ⁻¹)	3.47 ± 0.50 ^{††#}	2.97 ± 0.51 [#]	3.29 ± 0.59	3.91 ± 0.56 ^{*†#}	3.43 ± 0.51 ^{*#}	3.72 ± 0.50 [*]
$CO_{\max}$ (L · min ⁻¹)	23.5 ± 3.1	21.1 ± 2.2	23.5 ± 3.8	25.6 ± 3.0	24.3 ± 3.2 [*]	24.9 ± 3.8
HR_{peak} (bpm)	189 ± 11	187 ± 12 [#]	193 ± 15	187 ± 8	182 ± 9 [#]	189 ± 8
SV_{peak} (mL)	124 ± 15	113 ± 12	121 ± 16	137 ± 18	134 ± 17 [*]	133 ± 22
Anaerobic threshold (mL · kg ⁻¹ · min ⁻¹)	41.5 ± 6.0 ^{††#}	35.8 ± 4.9 [#]	40.0 ± 7.0 [‡]	44.9 ± 4.1 ^{††}	38.0 ± 5.2 [#]	43.2 ± 4.0 [‡]
$V_{E\text{ peak}}$ (L · min ⁻¹)	141 ± 20 [‡]	144 ± 27 [‡]	137 ± 17 [‡]	160 ± 20 [*]	164 ± 24 [*]	154 ± 16 [*]

Values are mean $\pm$ SD.

* Significant difference to values before heat acclimation

Significant difference to heat values

† Significant difference to hypoxic values

‡ Significant difference to trained participants, $p < 0.05$

Several variables that are associated with aerobic exercise performance are depicted in Table 2.2. $\dot{V}O_{\max}$ was increased in the untrained group by $15.4 \pm 12.5\%$ ($+3.2 \pm 2.4 \text{ L} \cdot \text{min}^{-1}$, $p = 0.011$) in HYP and by $10.0 \pm 12.7\%$ ($+2.1 \pm 3.1 \text{ L} \cdot \text{min}^{-1}$) in NOR even though that change did not reach statistical significance ($p = 0.24$). The minor changes in the trained group were $6.9 \pm 7.8\%$ ($1.6 \pm 2.0 \text{ L} \cdot \text{min}^{-1}$, $p = 0.73$), $0.9 \pm 8.1\%$ ($0.2 \pm 1.9 \text{ L} \cdot \text{min}^{-1}$, $p = 1.0$) and $2.9 \pm 15.1\%$ ($0.3 \pm 3.5 \text{ L} \cdot \text{min}^{-1}$, $p = 1.0$) in NOR, HYP and HE, respectively. Similar to $\dot{V}O_{2\max}$, the initial fitness level was “moderately” correlated with $\Delta\dot{V}O_{\max}$ in NOR ($r = -0.48$, $p = 0.038$) but not in HE ($r = -0.16$, $p = 0.52$). Surprisingly, no main effect of fitness level was observed even when $\dot{V}O_{\max}$ was normalized to BSA ($p = 0.25$). SV_{peak} increased by $17.9 \pm 9.8\%$ ($20.2 \pm 10.7 \text{ mL}$, $p < 0.01$) post-training in HYP for the untrained individuals whereas the respective change in NOR was again deemed not-significant ($11.3 \pm 11.8\%$, $13.5 \pm 15.4 \text{ mL}$, $p = 0.18$). HR_{peak} was consistently lower ($p < 0.001$) when participants were exercising in HYP with the exception of trials held in NOR for the trained group: $p = 0.81$ pre- and $p = 0.56$ post-training). Additionally, HR_{peak} was lower post-training across groups/environmental conditions ($p < 0.01$). As expected, anaerobic threshold (expressed in $\text{mL} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$) was higher in trained individuals (main effect of fitness level, $p < 0.001$) with the exception of exercise in HYP (pre: $p = 0.094$ and post-training: $p = 0.24$). Peak $\dot{V}_E$ was potentiated only in untrained individuals post-training across environmental conditions ($p < 0.01$). Maximal exercise S_pO_2 was higher in NOR and HE compared to HYP across time and fitness levels ($p < 0.001$). During maximal exercise in HYP, trained participants displayed a lower S_pO_2 before pre- (80.5% (74-83) vs 84.5% (76-87), $p = 0.045$) but not post-training (79% (75-84) vs 82.5% (68-87), $p = 0.105$) compared to the untrained.

2.2.3. Submaximal Exercise

Table 2.3 provides cardiorespiratory responses during the 30-min steady-state exercise in both trained and untrained groups. Post-training HR was lower in all environmental conditions and training groups ($p < 0.05$). The expected increases in HR were observed when participants from both groups were exercising in HYP and HE compared to NOR and they were persistent over time ($p < 0.01$). CO values were at least $1.5 \text{ L} \cdot \text{min}^{-1}$ lower in NOR than in HYP and HE across training groups ($p < 0.001$). However, post-hoc tests revealed this increase only became statistically significant for untrained individuals post-training in HYP ($p = 0.012$). Given the previously described decreases in HR with no parallel changes in CO, it comes as no surprise that SV was enhanced in the untrained participants post-training in HYP ($+17 \pm 10 \text{ mL}$, $p = 0.010$) whereas the same tendency was observed in NOR ($+14 \pm 9 \text{ mL}$, $p = 0.070$). Similar to SV, a main effect for time was observed on GME ($p = 0.002$). However, the *post-hoc* tests only revealed an increased GME in HYP for trained individuals ($p = 0.027$). Trained participants had a higher ventilation ($\dot{V}_E$) compared to their untrained counterparts ($p < 0.01$). A higher $\dot{V}_E$ was also observed when participants exercised in HYP ($P < 0.001$) across time. Interestingly, the

ventilatory response was attenuated post-training only in HYP (interaction effect between environmental condition and time: $p < 0.001$) for both trained ($+5.7 \pm 6.0 \text{ L} \cdot \text{min}^{-1}$, $p = 0.009$) as well as untrained ($+5.3 \pm 2.2 \text{ L} \cdot \text{min}^{-1}$, $p = 0.003$) individuals. Across fitness levels and environmental conditions, participants were rating the exercise workload as lighter post-training ($p < 0.001$). Participants perceived the exercise as harder in HYP than NOR or HE across time ($p < 0.001$). S_pO_2 was higher in NOR and HE compared to HYP across time and fitness levels ($p < 0.001$).

Table 2.3: Cardiorespiratory responses and RPE during constant-work rate cycling before and after heat acclimation for the normoxic, hypoxic and heat trials for the trained ($n = 10$) and the untrained group ($n=10$).

TRAINED	Pre-training			Post-training		
	NOR	HYP	HE	NOR	HYP	HE
HR (bpm)	$141 \pm 9^{\dagger\#}$	154 ± 12	154 ± 14	$132 \pm 12^{*\dagger\#}$	$144 \pm 10^*$	$143 \pm 8^*$
CO ($\text{L} \cdot \text{min}^{-1}$)	15.4 ± 3.3	17.8 ± 2.7	17.2 ± 2.4	15.7 ± 3.1	17.5 ± 3.0	17.3 ± 2.6
SV (mL)	110 ± 25	116 ± 18	112 ± 14	119 ± 24	122 ± 23	121 ± 17
VO ₂	2299	2373	2279	2234	2194	2298
($\text{mL} \cdot \text{min}^{-1}$)	± 297	± 258	± 222	± 260	$\pm 227^*$	± 297
GME (%)	19.1 ± 1.6	18.4 ± 1.2	19.4 ± 3.0	19.5 ± 1.6	$19.7 \pm 1.4^*$	19.1 ± 1.9
V _E ($\text{L} \cdot \text{min}^{-1}$)	55.2	72.6	56.9	53.4	66.9	57.0
¹⁾	$\pm 6.7^{\dagger}$	$\pm 7.5^{\#}$	± 7.1	$\pm 6.6^{\dagger}$	$\pm 8.6^{*\#}$	± 8.2
S _p O ₂ (%)	99	81.5	99	98.5	82	99
	(95-100)	(72-88)	(97-100)	(98-100)	(77-85)	(98-100)
RPE	13.4 ± 2.6	14.9 ± 1.9	13.4 ± 1.8	$10.8 \pm 1.6^{*\dagger}$	13.1 ± 1.7	$11.4 \pm 2.0^*$
UNTRAINED						
D						
HR (bpm)	$136 \pm 10^{\dagger\#}$	158 ± 9	157 ± 11	$123 \pm 13^{*\dagger\#}$	$144 \pm 11^*$	$144 \pm 10^*$
CO ($\text{L} \cdot \text{min}^{-1}$)	14.8 ± 1.9	16.8 ± 2.6	17.3 ± 3.0	14.6 ± 2.8	$17.8 \pm 3.4^{\dagger}$	16.3 ± 3.0
¹⁾						
SV (mL)	107 ± 15	106 ± 14	107 ± 26	120 ± 20	$123 \pm 20^*$	113 ± 28
VO ₂	1875	1973	1953	1863	1872	1915
($\text{mL} \cdot \text{min}^{-1}$)	± 244	± 263	± 278	± 269	± 269	± 305
GME (%)	19.4 ± 1.9	18.2 ± 0.9	18.0 ± 1.4	19.4 ± 1.1	19.2 ± 0.8	19.0 ± 0.8
V _E ($\text{L} \cdot \text{min}^{-1}$)	44.3	61.1	48.9	45.5	55.9	47.2
¹⁾	$\pm 5.7^{\dagger\#}$	$\pm 9.4^{\#}$	± 7.5	$\pm 6.0^{\dagger}$	$\pm 9.8^{*\#}$	± 7.1
S _p O ₂ (%)	99	84	99	99	84.5	99
	(98-100)	(76-89)	(97-100)	(97-100)	(70-89)	(98-100)
RPE	12.7 ± 2.4	14.4 ± 2.8	14.3 ± 2.9	$8.8 \pm 1.6^{*\dagger}$	$11.5 \pm 2.3^*$	$10.2 \pm 2.0^*$

Values are mean $\pm$ SD except for S_pO₂ median (range)

* Significant difference to values before heat acclimation

Significant difference to heat values

† Significant difference to hypoxic values

‡ Significant difference to trained participants, $p < 0.05$

2.2.4. Thermoregulation

An overview of the thermoregulatory variables during the 30-min submaximal exercise is shown in Table 2.4. Although there was a main effect of time ($p < 0.01$), *post-hoc* tests reported only a tendency for the resting T_{re} to be lower post-training in HE only for the trained participants (37.24 ± 0.19 vs 37.02 ± 0.23 °C, $p = 0.089$). In HE, ΔT_{re} was higher for the trained compared to the untrained group across time ($p < 0.01$) with trained individuals increasing their T_{re} ~ 0.5 °C more than the untrained. A similar tendency was observed in HYP pre-training ($p = 0.060$). While ΔT_{re} was consistently higher ($p < 0.05$) in HE compared to NOR for trained participants. ΔT_{re} was not affected by environmental condition in the untrained participants ($p > 0.05$). T_{sk} was higher ($p < 0.001$) in HE compared to NOR and HYP across time and participant groups. As exercise progressed, T_{sk} increased from baseline levels across environments ($p < 0.001$). T_{sk} remained lower in post- compared to pre-training ($p < 0.001$, ~ 0.2 °C in HE to ~ 0.4 °C in NOR and HYP).

Table 2.4: Thermoregulatory responses at rest and during the constant-work rate cycling before and pre- and post-training for the trained ($n = 10$) and untrained ($n = 10$) groups in normoxic, hypoxic and heat trials.

TRAINED	Pre-training						Post-training					
	NOR		HYP		HE		NOR		HYP		HE	
$T_{re \text{ rest}}$ (°C)	37.29 ± 0.26		37.23		37.24		37.12		37.03		37.02	
			0.18		0.19		0.29		0.19		0.23	
ΔT_{re} (°C)	$0.64 \pm 0.12^{\#†}$		0.84 ± 0.25		0.98 ± 0.33		0.66		0.69 ± 0.21		0.86 ± 0.24	
							$0.15^{\#}$					
H_{prod} (W kg ⁻¹)	8.4 ± 0.8		8.8 ± 0.7		8.3 ± 0.9		8.2 ± 0.8		$8.0 \pm 0.5^*$		8.4 ± 0.8	
T_{sk} (°C)	$33.8 \pm 0.7^{\#}$		$33.6 \pm 0.5^{\#}$		36.3 ± 0.3		$33.7 \pm 0.4^{\#}$		$33.6 \pm 0.5^{\#}$		36.1 ± 0.3	
ΔT_{f-f} (°C)	0.7 ± 2.0		0.7 ± 4.0		-0.4 ± 0.8		-0.2 ± 2.8		0.5 ± 2.9		1.2 ± 1.9	
$\dot{m}_{sw, peak}$ (g · m ⁻² · min ⁻¹)	1.16 ± 0.34		0.90 ± 0.40		1.14 ± 0.47		0.97		0.91		1.49 ± 0.33	
							$0.37^{\#}$		$0.33^{\#}$			
$\dot{m}_{sw \text{ gain}}$ (g · m ⁻² · min ⁻¹ · °C ⁻¹)	1.20 ± 0.70		1.06 ± 0.45		1.16 ± 1.10		1.16 ± 0.52		1.02 ± 0.46		1.48 ± 0.62	
H_{prod} (W m ⁻²)	326 ± 36		340 ± 30		321 ± 35		316 ± 31		$312 \pm 24^*$		325 ± 36	

Whole-body	0.60 ± 0.10 [#]	0.60	±	0.81 ± 0.14	0.63	±	0.57	±	1.01 ± 0.28
sweat rate (L)		0.15 [#]			0.11 [#]		0.15 [#]		
H _{prod} (W)	640 ± 87	666 ± 70		629 ± 78	620 ± 77		611 ± 64*		639 ± 90
Threshold T _{re} for	37.52 ± 0.24	37.41	±	37.38	±	37.28	±	37.16	±
sweating (°C)		0.16		0.16		0.29		0.21	0.18*
UNTRAINED									
T _{re rest} (°C)	37.27 ± 0.37	37.33	±	37.35	±	37.30	±	37.21	±
		0.31		0.21		0.29		0.15	0.24
ΔT _{re} (°C)	0.37 ± 0.21	0.49 ± 0.20		0.48 ± 0.23 [‡]	0.42 ± 0.20		0.46 ± 0.15		0.37 ± 0.13 [‡]
H _{prod} (W kg ⁻¹)	6.1 ± 0.6 [‡]	6.6 ± 0.6 [‡]		6.6 ± 0.8 [‡]	6.1 ± 0.7 [‡]		6.2 ± 0.6 [‡]		6.3 ± 0.5 [‡]
T _{sk} (°C)	33.4 ± 0.5 [#]	33.3 ± 0.8 [#]		36.2 ± 0.3	33.2 ± 0.6 [#]		33.5 ± 0.6 [#]		35.8 ± 0.3
ΔT _{ff} (°C)	1.2 ± 3.6	1.6 ± 3.3		0.5 ± 1.1	0.9 ± 3.3		1.9 ± 4.0		1.2 ± 4.0
m _{sw,peak} (g · m ⁻² · min ⁻¹)	1.08 ± 0.43	0.88 ± 0.19		1.20 ± 0.21	0.70	±	1.00 ± 0.35		1.28 ± 0.39
					0.25 [#]				
m _{sw} gain (g · m ⁻² · min ⁻¹ · °C ⁻¹)	2.59 ± 1.20	1.73 ± 0.77		2.32 ± 1.22	1.42 ± 0.91		1.59 ± 1.00		2.10 ± 1.46
H _{prod} (W m ⁻²)	252 ± 22 [‡]	271 ± 19 [‡]		270 ± 28	251 ± 25 [‡]		254 ± 22 [‡]		258 ± 24 [‡]
Whole-body	0.46 ± 0.12 [#]	0.46	±	0.69 ± 0.10	0.56	±	0.50	±	0.81 ± 0.13
sweat rate (L)		0.07 [#]			0.12 [#]		0.12 [#]		
H _{prod} (W)	517 ± 71	557 ± 74		549 ± 83	515 ± 76		522 ± 76		530 ± 83
Threshold T _{re} for	37.41 ± 0.24	37.45	±	37.38	±	37.46	±	37.33	±
sweating (°C)		0.31		0.17		0.18		0.13	0.25

T_{re} resting rectal temperature, ΔT_{re} difference from resting value in rectal temperature, H_{prod} metabolic heat production, T_{sk} skin temperature, ΔT_{ff} difference between forearm and fingertip skin temperatures, m_{sw} sweating rate. Values are mean ± SD

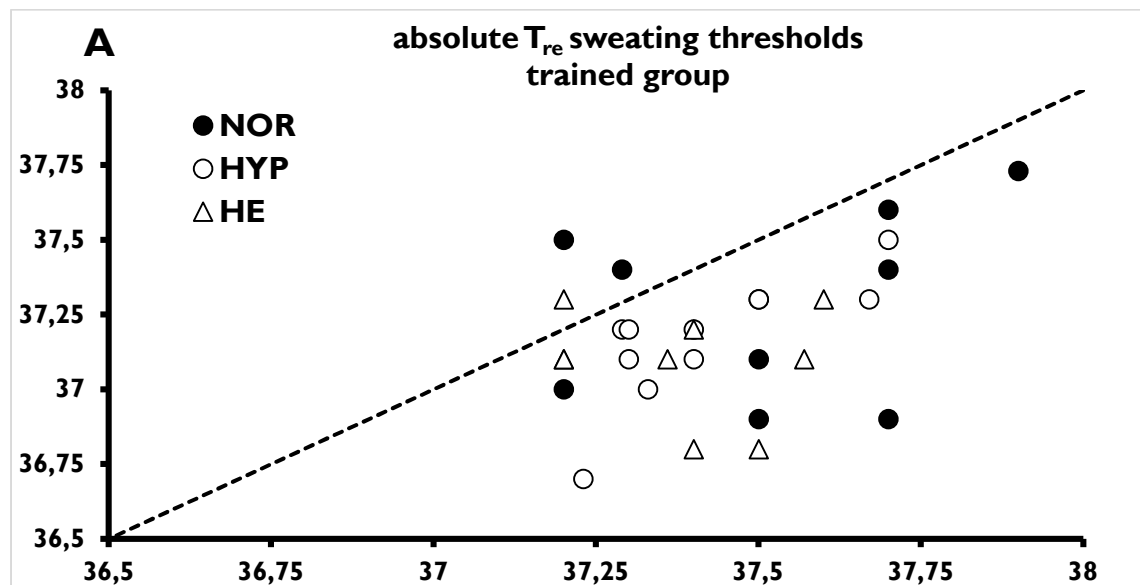
* Significant difference to pre-training values

Significant difference to heat values

† Significant difference to hypoxic values

‡ Significant difference to trained participants, $p < 0.05$

Metabolic heat production (H_{prod}) was lower post-training across groups ($p < 0.01$). Following the post-training increase in GME in trained people in HYP, H_{prod} was lower ($p < 0.01$) in that condition post-training in trained individuals. Only when normalized to body weight ($\text{W} \cdot \text{kg}^{-1}$, $p < 0.01$) or body surface area ($\text{W} \cdot \text{m}^{-2}$, $p < 0.05$), H_{prod} was consistently lower in untrained versus trained individuals across trials. The only exception was pre-training in HE where trained and untrained people produced a similar amount of metabolic heat per body surface area ($p = 0.11$). Whole-body sweat rate was higher ($p < 0.01$) in both HE trials across participants and time. Despite there being a main effect of time ($p = 0.011$), a higher sweat loss was not confirmed in HE post-training as the changes of $0.19 \pm 0.25 \text{ L}$ ($p = 0.11$) for the trained and $0.12 \pm 0.12 \text{ L}$ ($p = 0.19$) for the untrained did not reach statistical significance.



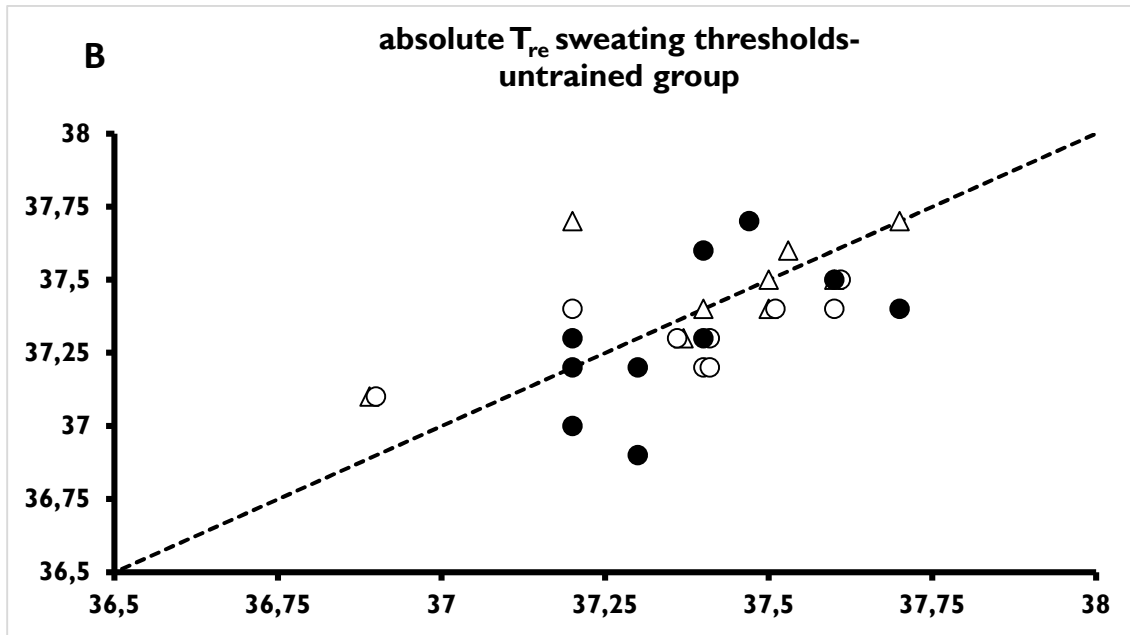


Figure 2.2: Pre- and post-training individual data for absolute T_{re} sweating thresholds in normoxia (closed circles), hypoxia (open circles), and heat (triangles) in the trained (A) and the untrained (B) individuals.

Figure 2.2 presents individual data against the line of no difference (before and after the 10-day training period) for T_{re} sweating threshold in both groups. The rectal temperature threshold for sweating (expressed in absolute values of T_{re}) decreased post-training in HE (-0.29 ± 0.26 °C, $p = 0.028$), NOR (-0.24 ± 0.32 °C, $p = 0.12$) and HYP (-0.26 ± 0.14 °C, $p = 0.08$) in the trained group. When rectal temperature sweating thresholds were expressed as relative changes from the resting T_{re} , sweating was initiated at a lower ΔT_{re} in HE in the trained group ($p < 0.01$) and that untrained participants initiated sweating at a lower T_{re} than their trained counterparts ($p = 0.043$). The gain of the sweating response was higher in the untrained group compared to the trained group ($p < 0.01$) attaining values higher than $2 \text{ mg} \cdot \text{cm}^{-2} \cdot \text{min}^{-1} \cdot \text{°C}^{-1}$ in HE for the untrained participants, whereas trained individuals demonstrated typical values ranging between 1.02 ± 0.46 and $1.48 \pm 0.62 \text{ mg} \cdot \text{cm}^{-2} \cdot \text{min}^{-1} \cdot \text{°C}^{-1}$. Finally, post-training peak sweat rate in HE was higher in both groups (1.49 ± 0.33 vs $0.97 \pm 0.37 \text{ mg} \cdot \text{cm}^{-2} \cdot \text{min}^{-1}$, $p = 0.011$ for the untrained and 1.28 ± 0.39 vs $0.70 \pm 0.25 \text{ mg} \cdot \text{cm}^{-2} \cdot \text{min}^{-1}$, $p < 0.01$ for the trained individuals) compared to NOR but higher only in the trained people compared to the same time point in HYP ($0.91 \pm 0.33 \text{ mg} \cdot \text{cm}^{-2} \cdot \text{min}^{-1}$, $p < 0.01$).

2.2.5. Hematological Results and Vascular Volumes

Table 2.5 presents the hematological responses as measured over the course of the training protocol. Hematocrit decreased below baseline values ($44.7 \pm 1.9\%$) already by day 2 in trained participants ($42.9 \pm 2.1\%$, $p = 0.030$) whereas it took longer in the untrained participants with the value on day 6 ($41.5 \pm 3.1\%$) being significantly reduced compared to baseline ($44.3 \pm 3.0\%$, $p < 0.001$). A similar pattern was observed for the hemoglobin concentration. With pre-training values as a reference, hemoglobin concentration was lower already from day 6 onwards in both trained (146 ± 8 vs $155 \pm 6 \text{ g} \cdot \text{L}^{-1}$, $p < 0.01$) and untrained (146 ± 9 vs $154 \pm 9 \text{ g} \cdot \text{L}^{-1}$, $p < 0.01$) groups. Based on the above, PV expansion reached statistical significance on day 6 in the trained ($+16.2 \pm 11.2\%$, $p < 0.001$) and

untrained individuals ($+14.5 \pm 8.7\%$, $p < 0.001$) and remained in higher levels by day 10 ($+12.2 \pm 10.8\%$, $p < 0.01$ for the untrained and $+10.7 \pm 7.2$, $p = 0.032$ for the trained). Even though the increases were of a lower magnitude, blood volume followed the kinetics of PV being elevated by day 6, both in the trained ($+5.9 \pm 4.1\%$, $p < 0.001$) and in the untrained participants ($+5.2 \pm 3.8\%$, $p < 0.001$). It remained stable from that time point until the training protocol was completed ($+5.0 \pm 4.4\%$, $p = 1.0$ for the untrained and $+4.1 \pm 2.5\%$, $p = 0.83$ for the trained). Red cell volume remained unaffected across participants despite a main effect observed for time ($p = 0.017$) with values reaching their lowest by day 6 for both groups; $-1.11 \pm 1.40\%$, ($p = 0.17$) and $-1.56 \pm 1.71\%$ ($p = 0.71$) for the trained and the untrained groups, respectively. EPO concentration increased by day 6 in the untrained people ($p = 0.009$), but had returned to baseline by day 11 ($p = 0.48$). In contrast, the trained group demonstrated a tendency for lower EPO concentration on day 11 compared to baseline values ($p = 0.074$). With the exception of day 6 ($p = 0.94$), untrained individuals had higher values of EPO concentration compared to the trained group ($p < 0.05$). The training protocol did not affect intracellular HSP70 in the trained ($p = 0.43$) or untrained individuals ($p = 0.69$). Similarly, HSP90 remained unchanged over the training days in trained ($p = 0.36$) and untrained ($p = 0.145$) individuals.

TRAINED	Pre-training	Day 2	Day 6	Post-training
Erythrocytes ($10^{12} \cdot \text{L}^{-1}$)	5.1 ± 0.3	4.9 ± 0.3	$4.7 \pm 0.4^*$	$4.8 \pm 0.3^*$
Hemoglobin ($\text{g} \cdot \text{L}^{-1}$)	155 ± 6	150 ± 6	$146 \pm 8^*$	$149 \pm 6^*$
Hematocrit (%)	44.7 ± 1.9	$42.9 \pm 2.1^*$	$41.8 \pm 2.6^*$	$42.7 \pm 1.6^*$
Plasma EPO ($\text{mIU} \cdot \text{mL}^{-1}$)	11.1 (0.02-156.1)	14.9 (0.9-140.8)	21.7 (4.2-127.4)*	4.5 (0.02-32.2)
¹⁾				
HSP72 ($\text{ng} \cdot \text{mL}^{-1}$)	0.64 (0.20-3.25)	1.19 (0.20-4.54)	1.32 (0.20-7.32)	0.56 (0.28-8.44)
HSP90 ($\text{ng} \cdot \text{mL}^{-1}$)	0.08 (0.01-1.20)	0.23 (0.02-3.59)	0.19 (0.05-0.70)	0.09 (0.02-1.28)
Plasma volume (%)	-	9.4 ± 5.7	$16.2 \pm 11.2^*$	$10.7 \pm 7.2^*$
UNTRAINED				
Erythrocytes ($10^{12} \cdot \text{L}^{-1}$)	5.1 ± 0.3	4.9 ± 0.3	$4.8 \pm 0.4^*$	$4.8 \pm 0.3^*$
Hemoglobin ($\text{g} \cdot \text{L}^{-1}$)	154 ± 11	150 ± 9	$146 \pm 10^*$	$148 \pm 7^*$
Hematocrit (%)	44.3 ± 3.0	42.8 ± 2.6	$41.5 \pm 3.1^*$	$42.1 \pm 2.5^*$
Plasma EPO ($\text{mIU} \cdot \text{mL}^{-1}$)	3.4 (0.01-22.9)#	3.1 (0.01-25.2)#	20.1 (5.0-39.3)	0.3 (0.01-23.6)*#
¹⁾				
HSP72 ($\text{ng} \cdot \text{mL}^{-1}$)	0.67 (0.20-4.12)	2.16 (0.20-3.36)	1.81 (0.20-5.57)	0.46 (0.20-6.86)
HSP90 ($\text{ng} \cdot \text{mL}^{-1}$)	0.02 (0.01-0.89)	0.32 (0.04-1.60)	0.42 (0.05-2.96)	0.08 (0.01-3.43)
Plasma volume (%)	-	7.3 ± 10.0	$14.8 \pm 8.7^*$	$12.2 \pm 10.8^*$

EPO erythropoietin, HSP72 heat shock protein 72, HSP90 heat shock protein 90. Values are mean $\pm$ SD except for plasma EPO, HSP72 AND HSP90 data which are presented as median (range).

* Significant difference to pre-training

Significant difference to trained individuals, $p < 0.05$

Table 2.5: Resting hematological variables pre-, on days 2 and 6 and post-training for the trained (n=10) and the untrained individuals (n=10).

2.3. Discussion

The main finding of the present study is that a 10-d moderate-intensity training protocol increases maximal aerobic power only in untrained individuals in thermoneutral normoxic and hot conditions indicating that a training effect was not a significant factor in participants of a high fitness level.

2.3.1. Aerobic Capacity

Exercise training has been widely documented to elicit several cardiovascular/ adaptations that contribute to improvements in aerobic performance. Whether tangible benefits can be observed after only 10 days of training remains unresolved. A training effect can be defined as any physiological adaptation that enhances aerobic performance. Given that aerobic performance is determined by $\dot{V}O_{2\max}$, exercise economy and anaerobic threshold (Bassett & Howley, 2000), a training effect should be translated to an improvement in any of these indices.

We report a net $\dot{V}O_{2\max}$ increase of +9.2% in NOR and +10.2% in HE in untrained individuals after the completion of 10 daily training sessions. Hickson, Hagberg, Ehsani, and Holloszy (1981) trained recreationally active humans and reported a higher $\dot{V}O_{2\max}$ after only 18 training sessions. In a similar fashion, Montero and Lundby (2017) demonstrated an average $\dot{V}O_{2\max}$ increase of ~150mL in untrained participants following 12 training sessions (2 training sessions per week for 6 weeks) at 65% W_{peak} , an intensity slightly higher than the one prescribed in the present study. In contrast, Daussin et al. (2007) were unable to detect $\dot{V}O_{2\max}$ increases in sedentary individuals using a traditional continuous-training protocol with the exercise intensity at 60% W_{peak} . The same participants improved their $\dot{V}O_{2\max}$ when the same amount of work was performed in interval sessions of higher intensity. The results of this study were not surprising as the continuous-training group trained as little as 20 minutes per session. Moreover, recent HeA/HyA studies that involved control groups training for 10-28 days in normoxic thermoneutral conditions failed to demonstrate any ergogenic effect (Karlsen et al., 2015; Lorenzo et al., 2010; Siebenmann et al., 2012). In light of the present results, these findings might be attributed to the different fitness level of the participants since in the former studies individuals were less fit. This possibility is further supported by the “very large” correlation between initial fitness level and training-induced $\dot{V}O_{2\max}$ gains reported in the present study.

From a mechanistic standpoint, $\dot{V}O_{2\max}$ is governed by arterial oxygen delivery and extraction. In normoxic conditions, arterial oxygen delivery is constrained by $CO_{\max}$. The blood volume expansion observed in both groups already by day 6 could theoretically increase the preload of the heart via the Frank-Starling mechanism and subsequently lead to higher SV_{peak} and $CO_{\max}$ values (Bonne, Doucende, et al., 2014). Provided that trained individuals possess a higher baseline PV, untrained individuals would be expected to benefit more from such a hematological adaptation (Coyle et al., 1990). The respective average $CO_{\max}$ increases of +2.1 and +1.4 L · min⁻¹ (+14 and +20 mL for SV_{peak}) in NOR and HE for the untrained group support this argument, especially in HE where the amount of blood redirected to the skin is enlarged (as indirectly evidenced by high T_{sk} and low $\Delta T_{\text{f-f}}$ values). In HYP, arterial oxygen content is the limiting factor for $\dot{V}O_{2\max}$. Despite a lower hemoglobin concentration post-training, $CO_{\max}$ was enhanced by +3.2 L · min⁻¹ counteracting the fall in arterial oxygen content. Along the same line, the contribution of an enhanced arteriovenous oxygen difference to $\dot{V}O_{2\max}$ gains has been emphasized over time (Ekblom, Astrand, Saltin, Stenberg, & Wallstrom, 1968; Murias, Kowalchuk, & Paterson, 2010). Our inability to evoke clear hemodynamic adaptations in the admittedly

short time frame might be explained by the low exercise intensity, since studies that succeeded in elevating $\dot{V}O_{2\max}$ (Daussin et al., 2007; Ekblom et al., 1968; Helgerud et al., 2007) elicited HR above 90% of maximal values even for short intervals. However, the aim of the present study was not to provide the participants with a training stimulus that would stimulate cardiovascular adaptations. On the contrary, we decided to reproduce the exercise intensity that is usually selected for fixed-workload heat (Lorenzo et al., 2010) or even hypoxic (Sotiridis et al., 2018, Chapter 3) -acclimation protocols. Secondly, this exercise intensity has been proposed (Gibson, Willmott, James, Hayes, & Maxwell, 2017) and selected (Sotiridis, Debevec, Ciuha, Eiken, & Mekjavic, 2019, Chapter 4) to potentiate the targeted increase in T_e during the initial 30-min stage of controlled-hyperthermia heat-acclimation protocols. Finally, given the fact that recent controlled-hyperthermia protocols (Gibson, Mee, et al., 2015; Rendell et al., 2017; Sotiridis et al., 2019, Chapter 4) have reported an average power output of $\sim 30\%$ W_{peak} , a training intensity that would provide the participants with a similar training volume/mechanical strain, but over a lower duration (60 min instead of 90 min) was preferable.

Whether HeA protocols could potentiate an increase in mechanical efficiency (Sawka et al., 1983; Sotiridis et al., 2019, Chapter 4) or not (Karlsen et al., 2015), as measured in thermoneutral conditions, is still under debate. The increases in mechanical efficiency following HeA protocols have been claimed to be due to a lower fitness level of the participants or familiarization with the experimental set-up (Nybo & Lundby, 2016). Submaximal $\dot{V}O_2$ was not altered in the untrained group rendering a potential influence of a 10-day training protocol on GME unlikely in NOR. Sawka et al. (1983) also reported an unaltered rate of metabolism after relatively trained participants underwent a 4-week physical training program at 75% $\dot{V}O_{2\max}$. Nevertheless, the rate of metabolism increased in the same participants when a 10-day HeA preceded physical training. These data further reinforce the possibility that any HeA-induced increase in GME (Sotiridis et al., 2019, Chapter 4) might be attributed to an interactive effect of the heat and exercise stimuli and could be explained by a Q_{10} effect; a lower exercise T_{re} resulted in a lower metabolic rate. In the present study, exercise T_{re} remained unchanged and consequently metabolic rate should not change either. Paradoxically, GME was enhanced in HYP only in the trained group. A higher respiratory quotient (0.90 ± 0.03 vs 0.94 ± 0.04 , data not shown) suggests that trained participants switched to a more economic fuel (CHO as opposed to fat) when exercising in HYP post-training in addition to limiting respiratory work by virtue of a blunted V_E .

Expectedly, anaerobic threshold did not change either since an exercise intensity higher than 70% $\dot{V}O_{2\max}$ seems necessary for the elevation of the (ventilatory) anaerobic threshold (Poole & Gaesser, 1985). Finally, a “large” correlation observed between baseline values and subsequent increases in W_{peak} is another indication of the differential effects of the selected training protocol in participants of a different fitness level. Given that the anaerobic threshold remained unchanged, an augmented anaerobic capacity could mediate the improvements in W_{peak} in trained individuals. A learning effect could be safely eliminated since by including a preliminary test, practice gains seem to be limited in repeated performance tests independent of the athletic status of the individuals (Hopkins, Schabort, & Hawley, 2001). Taken collectively, the so-called “training component” claimed to be present in previous acclimation protocols should be evaluated in view of $\dot{V}O_{2\max}$ rather than anaerobic threshold or even GME gains in NOR.

2.3.2. Thermoregulation

Given that almost 80% of the energy expenditure during exercise is converted into heat, it seems reasonable that training even under thermoneutral conditions could impose a

substantial thermal burden in the participants. Heat-acclimation protocols utilize a combination of endogenous heat production and environmental heat load to stimulate desired adaptations. In the present study, we attempted to delineate the effects of exercise training *per se* on thermoregulatory adaptations. Our previous studies clearly demonstrate that a hypoxia-induced higher relative exercise intensity does not alter thermoregulatory responses compared to NOR in unacclimated individuals (Sotiridis et al., 2019; Sotiridis et al., 2018, Chapters 3 and 4). Trained participants had to overcome a higher ΔT_{re} increase during submaximal exercise. This is not surprising given that ΔT_{re} is determined by H_{prod} divided by body mass (Jay et al., 2011). H_{prod} expressed in $W \cdot kg^{-1}$ was consistently higher in trained individuals since they were exercising at higher absolute workloads and they were of a lower body mass. With regards to the sweating response, sweating was initiated at a lower T_c post-training only in the trained participants. Paradoxically, no such change was observed for the untrained participants who, in contrast to the trained individuals, had a higher rate of sweat secretion for any given ΔT_{re} . These findings seem in sharp contrast with the long-held tenet that humans demonstrate a decreased threshold temperature for the onset of sweating (Roberts et al., 1977) as well as an increased gain of the sweating response (Nadel et al., 1974) after participating in a training protocol. Evaporative heat loss has recently been shown to be superior in trained individuals only when a certain threshold of net heat load is exceeded (Lamarche, Notley, Poirier, & Kenny, 2018). Going one step further, it could be speculated that trained participants were training at an intensity sufficiently high to stimulate evaporative heat loss in each session whereas untrained participants did not reach that threshold. In view of a low ΔT_{re} , untrained participants might have not been sufficiently thermally challenged (M.J. Tipton, 2019). Indeed, a similar effect has been observed in the adaptive response to HeA whereby participants that were exercising at a lower H_{prod} displayed a lower reduction in T_{re} during a heat-stress test (Corbett, Rendell, Massey, Costello, & Tipton, 2018). Unfortunately, measures of whole body sweat loss were not obtained during the training sessions. Even a daily exercise-induced increase of T_{re} to 38 °C does not seem capable of potentiating the forehead sweating response unless T_{sk} is simultaneously markedly increased, i.e. participants training in hot conditions (Regan et al., 1996). Moreover, the higher gain of the sweating response in the untrained group may have come as a result of the analysis used; untrained people demonstrated a lower ΔT_{re} which by definition magnifies the gain of the sweating response. As a result, caution should be taken when the classic analysis of the components of the sweating response (threshold, sensitivity, peak value) is undertaken between groups when differences in the response are relative to ΔT_{re} . Jay et al. (2011) have elegantly demonstrated that the gain of the sweating response is not as sensitive an index to distinguish between trained and untrained participants exercising at metabolic heat productions differing as much as $\sim 120 W \cdot m^{-2}$ which is double our respective between-groups difference in NOR (Table 2.3). Finally, the higher values of whole-body sweat rate and peak sweat rate in HE across groups reflect the increased T_{sk} in that environmental condition. It has been elegantly shown that for a given T_c , local thermosensitivity is augmented when T_{sk} increases in 1°C increments (Nadel, Bullard, & Stolwijk, 1971). Taken together, a rather high net thermal load – accomplished by increasing either exercise workload or ambient heat – is a prerequisite for systemic thermoregulatory adaptations to occur after such short-term protocols as reflected in the trained participants.

2.3.3. Hematology

A limitation of the present study is that blood volumes were estimated from the changes in hemoglobin concentration and hematocrit. Red cell volume expansion has been claimed to partly account for blood volume expansion following the first ~8-10 training sessions

(Sawka et al., 2000). As a result, we are quite confident that the reported PV expansion by day 6 constitutes a factual phenotypic adaptation mediated by increases in plasma renin activity and antidiuretic hormone at such an early stage (Convertino, Brock, Keil, Bernauer, & Greenleaf, 1980) and was not overestimated by any erythropoietic effect. The finding that only untrained individuals demonstrated a higher EPO response by day 6 might be attributed to the low degree of adaptation to exercise training established by that timepoint and further suggests that the EPO response is potentiated in the untrained state versus trained state when humans are subjected to a similar exercise stimulus in terms of duration and relative intensity. Alternatively, an increased EPO response may serve to counteract the concurrent decrease in hematocrit levels which was stimulated by PV expansion (Montero et al., 2017). This possibility, however, is not supported by the fact that the trained individuals expanded their PV, with EPO remaining unaffected and our inability to correlate changes in hematocrit with EPO (data not shown). Intracellular measures of heat stress (HSP70 and 90) remained unaffected by the applied training protocol. During submaximal exercise in normoxic conditions, T_{re} plateaued in all but one untrained individuals below 38 °C both pre- and post-training. Thus, it can be assumed that T_{re} did not exceed 38.5 °C during the 60-min training sessions. McClung et al. (2008) have reported increases in the baseline levels of intracellular HSPs following a successful 10-day HeA protocol comprising daily exercise (treadmill walking) in a hot environment (T_a 49°C, 20% RH). Following training/HeA protocols, the increased expression of HSPs is preceded by an augmented HSP mRNA transcription tightly linked to increases in T_c (Gibson, Tuttle, Watt, Maxwell, & Taylor, 2016). Consequently, the lack of changes in the systemic thermoregulatory responses that is also reflected at the baseline levels of HSPs further implicates an insufficient heat strain for the untrained group (see also *Thermoregulation*). The marginal thermoregulatory adaptations observed in the trained cohort (reduced sweating threshold and resting T_{re} , higher peak and whole-body sweat rate in HE) were not accompanied by respective increases in HSPs. Despite higher T_{re} attained during the training sessions compared to the untrained group, the magnitude of the increases in T_{re} may not have been sufficient to upregulate HSP gene expression. Alternatively, a large proportion of the signalling for gene transcription is unaccounted for by T_c alone. Heat shock factor, the major regulator of the heat shock gene expression, could be activated by increased metabolic stress. Nevertheless, the cardiovascular strain was similar between groups during exercise sessions. To further add to the complexity, differences in the HSP mRNA expression between trained and untrained individuals have been dissociated from the respective between-groups differences in intracellular HSPs (Shin et al., 2004). Further research is needed to explore whether the exercise training-associated thermal stress is capable of inducing adaptations at a cellular level.

Chapter 3

Exercise Cardiorespiratory and Thermoregulatory Responses in Normoxic, Hypoxic, and Hot Environment Following 10-Day Continuous Hypoxic Exposure

Many outdoor activities expose humans to a variety of environmental stressors that are usually concomitant with an elevated activity-related metabolic stress (i.e. a specific exercise modality). The effect of environmental stressors on physiological systems has traditionally been studied under laboratory conditions, whereby the effect of an environmental stressor is examined independently, or in combination with an additional stressor. The latter approach has been used to establish the manner in which different stressors interact (i.e. synergistic, additive, etc.). Such investigations, though fundamental to our understanding of the action of environmental factors on the regulation within physiological systems, do not account for the process of acclimation that occurs during prolonged exposure to one or more environmental stressors, since in “real-life” scenarios they are seldom experienced in isolation (M.J. Tipton, 2012). This has initiated a new avenue of research, whereby the effects of acclimation to one environmental stressor are also examined during subsequent exposure to other stressors. It has long been suggested that acclimation to heat can induce protective responses to other stressors such as cold (Hessemer et al., 1986) or ischemia (Levi et al., 1993). This phenomenon is termed cross-acclimation when physiological strain is attenuated and mostly underlined by the activation of common molecular pathways. It is of note however, that cross-acclimation may not always be beneficial, and can result in either positive or negative effects (A. C. White et al., 2016). For example, during exercise in the heat, hypoxia-induced angiogenesis would benefit maximal skin blood flow, whereas the concomitant PV contraction could threaten cardiovascular stability.

The activation of common pathways for heat and hypoxia has only recently received limited attention. Nevertheless, two of the key contributors to both heat and hypoxia acclimation responses are heat shock proteins (HSPs) and hypoxia-inducible factors (i.e. HIF-1). Both promote molecular responses during acclimation and contribute to the systemic physiological response (Ely, Lovering, Horowitz, & Minson, 2014). Hypoxia-related HSPs and HIF-1 activation initiates a cascade of molecular adaptations that might

provide benefits in hot conditions and counteract the acclimation-induced PV contraction. In particular, HIF-1 activation leads to increased EPO production, increased sensitivity of the carotid chemoreceptors to oxygen, enhanced inducible nitric oxide synthase (iNOS) and glucose transporters-1 (GLUT1) activity and angiogenesis (Ely et al., 2014). On the other hand, HSP90 enhances PV expansion (Nielsen et al., 1993) and stimulates endothelial nitric oxide synthase (eNOS) activity. All the above noted molecular responses could improve convective oxygen transport and consequently enhance exercise performance in the heat and/or hypoxia.

The scientific evidence regarding the effect of hypoxic exposure and/or training on normoxic/hypoxic performance remains controversial. Some strategies of HyA offer benefits in terms of normoxic and hypoxic performance (Katayama, Matsuo, Ishida, Mori, & Miyamura, 2003; Levine & Stray-Gundersen, 1997), while others do not (Debevec et al., 2010; Morton & Cable, 2005). HyA *per se*, without concomitant exercise training, does not seem to alter normoxic or hypoxic $\dot{V}O_{2\max}$ and respective W_{peak} (Keramidas, Kolegard, et al., 2016; Kounalakis et al., 2013). Despite increases in both hemoglobin concentration and S_pO_2 , hypoxic $\dot{V}O_{2\max}$ remains lower than normoxic, both acutely and after a lengthy hypoxic stimulus mainly as a result of lower CO (Calbet et al., 2002). Nonetheless, physical activity is another factor that could promote acclimation to hypoxia. Residing in hypoxia combined with training under normoxic conditions (e.g. sleep high-train low protocols (Levine & Stray-Gundersen, 1997)) could result in enhanced convective oxygen transport (Bailey & Davies, 1997) as well as augment non-hematological factors (i.e. mitochondrial efficiency and muscle buffering capacity) (Gore, Clark, & Saunders, 2007), thus leading to enhanced aerobic performance.

During exercise, particularly in warm conditions, the efficiency of the thermoregulatory system to dissipate heat is fundamental for optimal performance. Adaptations of the autonomic thermoregulatory responses for heat loss are initiated by regular increases in body temperature, either due to physical exercise, and/or exposure to heat. Thus, an aerobically trained athlete already possesses a certain degree of heat acclimation, essential in preventing/postponing excessive elevations in T_c that may hinder performance (Cadarette, Sawka, Toner, & Pandolf, 1984). The efficiency of exercise temperature regulation, specifically heat loss responses, relate to the initiation of sweating (T_c thresholds), the responsiveness of the sweat glands to changes in mean body temperature (the magnitude of the sweating response to a change in T_c ; the gain of the response) and the overall capacity of these glands to secrete sweat (N. A. Taylor, 2014). Hypovolemia has been shown to impair conductive and evaporative heat loss (Fortney et al., 1981). In the event that exercise is performed in a hypoxic environment, one needs to consider hypoxia as a non-thermal factor that can modify heat loss responses to thermal factors (i.e. body temperature). We have previously demonstrated (Kacin et al., 2007; Kounalakis et al., 2012) a hypoxia-induced potentiation of the forehead sweating response during exercise. The exercise sweating response under normoxia was enhanced after a 4-wk “sleep high-train low” regimen. In this case, the combined metabolic and hypoxic adaptation resulted in a synergistic response: augmented heat loss and decreased relative workload.

Surprisingly, no study to date investigated the effect of continuous HyA on exercise performance and thermoregulatory responses in the heat.

3.1. Methods

3.1.1. Participants

Twelve healthy male participants with a mean $\pm$ SD age of 23.8 ± 2.0 yrs and stature of 182.5 ± 6.9 cm were recruited for the present study. During the selection procedure participants completed a panel interview, and underwent a test of maximal aerobic capacity to establish their normoxic $\dot{V}O_{2\max}$, and normoxic W_{peak} . For the participants that were included in the study, their normoxic W_{peak} was used for setting the steady-state workload for the pre- and post-acclimation tests (40% of the W_{peak} attained during the preliminary test).

All participants were near-sea level residents, non-smokers and had not been exposed to altitudes $>1500\text{m}$ during the month preceding the study. None of them had a history of any cardiorespiratory or hematological disease. They were instructed to avoid any caffeine and alcohol containing beverages throughout the study protocol. Prior to inclusion in the study, the participants provided a written informed-consent and were extensively informed regarding the study protocol as well as the possible risks involved.

3.1.2. Study Design

As shown in Figure 3.1, the study comprised three parts: a) Pre-acclimation tests: Prior to the 10-d HyA, the participants conducted three exercise tests on separate days. b) HyA: Participants were confined to a hypoxic facility for 10 days (240 hrs). They conducted 60 min of cycle ergometry at a HR corresponding to 50% of hypoxic W_{peak} each day. c) Post-acclimation tests: All three exercise tests conducted prior to the onset of acclimation (pre-acclimation tests) were repeated upon completion of the 10-d acclimation period. For all participants, the first pre-acclimation test was always the normoxic test. Post-acclimation, 8 out of 12 first trials were hypoxic. The order of the remaining trials was counterbalanced.

Figure 3.1: Outline of the experimental protocol.

3.1.3. Hypoxic Acclimation

The HyA protocol was performed in the hypoxic facility of the Planica Nordic Centre (Slovenia) situated at an altitude of 940m. All pre- and post- acclimation exercise tests were performed during the three days before (pre-) and three days after (post-) the period of HyA, respectively, in a laboratory situated at 300 m above sea level (Ljubljana, Slovenia).

Each participant was confined to the hypoxic area of the facility for exactly 10 days. They lived continuously (24 hours per day) at a simulated altitude of approximately 4000 m which was achieved by maintaining the fraction of inspired oxygen (F_{iO_2}) at $13.65 \pm 0.35\%$ in the facility situated at 940 m, resulting in P_{iO_2} of 87 ± 3 mmHg. Hypoxia within the facility was achieved using an oxygen dilution system (B-cat, Tiel, the Netherlands), based on the Vacuum-Pressure Swing Adsorption principle. The oxygen levels in each room were monitored and recorded continuously using oxygen sensors (PGM-1100, Rae Systems, San Jose, California, USA).

The average values for T_a , RH and atmospheric pressure in the hypoxic facility were 24.5 ± 0.7 °C, 49 ± 6 %, and 687 ± 6 mmHg, respectively. Resting values of S_pO_2 , HR, arterial pressure, tympanic temperature as well as Lake Louise Mountain Sickness Scores were recorded each morning. The participants performed one 60-min exercise training session per day. Exercise training was conducted on mountain bikes mounted on resistance trainers (Elite Cycling, Italy). The exercise intensity was set according to each participant's exercise HR, measured with a HR monitor (Wristox 3100 Nonin, Plymouth, Massachusetts,

PRE			Hypoxic Acclimation										POST		
D-3	D-2	D-1	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D+1	D+2	D+3
·3 SSTs ·3 $\dot{V}O_{2max}$ tests: 1.normoxia 2.hypoxia 3.heat			·240 hours, $F_iO_2 = 13.65 \pm 0.35 \%$ ·Daily exercise Training for 1h at 50% hypoxic W_{peak}										·3 SSTs ·3 $\dot{V}O_{2max}$ tests: 1.normoxia 2.hypoxia 3.heat		
SST : Steady State Test that comprises 30min cycling in normoxic 40% W_{peak}															

USA). S_pO_2 and RPE were recorded at 10-min intervals. During the daily exercise regimen, exercise intensity was regulated so that participants' exercise HR corresponded to that observed at 50% of hypoxic W_{peak} measured in the pre-acclimation test. Participants had

ad libitum access to food and water and had the same daily menu throughout their sojourn at the Planica Nordic Centre. The individuals' nutritional intake was self-recorded and analyzed using the Open Platform for Clinical Nutrition software (www.opkp.si). Participants were accommodated in double bedrooms for the duration of the study, and were able to socially interact with each other throughout.

During their 10-d sojourn at the Planica Nordic Centre participants followed a daily schedule of lights-out at 2300 hrs, and waking up at 0700 hrs. Upon waking, morning measurements of HR and S_pO_2 were obtained using finger pulse oxymeter (Wristox 3100, Nonin, Plymouth, Massachusetts, USA), and arterial pressure using an automatic sphygmomanometer (Withings, Cambridge, USA) with the participants in a supine position. To assess the presence of altitude sickness, participants completed the Lake Louise Mountain Sickness Questionnaire on a daily basis. Body composition was derived on day 1 and day 10 of HyA using Dual X-ray absorptiometry (DEXA, Hologic Discovery QDR, Hologic Inc., Bedford, Massachusetts, USA).

3.1.4. Blood Sampling and Biochemical Analysis

Blood samples were obtained from an antecubital vein of the participants on the first day of exercise testing both before and after HyA (see Figure 3.1, D-3 and D+1, respectively). The participants fasted overnight prior to the procedure and rested in a seated position for 10 min prior to sampling. Samples were analyzed for hematocrit, hemoglobin, ferritin and reticulocyte concentration and erythrocyte count using an automated laser-based hematology analyzer ADVIA 120 (Siemens, München, Germany). Additional blood samples were also immediately stored in BD Vacutainers (K2E, Becton–Dickinson, New Jersey, USA) and centrifuged (10 min at 3,000 rpm). The obtained plasma was frozen to $-80\text{ }^{\circ}\text{C}$ and stored for further analysis. EPO, HIF-1 α , HSP72 and HSP90 concentrations were determined using sandwich enzyme-linked immunoassay. Optical density was quantified on a microplate reader Biotek ELX 808 (Biotek Instruments, Winooski, VT, USA) set at 450 nm and corrected at 630 nm. The renin and aldosterone concentrations were determined in 50 μl of plasma using a solid phase enzyme-linked immunosorbent assay (ELISA) based on the competition principle (IVD, Aldosterone/Renin ELISA, IBL, Germany). The quantification of the optical density was performed on a microplate reader BioTek (BioTek instruments, Elx808TM) set at 450 nm. All techniques were performed in accordance with the protocol provided by the manufacturer. Samples and standards were assayed in duplicate, except for HSP72, whereas two microplates were used for each participant's samples in order to minimize any effects of variability between the plates. The estimated coefficients of variation for the analysis were 5.5, 5.0, 5.2, 9.4 and 13.8% for HIF-1 α , HSP90, renin, aldosterone and EPO, respectively. The sensitivity of the measurements was 0.156 ng.mL^{-1} , 0.09 ng.mL^{-1} , 0.05 ng.mL^{-1} , 5.7 pg.mL^{-1} , 0.81 pg.mL^{-1} and 1.1 mIU.mL^{-1} for HIF-1 α , HSP72, HSP90, renin, aldosterone and EPO, respectively. Blood and PV changes were determined according to the recommendations of Dill and Costill (1974).

3.1.5. Exercise Testing

All exercise trials were conducted on a cycle ergometer (Daum, Electronic, Furth, Germany) and consisted of two stages; a 30-min steady-state exercise followed by an incremental exercise to exhaustion. During exercise, the participants pedaled at a preferred cadence (between 60 and 90 rpm), which they maintained via visual and verbal feedback throughout the trial. Before (pre) and after (post) the 10-d HyA, the participants conducted three trials on consecutive days. In one condition (NOR), the participants

breathed room air ($P_{iO_2} = 143 \pm 1$ mmHg) and exercised in thermoneutral conditions ($T_a = 22.7 \pm 0.8$ °C and $RH = 48 \pm 2$ %). In the second condition (HYP), they breathed a hypoxic gas mixture ($P_{iO_2} = 92 \pm 3$ mmHg) and exercised in thermoneutral conditions ($T_a = 22.5 \pm 0.3$ °C and $RH = 48 \pm 1$ %). In the third condition (HE), they inspired room air ($P_{iO_2} = 142 \pm 2$ mmHg), but exercised in a hot environment ($T_a = 35.9 \pm 1.0$ °C and $RH = 50 \pm 2$ %) (HE). Participants' $\dot{V}O_{2max}$ and W_{peak} were determined during the preliminary inclusion/exclusion tests.

A metabolic cart (Quark CPET, Cosmed, Rome, Italy) was used to obtain breath-by-breath respiratory responses during the trials. Before each trial, the oxygen and CO_2 analyzers were calibrated using two different gas mixtures, and the pneumotachograph was calibrated with a 3-L syringe, in accordance with the manufacturer's recommendation.

HR and S_pO_2 were monitored using a telemetry system (Polar S810i, Kempele, Finland) and fingertip oxymeter (Nellcor, BCI 3301, Boulder, USA), respectively. Participants were instructed to relax their hands during S_pO_2 measurement. RPE were obtained using a Borg scale (Borg, 1970). During the exercise training trials, all participants wore shorts and athletic shoes. Their naked body mass was measured before and after each exercise test, and training sessions on a weighing scale (0.05 kg) (TPT 5N, Libela ELSI, Celje, Slovenia).

T_{re} was measured with a thermistor probe (MSR 12, Henggart, Switzerland) inserted 10 cm beyond the anal sphincter. Skin temperatures were measured at four sites (chest, arm, thigh and calf) using skin thermistors (MSR 12, Henggart, Switzerland) attached to the skin with a single piece of adhesive tape (Tegaderm, 3M, Saint Paul MS, USA). The assessment of calf, thigh, chest, and arm skin temperature enabled the calculation of T_{sk} (Ramanathan, 1964). The ventral fingertip and the forearm temperature were also measured. ΔT_{ff} is considered a valid index of cutaneous vasomotor tone even during steady-state exercise (Keramidas et al., 2013), whereby values of $\Delta T_{ff} > 2$ °C indicate vasoconstriction (House & Tipton, 2002). Minute mass flow of secreted sweat ($\dot{m}_{sw}$) was measured with a ventilated capsule placed on the forehead. Forced evaporation of sweat under the capsule (surface area 4.8 cm²) was achieved by a constant flow of air (0.9 L · min⁻¹) through the capsule. Minute mass flow of secreted sweat was estimated each minute from the difference between the temperature and the humidity of inflowing and outflowing air. Air temperature and RH were measured with thermistors and humidity sensors (MSR 12, Henggart, Switzerland).

3.1.6. Steady State and Incremental Exercise to Exhaustion

As mentioned, all exercise tests commenced with a steady-state exercise, followed by an incremental-load exercise to exhaustion. Following a 2-min rest period, the participants commenced with a 2-min warm up (90 W). Thereafter, they continued cycling for 30-min at a steady-state work rate equivalent to 40% of their normoxic W_{peak} attained in the preliminary test. This workload was chosen on the basis of our previous experience that most participants are unable to complete 30 minutes of cycle ergometry at higher relative workloads in HYP (Kacin et al., 2007; Kounalakis et al., 2012). For each participant, the absolute workload was the same in all three exercise trials, so as to avoid differences in exercise-induced heat production. Immediately following the 30-min steady-state exercise protocol, participants performed an incremental-load exercise to exhaustion. It commenced with a work rate of 100 W for 2 min, thereafter increasing by 20 W every minute until volitional exhaustion (inability to maintain the cycling cadence above 60 rpm). During each trial, HR, S_pO_2 and sweat rate data were recorded at minute-intervals. RPE values were recorded at 5-min and 2-min intervals during the steady state and the incremental-load exercise, respectively. Attainment of $\dot{V}O_{2max}$, defined as the highest $\dot{V}O_2$ averaged over 60s, was confirmed when the participants met at least three of the following criteria, listed

in priority order: (a) exhaustion leading to inability to maintain exercise at a given work rate (cycling cadence lower than 60 rpm), (b) a plateau in $\dot{V}O_2$, (c) a subjective RPE near maximal (Borg scale rating greater than 17) and (d) respiratory quotient greater than 1.10.

W_{peak} was calculated according to the following equation:

$W_{\text{peak}} = \text{work rate of last stage completed} + [(\text{work rate increment})^*(\text{time into current stage/duration of the stage in seconds})]$

3.1.7. Data Analysis

Statistical analyses were performed using Statistica 5.0 (StatSoft, Tulsa, OK). Cardiorespiratory and temperature data collected during steady-state exercise were averaged in 10-min segments and analyzed for the last ten minutes of the steady-state exercise, unless stated otherwise. A two-way analysis of variance (NOR-HYP-HE, pre-post) with repeated measures was used to define the effect of acclimation on exercise responses in each environmental condition. A Tukey post-hoc test was employed to assess specific differences when a main effect had been observed. In the event that the statistical analysis revealed a non-normal distribution of the data, nonparametric statistic tests were employed. Such was the case for hematological variables (specifically, HSP72 and EPO), which were analyzed using the Wilcoxon non-parametric test. RPE and ΔT_{re} were analyzed using the Friedman non-parametric test. The sweat rate and temperature data were analyzed only for the steady-state exercise. Least square linear regression was used to determine the mean T_{re} threshold for initiation of sweating, as well as the mean slope relating sweating to T_{re} . The reported values are averages of the individual thresholds and slopes derived from linear regression parameters. Data are presented as mean $\pm$ SD unless indicated otherwise. The alpha level of significance was set *a priori* at 0.05.

3.2. Results

3.2.1. Hypoxic Acclimation

All twelve participants successfully completed the HyA protocol, as well as all of the pre- and post-acclimation trials. During the course of HyA, the resting mean S_pO_2 increased (day 1: 86 ± 5 , day 10: 89 ± 2 , $p = 0.03$) whereas the resting HR decreased (day 1: 75 ± 12 , day 10: 60 ± 13 , $p < 0.001$). The Lake Louise Mountain Sickness Scores indicated that all but one participant initially showed symptoms of acute mountain sickness (Lake Louise Score ≥ 3) on the first day of acclimation to the hypoxic environment. However, the symptoms were alleviated by the second day (the median score was 4.5 and 1.5 for the first and the second day of HyA, respectively).

3.2.2. Body Composition and Energy Intake

While whole body mass was reduced ($P = 0.02$) following HyA (pre: 77.5 ± 9.0 kg vs post: 76.6 ± 8.7 kg), the fat mass was increased (pre: 12.7 ± 2.4 kg vs post: 13.2 ± 2.3 kg, $p = 0.01$). There was a decrease ($p < 0.001$) in fat-free mass (1.4 ± 1.0 kg) and an increase ($p < 0.001$) in the body fat percentage during the acclimation period (pre: 16.4 ± 1.9 % vs post: 17.2 ± 1.8 %). The energy intake did not change during the acclimation protocol ranging from 2482 ± 721 (day 3) to 3181 ± 613 kcal (day 9) (Table 3.1). Carbohydrate intake, expressed as daily intake percentage, remained unaltered ($\sim 45\%$) being the lowest on day 2 (43%) and the highest on day 7 (48%).

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Energy										
Intake	2704 $\pm$ 98	2575 $\pm$ 37	2482 $\pm$ 72	2935 $\pm$ 78	2784 $\pm$ 83	2760 $\pm$ 41	3010 $\pm$ 62	3039 $\pm$ 57	3181 $\pm$ 61	2494 $\pm$ 61
(kcal $\cdot$ day $^{-1}$)	3	7	1	3	2	0	3	0	3	7
Fluid										
Intake	2286 $\pm$ 97	2305 $\pm$ 93	2212 $\pm$ 73	2371 $\pm$ 73	2279 $\pm$ 91	2519 $\pm$ 87	2244 $\pm$ 82	2651 $\pm$ 90	2332 $\pm$ 72	1873 $\pm$ 58
(mL $\cdot$ day $^{-1}$)	5	7	0	0	5	1	5	2	8	6
Carbohydrate (g $\cdot$ day $^{-1}$)	305 $\pm$ 128	271 $\pm$ 53	286 $\pm$ 115	353 $\pm$ 160	305 $\pm$ 83	326 $\pm$ 85	360 $\pm$ 78	325 $\pm$ 71	372 $\pm$ 127	274 $\pm$ 104
Fat (g $\cdot$ day $^{-1}$)	100 $\pm$ 39	107 $\pm$ 29	93 $\pm$ 23	106 $\pm$ 30	109 $\pm$ 104	104 $\pm$ 22	111 $\pm$ 32	125 $\pm$ 40	117 $\pm$ 19	96 $\pm$ 21
Protein (g $\cdot$ day $^{-1}$)	142 $\pm$ 63	131 $\pm$ 33	122 $\pm$ 41	138 $\pm$ 41	143 $\pm$ 53	130 $\pm$ 34	139 $\pm$ 30	150 $\pm$ 51	156 $\pm$ 37	130 $\pm$ 24

Values are mean $\pm$ SD.

Table 0.1: Average daily (acclimation days 1 to 10) energy (kcal $\cdot$ day $^{-1}$), fluid (mL $\cdot$ day $^{-1}$) and macronutrient (g $\cdot$ day $^{-1}$) intakes during the HyA protocol

3.2.3. Steady-State Exercise

3.2.3.1. Cardiorespiratory Responses and Rating of Perceived Exertion (RPE)

At rest, none of the measured cardiorespiratory variables (except for S_pO_2) showed any inter-trial differences, neither pre- nor post-acclimation. The following results section will therefore focus on inter-trial comparisons and comparisons of the values between pre- and post-acclimation. HR decreased from pre- to post-acclimation in both NOR (-11 ± 7 bpm) ($p = 0.02$) and HYP (-13 ± 7 bpm) ($p = 0.01$), whereas it remained unchanged in HE ($p = 1.0$). Independent of the acclimation state, HR was lower in NOR compared to both HYP ($p < 0.001$) and HE (pre: $p = 0.01$, post: $p < 0.001$). VO_2 decreased by 9.7% ($p < 0.001$) from pre- to post-acclimation in HYP. Pre-acclimation VO_2 in HE was lower than both in NOR ($p = 0.05$) and HYP ($p = 0.01$) (Table 3.2). VO_2 did not change ($p = 0.3$) from pre- to post-acclimation in NOR. Higher V_E was noted in HE ($\sim 15\%$) (pre: $p = 0.002$, post: $p < 0.001$) and HYP ($\sim 26\%$) ($p < 0.001$) compared to NOR, with the value in HYP being greater than in HE both pre- and post-acclimation ($p < 0.001$) (Table 3.2). Pre- to post-acclimation, V_E was elevated ($p < 0.001$) only in HE, with a tendency, albeit not significant, of an increase in the HYP ($p = 0.07$). The S_pO_2 levels were consistently lower ($p < 0.001$) in HYP compared to both NOR and HE, but were elevated ($p < 0.001$) after HyA in HYP by $8 \pm 2\%$ (Table 3.2). $P_{ET}CO_2$ values decreased ($p < 0.001$) following the protocol in all trials (Table 3.2). $P_{ET}CO_2$ was consistently higher ($p < 0.001$) in NOR compared to HYP and HE. RPE values were lower after the acclimation protocol in all conditions ($p = 0.01$). Exercise was perceived as easier ($p = 0.01$) post-acclimation in NOR compared to HYP and HE (Table 3.2).

Table 3.2: Cardiorespiratory responses and RPE in the last ten minutes of the constant-work rate cycling before and after HyA for the normoxic, the hypoxic and the trial in the heat. (n = 12).

3.2.3.2. Thermoregulatory Responses

HyA provoked a main effect on T_{re} both at rest ($p = 0.01$) and during exercise ($p = 0.01$). However, no post-hoc differences were noted.

T_{sk} was higher ($p < 0.001$) in HE than in HYP and NOR independent of the acclimation (Table 3.3). Moreover, pre-acclimation NOR T_{sk} was higher ($p = 0.01$) than pre-acclimation HYP T_{sk} and also than post-acclimation NOR T_{sk} (33.5 ± 0.7 pre vs 32.6 ± 0.8 post NOR, $p < 0.001$) (Table 3.3).

Exercise $\dot{m}_{sw}$ increased in all six trials ($p < 0.001$) and reached asymptotic values ~15-20 min after the initiation of exercise (Figure 3.2). Exercise $\dot{m}_{sw}$ was significantly elevated in HE compared to NOR ($p < 0.003$) and HYP ($p = 0.01$) only after the acclimation

	Pre-HyA			Post-HyA		
	NOR	HYP	HE	NOR	HYP	HE
HR (bpm)	136 $\pm$ 13	[†] 154 $\pm$ 13	[†] 148 $\pm$ 15	[*] 125 $\pm$ 9	[*] [†] 141 $\pm$ 11	[†] 148 $\pm$ 14
VO ₂ (mL $\cdot$ min ⁻¹)	2175 $\pm$ 194	2208 $\pm$ 262	^{†‡} 2065 $\pm$ 207	2101 $\pm$ 189	[*] 2013 $\pm$ 208	2101 $\pm$ 204
P _{ET} CO ₂ (mmHg)	43.3 $\pm$ 2.3	[†] 38.1 $\pm$ 3.5	[†] 37.5 $\pm$ 2.5	[*] 39.5 $\pm$ 1.0	[*] [†] 33.3 $\pm$ 2.4	[*] [†] 33.5 $\pm$ 1.8
V _E (L $\cdot$ min ⁻¹)	49.8 $\pm$ 5.7	[†] 62.8 $\pm$ 8.8	^{†‡} 54.6 $\pm$ 7.5	52.9 $\pm$ 5.8	[†] 66 $\pm$ 6.9	[*] ^{†‡} 60.4 $\pm$ 7.9
S _p O ₂ (%)	[†] 95.0 $\pm$ 1.3	76.8 $\pm$ 4.8	[†] 94.6 $\pm$ 1.0	95.8 $\pm$ 0.8	[*] [†] 84.8 $\pm$ 3.7	[†] 95.6 $\pm$ 0.8
% of W _{peak}	41.9 $\pm$ 1.6	[†] 49.4 $\pm$ 3.2	^{†‡} 44.5 $\pm$ 2.9	40.7 $\pm$ 1.4	[†] 47.6 $\pm$ 2.7	^{†‡} 44.9 $\pm$ 3.0
RPE	12(9-15)	13(11-15)	12(10-15)	[*] 11(7-12)	[*] [†] 12(10-14)	[*] [†] 11(8-13)

Values are mean $\pm$ SD except for RPE median (range).

* Significant difference to values before HyA

† Significant difference to normoxic values

‡ Significant difference to hypoxic values, $p < 0.05$

protocol reaching values of ~12 g m⁻² min⁻¹ (Figure 3.2B). In all given exercise conditions, absolute sweating thresholds remained unchanged (Table 3.3). It is of note that the post-acclimation sweating thresholds tended to be lower (by 0.2, 0.1 and 0.1 °C in NOR, HYP and HE, respectively), albeit the differences were not significant ($p > 0.8$). No significant differences ($p > 0.3$) were found in the gain of the sweating response independent of the acclimation or the environmental conditions. Values ranged from 19.3 ± 8.2 (pre-acclimation HYP) to 27.1 ± 10.3 g $\cdot$ m⁻² $\cdot$ min⁻¹ $\cdot$ °C⁻¹ (pre-acclimation NOR) (Table 3.3).

The pre-acclimation NOR $\Delta T_{\text{f-f}}$ was greater than in HE ($p = 0.02$). $\Delta T_{\text{f-f}}$ was lower after HyA in HYP ($p = 0.04$) and tended to be lower in NOR ($p = 0.07$) (Table 3.3 and Figure 3.3). Both pre- and post-acclimation, the difference in body mass was higher in HE than in HYP (pre: $p = 0.002$, post: $p = 0.01$) and NOR ($p = 0.01$) (Table 3.3).

Table 3.3: Thermoregulatory responses at rest and during the last ten minutes of the constant-work rate cycling before and after HyA for the normoxic, the hypoxic and the heat trial ($n = 12$).

	Pre-HyA			Post-HyA		
	NOR	HYP	HE	NOR	HYP	HE
$T_{re\ rest}$ (°C)	37.6±0.3	37.5±0.2	37.4±0.6	37.3±0.4	37.2±0.3	37.3±0.3
$T_{re\ exer}$ (°C)	38.1±0.2	38.0±0.3	38.0±0.3	37.8±0.4	37.9±0.3	37.8±0.3
T_{sk} (°C)	33.5±0.7	[†] 32.9±0.7	[‡] 36.6±0.3	[*] 32.6±0.8	32.6±0.6	[‡] 36.3±0.4
ΔT_{f-f} (°C)	0.88(-2.22- +10.59)	0.32(-2.07- +10.60)	[†] 0.26(-0.73- +0.78)	-0.54(-1.81- +4.64)	[*] 0.07(-2.1- +5.99)	0.2(-1.53-1.05)
$\dot{m}_{sw}$ (g · m ⁻² · min ⁻¹)	7.0±2.8	7.3±2.2	9.6±2.6	5.9±3.1	6.6±1.8	[‡] 10.5±2.6
$\dot{m}_{sw\ gain}$ (g · m ⁻² min ⁻¹ · °C ⁻¹)	27.1±10.3	19.3±8.2	19.4±7.0	20.3±6.1	22.7±15.6	21.5±12.6
Threshold T_{re} for sweating (°C)	37.6±0.2	37.5±0.2	37.4±0.3	37.4±0.4	37.3±0.3	37.3±0.3
Difference in body mass (%)	-0.65±0.25	-0.61±0.39	[‡] -0.96±0.14	-0.65±0.12	-0.65±0.11	[‡] -0.94±0.19

T_{re} rectal temperature, T_{sk} mean weighted skin temperature from four sites, ΔT_{f-f} difference between forearm and fingertip skin temperatures, $\dot{m}_{sw}$ sweating rate. Sweat rate production values $\pm \dot{m}_{sw}$ are averages of the 30 min of exercise. Values are mean $\pm$ SD except for ΔT_{f-f} median (range)

* Significant difference to values before HyA

† Significant difference to normoxic values

‡ Significant difference to hypoxic values, $p < 0.05$

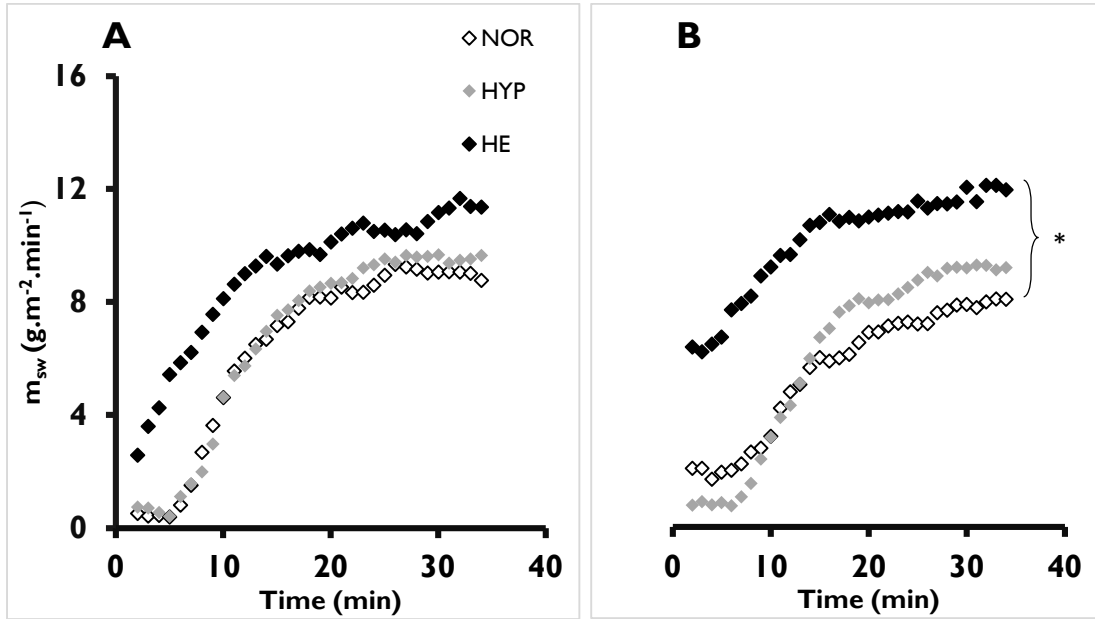


Figure 3.2: Temporal response of the mean forehead sweating rate ($\dot{m}_{sw}$, $\text{g} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$) during exercise in the normoxic (NOR), hypoxic (HYP), and heat (HE) trials before (A) and after (B) HyA ($n = 12$). * $P < 0.05$.

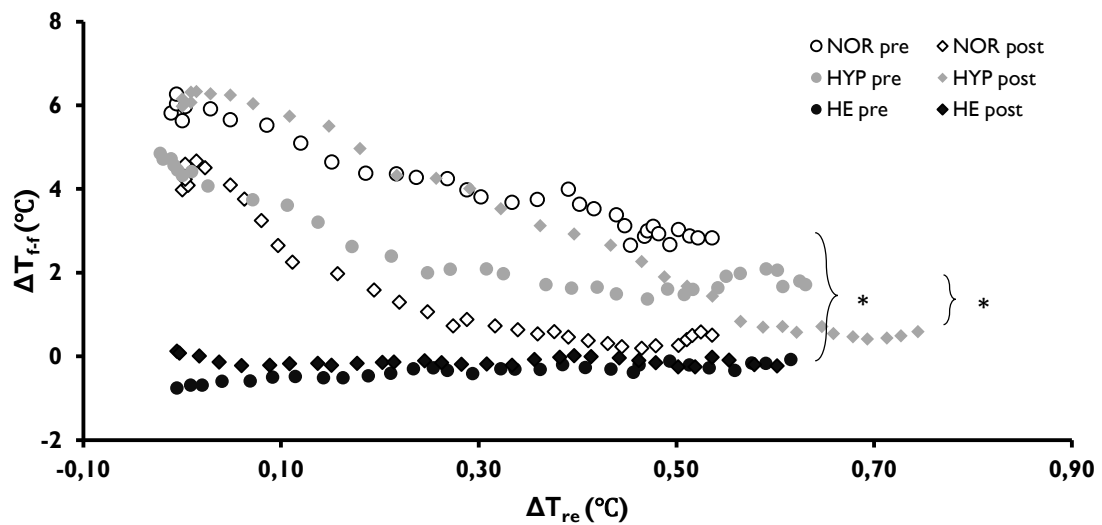


Figure 3.3: Mean changes in the forearm-fingertip skin temperature gradient (ΔT_{f-f} , $^{\circ}\text{C}$) as a function of the relative change in rectal temperature (ΔT_{re} , $^{\circ}\text{C}$) during the normoxic,

hypoxic and heat trials before and after HyA (n=12) *p < 0.05. SD bars are omitted for the purpose of clarity.

3.2.4. Hematological Data

The HyA protocol induced marked overall changes in the hematological variables (Table 3.4). The erythrocyte and hemoglobin concentration were increased as PV contracted. As a result, the hematocrit was higher post-acclimation. A decrease in ferritin and an increase in reticulocyte concentration were noted as a result of the acclimation. On the contrary, the 52% increase in plasma EPO did not reach statistical significance. No changes were observed on the renin-angiotensin-aldosterone axis or the HSP90 concentration pre- to post-acclimation. Interestingly, the HIF-1 α concentration was lower while the plasma HSP72 expression was higher after the protocol.

Table 3.4: The measured hematological variables pre and post the 10-day HyA.

	Pre-HyA	Post-HyA	p-value
Erythrocytes ($10^{12}.L^{-1}$)	5.2 ± 0.3	5.6 ± 0.3	< 0.001
Hemoglobin (g.L ⁻¹)	152 ± 6.8	168.4 ± 7.5	< 0.001
Hematocrit (%)	45 ± 2	49 ± 2	< 0.001
Ferritin ($\mu g.L^{-1}$)	75.1 ± 24.6	45 ± 14.1	< 0.001
Reticulocytes ($10^9.L^{-1}$)	66 ± 12	118 ± 21	< 0.001
Plasma Erythropoietin (mIU.mL ⁻¹)	7.84 (3.6-28.4)	12.83(1.1-51.5)	0.347
Hypoxia-Inducible factor 1a (HIF-1a) (ng.mL ⁻¹)	1.81 ± 0.25	1.55 ± 0.25	0.014
Heat Shock Protein 72 (HSP72) (ng.mL ⁻¹)	0.63 (0.1-7.16)	0.87(0.12-8.25)	0.005
Heat Shock Protein 90 (HSP90) (ng.mL ⁻¹)	32.6 ± 9.1	31.7 ± 7.4	0.700
Renin (pg.mL ⁻¹)	21.3 ± 18.7	21.7 ± 10.8	0.951
Aldosterone (pg.mL ⁻¹)	137 ± 72	154 ± 68	0.439
Plasma volume changes (%)	-	-15.8 ± 3.4	-

Values are mean $\pm$ SD except for plasma EPO and HSP72 median (range).

With regard to the individual data (Figure 3.4), HSP72 plasma concentration increased in 10 out of 12 participants. Of note, two participants had a baseline HSP72 plasma concentration above 6 ng.mL⁻¹ (6.2 and 7.16 ng.mL⁻¹, respectively) while the rest entered the study with their HSP72 plasma concentration below 2 ng.mL⁻¹. HSP90 concentration decreased in 6 and increased in the other 6 participants. EPO plasma concentration was higher in 7 and lower in 4 participants post-acclimation. The individual pre- to post-acclimation difference in EPO plasma concentration ranged from -9 to +45 mIU.mL⁻¹ indicating a significant individual variability in the acclimation response.

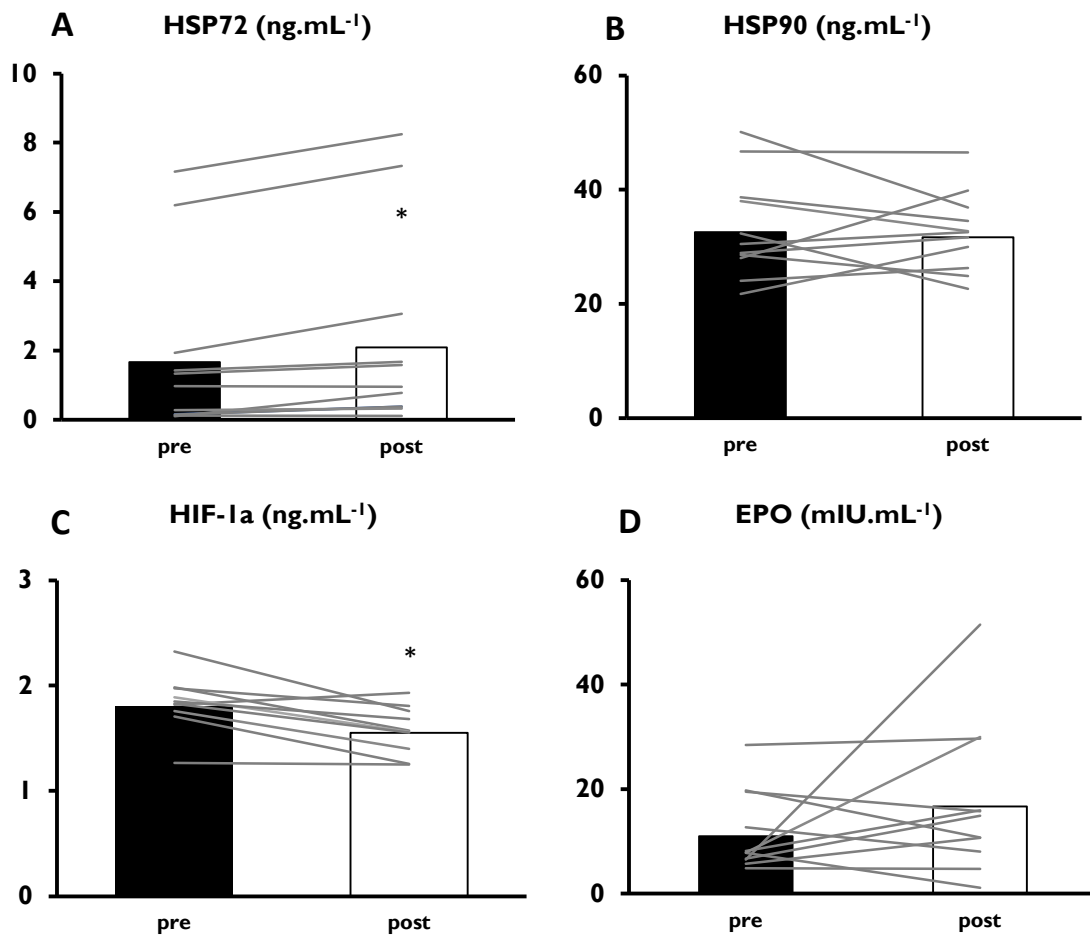


Figure 3.4: Pre- and post-acclimation mean and individual data for extracellular (A) Heat shock protein 72 (HSP72), (B) Heat shock protein 90 (HSP90), (C) Hypoxia-inducible factor 1a (HIF-1a), and (D) Erythropoietin (EPO). *P < 0.05.

3.2.5. Cardiorespiratory Responses to Incremental Exercise to Exhaustion

Maximal HR remained unchanged, and was independent of conditions ranging from 180 ± 8 (post-acclimation HYP) to 191 ± 7 (pre-acclimation HE). Peak $\dot{V}_E$ was significantly higher post-acclimation ($p = 0.02$) only in NOR compared to HE. $\dot{V}O_{2\max}$ was significantly higher ($p < 0.001$) in NOR compared to HYP and HE, both before and after the acclimation protocol. $\dot{V}O_{2\max}$ increased ($p = 0.002$) by 9% from pre- to post-acclimation in NOR, while it remained unchanged in HYP (-2%, $p = 0.90$) and HE (+5.8%, $p = 0.16$). However, due to the reduction in body mass after the acclimation protocol, $\dot{V}O_{2\max}$ expressed relative to body mass increased ($p = 0.03$) by 7.9% from pre- to post-acclimation also in HE, whereas no differences were found in HYP (pre: $39.9 \pm 3.8 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ vs post: $39.4 \pm 5.1 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $p = 1.0$) (Table 3.5). Only one participant in HE and one in NOR had a lower $\dot{V}O_{2\max}$ value post-acclimation (Figure 3.5A). Following the same pattern as $\dot{V}O_{2\max}$, W_{peak} expressed either in absolute (W) or relative values ($\text{W} \cdot \text{kg}^{-1}$ body mass) was significantly higher in NOR compared to HYP ($p < 0.001$) or HE (pre: $p = 0.01$, post: $p < 0.001$) and in HE compared to HYP (pre: $p < 0.001$, post: $p = 0.02$) independent of the acclimation (Table 3.5). In 9 out of 12 participants, a pre- to post-acclimation increase in W_{peak} was observed in HYP (Figure 3.5B).

Table 3.5: Maximal cardiorespiratory responses and peak work rate during the incremental exercise to exhaustion before and after HyA for the normoxic, hypoxic and heat trials ($n = 12$).

	Pre-HyA			Post-HyA		
	NOR	HYP	HE	NOR	HYP	HE
HR _{max} (bpm)	190 ± 9	188 ± 10	191 ± 7	181 ± 7	180 ± 8	190 ± 9
$\dot{V}O_{2\max}$	3786 ± 3134	3272 ± 296	4151 ± 3070	3474 ± 267		
(mL · min ⁻¹)	388	315	501			
$\dot{V}O_{2\max}$	48.3 ± 5.6	39.9 ± 3.8	41.9 ± 4.5	53.5 ± 6.1	39.5 ± 5.1	45.2 ± 5.2
(mL · kg ⁻¹ · min ⁻¹)						
$\dot{V}_E$ peak (L · min ⁻¹)	151 ± 28	150 ± 26	149 ± 32	163 ± 26	158 ± 25	148 ± 26
W_{peak} (W)	345 ± 37	293 ± 25	325 ± 32	355 ± 38	304 ± 31	323 ± 31

W_{peak} ($\text{W} \cdot \text{kg}^{-1}$) 4.4 ± 0.4 $^{\dagger}3.8 \pm 0.3$ $^{\dagger\dagger}4.2 \pm 0.4$ 4.6 ± 0.4 $^{\dagger}3.9 \pm 0.3$ $^{\dagger\dagger}4.2 \pm 0.4$

HR_{max} maximal heart rate, $\dot{V}O_{2\text{max}}$ maximal aerobic power, $V_{E\text{ peak}}$ peak respiratory rate, W_{peak} peak work rate. Values are mean $\pm$ SD.

* Significant difference to values before HyA

† Significant difference to normoxic values

†† Significant difference to hypoxic values, $p < 0.05$.

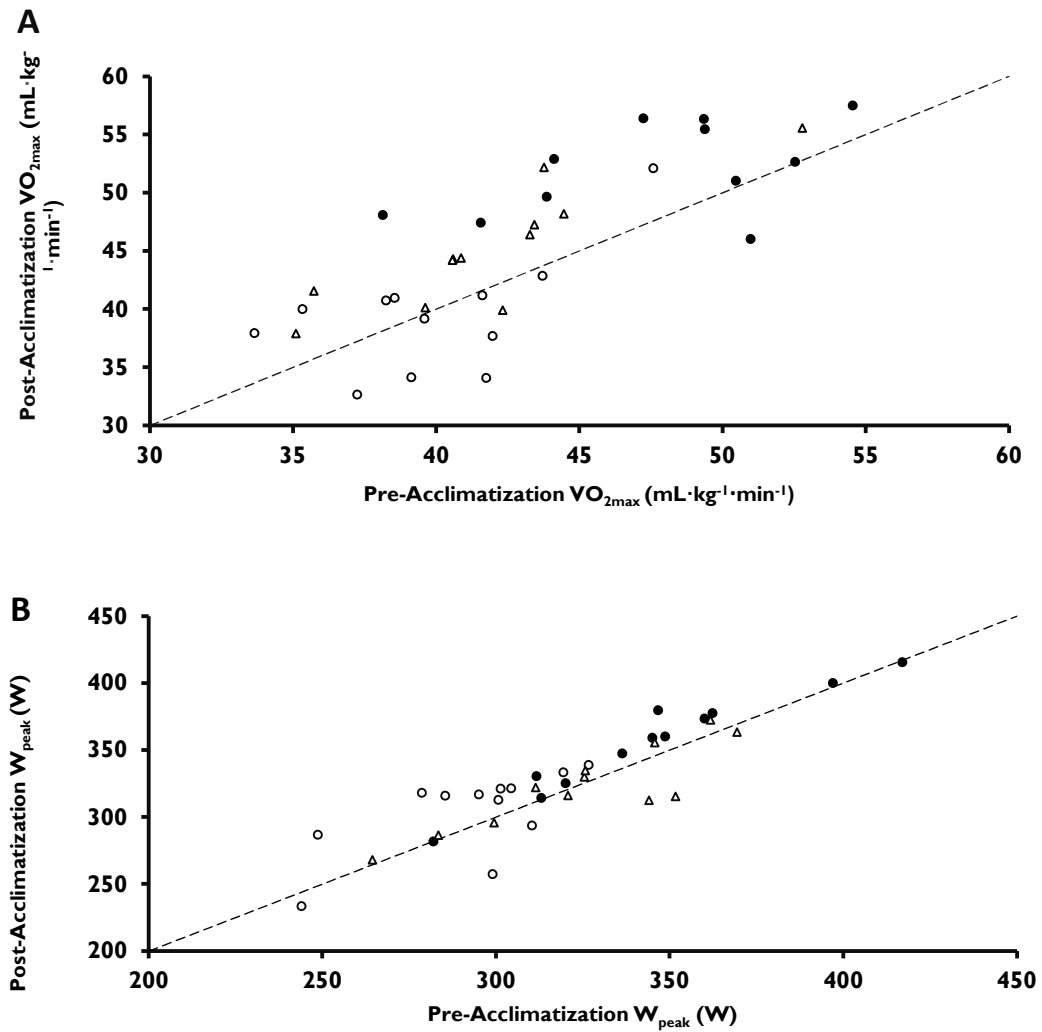


Figure 3.5: Pre- and post-acclimation individual data for (A) maximal aerobic power ($\dot{V}O_{2\text{max}}$, $\text{mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) and (B) Peak Power Output (W_{peak} , Watts) in normoxia (closed circles), hypoxia (open circles) and in the heat (triangles).

3.3. Discussion

The principal finding of the present study is that a 10-day normobaric hypoxic acclimation with daily normobaric hypoxic exercise improved maximal aerobic power and did not impair exercise performance (W_{peak}) in normoxia and heat. Hypoxic acclimation did not affect exercise thermoregulatory responses in NOR, HYP and HE. There appears to be no apparent beneficial nor negative influence of prolonged hypoxic acclimation to the thermoregulatory responses in a hot environment.

3.3.1. Acute Responses

3.3.1.1. Cardiorespiratory Responses and Exercise Performance

Our data demonstrate that heat and hypoxic stress reduced individual $\dot{V}O_{2\text{max}}$ and W_{peak} relative to the level achieved in the thermoneutral normoxic environment. These findings are in agreement with other studies that have compared exercise performance in normoxic versus hypoxic conditions (Debevec et al., 2010; Kounalakis et al., 2013; Peltonen, Tikkanen, & Rusko, 2001) or thermoneutral versus hot conditions (Sawka et al., 1985; Wingo & Cureton, 2006).

Concerning the incremental exercise to exhaustion in the heat, it is long known that heat stress can displace blood from contracting muscles to the cutaneous vasculature. Effective blood volume and subsequently venous return and CO will decrease. The lower CO could reduce arterial oxygen delivery and in turn reduce aerobic energy turnover within the exercising muscles, thereby inducing peripheral fatigue (Rowell, 1977). While no direct measurement of skin blood flow was conducted in our experiment the $\Delta T_{\text{f-f}}$ provided an index of peripheral perfusion. Thus, vasodilation is reflected in $\Delta T_{\text{f-f}}$ values equal to, or less than zero (House & Tipton, 2002), similar to what we recorded in the HE condition.

During the exercise tests, S_pO_2 was lower in HYP, and thus limited $\dot{V}O_{2\text{max}}$, compared to HE and NOR. Another mechanism that contributed to the reduced ability of our participants to perform maximal work in both HE and HYP could be the increased work of breathing. In HYP, augmented V_E serves to increase the oxygen partial pressure in the lungs and thus increase the partial pressure of the oxygen gradient between the lungs and blood. The elevated ventilatory response in HYP also augments the oxygen delivery to the pulmonary circulation. On the contrary, the main purpose of the hyperventilation observed in HE is to promote respiratory heat loss (M. D. White, 2006), since S_pO_2 is normal. We recorded a consistently higher V_E in HE and HYP compared to NOR for the same absolute workload. Increased V_E has been connected to a reduced exercise performance in acute hypoxia (Amann, Pegelow, Jacques, & Dempsey, 2007).

3.3.1.2. Thermoregulatory Responses

Thermoregulatory responses did not differ between the environmental conditions in the present study. Not surprisingly, T_{sk} was consistently higher in HE compared to NOR and HYP. The magnitude of T_{re} elevation during steady-state exercise was largely independent of T_a , as has been shown before (Stolwijk, Saltin, & Gagge, 1968). In accordance with other studies, T_{re} was not different when participants exercised in acute hypoxia (Coombs et al., 2017; Greenleaf et al., 1969). T_{re} is elevated when heat production outweighs heat loss. Heat production depends on the absolute work rate which was the same in our three environmental conditions. As a result, the lack of differences in T_{re} between environments suggests a similar balance of heat loss and heat production in each condition. It has been

argued that the increased sweating observed in acute hypoxia coincided with a reduced vasodilation so that heat loss was maintained at the same level (Kounalakis et al., 2012). However, our data show no differences in sweating and in ΔT_{fe} between NOR and HYP.

In HE, as T_a was almost equal to T_{sk} , insensible heat exchange (evaporation) accounted for essentially all of the heat loss. Nevertheless, our data show a significant difference in $\dot{m}_{\text{sw}}$ between HE and the thermoneutral environments only after the acclimation protocol. Moreover, the decrease in body mass, which is a valid index of the whole body sweat production, was consistently greater in HE.

The gain of the sweating response ($\Delta \dot{m}_{\text{sw}} / \Delta T_{\text{re}}$) and T_{re} sweating threshold did not change between the environmental conditions, which is in contrast with our previous findings (Kacin et al., 2007; Kounalakis et al., 2012), but in line with other studies (Coombs et al., 2017; Low, Bailey, Timothy Cable, & Jones, 2016) examining the acute effect of hypoxia on thermoregulatory responses. The former studies imply that the forehead sweating response can be influenced by non-thermal physiological responses associated with increased physical strain and sympathoactivation (Mekjavic & Eiken, 2006). The increased sympathetic drive as a response to acute hypoxia was verified by our steady-state exercise data such as increased HR and V_E (RPE higher only post-acclimation), but the higher relative work rate in HYP did not produce augmented sweating responses.

We matched the absolute work rate between conditions with the aim of maintaining the exercise-related heat production constant. Recently, Coombs et al. (2017) found no differences in the sweating responses during exercise in neutral versus hot hypoxic conditions. Their experimental design controlled for heat production, but not absolute work rate. The authors reasoned that heat production for a fixed workload is not the same between normoxia and hypoxia, because of an altered mechanical efficiency. Nevertheless, in their study absolute workload did not differ more than 3 W between trials matched for heat production.

Another mechanism that could be of significance for temperature regulation in HE and HYP is respiratory heat loss. For the same T_a ($\sim 23^\circ\text{C}$), respiratory heat loss becomes a linear function of V_E (Cain, Livingstone, Nolan, & Keefe, 1990). When oxygen supply to the brain is reduced, peripheral chemoreceptors located on carotid bodies initiate a reflex, which enhances the activity of the inspiratory muscles, increasing V_E . Indeed, V_E was higher in HYP ($\sim 25\%$) compared to NOR. However, Coombs et al. (2017) reported an only slightly higher (6 W) respiratory heat loss in hot hypoxic conditions compared to normoxia. Due to the minor contribution to overall heat exchange during exercise, the respiratory heat loss may be disregarded.

3.3.2. Consequences of Hypoxic Acclimation

3.3.2.1. Cardiovascular Responses and Exercise Performance

Our data demonstrate an increased $\dot{V}O_{2\text{max}}$ in HE and NOR, but not in HYP after HyA. Interestingly, this augmented $\dot{V}O_{2\text{max}}$ did not mediate any improvements in exercise performance, as reflected in the post-acclimation W_{peak} in any of the tested environmental conditions. Despite the fact that 6 participants had a lower $\dot{V}O_{2\text{max}}$ in HYP after acclimation (Figure 3.5A), only three of them performed worse after HyA. To compensate for the reduced aerobic capacity, participants may have relied more on anaerobic glycolysis. Greater muscle buffering and increased lactic acid production and tolerance have been noted among adaptations to hypoxia (Gore et al., 2007). That 50% of the participants had a lower $\dot{V}O_{2\text{max}}$ in HYP after the acclimation may also be a result of individual variation in the responses to HyA (Chapman et al., 1998).

Given the low intensity and duration, our exercise protocol was not expected to importantly affect $\dot{V}O_{2\max}$ (Wenger & Bell, 1986). Exercise was perceived as less than hard (RPE < 15) by the participants over HyA. The progressively lower resting HR over the 10-d acclimation protocol was most likely due to the attenuated sympathetic activity and catecholamine response induced by the daily exercise stressor. In light of this evidence, we do not believe that training-mediated adaptations are responsible for the $\dot{V}O_{2\max}$ elevation in NOR and HE. However, a training camp effect cannot be excluded if we consider our participants' average fitness level and the accumulated training volume. Our previous data (Kounalakis et al., 2013) from another study performed at the same facility following the same confinement protocol (240 hours combined with the same training stimulus) in normoxia did not demonstrate any differences in the examined cardiorespiratory or hematological parameters.

It is also of note that our participants were maximally tested during the three days preceding and following the 10-day HyA protocol. Residual fatigue may have influenced the performance of our participants especially during the post-acclimation tests. However, the increased oxygen-carrying capacity achieved with HyA does seem to be blunted after the first week upon return to sea level as a result of pronounced neocytolysis (Chapman, Laymon Stickford, Lundby, & Levine, 2014). Thus, we opted for running the performance tests as soon as possible following the cessation of HyA.

Concerning HyA effect, HIF and VEGF-induced angiogenesis may have facilitated oxygen delivery to tissues (Ely et al., 2014) which would explain, in part, the increased $\dot{V}O_{2\max}$ in NOR and HE. Calbet et al. (2003) have investigated the underlying mechanisms responsible for the lower $\dot{V}O_{2\max}$ in hypoxia both acutely and even after a very strong hypoxic stimulus (participants lived at an altitude of 5260 m for 10 weeks). They tracked changes in the main steps of the oxygen transport system and suggested that their protocol raised both hemoglobin concentration and S_pO_2 leading to a normalized arterial oxygen concentration similar to our results. Nonetheless, $\dot{V}O_{2\max}$ failed to recover due to an unchanged reduction in CO and redistribution of blood to non-contracting tissues. This seems in line with other studies (Keramidas, Kolegard, et al., 2016; Kounalakis et al., 2013) showing that PV contraction results in a lower preload which together with the reduction in maximal HR limit CO and subsequently $\dot{V}O_{2\max}$ after a prolonged HyA. Indeed, lower maximal HR (8-10 bpm after acclimation, albeit not statistically significant) and PV contraction (~16%) were observed post-acclimation.

After HyA, peak V_E increased in NOR but not in HE. An exponential relationship between pulmonary ventilation and the associated oxygen cost of ventilation during exercise has previously been established (Vella, Marks, & Robergs, 2006). Up to ~15% of CO is directed to respiratory muscles during maximal exercise; most of it probably diverted from the legs (Harms et al., 1998). Indeed, up to 37% of the acclimation-induced gain in $\dot{V}O_{2\max}$ may be employed to metabolically cover the respiratory muscles work (Wilhite, Mickleborough, Laymon, & Chapman, 2013). Thus, the lack of differences in NOR W_{peak} could be explained by a disproportionate increase in the oxygen cost of ventilation. On the contrary, the respiratory limitation (i.e. the inability of V_E to increase post-acclimation) observed at maximal exercise in HE may be a consequence of the competition for blood flow between respiratory muscles, locomotor muscles and the skin. Skin blood flow was most probably not reduced as the T_c - T_{sk} gradient did not change after the acclimation in HE. To our knowledge, the only study that examined the effect of T_a on inspiratory muscle fatigue found that exercise in the heat did not exacerbate inspiratory muscle fatigue in trained males (Romer, Bridge, McConnell, & Jones, 2004) leading us to the conclusion that CO to the respiratory muscles did not change either. One could thus hypothesize that, given the fact that CO decreases after a HyA, the percentage of CO reserved for the skeletal

muscles may have been reduced explaining our inability to find an increased W_{peak} despite an increased $\dot{V}O_{2\text{max}}$ in HE.

The fact that the profound PV contraction did not limit W_{peak} in any environmental condition warrants a mention. From a practical standpoint, this is the first study to date demonstrating that recreationally active humans (athletes/soldiers) can undergo continuous HyA protocol and by doing that not compromising their exercise performance in a hot environment.

3.3.2.2. Thermoregulatory Responses

Thermoregulatory responses were slightly improved after the HyA protocol in NOR. PV contraction has been shown to reduce skin blood flow (Fortney et al., 1981). However, convective heat loss can be supported by other physiological mechanisms. Specifically, T_{sk} was reduced in NOR. The wider $T_{\text{c}}-T_{\text{sk}}$ gradient maintained the magnitude of convective heat loss for a lower amount of skin blood flow. ΔT_{ff} was, or tended to be lower compared to pre-acclimation in HYP and NOR (lower ΔT_{ff} implies vasodilation). Vasodilation also promotes convective heat loss. At a molecular level, HIF-induced angiogenesis and enhanced iNOS activity could augment peripheral perfusion (Ely et al., 2014) present in both NOR and HYP in agreement with our previous findings (Kounalakis et al., 2012).

On the other hand, no significant effect of the HyA protocol was observed in the gain of the sweating response. From a physiological standpoint, sweat production may have been hindered by the lower estimated PV after the acclimation as hypovolemia is correlated with decreased gain of sweating (Fortney et al., 1981). This finding comes in contrast to the increased sweating gain in NOR after a 4-week LHTL protocol observed in our previous study (Kounalakis et al., 2012). Another factor that could have prevented any improvements in the sweating response was dehydration. However, the water intake was not suppressed during the acclimation protocol (data not presented). Moreover, body weight changes can reflect sweat losses during short-duration exercise (American College of Sports et al., 2007). In our trials, the exercise testing duration did not exceed 50 minutes and the biggest individual decrease in total mass, including HE, was 1.2%, excluding the possibility that our participants had a water deficit.

The exercise stimulus employed during the acclimation was insufficiently high for thermoregulatory adaptations. To obtain thermoregulatory benefits as a result of training, individuals must adequately stimulate thermoregulatory effector responses, i.e. they must maintain T_{re} at an elevated level for a sustained period of time. In the present study, we opted for a rather low training intensity since we did not want the metabolic stressor (exercise) to induce significant thermoregulatory adaptations. Normobaric HyA protocols require that participants be confined in a restricted area (i.e. a hotel floor) for consecutive days limiting their involvement in daily activities (Siebenmann et al., 2012). Thus, the main aim of the exercise training inclusion was to compensate for this reduction in habitual energy expenditure. Lastly, a linear relationship was assumed between the sweating response and T_{re} . Future studies should consider using a more robust index for the sweating response such as the area under the curve or assume an exponential relationship between $\dot{m}_{\text{sw}}$ and T_{re} .

3.3.2.3. Hematological Data

There is an ongoing discussion over the standardization of the hypoxic dose applied in acclimation protocols in order to induce ventilatory and hematological adaptations (Garvican-Lewis et al., 2016). When an intermittent protocol is employed, a minimum of

3 weeks was required for accelerated erythropoiesis to occur (Wilber, Stray-Gundersen, & Levine, 2007). However, a recent meta-analysis suggested that the erythropoietic effect of the HyA may be a function of both the hypoxic stimulus and the duration of the protocol (Garvican-Lewis et al., 2016) independent of the type of the acclimation (continuous vs intermittent). Indeed, the increased hematocrit and hemoglobin, erythrocyte, reticulocyte concentrations suggest that the hypoxic stimulus (960 km h) induced hematological acclimation.

Hemoconcentration is one of the most rapid responses to hypoxic exposure. The PV contraction was of a similar magnitude to the values of recent studies that employed a similar hypoxic stimulus (Keramidas, Mekjavic, et al., 2016; Siebenmann, Cathomen, et al., 2015). However, the acclimation-induced differences in PV were graded quantitatively by comparing PV changes as estimated indirectly from changes in hemoglobin concentration and hematocrit (Dill & Costill, 1974). It should be taken into consideration that any erythropoietic effect occurring during HyA may have produced an overestimation of the actual PV contraction. The use of a direct tracer-dilution method should be considered to study PV changes in future HyA studies. Unfortunately, we were not able to measure Hbmass, the primary index of the hematopoietic effect of any HyA.

The degree of PV contraction could be related to changes in plasma renin concentration/activity. Previous findings have shown that renin activity could be either reduced (Siebenmann, Cathomen, et al., 2015) or remain unchanged (Keramidas, Mekjavic, et al., 2016) during hypoxic protocols. It has been suggested (Siebenmann, Robach, & Lundby, 2017) that whether or not the renin-angiotensin-aldosterone axis is suppressed upon exposure to hypoxia depends on the rate of altitude ascent. Enhanced renin-angiotensin-aldosterone axis activity would blunt the diuresis-mediated fluid loss observed at altitude. Renal sympathetic nerves innervate the juxtaglomerular cells. It is through this pathway that hypoxia stimulates renin secretion. In the current study that included a rapid (almost immediate) ascent to ~4,000 m, renin concentration remained unchanged pre- to post-acclimation, which would explain the small water loss. Besides the rapidity of the ascent, participants were involved in daily cycling exercise sessions during their acclimation. Exercise training enhances plasma renin activity leading to an increased blood volume (Convertino et al., 1980). This putative exercise-induced increase could also minimize the altitude-mediated decrease in renin concentration.

We did not find any significant change in EPO after HyA. Indeed, our results are in agreement with previous studies focusing on the time course of hematopoiesis upon continuous hypoxic exposure (Berglund, Gennser, Ornhaugen, Ostberg, & Wide, 2002; Debevec et al., 2012; Debevec et al., 2014; Gunga, Kirsch, Rucker, & Schoberberger, 1994; Keramidas, Mekjavic, et al., 2016). EPO concentration seems to reach its maximal value by the fourth day of hypoxic exposure, and thereafter returns to levels slightly above baseline. Upon completion of our 10-d HyA protocol, the average EPO values were still higher $\approx 52\%$ compared to pre-acclimation values. However, this increase was not statistically significant, predominantly as a result of the large individual variability in EPO responses (Figure 3.4D). The individual variability can be potentially attributed to the natural circadian variation reported for EPO (Debevec et al., 2012; Klausen, Dela, Hippe, & Galbo, 1993). We suspect that had the hypoxic exposure been longer, the EPO concentration would have returned to pre-exposure levels.

There is clear evidence that intermittent (L. Taylor et al., 2011) and/or acute hypoxic (L. Taylor et al., 2010) as well as intense physical training (Liu et al., 2000) protocols augment HSP72 expression. Indeed, we also observed an increase in plasma HSP72 concentration after HyA probably as a result of increased sympathetic activation, i.e. the stress response (Johnson & Fleshner, 2006). To our knowledge, only one study to date examined the extracellular HSP72 and HIF-1 α responses to a continuous hypoxic exposure

(Lee et al., 2016). During HyA that also included daily 60-min training sessions, the researchers found that both proteins were increased post-acclimation. In their study, the time-trial performance was improved in hypoxia, whereas the physiological strain index during a heat test did not change. Unfortunately, the researchers did not employ a performance test in HE.

Unlike the HSP72 response, HIF-1 α plasma concentration was lower post-acclimation in our study showing smaller individual variability. The major limiting factor in our attempt to correlate systemic responses with changes at a molecular level is the extracellular sampling site, especially for the HIF-1 α , known to be mostly expressed intracellularly via Von-Hippel Lindau/proteasome pathway and prolyl hydroxylases stabilization. Nevertheless, the induction of HIF-1 α in response to hypoxia is found to be instantaneous (Jewell et al., 2001). The possibility that the 90-min period between exiting the hypoxic environment, and obtaining the first blood sample blunted the HIF-response cannot be excluded. Unfortunately, we were not able to keep track of the cellular kinetics as blood samples were obtained once before and once after HyA.

Chapter 4

Heat Acclimation does not Affect Maximal Aerobic Power in Normoxic or Hypoxic Conditions

HeA develops upon repeated exposure to environmental conditions that are sufficiently stressful to elevate core and skin temperature (Regan et al., 1996) and induce profound sweating (Hessemer et al., 1986). Thus, an efficient HeA protocol can comprise both, a metabolic/endogenous stressor (i.e. physical work/exercise) to raise T_c , and an exogenous stressor (i.e. heat exposure) to raise skin temperature. Fox et al. (1963) were the first to introduce the controlled hyperthermia HeA strategy in which the endogenous thermal stress is standardized to maintain a given level of thermal strain, as reflected in the elevation of T_c during the course of the adaptation.

It has been suggested that heat-induced adaptations may also benefit an individual exposed to another stressor, such as hypoxia. This phenomenon, whereby adaptation to one environmental stressor may positively modulate responses to another stressor, has been termed cross-adaptation (Hessemer et al., 1986). At a cellular level, heat shock proteins (HSP72 and HSP90) and HIF-1 α have been identified as key cellular drivers of this cross-stressor response. Enhanced HSP synthesis normally results from elevated T_c (Gibson et al., 2016). HSP-90 augments vasodilation in acclimated individuals (Fujii, Zhang, McNeely, Nishiyasu, & Kenny, 2017). HIF-1 α , on the other hand, is stimulated by exposure to systemic hypoxia and mediates molecular adaptations to upregulate angiogenesis, erythropoiesis (Goldberg & Schneider, 1994) and respiration (Wilson, Roy, & Lahiri, 2005). HSP90 interacts with the oxygen-dependent HIF-1 α subunit stabilizing HIF-1 α even in normoxic conditions (Salgado, White, Schneider, & Mermier, 2014). Other physiological adaptations underlying HeA at a systemic level may be secondary to the expanded PV and include improved exercise economy (Sawka et al., 1983), greater SV, lower HR (Lorenzo et al., 2010), increased ventricular compliance and myocardial efficiency (Horowitz et al., 1986). With regards to thermoregulatory responses, HeA results in an increased sweat rate at a given T_c . This is accomplished by a shift of the T_c threshold for sweating to a lower temperature (Nadel et al., 1974; Wyndham, 1967), and an increased sweating response for a given change in T_c (Patterson et al., 2004; Roberts et al., 1977; Wyndham, 1967).

Few studies to date have investigated the potential of HeA to improve exercise performance in acute hypoxia (Heled et al., 2012; Lee et al., 2016; A. C. White et al., 2016). The first study to test this cross-tolerance approach was conducted by Heled et al. (2012) who observed a delayed onset of blood lactate accumulation during hypoxic exercise in a group of heat-acclimated trained males. Subsequently, A. C. White et al. (2016) examined

whether 10 days of HeA would improve exercise performance and $\dot{V}O_{2\max}$ during acute hypobaric hypoxia (4350 m) in trained men. $\dot{V}O_{2\max}$ remained unaffected by HeA at 4350 m or 1600 m whereas there was a tendency for an improved 16 km time trial performance at 4350 m after HeA. Lee et al. (2016) had three groups of male participants train for 10 days in thermoneutral normoxic, hypoxic or hot conditions and reported an improved time-trial performance for the heat-acclimated group in hypoxia. The above studies used the traditional fixed-work rate protocol to induce HeA and then tested exercise performance in hypoxia. It appears, however, that no study to date has employed the controlled-hyperthermia technique where the adaptation impulse is maintained following a progressive increase in the overload (N. A. Taylor, 2014). Only Gibson, Turner, et al. (2015) employed a controlled-hyperthermia protocol to investigate whether HeA would attenuate physiological strain during subsequent acute hypoxic exposure (at rest and two submaximal exercise intensities). Lower HSP72 mRNA values together with an increased oxygen pulse, a lower HR and preserved S_pO_2 were reported post-HeA but no measures of exercise performance or $\dot{V}O_{2\max}$ were obtained.

There is a growing interest in whether HeA enhances performance in thermoneutral conditions (Karlsen et al., 2015; Keiser et al., 2015; Lorenzo et al., 2010; Rendell et al., 2017) in trained individuals. Lorenzo et al. (2010) selected a 10-day traditional fixed work rate protocol and observed increased $\dot{V}O_{2\max}$ and increased time-trial performance after HeA during exercise both in the heat and cool-temperate conditions. The increased aerobic performance observed in that study was attributed to several cardiovascular (increased CO, PV expansion, increased exercise economy, increased lactate threshold) as well as thermoregulatory (lower skin temperature and earlier onset of sweating lowering skin blood flow) adaptations. Subsequent studies attempted to reinforce those results. Rendell et al. (2017) used an 11-day controlled-hyperthermia HeA protocol and noted increased performance measures, such as lactate threshold, W_{peak} and total work done, but no differences in $\dot{V}O_{2\max}$ or GME in temperate conditions. Their results were not affected by the addition of mild nocturnal hypoxia (for a discussion on the sufficiency of the applied hypoxic dose see [Chapter 7](#)). On the other hand, HeA protocols that involved either 2-week outdoor (Keiser et al., 2015) or 10-day lab-based (Karlsen et al., 2015) training in the heat on well-trained cyclists reported no effects on $\dot{V}O_{2\max}$, time-trial performance, W_{peak} , CO or GME in temperate conditions. Nevertheless, none of the above studies evaluated the localized sweating response (threshold, gain) whereas only Keiser et al. (2015) measured central hemodynamics, i.e. CO and SV. Furthermore, the high inter-individual variability reported in the adaptive responses to HeA (Corbett et al., 2018) may not be minimized when using independent (control) group (Karlsen et al., 2015; Lorenzo et al., 2010) or even cross-over designs (Keiser et al., 2015; Rendell et al., 2017) as opposed to within-individual repeated measures designs.

4.1. Methods

4.1.1. Participants

Twelve healthy males with a mean (SD) age of 22.3 ± 2.8 years, baseline $\dot{V}O_{2\max}$ of 54.7 (5.7) $\text{mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (range: $41.2 - 61.0$) and body fat percentage of $15.4 \pm 2.2\%$ were recruited for the present study. Participants ranged from moderately active to trained. This sample size is consistent with previous work in this area that has identified differences in key cardiovascular and exercise performance indices in thermoneutral normoxic (Lorenzo et al., 2010) or hypoxic conditions (A. C. White et al., 2016). All participants were near-sea level residents, non-smokers and had not been exposed to altitudes $>1500\text{m}$ or

temperatures >30 °C for at least 1 month prior to the start of the study. None had a history of any cardiorespiratory or hematological disease. They were instructed to abstain from caffeine and alcohol consumption during the whole study protocol. They were extensively informed regarding the study protocol as well as the potential risks involved. During the pre-screening procedure the participants completed a graded exercise test on a cycle ergometer to establish their normoxic $\dot{V}O_{2\max}$ and W_{peak} . For the participants that were included in the study, the measured normoxic W_{peak} was used to determine their steady-state workload during the pre- and post-HeA tests (40% of the normoxic W_{peak} attained during the preliminary test).

4.1.2. Study Outline

The study comprised three parts: a) Pre-HeA tests: Prior to the 10-d HeA, the participants conducted three exercise tests on three consecutive days. The order of the trials was randomized and counterbalanced to avoid any order related bias, b) Heat Acclimation (HeA): Participants conducted daily 90-min training sessions for 10 days, in a climatic chamber where T_a and RH were set at 35 °C and 50%, respectively. During the 90-min cycle ergometry T_{re} was maintained at 38.5 °C, and c) Post-HeA tests: All three exercise tests conducted prior to the onset of acclimation (pre-HeA tests) were repeated upon completion of the 10-d acclimation period. For a given participant, the order of the exercise tests was the same as in the pre-acclimation period. The six exercise tests for each participant were held at the same time of the day (± 1 hour). The daily training sessions were also performed at the same time of the day (± 1 hour).

4.1.3. Heat Acclimation

HeA was achieved with the controlled-hyperthermia technique (isothermal clamping) (Fox et al., 1963). During all HeA exposures, a combination of exercise (metabolic) and heat stress was used to elevate and maintain a target T_{re} . The HeA protocol consisted of ten 90-min daily sessions of cycle ergometry starting at a workload corresponding to 50% of participants' normoxic W_{peak} so that the target T_{re} of 38.5 °C was reached within 30-45 min of exercise (Gibson et al., 2017). Once the target T_{re} was attained, power was adjusted every 5 min ($\pm 10\%$ W_{peak}) to maintain T_{re} at the desired level. Participants were instructed to drink 500 mL of water two hours before training, but had no access to food and water during the training sessions. Fluid-regulatory strain that comes from permissive dehydration is known to improve functional adaptations observed after a HeA protocol (Garrett et al., 2014).

Participants performed their training in the climatic chamber (IZR d.o.o., Škofja Loka, Slovenia). The average values for T_a , RH and atmospheric pressure in the environmental chamber during the training sessions were 35.0 ± 0.1 °C, $56.4 \pm 3.2\%$ and 689 ± 2 mmHg, respectively. Ambient air was flushed inside the chamber during the training sessions to ensure normoxic and normocapnic conditions. The wind speed was less than $0.5 \text{ m} \cdot \text{s}^{-1}$. Resting values of S_pO_2 and HR as well as tympanic temperature were recorded 15 min before each training session. T_{re} was measured continuously during the training sessions using a rectal thermistor probe (MSR 12, Henggart, Switzerland) inserted 10 cm beyond the anal sphincter. Exercise HR and S_pO_2 were assessed using a finger pulse oximeter (Wristox 3100 Nonin, Plymouth, Massachusetts, USA) at 5-min and 10-min intervals, respectively. RPE were recorded at 10-min intervals. During the training sessions participants exercised on mountain bikes mounted on resistance trainers (Elite Cycling, Italy). Each participant also conducted five of the training sessions on a mechanically braked cycle ergometer (Monark Ergomedic 884E, Sweden), so that the exact exercise

workload during the training sessions could be recorded. Participants trained in groups of 2 or 4 under strict supervision of the researchers.

4.1.4. Blood Sampling and Biochemical Analysis

Blood samples were obtained from an antecubital vein on the first day of exercise testing both before and after HeA. The participants were overnight fasted prior to the procedure and rested in a seated position for at least 10 min prior to the blood draw. Samples were analysed for haematocrit, haemoglobin, ferritin and reticulocyte concentration and erythrocyte count using an automated laser-based haematology analyser ADVIA 120 (Siemens, Munich, Germany). Additional blood samples were also immediately stored in BD Vacutainers (K2E, Becton–Dickinson, New Jersey, USA) and centrifuged (10 min at 3,000 rpm). The obtained plasma was frozen to -80°C and stored for further analysis. Erythropoietin (EPO), HIF-1 α , HSP72 and HSP90 concentrations were determined using sandwich enzyme-linked immunoassays. Optical density was quantified on a microplate reader Biotek Elx808TM (Biotek Instruments, Winooski, VT, USA) set at 450 nm and corrected at 630 nm. Renin and aldosterone concentrations were determined in 50 μl of plasma using a solid phase ELISA based on the competition principle (IVD, Aldosterone/Renin ELISA, IBL, Germany). The quantification of the optical density was performed on a microplate reader Biotek Elx808TM (Biotek Instruments, Winooski, VT, USA) set at 450 nm. All techniques were performed in accordance with the protocol provided by the manufacturer. Samples and standards were assayed in duplicate, except for HSP72. The estimated coefficients of variation (%) for the analysis were 12, 6.5, 2.7, 5.6 and 18.4% for HIF-1 α , HSP90, renin, aldosterone and EPO, respectively. The sensitivity of the measurements was $0.156\text{ ng} \cdot \text{mL}^{-1}$, $0.09\text{ ng} \cdot \text{mL}^{-1}$, $0.05\text{ ng} \cdot \text{mL}^{-1}$, $5.7\text{ pg} \cdot \text{mL}^{-1}$, $0.81\text{ pg} \cdot \text{mL}^{-1}$ and $1.1\text{ mIU} \cdot \text{mL}^{-1}$ for HIF-1 α , HSP72, HSP90, renin, aldosterone and EPO, respectively. PV changes were determined according to the recommendations of Strauss et al. (1951).

4.1.5. Exercise Testing

All the pre- and post-acclimation exercise trials were conducted in a laboratory situated at 300 m above sea level (Ljubljana, Slovenia). The trials were conducted on a cycle ergometer (Daum, Electronic, Furth, Germany) and comprised two stages; a 30-min steady-state exercise immediately followed by an incremental exercise to exhaustion. During exercise, each participant pedalled at a preferred cadence (between 60 and 90 rpm), which they maintained via visual and verbal feedback throughout the trial. Before (Pre) and after (Post) the 10-d HeA, participants conducted three trials on the three consecutive days immediately preceding and following HeA protocol. In the normal temperature and normoxic condition (NOR), participants breathed room air ($P_{\text{iO}_2} = 143 \pm 1\text{ mmHg}$, $F_{\text{iO}_2} = 20.8 \pm 0.1\%$) and exercised in thermoneutral conditions ($T_{\text{a}} = 23.0 \pm 0.1^{\circ}\text{C}$ and $\text{RH} = 50.9 \pm 0.9\%$). In the hypoxic condition (HYP), they inspired a hypoxic gas mixture ($P_{\text{iO}_2} = 92 \pm 3\text{ mmHg}$, $F_{\text{iO}_2} = 13.3 \pm 0.5\%$) and exercised in thermoneutral conditions ($T_{\text{a}} = 23.0 \pm 0.01^{\circ}\text{C}$ and $\text{RH} = 50.5 \pm 0.5\%$). In the hot condition (HE), the participants inspired room air ($P_{\text{iO}_2} = 142 \pm 1\text{ mmHg}$, $F_{\text{iO}_2} = 20.7 \pm 0.1\%$), but exercised in a hot environment ($T_{\text{a}} = 34.97 \pm 0.02^{\circ}\text{C}$ and $\text{RH} = 50.02 \pm 0.62\%$). As stated earlier, the order of the exercise trials was randomized and counterbalanced. Body composition was assessed once during the 10-d HeA protocol using DEXA (Hologic Discovery QDR, Hologic Inc., Bedford, Massachusetts, USA).

A metabolic cart (Quark CPET, Cosmed, Rome, Italy) was used to obtain the breath-by-breath respiratory responses during the exercise trials. Before each trial, the oxygen and

CO₂ analysers were calibrated using two different gas mixtures, and the pneumotachograph was calibrated with a 3-L syringe, in accordance with the manufacturer's recommendation.

HR was derived from electrocardiographic recordings with electrodes in a precordial position. CO was determined using electrical impedance cardiography (Physioflow Q-Link, Manatec Biomedical, Paris, France). The method measures changes in thoracic impedance during cardiac ejection to estimate SV. Six electrodes were placed at the base of the neck and on the xiphoid process. Electrical impedance cardiography has been validated against the direct Fick method during maximal incremental exercise in healthy participants (Siebenmann, Rasmussen, et al., 2015). S_pO₂ was recorded using a fingertip oximeter (Nellcor, BCI 3301, Boulder, USA). Participants were instructed to relax their hands during measurement of S_pO₂. RPE was obtained using a Borg scale (6-20). During all trials the participants wore only shorts and athletic shoes. The body mass was measured before and after each exercise testing on a weighing scale (± 0.05 kg) (TPT 5N, Libela ELSI, Celje, Slovenia).

Skin temperatures were measured at four sites (chest, arm, thigh and calf) using thermistors (MSR 12, Henggart, Switzerland) attached to the skin with a single piece of adhesive tape. The assessment of calf, thigh, chest and arm skin temperature enabled the calculation of T_{sk} (Ramanathan, 1964). The ventral fingertip and forearm temperatures were also measured. ΔT_{f-f} has been validated as an index of vasomotor tone during steady-state exercise (Keramidas et al., 2013). Minute mass flow of secreted sweat ($\dot{m}_{sw}$, g · m⁻² · min⁻¹) was measured with a ventilated capsule placed on the forehead. Forced evaporation of sweat under the capsule (surface area 4.8 cm²) was achieved by a constant flow of air (1.0 L · min⁻¹) through the capsule. Minute mass flow of secreted sweat was estimated each minute from the difference between the temperature and the humidity of inflowing and outflowing air. Air temperature was measured with thermistors (LM35, National Semiconductor Corp., Santa Clara, CA, USA) and the RH with capacitance hygrometers (Valvo air humidity sensor, Valvo-Philips GmbH, Hamburg, Germany).

4.1.6. Steady-State and Incremental Exercise

Following a 2-min rest period, participants commenced pedalling on the cycle ergometer. During the first 2 min the load was set at 90 W (warm-up). Thereafter, they cycled for 30 min at a steady-state work rate equivalent to 40% of their NOR W_{peak} attained during the preliminary testing. This intensity was chosen on the basis of our previous experience that most participants are unable to complete 30 minutes of cycle ergometry while exercising in hypoxic conditions at an absolute workload equal to 50% NOR W_{peak} (Kounalakis et al., 2012). In order to avoid differences in exercise heat production, the absolute and not the relative external workload was the same in all three exercise trials (the same power output was employed for a specific participant across environmental conditions, see also discussion). Our experimental design permitted that each participant served as their own control to eliminate variation in other factors that are shown to affect T_c and the sweating response and arise when using a between-subject experimental design, such as body surface area, body mass and adiposity (Cramer & Jay, 2014). Immediately following the 30-min steady-state exercise protocol, participants performed an incremental-load exercise to exhaustion. The initial work rate was set at 100 W (2 min), thereafter increasing by 20 W every minute until volitional exhaustion (inability to maintain the cycling cadence above 60 rpm). During each trial, S_pO₂ and sweat rate data were recorded at minute intervals whereas HR, CO and SV were measured every 10 s but averaged to minute intervals. RPE values were recorded at 5-min and 2-min intervals during the steady-state and the incremental-load exercise, respectively. Attainment of $\dot{V}O_{2max}$, defined as the highest $\dot{V}O_2$ averaged over 60s, was confirmed when participants met at least three of the following

criteria, listed in order of priority: (a) severe fatigue or exhaustion leading to inability to maintain exercise at a given work rate (cycling cadence lower than 60 rpm), (b) a plateau in $\dot{V}O_2$, as indicated by the breath-by-breath values, despite an increase in power output (c) a subjective RPE near maximal (Borg scale rating greater than 17) and (d) respiratory quotient greater than 1.10. W_{peak} was calculated according to the following equation:

$$W_{\text{peak}} = \text{work rate of last stage completed} + [(\text{work rate increment}) * (\text{time into current stage}/60)]$$

4.1.7. Data Analysis

Statistical analyses were performed using Statistica 5.0 (StatSoft, Tulsa, OK). Cardiorespiratory and temperature data collected during steady-state exercise were analysed for the last twenty minutes of the steady-state exercise, unless stated otherwise. A two-way ANOVA (Environmental condition: NOR-HYP-HE; HeA: pre-post) with repeated measures was used to define the effect of acclimation on exercise responses in each environmental condition. When ANOVA revealed a significant F-ratio for interaction and/or main effect, pairwise comparisons were performed with Tukey honestly significant difference post hoc test. Haematological variables were analysed using paired t-tests. In the event that the statistical analysis revealed a non-normal distribution of the data, a nonparametric test was performed. Such was the case for HSP72 plasma concentration values, which were analysed using the Wilcoxon non-parametric test. RPE and gains of the sweating response (relative change in $\dot{m}_{\text{sw}}$ as a function of the relative change in T_{re}) were analysed using the non-parametric Friedman test. The sweat rate and temperature data were analysed only for the steady-state exercise. Least square linear regression was used to determine the mean T_{re} threshold for initiation of sweating, as well as the gain of the sweating response. The reported values are averages of the individual thresholds and slopes derived from linear regression parameters. Vasodilation thresholds were identified using $\Delta T_{\text{f-f}}$ inflection points (House & Tipton, 2002). The ventilatory threshold was defined as the $\dot{V}O_2$ that corresponded to the departure of carbon dioxide production ($\dot{V}CO_2$) from a line of identity drawn through a plot of $\dot{V}CO_2$ vs. $\dot{V}O_2$ (V-slope method) (Beaver, Wasserman, & Whipp, 1986). Data are presented as mean $\pm$ SD unless indicated otherwise. The alpha level of significance was set *a priori* at 0.05. For the parameters with statistical differences, the observed statistical analysis power revealed by the repeated measures ANOVA with an alpha set at 0.05 ranged between 0.80 and 1.

4.2. Results

4.2.1. Heat Acclimation

All twelve participants successfully completed HeA protocol, as well as all of the pre- and post-HeA exercise trials. The average T_{re} was not different between training sessions ($P = 0.23$) ranging between $38.4\text{ }^{\circ}\text{C}$ and $38.6\text{ }^{\circ}\text{C}$ (Table 4.1). The average HR remained unchanged ($P = 0.39$) for all the training sessions. The workload accomplished ranged between 30 ± 6 (day 3) and $34 \pm 4\%$ normoxic W_{peak} (day 7) ($P = 0.98$). There was a significant increase ($P = 0.013$) in the whole body sweat rate from day 1 ($1.6 \pm 0.6\text{ L} \cdot \text{h}^{-1}$) to day 10 ($1.9 \pm 0.7\text{ L} \cdot \text{h}^{-1}$).

4.2.2. Steady-State Exercise

4.2.2.1. Cardiorespiratory Responses and Ratings of Perceived Exertion (RPE)

HR was lower ($P < 0.001$) post-HeA by $8.7 \pm 5.8\%$ in NOR, $6.6 \pm 4.6\%$ in HYP and $10.0 \pm 5.3\%$ in HE. HR was consistently lower ($P < 0.001$) in NOR compared to both HYP and HE, the difference ranging from 10 to 20 bpm. Post-HeA, HR was higher ($P < 0.05$) in HE than in HYP. No main effect or interaction was observed in CO ($P = 0.71$) or SV ($P = 0.33$) (Table 4.2). Figure 4.1 presents percentage changes from pre-HeA of several cardiorespiratory parameters in all environmental conditions. The exercise economy, defined as the reciprocal of $\dot{V}O_2$ at the standardized workload ($40\% W_{peak}$) performed during the steady-state exercise, was higher ($P = 0.003$) post-HeA across environmental conditions (respective changes in submaximal $\dot{V}O_2$ in NOR: $-6.3 \pm 3.7\%$, HYP: $-3.9 \pm 5.8\%$, HE: $-6.2 \pm 5.7\%$, Figure 4.1). The ventilatory threshold increased in NOR pre- to post-HeA ($+6.8 \pm 11.3\%$; $P = 0.04$) from 39.4 ± 5.8 to $41.7 \pm 4.8\text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (Figure 4.1).

$P_{ET}\text{ CO}_2$ was higher ($P < 0.001$) in NOR than in HYP and HE over HeA (Table 4.2). Across HeA status, $\dot{V}_E$ was elevated by $\sim 10\text{ L} \cdot \text{min}^{-1}$ in HYP ($P < 0.001$) compared to both NOR and HE (Table 4.2). Nevertheless, $\dot{V}_E$ was lower by $\sim 3.5\text{ L} \cdot \text{min}^{-1}$ post-HeA ($P < 0.01$) across environmental conditions. The S_pO_2 levels were lower ($P < 0.001$) in HYP compared to both NOR and HE independent of the acclimation state averaging $\sim 78\%$ in HYP and $\sim 95\%$ in NOR and HYP (Table 4.2).

Table 4.1: Average daily exercise responses during HeA (n=12).

Repeated measures one-way ANOVA was performed

	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	p-value
VO ₂ max (l·min ⁻¹)	38.6±0.2	38.5±0.2	38.5±0.1	38.5±0.1	38.6±0.2	38.5±0.1	38.5±0.1	38.4±0.2	38.5±0.1	38.5±0.1	0.23
HR (b·min ⁻¹)	141±12	139±11	135±11	141±13	138±14	141±11	139±12	141±9	140±11	138±10	0.39
Load (kg)	112±14	-	108±24	-	117±17	-	121±21	-	117±18	114±20	0.98
Load (W)	31±3	-	30±6	-	33±6	-	34±4	-	31±3	31±5	0.98
Exercise rate (kg·min ⁻¹)	75.8±6.7	75.7±6.6	75.8±6.3	75.8±6.8	75.8±6.3	75.9±6.7	75.7±6.3	75.3±6.6	75.2±6.2	75.5±6.1	0.22
Body rate (l·min ⁻¹)	1.6±0.6	1.6±0.5	1.7±0.8	1.7±0.6	1.8±0.5	1.8±0.5	1.8±0.4	1.8±0.7	1.8±0.5	1.9±0.7	0.013

Table 4.2: Cardiorespiratory responses and RPE in the last 20 of the 30 minutes of constant-work rate cycling before and after heat acclimation for the normoxic, the hypoxic and the heat trial (n = 12).

	Pre-heat acclimation			Post- heat acclimation		
	NOR	HYP	HE	NOR	HYP	HE
HR (bpm)	136 ± 10 ^{#†}	153 ± 8	150 ± 9	123 ± 8 ^{*#†}	143 ± 6 ^{*#}	134 ± 10 [*]
CO (L · min ⁻¹)	16.2 ± 1.7	17.9 ± 3.4	16.9 ± 2.2	15.6 ± 1.8	17.2 ± 2.6	16.9 ± 1.7
SV (mL)	121 ± 19	117 ± 23	113 ± 16	128 ± 16	120 ± 17	127 ± 16
VO ₂ (mL · min ⁻¹)	2334 ± 224	2314 ± 266	2285 ± 191	2185 ± 209 [*]	2219 ± 254 [*]	2162 ± 250 [*]
GME (%)	18.1 ± 1.0	18.5 ± 0.8	18.6 ± 1.5	19.4 ± 1.2 [*]	19.2 ± 1.2	19.5 ± 1.2 [*]
VT (mL · kg ⁻¹ · min ⁻¹)	39.4 ± 5.8 ^{#†}	33.0 ± 3.0 [#]	35.7 ± 3.5	41.7 ± 4.8 ^{*#†}	32.9 ± 3.6 [#]	36.8 ± 4.4
P _{ET} CO ₂ (mmHg)	43 ± 2 ^{#†}	36 ± 2 [#]	40 ± 1	42 ± 2 ^{#†}	37 ± 2	39 ± 2
V _E (L · min ⁻¹)	55.1 ± 7.7 [†]	66.9 ± 10.5 [#]	56.6 ± 5.7	51.5 ± 8.3 ^{*†}	63.2 ± 10.1 ^{*#}	52.7 ± 8.1 [*]
S _p O ₂ (%)	95.0 ± 0.8 [†]	78.4 ± 4.2 [#]	94.6 ± 0.8	95.6 ± 0.8 [†]	77.4 ± 4.9 [#]	95.1 ± 0.6
RPE	11 (9.5-14) ^{#†}	12.5 (12-15)	13 (10-14)	10.5 (9-13) [#]	11(9.5-13.5) ^{*#}	10 (8-11) [*]

Values are mean ± SD except for RPE median (range), VT ventilatory threshold

* Significant difference to values before heat acclimation

Significant difference to heat values

† Significant difference to hypoxic values, p < 0.05.

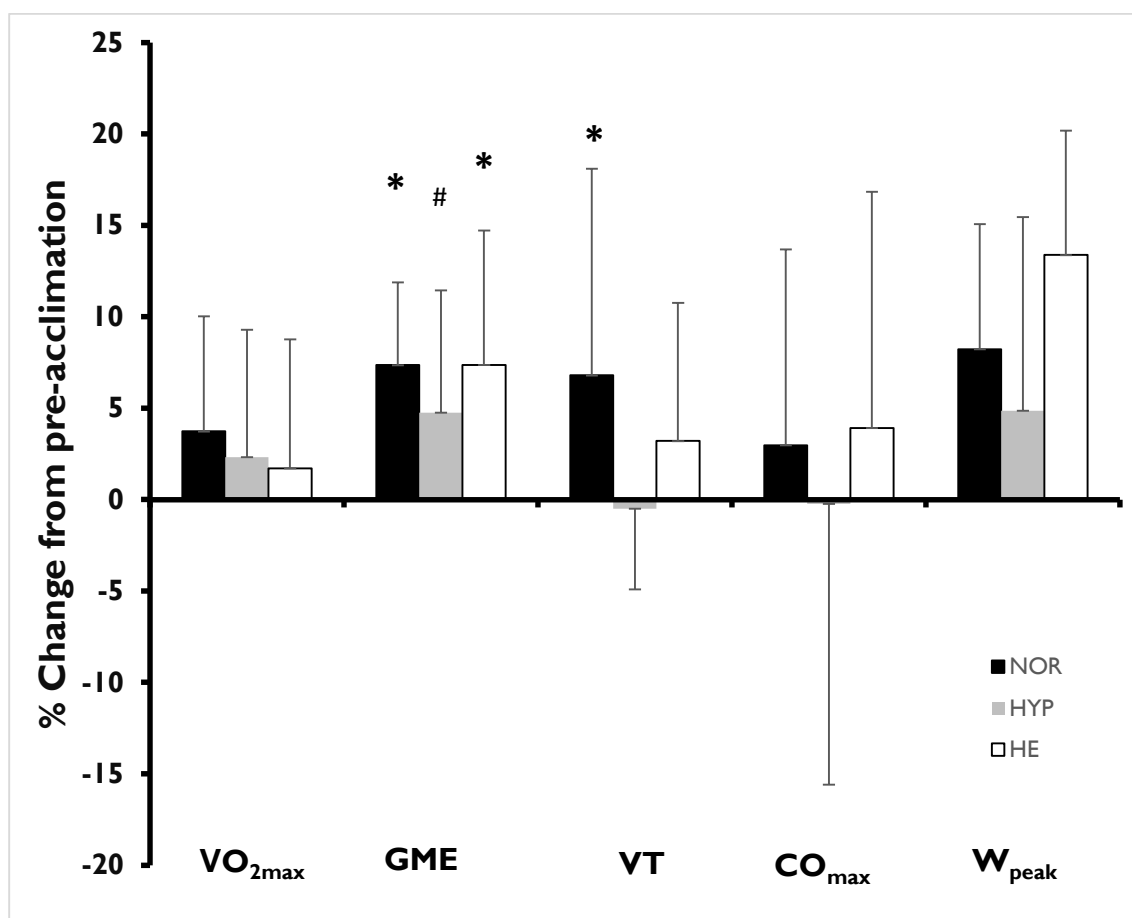


Figure 4.1: Cardiorespiratory and performance variables as percentage changes from pre-acclimation. Values are means $\pm$ SD. VO_{2max} maximal aerobic power, GME gross mechanical efficiency, VT ventilatory threshold, CO_{max} maximal cardiac output, W_{peak} peak power output. * $P < 0.05$, # $P = 0.07$ vs. pre-HeA within environmental condition.

4.2.2.2. Thermoregulatory Responses

Resting T_{re} was lower ($P < 0.01$) post-HeA by 0.3 ± 0.3 °C, 0.3 ± 0.3 °C and 0.4 ± 0.3 °C in NOR, HYP and HE, respectively (Table 4.3). However, during exercise there was no

main/interaction effect ($P = 0.63$) in ΔT_{re} , averaging ~ 0.5 °C in HE and HYP and ~ 0.6 °C in NOR. T_{sk} was higher ($P < 0.001$) in HE over HeA (Table 4.3). Even though there was a main effect of HeA on T_{sk} ($P < 0.01$), the post-hoc tests did not show any differences ($P > 0.05$). No main effect or interaction was observed in the ΔT_{f-f} response ($P = 0.43$), which ranged from -0.6 ± 0.7 °C in NOR post-HeA to 0.2 ± 0.7 °C in HE, again post-HeA.

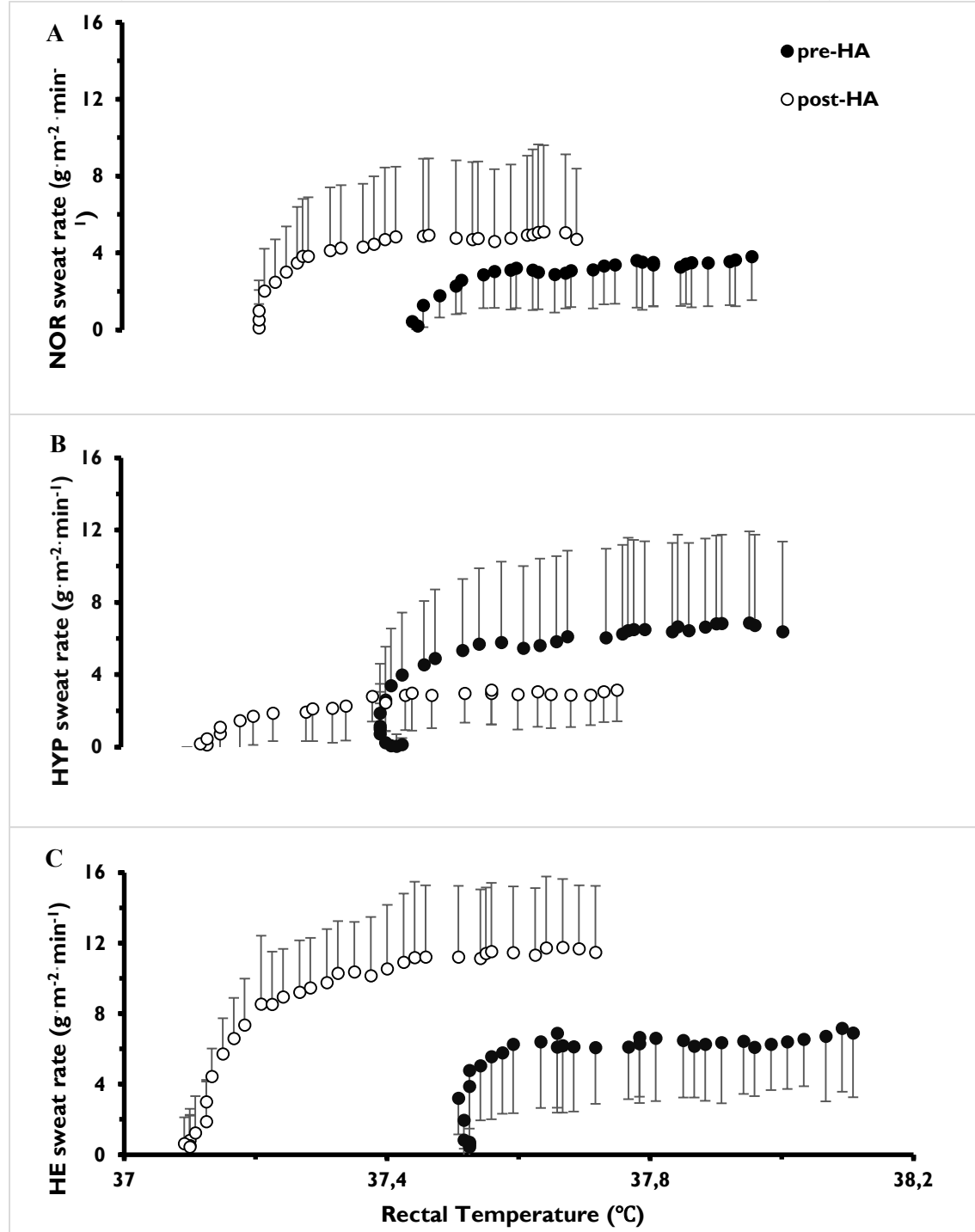


Figure 4.2: Mean changes in the rate of forehead sweating plotted as a function of changes in T_{re} , in (A) normoxic, (B) hypoxic and (C) heat trials both before (●) and after (○) heat acclimation protocol.

Table 4.3: Thermoregulatory responses at rest and during the last 20 of the 30 minutes of the constant-work rate cycling before and after heat acclimation for the normoxic, the hypoxic and the heat trials ($n = 12$).

	Pre-heat acclimation				Post-heat acclimation			
	NOR		HYP		HE		NOR	
$T_{re\ rest} (^{\circ}C)$	37.5 ± 0.4		37.4 ± 0.3		37.5 ± 0.3		$37.2 \pm 0.4^*$	
$\Delta T_{re} (^{\circ}C)$	0.5 ± 0.1		0.6 ± 0.2		0.6 ± 0.2		0.5 ± 0.1	
$T_{sk} (^{\circ}C)$	$34.4 \pm 0.6^{\#}$		$34.2 \pm 0.8^{\#}$		36.7 ± 0.5		$34.0 \pm 0.6^{\#}$	
$\Delta T_{if} (^{\circ}C)$	-0.5 ± 1.0		-0.3 ± 1.7		0.1 ± 1.0		-0.6 ± 0.7	
$H_{prod} (W)$	656 ± 59		640 ± 74		635 ± 48		$602 \pm 59^*$	
$H_{prod} (W \cdot m^{-2})$	334 ± 19		325 ± 25		323 ± 21		$307 \pm 23^*$	
$H_{prod} (W \cdot kg^{-1})$	8.7 ± 0.6		8.5 ± 0.6		8.4 ± 0.7		$8.0 \pm 0.6^*$	
$\dot{m}_{sw, peak} (g \cdot m^{-2} \cdot min^{-1})$	$3.8 \pm 2.3^{\# \dagger}$		7.3 ± 5.2		8.2 ± 3.4		5.4 ± 3.7	
$\dot{m}_{sw} \text{ gain } (g \cdot m^{-2} \cdot min^{-1} \cdot ^{\circ}C^{-1})$	$8.2 \pm 5.2^{\#}$		$11.8 \pm 8.5^{\#}$		25.2 ± 8.2		14.5 ± 11.9	
Sweating Threshold	37.5 ± 0.4		37.4 ± 0.3		37.5 ± 0.3		$37.2 \pm 0.4^*$	
$T_{re} (^{\circ}C)$					0.3			
Vasodilation	37.5 ± 0.4		37.4 ± 0.3		$-$		$37.2 \pm 0.4^*$	
Threshold $T_{re} (^{\circ}C)$					$-$			

T_{re} resting rectal temperature, ΔT_{re} difference from resting value in rectal temperature, T_{sk} weighted mean skin temperature, ΔT_{f-f} difference between forearm and fingertip skin temperatures, H_{prod} metabolic heat production, $\dot{m}_{sw}$ sweating rate. Values are mean $\pm$ SD.

* Significant difference to values before heat acclimation

Significant difference to heat values

† Significant difference to hypoxic values, $p < 0.05$.

Exercise $\dot{m}_{sw}$ increased in all conditions ($P < 0.001$) and reached asymptotic values ~ 5 -10 min before the end of the steady-state exercise. Figure 4.2 presents the $\dot{m}_{sw}$ responses relative to the changes in T_{re} . Sweating and vasodilation thresholds were identical and lower ($P < 0.01$) post-HeA in all environmental conditions (Table 4.3).

4.2.3. Incremental Exercise to Exhaustion

Despite a main effect for HeA ($P = 0.029$), the changes observed in $\dot{V}O_{2max}$ in NOR (+3.5%, $P = 0.29$) and HE (+3.2%, $P = 0.46$) did not reach statistical significance (Figure 4.1). $\dot{V}O_{2max}$ was significantly higher in NOR compared to HYP ($P < 0.001$) and HE ($P < 0.01$) and in HE compared to HYP ($P < 0.001$) across HeA (Figure 4.3, Table 4.4).

Table 4.4: Maximal cardiorespiratory responses and peak power output (mean $\pm$ SD) during the incremental exercise to exhaustion before and after HeA for the normoxic, the hypoxic and the heat trial ($n = 12$).

Unlike $\dot{V}O_{2\max}$, clear improvements ($P < 0.001$ for factor HeA, $P = 0.001$ for interaction effect) in W_{peak} were induced by HeA protocol in NOR ($P < 0.001$) and HE ($P < 0.001$) (Figure 4.1, Table 4.4). In particular, W_{peak} was elevated after HeA by 26 ± 22 ($+8.2 \pm 6.9\%$) and 41 ± 21 W ($+13.4 \pm 6.8\%$) in NOR and HE, respectively. It is of note that all participants increased their W_{peak} in HE, and none decreased in NOR with 9 participants increased and 3 maintaining (Figure 4.3). In HYP, W_{peak} was not affected by HeA ($+4.9 \pm 10.6\%$, $P = 0.14$).

	Pre-heat acclimation			Post-heat acclimation		
	NOR	HYP	HE	NOR	HYP	HE
$\dot{V}O_{2\max}$ ($\text{mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	$54.7 \pm 5.7^{\# \dagger}$	$44.0 \pm 4.3^{\#}$	49.8 ± 5.0	$56.7 \pm 6.1^{\# \dagger}$	$44.9 \pm 3.6^{\#}$	51.4 ± 5.6
$V_{E \text{ peak}}$ ($\text{L} \cdot \text{min}^{-1}$)	171 ± 24	169 ± 28	152 ± 27	180 ± 22	177 ± 22	171 ± 26
W_{peak} (W)	$336 \pm 40^{\# \dagger}$	$282 \pm 28^{\#}$	306 ± 24	$363 \pm 38^{\# \dagger}$	$294 \pm 26^{\#}$	$347 \pm 34^*$
$HR_{\max}$ (bpm)	190 ± 6	$187 \pm 8^{\#}$	193 ± 8	$187 \pm 7^{\dagger}$	$182 \pm 8^{\#}$	190 ± 9
$CO_{\max}$ ($\text{L} \cdot \text{min}^{-1}$)	24.2 ± 3.6	23.5 ± 3.9	24.2 ± 3.2	24.8 ± 3.6	23.2 ± 3.7	25.0 ± 3.4
$CO_{\max} / W_{\text{peak}}$ ($\text{mL} \cdot \text{min}^{-1} \cdot \text{W}^{-1}$)	$72.3 \pm 9.3^{\dagger}$	83.7 ± 12.2	79.0 ± 8.4	$68.4 \pm 7.4^{\dagger}$	78.6 ± 7.8	$72.1 \pm 8.0^*$
$SV_{\max}$ (mL)	133 ± 22	134 ± 24	130 ± 21	146 ± 21	137 ± 21	142 ± 22

$\dot{V}O_{2\max}$ maximal aerobic power, $V_{E \text{ peak}}$ peak respiratory rate, W_{peak} peak power output, $HR_{\max}$ maximal heart rate, $CO_{\max}$ maximal cardiac output, $SV_{\max}$ maximal stroke volume. Values are mean $\pm$ SD.

* Significant difference to values before heat acclimation

Significant difference to heat values

† Significant difference to hypoxic values, $p < 0.05$

No main effect or interaction was observed in $CO_{\max}$ ($P = 0.55$) (Table 4.4, Figure 4.1). Maximal HR was lower post-HeA in HYP ($P = 0.01$) (Table 4.4). Furthermore, maximal HR in HYP was lower than in NOR ($P = 0.01$) and HE ($P < 0.001$) post-HeA and also lower than in HE pre-HeA ($P < 0.001$). Even though there was a main effect of HeA on maximal SV ($P = 0.023$), the post-hoc tests displayed that the average increases of 13 and 12 mL observed pre- to post-HeA in NOR and HE, respectively, were not statistically significant. When we expressed $CO_{\max}$ normalized to W_{peak} , there was a tendency for the

HE values to be lower post-HeA ($P = 0.089$) (Table 4.4). Moreover, CO_{max} normalized to W_{peak} was consistently higher in HYP than NOR ($P < 0.01$).

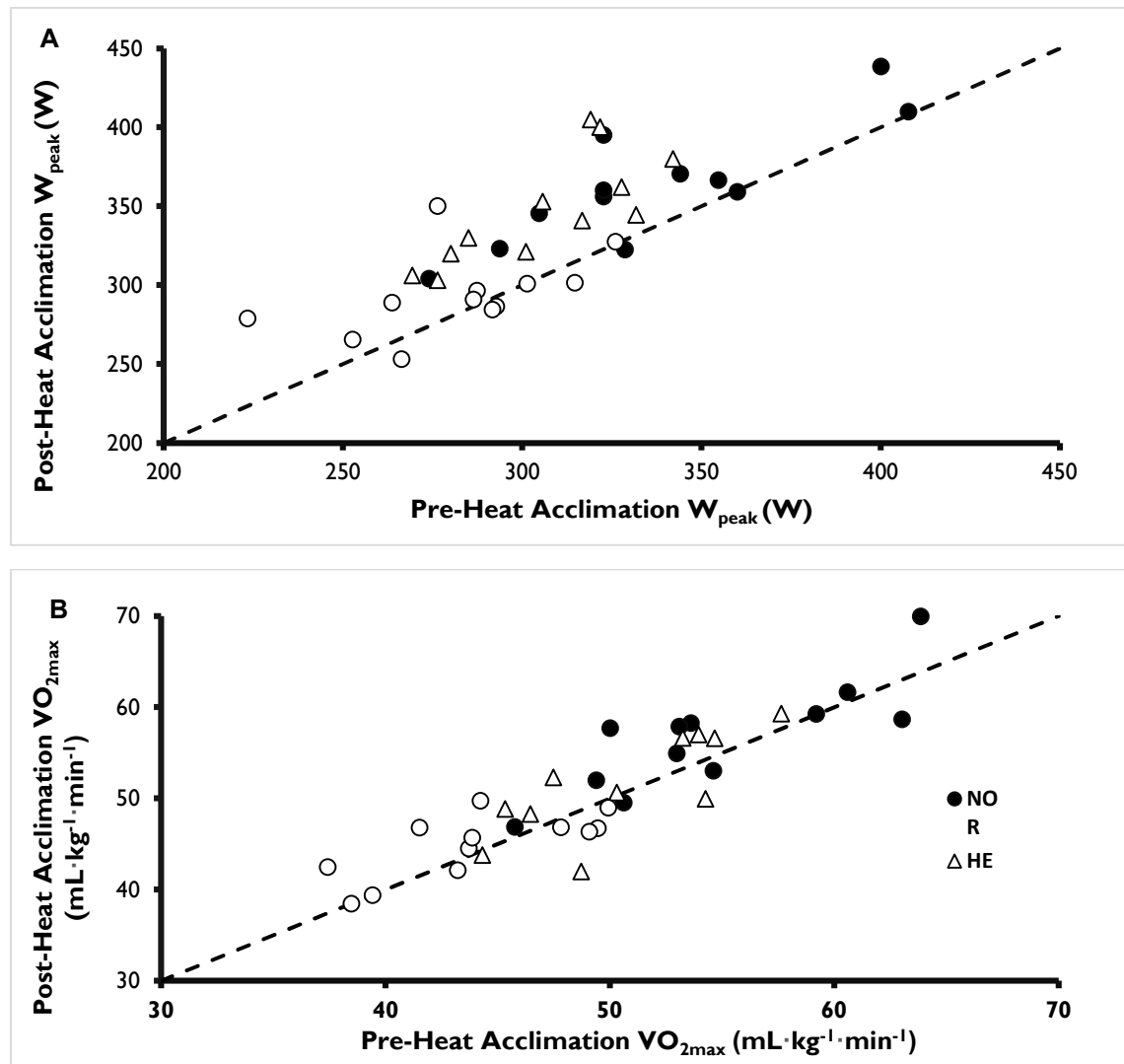


Figure 4.3: Pre- and post-acclimation individual data for performance variables in NOR (●), HYP (○) and HE (△). (A) Peak Power Output (W_{peak}), and (B) maximal aerobic power ($\text{VO}_{2\text{max}}$). The dashed line represents the line of equality.

4.2.4. Hematological Data

HeA induced significant overall changes in the hematological variables (Table 4.5). As expected, PV expansion was noted in 10 out of 12 participants resulting in an average expansion of +3.7%. The exception was one participant, in whom PV contracted by -7.7%. The PV expansion led to a decreased concentration of erythrocytes, hemoglobin, ferritin and reticulocytes. HIF-1 α concentration and hematocrit tended to decrease post-HeA. No changes were observed in the renin-aldosterone-axis or EPO. Surprisingly, HSP90 plasma

concentration was reduced post-acclimation while HSP72 plasma concentration did not change.

Table 4.5: Resting hematological variables pre- and post-heat acclimation (n=12).

	Pre-heat acclimation	Post-heat acclimation	p-value
Erythrocytes ($10^{12} \cdot \text{L}^{-1}$)	5.0 ± 0.3	4.8 ± 0.4	0.011
Hemoglobin ($\text{g} \cdot \text{L}^{-1}$)	151.0 ± 8.0	145.9 ± 9.1	0.048
Hematocrit (%)	43 ± 2	42 ± 2	0.086
Ferritin ($\mu\text{g} \cdot \text{L}^{-1}$)	66.1 ± 39.4	45.6 ± 24.7	0.008
Reticulocytes ($10^9 \cdot \text{L}^{-1}$)	72.3 ± 14.6	58.7 ± 10.9	0.029
Plasma EPO ($\text{mIU} \cdot \text{mL}^{-1}$)	9.5 ± 7.3	12.1 ± 14.1	0.30
HIF-1 α ($\text{ng} \cdot \text{mL}^{-1}$)	2.1 ± 1.5	2.0 ± 1.6	0.056
HSP72 ($\text{ng} \cdot \text{mL}^{-1}$)	0.7 (0.4-3.3)	0.6 (0.5-3.0)	0.906
HSP90 ($\text{ng} \cdot \text{mL}^{-1}$)	44.1 ± 13.0	31.4 ± 11.6	0.005
Renin ($\text{pg} \cdot \text{mL}^{-1}$)	21.6 ± 13.5	19.1 ± 9.7	0.37
Aldosterone ($\text{pg} \cdot \text{mL}^{-1}$)	197 ± 170	232 ± 199	0.24
Plasma volume (%)	-	$+3.7 \pm 5.1$	-

EPO erythropoietin, HIF-1 α hypoxia-inducible factor 1 α , HSP72 heat shock protein 72, HSP90 heat shock protein 90. Values are mean $\pm$ SD except for HSP72 data which is presented as median (range).

4.3. Discussion

According to the theory of cross-adaptation, adaptation to heat may beneficially influence the exercise responses when humans are exposed to hypoxia. Investigation of the manner in which such cross-adaptation affects maximal aerobic power and exercise thermoregulatory function was the principal aim of this study. Our results demonstrate that a 10-day controlled-hyperthermia heat acclimation protocol did not increase maximal aerobic power in thermoneutral normoxic or hypoxic conditions. However, heat acclimation augmented the sweating response in normoxia and heat, but attenuated it in hypoxia. Exercise capacity, as assessed by W_{peak} , was improved in normoxic, but not hypoxic conditions. These data do not support a beneficial carry-over effect between training in the heat and subsequent responses in a hypoxic environment.

4.3.1. Heat Acclimation

Our data clearly show that the employed HeA protocol provoked most of the expected physiological adaptations while exercising in hot conditions; submaximal HR was lower (Table 4.2), resting and exercising T_{re} were lower (Table 4.3), higher whole body (Table 4.1) and forehead (Figure 4.2) sweat rates were observed as plasma and blood volume expanded, vasodilation, as indicated by $\Delta T_{\text{f-f}}$, and sweating were stimulated at a lower T_{c} (Table 4.3).

4.3.2. Cardiorespiratory Responses

There is an ongoing discussion regarding the ability of HeA to facilitate $\dot{V}O_{2\text{max}}$ and exercise performance in thermoneutral/cool conditions (Minson & Cotter, 2016; Nybo & Lundby, 2016). We did not find significant differences in $\dot{V}O_{2\text{max}}$ in NOR despite an expanded resting PV post-HeA. Given the fact that no changes have been reported in arteriovenous difference pre- to post-HeA (Nielsen, Strange, Christensen, Warberg, & Saltin, 1997), our finding that CO_{max} did not change ($23\text{--}25 \text{ L} \cdot \text{min}^{-1}$) after HeA could account for this result in agreement with others (Keiser et al., 2015). CO_{max} did not change as the lowered HR_{max} may have offset the marginally higher SV_{max} post-HeA across environmental conditions. An exercise intensity not lower than 90% HR_{max} is necessary for improvements in SV_{max} and subsequently convective oxygen delivery (Helgerud et al., 2007). Even if CO_{max} was elevated, it should also outweigh the hypervolemic hemodilution (151 vs $146 \text{ g hemoglobin} \cdot \text{L}^{-1}$) after HeA to increase $\dot{V}O_{2\text{max}}$ (Coyle et al., 1990). Interestingly, Lorenzo et al. (2010) mentioned an increase of 8.9% in NOR CO_{max} due to an increased SV_{max} and a 6.5% PV expansion using a traditional fixed work rate HeA protocol for 10 days. To our knowledge, this is the only well-controlled study to report a positive effect of HeA on $\dot{V}O_{2\text{max}}$ in NOR in a trained population whereas others report either trivial changes (Keiser et al., 2015; Rendell et al., 2017; Shvartz et al., 1977) or improvements in participants of lower fitness level (Sawka et al., 1985; Shvartz et al., 1977). From a coaching perspective, the two participants that entered the study with the highest fitness level ($\sim 63 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) displayed a very different response to HeA (one improved his $\dot{V}O_{2\text{max}}$ by 6 and the other impaired by $5 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ post-HeA, see Figure 4.3) verifying recent findings that the variance in HeA responses does not relate to aerobic capacity at the individual level (Corbett et al., 2018).

We also confirm no difference in $\dot{V}O_{2\text{max}}$ in HYP post-HeA (A. C. White et al., 2016). The fact that CO_{max} was not different between NOR and HYP can be attributed to the employed simulated altitude as even higher altitudes are required for CO_{max} to be acutely reduced (Calbet et al., 2003). As a result, $\dot{V}O_{2\text{max}}$ in HYP is reduced in the same proportion

that arterial oxygen content is reduced (Mollard, Woorons, Letournel, Lamberto, et al., 2007). Arterial oxygen content did not increase post-HeA as peak $\dot{V}_E$ and consequently S_pO_2 remained unchanged and hemoglobin concentration decreased due to PV expansion.

We are aware that the absence of a sport-specific performance measure (e.g. a time trial) poses a major limitation in our attempt to explore exercise capacity in the present study. Participants had to complete the post-HeA tests as soon as possible so that the decay of HeA-induced adaptations was minimized. Therefore, W_{peak} obtained from the graded $\dot{V}O_{2max}$ tests was used as a surrogate index of exercise performance. We report an improved W_{peak} in both NOR and HE. This further supports the majority of the studies on this topic (Lorenzo et al., 2010; Nielsen et al., 1993; Rendell et al., 2017). The trivial increases in $\dot{V}O_{2max}$ in HE and NOR (~4%) cannot account for the disproportional increases in W_{peak} . Moreover, HeA has been suggested by some (Sawka et al., 1983), but not all (Karlsen et al., 2015; Rendell et al., 2017) to improve the efficiency of oxidative phosphorylation at the cellular level. Indeed, our observed higher exercise economy could mediate W_{peak} improvements after HeA protocols not only in NOR and HE, but also in HYP (A. C. White et al., 2016). Thermoregulatory adaptations, initiated by elevated T_c , are present even during training in cool conditions. Therefore, the contribution of HeA *per se* on components of aerobic performance (e.g. exercise economy) remains very challenging to delineate. Past studies addressed this by employing a separate control group that undertook training in thermoneutral (Regan et al., 1996) or even cool conditions to suppress heat loss responses (Hessemer et al., 1986), but no data on exercise economy were presented. The limited effect of HeA on W_{peak} in HYP could be attributed to the low sensitivity of a graded test to exhaustion, i.e. a reduced signal-to-noise ratio with the signal being the improvement in W_{peak} (lower compared to NOR and HE) and the noise being the variability in the performance index as assessed by graded exercise tests (similar across conditions) (Currell & Jeukendrup, 2008). Furthermore, we do not believe that the higher relative workload in HYP (compared to NOR and HE) could nullify the performance improvements in that condition since all participants were exercising at an intensity lower than their ventilatory threshold (data not shown). As exercise performance was not the primary variable of interest in the present study and despite the relatively high fitness level of the participants, the absence of a matched control group or a lead-in period of supervised training as well as the higher exercise economy support the possibility of a training effect on W_{peak} . Even in the presence of a training effect, the differential effects of HeA between NOR and HYP (on W_{peak} and exercise economy) indicate a greater potential for the adaptive responses to HeA to prove beneficial in normoxic rather hypoxic conditions.

4.3.3. Thermoregulatory Responses

Both resting and exercise T_{re} were lower post-HeA independent of the environmental conditions. Such a reduction in T_{re} serves as a classic index of heat adaptation, reflecting improved heat loss, and has previously been reported in all controlled-hyperthermia HeA studies (Patterson et al., 2004; Rendell et al., 2017). Several characteristics of sudomotor function are susceptible to adaptation to the heat stressor. Firstly, sweating was initiated at a lower T_{re} post-HeA independent of the environmental condition, and higher sweat rates were sustained (except in HYP) throughout exercise (Figure 4.2). The lower T_{re} thresholds appear to be primarily related to lower resting T_{re} and may reflect direct hypothalamic effects of repeated T_c elevation (Shido, Sugimoto, Tanabe, & Sakurada, 1999). When expressing the sweating thresholds as relative changes in T_{re} (ΔT_{re}), no effect of HeA on the sweating thresholds was observed. Interestingly, the decrement in resting T_c observed after HeA has been claimed to benefit subsequent endurance performance in

thermoneutral conditions (Hessemer, Langusch, Bruck, Bodeker, & Breidenbach, 1984) demonstrating another possible mechanism for the observed increase in W_{peak} in NOR.

Moreover, the gain of the sweating response has been reported to increase in both NOR (Roberts et al., 1977) and HE (Patterson et al., 2004) after exercise-induced HeA protocols. The increase in the gain of sweating is thought to occur due to anatomical changes such as sweat gland hypertrophy (volume, length, secretory tubule diameter) – accompanied by functional changes, i.e. enhanced cholinergic sensitivity of the sweat glands (Sato & Sato, 1983). However, in our study the pre- to post-HeA differences in the gains of the sweating response were not statistically significant, possibly due to the rather high baseline aerobic fitness level of our participants (Nadel et al., 1974). Paradoxically, HeA protocol impaired the forehead sweating response in HYP (Figure 4.2b). Sweat redistribution toward the limbs does not compensate for the reduction in evaporative heat loss after HeA (Patterson et al., 2004). Central thermoregulatory mechanisms could regulate the magnitude of heat loss, such that the observed reduction of heat loss via evaporation is compensated by an increase in heat loss by conduction. The similar decreases in T_{re} and T_{sk} pre- to post-HeA observed in the present study did not affect the $T_{\text{re}}-T_{\text{sk}}$ gradient in sharp contrast to the widened gradient post-HeA reported in the results of Lorenzo et al. (2010). Thus, conductive heat loss was not augmented post-HeA in HYP.

We decided to match the absolute work rate between trials with the aim of maintaining a similar exercise-related heat production. Recently, Coombs et al. (2017) found no differences in the sweating responses during exercise in neutral versus hot hypoxic conditions. Their experimental design controlled for heat production, but not absolute work rate. The authors reasoned that heat production for a fixed workload is not the same between normoxia and hypoxia, because of an altered mechanical efficiency. In their study absolute workload did not differ by more than 3 W between trials matched for heat production. For all environmental conditions, the pre- and post-HeA submaximal $\dot{V}O_2$, that largely determines metabolic heat production, was the same. However, the post-HeA $\dot{V}O_2$ in all conditions was lower compared to the pre-HeA values. Body mass and surface area remained unaltered after HeA. Provided that the indices of metabolic heat production normalized to either body mass, or body surface area dictate changes in T_c and local sweat rate, respectively (Jay & Cramer, 2015), our decision may have slightly underestimated metabolic heat production, and thus could partially contribute to the observed attenuated sweating response in HYP post-HeA. However, our data also suggest an enhanced sweating response in NOR and HE as well as similar exercise-induced increases in T_{re} pre- to post-HeA (Table 4.3). Thus, metabolic heat production does not appear to be the sole determinant of thermoregulatory responses during exercise in humans.

4.3.4. Hematological Data

The decreased haematocrit and haemoglobin, erythrocyte, reticulocyte concentrations result from the HeA-elicited PV expansion. That baseline extracellular HSP72 remained unaltered after the 10-day HeA is in agreement with recent findings that used either the controlled-hyperthermia technique (Magalhaes Fde et al., 2010) or the traditional fixed-work rate protocol (Yamada, Amorim, Moseley, Robergs, & Schneider, 2007) and even corrected the concentration values for PV expansion. Of note, the same researchers reported a higher HSP72 intracellular concentration in leukocytes (Magalhaes Fde et al., 2010) or peripheral blood mononuclear cells (Yamada et al., 2007), in agreement with others (Lee et al., 2016), indicating that comparing results among studies with different sampling sites (intracellular vs extracellular) might be problematic. Nevertheless, the HSP response to HeA has been suggested to be biphasic (Horowitz, 1998), with elevated basal HSP72 levels only after long-term HeA (~30 days). As a result, longer acclimation protocols

should be employed to further explore the potential of both cellular and systemic adaptations in humans.

Using a similar HeA protocol as in the present study, Rendell et al. (2017) reported an increase in EPO concentration, which is in contrast to our observation of no change in EPO concentration after the 10-day HeA. However, our average values fall in the same range (9.5 vs 8.3 mIU · mL⁻¹ pre; 12.1 vs 10.1 mIU · mL⁻¹ post). The high interindividual variability of EPO in our data renders the difference non-significant. The increases in EPO concentration seem to be initiated by the PV expansion and a lowered hematocrit similarly to what happens after two weeks of endurance training (Montero et al., 2017). There are conflicting reports on changes in resting plasma aldosterone levels after HeA protocols (Kirby & Convertino, 1986; Nielsen et al., 1993). Unfortunately, we were not able to monitor the hormonal responses during exercise and the participants' diet during the period of HeA in the present study. Thus, we cannot account for the unaffected resting plasma aldosterone levels, despite a calculated PV expansion. On the other hand, the unchanged plasma renin concentration seems in accordance with previous findings (Nielsen et al., 1993). HSP90 upregulates steroid hormone receptors and stabilizes HIF-1 α under normoxic conditions. As a consequence, the lower HSP90 levels after HeA may have blunted the aldosterone response and could be linked with the lower HIF-1 α concentration after HeA. Future studies that aim at estimating HIF-1 α activity from plasma samples should instead measure downstream proteins such as VEGF, EPO, GLUT-1 or even HIF-1 α mRNA as HIF-1 α plasma values most likely reflect cell death and destruction.

Chapter 5

No Ergogenic Effects of a 10-Day Combined Heat and Hypoxic Acclimation on Aerobic Performance in Normoxic Thermoneutral or Hot Conditions

Hypoxic acclimation has been shown to enhance $\dot{V}O_{2\max}$ in hypoxic (Schuler et al., 2007), normoxic thermoneutral (Levine & Stray-Gundersen, 1997) and hot ([Chapter 3](#)) conditions. These improvements are possibly mediated by augmented red cell volume (Levine & Stray-Gundersen, 2005) that in cooperation with the PV (PV) contraction serves to increase arterial oxygen content. Similarly, HeA has also gained in popularity among athletes as a potential ergogenic aid in aerobic performance in hot conditions. There is a disagreement however, over whether such a protocol increases aerobic performance in normoxic thermoneutral conditions (Lorenzo et al., 2010; Rendell et al., 2017) or not (Karlsen et al., 2015; Keiser et al., 2015). Potential HeA-induced ergogenic mechanisms include PV expansion (Lorenzo et al., 2010), an increase in exercise economy (Sawka et al., 1983) and/or power output at the lactate threshold (Lorenzo et al., 2010; Rendell et al., 2017). Thus, the separate physiological mechanisms associated with heat and hypoxic acclimation might act synergistically to influence aerobic performance in thermoneutral or hot conditions following heat and hypoxic acclimation. Attempts have been made to address this hypothesis by several previous studies detailed below, and it is also the principal aim of the current study.

The potential improvements that can be derived from such a combined hypoxic and HeA are associated with either predominantly hematological and cardiorespiratory modifications in the case of the former, and thermoregulatory modifications in the case of the latter. With regards to aerobic performance, several recent studies have employed live high-train low concomitant with heat protocols to explore the effects of this promising synergistic interaction. Buchheit et al. (2013) recruited professional football players that completed sport-specific training sessions in the heat and slept either at normobaric hypoxia (F_{iO_2} between 14.3 and 15.2%) or normoxia over the course of 12 days. PV and performance on the Yo-Yo Intermittent Recovery test were substantially higher in both groups whereas the between-groups difference in Hbmass (+3.2%) was higher than the typical error of measurement post-acclimation. The main limitation of the study was that

no $\dot{V}O_{2\max}$ measurements were obtained and the session RPE was used as an index of the training load. Using a counterbalanced, cross-over design, Rendell et al. (2017) had trained cyclists undergo a controlled-hyperthermia protocol while sleeping overnight in normoxia and moderate hypoxia ($F_{iO_2} = 15.6\%$) for 11 days. Hbmass remained unchanged as PV expanded following HeA. Work done in a time trial (+4%), W_{peak} (+4%) and lactate threshold were higher following HeA with no further differences when individuals had been sleeping in hypoxia. Finally, McCleave et al. (2017) hypothesized that the superposition of heat interval training with a 3-week live-high train-low hypoxic protocol would cause an additive effect on aerobic performance in well-trained runners. Paradoxically, the average observed increase in Hbmass (+3.8%) did not translate into enhanced 3-km time trial performance. This lack of improvement was attributed to a negative psychological response and the inability of the selected performance test to reflect an improved aerobic capacity. Surprisingly, only Rendell et al. (2017) measured $\dot{V}O_{2\max}$, the main determinant of aerobic capacity, in normoxic thermoneutral conditions. Examination of the thermoregulatory responses following a combined heat and hypoxia acclimation protocol by McCleave et al. (2018) revealed no change in the core temperature (T_c) response, PV and sweat rate. Buchheit et al. (2013) reported unchanged sweat rate, but a lower HR in the group that was sleeping in hypoxia. In contrast, Rendell et al. (2017) stated that adaptation to the heat stressor was not negated by adding nocturnal hypoxia as reflected in lower rectal temperature (T_{re}) and skin temperature, lower HR and an expanded PV. The difference could be explained by an insufficient hypoxic dose applied by the latter group ([Chapter 7](#)). Alternatively, the participants of the former studies that employed traditional fixed-work rate HeA protocols may have experienced an ever-diminishing thermal adaptation impulse (N. A. Taylor, 2014). Thus, the straightforward option to explore an ergogenic combination of heat and hypoxic acclimation is to conduct a continuous hypoxic confinement daily interspersed by controlled-hyperthermia training sessions in the heat. Such an acclimation protocol could also mitigate the decrement in exercise capacity in hot hypoxic conditions as reflected in a lower time to exhaustion at 66% W_{peak} (Girard & Racinais, 2014). Such a combined-stressor protocol could potentially benefit endurance athletes preparing for the Olympic Games in Tokyo, which are predicted to be the warmest Olympic Games ever.

5.1. Methods

5.1.1. Participants

Eight healthy recreationally active to trained males [average (range) $\dot{V}O_{2\max}$: 51.61 (44.7-59.8) $\text{mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, W_{peak} : 343 (304-390) W], not involved in high-level sport, participated in the present study. They refrained from travelling to altitudes > 2.500 m and were not exposed to temperatures > 30 °C during the four weeks prior to the study. None of them had a history of any cardiorespiratory or hematological disease. They were requested not to consume any caffeine- and/or alcohol-containing beverages throughout the period of the study.

Preceding their inclusion in the study, the participants provided a written informed consent to participate in the study and were informed regarding the study protocol as well as the possible risks involved. All applicants performed a preliminary normoxic maximal test on a cycle ergometer to familiarize themselves with the test protocol. W_{peak} as established from the preliminary test served to determine the submaximal workload for: 1) the subsequent pre- and post-acclimation tests (40% of the preliminary W_{peak}), 2) the time to exhaustion test in hot hypoxic conditions (80% of the preliminary W_{peak}) and 3)

the initial workload for the controlled-hyperthermia exercise sessions (see *Heat acclimation*).

5.1.2. Study Design

Participants performed a battery of criterion exercise performance tests prior to commencing the acclimation protocol, and immediately upon completion of the acclimation. These tests comprised a $\dot{V}O_{2\max}$ test conducted in three different environmental conditions on consecutive days. To assess exercise performance, they also performed a time to exhaustion test at a standardized workload in hot hypoxic conditions six hours after the first maximal test. Following the three days of testing, participants rested for 24 h before commencing the acclimation protocol, which was conducted at the Olympics Sport Centre Planica (Rateče, Slovenia). During the acclimation protocol they were confined to the hypoxic facility of the Olympic Sport Centre Planica, where they were exposed to a hypoxic environment for 10 days (a total of 230 hrs). During this period, they exercised daily in a hot normoxic (the Olympic Sport Centre is situated at an altitude of 940 m) environment for 90 min. The participants rested for the first day after exiting the hypoxic environment and then the criterion exercise tests were repeated. For a given participant, the order of the exercise tests was the same as in the pre-acclimation period. The maximal exercise tests for each participant were held at the same time of the day (± 1 hour) in a laboratory situated at 300 m above sea level (Ljubljana, Slovenia). The daily training sessions were also performed at the same time of the day (± 2 hours).

5.1.3. Hypoxic Acclimation

The hypoxic acclimation protocol was performed in the hypoxic facility of the Olympic Sports Centre Planica (Rateče, Slovenia) situated at an altitude of 940m. Each participant was confined to the hypoxic facility for more than 10 days (entering hypoxia in the morning of the first day and exiting in the evening of the eleventh day). They lived (22 hours per day) at a simulated altitude of approximately 4000 m achieved by maintaining F_iO_2 at 14.05 ± 0.46 % in the facility situated at 940 m, resulting in an inspired partial pressure of oxygen (P_iO_2) of 90.4 ± 2.3 mmHg. Hypoxia within the facility was achieved using an oxygen dilution system (B-cat, Tiel, the Netherlands), based on the Vacuum-Pressure Swing Adsorption principle. The oxygen levels in each room were monitored and recorded continuously using oxygen sensors (PGM-1100, Rae Systems, San Jose, California, USA). In the event that F_iO_2 in the room decreased by more than 0.5% of the preset value, the control system activated a fan, which delivered external ambient air into the room. As a result, F_iO_2 in the room would be increased rapidly. Once F_iO_2 increased above the preset value, the fan would be deactivated. This type of intervention was never activated, since the system was capable of maintaining F_iO_2 within the pre-set limits. The atmospheric pressure in the hypoxic facility was 690 ± 4 mmHg. Resting values of capillary oxyhemoglobin saturation (S_pO_2), HR as well as Lake Louise Mountain Sickness Scores were recorded each morning. Participants had *ad libitum* access to food and water and had the same daily menu throughout their sojourn at the Planica Nordic Centre. Participants were accommodated in double bedrooms for the duration of the study. During their 10-d sojourn at the Planica Nordic Center participants followed a daily schedule of lights-out at 2300 hrs, and waking up at 0700 hrs. Body composition was measured on days 1 and 10 of the hypoxic acclimation using DEXA (Hologic Discovery QDR, Hologic Inc., Bedford, Massachusetts, USA).

5.1.4. Heat Acclimation

HeA was achieved with the controlled-hyperthermia technique (isothermal clamping) (Fox et al., 1963). During all HeA exposures, a combination of exercise (metabolic) and heat stress was used to elevate and maintain high T_{re} and T_{sk} . HeA protocol consisted of ten 90-min daily sessions of cycle ergometry starting at a workload corresponding to 50% of participants' normoxic W_{peak} so that the target T_{re} of 38.5 °C was reached within 30-45 min of exercise (Gibson et al., 2017). Once the target T_{re} was reached, power was adjusted every 5 min to maintain T_{re} at the desired level. For reasons unrelated to the study, participants were passively heated for additional 2 hours after the training sessions 1 and 10. Participants were provided with 1.5 L of water during each exercise session in six doses of 250 mL at 15-min intervals. During HeA trials, T_a was monitored at 5-min intervals using a temperature sensor (MSR 145, 175 Henggart, Switzerland). The average T_a and RH in the laboratory during the training sessions were 37.4 ± 0.5 °C and 38.5 ± 1.7 %, respectively. The wind speed was less than $0.5 \text{ m} \cdot \text{s}^{-1}$. T_{re} was measured continuously during the training sessions using a rectal thermistor probe (MSR 145, 175 Henggart, Switzerland) inserted 10 cm beyond the anal sphincter. Exercise HR was assessed using finger pulse oximeters (Wristox 3100 Nonin, Plymouth, Minnesota, USA) at 10-min intervals. RPE were also recorded at 10-min intervals. During the training sessions participants exercised on mountain bikes mounted on resistance trainers (Elite Cycling, Italy). Participants trained in groups of 2 or 3 under strict supervision of the researchers.

5.1.5. Exercise Testing

All the pre- and post-training criterion exercise trials were conducted in a laboratory situated at 300 m above sea level (Ljubljana, Slovenia). The trials were conducted on a cycle ergometer (Daum, Electronic, Furth, Germany) and comprised two stages; a 30-min steady-state exercise immediately followed by an incremental exercise to exhaustion. During exercise, each participant pedaled at a preferred cadence (between 60 and 90 rpm), which they maintained via visual and verbal feedback throughout the trial. Before (Pre) and after (Post) the 10-day training protocol, participants conducted three trials on three consecutive days. In the normal temperature and normoxic condition (NOR), participants breathed room air (pre: $P_iO_2 = 144 \pm 2$ mmHg, post: $P_iO_2 = 143 \pm 1$ mmHg) and exercised in thermoneutral conditions (pre: $T_a = 23.67 \pm 0.19$ °C and $RH = 46.9 \pm 1.2\%$, post: $T_a = 23.41 \pm 0.23$ °C and $RH = 49.1 \pm 0.8\%$). In the hypoxic condition (HYP), they inspired a hypoxic gas mixture (pre: $P_iO_2 = 93 \pm 2$ mmHg, post: $P_iO_2 = 92 \pm 3$ mmHg) and exercised in thermoneutral conditions (pre: $T_a = 23.32 \pm 0.19$ °C and $RH = 45.8 \pm 5.6$ %, post: $T_a = 23.16 \pm 0.26$ °C and $RH = 50.2 \pm 0.8$ %). In the hot condition (HE), the participants inspired room air (pre: $P_iO_2 = 144 \pm 1$ mmHg, post: $P_iO_2 = 139 \pm 2$ mmHg), but exercised in a hot environment (pre: $T_a = 35.08 \pm 0.89$ °C, $RH = 42.0 \pm 2.8\%$, post: $T_a = 35.34 \pm 0.25$ °C and $RH = 46.8 \pm 1.5\%$). As stated earlier, the order of the exercise trials was randomized and counterbalanced. A resting day was scheduled immediately before and after the training protocol to minimize the occurrence of fatigue during the exercise tests.

A metabolic cart (Quark CPET, Cosmed, Rome, Italy) was used to obtain the breath-by-breath respiratory responses during the exercise trials. Metabolic energy expenditure was calculated during submaximal exercise using the following equation:

$$M \text{ (W)} = \dot{V}O_2 \frac{\left(\frac{RER-0.7}{0.3}\right)e_c + \left(\frac{1-RER}{0.3}\right)e_f}{60}$$

where RER respiratory exchange ratio, and e_c and e_f are the caloric equivalents per liter of oxygen for the oxidation of carbohydrates (21.13 kJ) and fats (19.62 kJ), respectively.

Metabolic heat production (H_{prod}) was estimated by subtracting the rate of mechanical work from metabolic energy expenditure. Before each trial, the oxygen and CO_2 analyzers were calibrated using two different gas mixtures, and the pneumotachograph was calibrated with a 3-L syringe, in accordance with the manufacturer's recommendation.

HR was derived from electrocardiographic recordings with electrodes in a precordial position. CO was determined using electrical impedance cardiography (Physioflow Q-Link, Manatec Biomedical, Paris, France), whereby changes in thoracic impedance during cardiac ejection are used to estimate SV. Six electrodes were placed at the base of the neck and on the xiphoid process. Electrical impedance cardiography has been validated against the direct Fick method during maximal incremental exercise in healthy participants (Siebenmann, Rasmussen, et al., 2015). $\text{S}_\text{p}\text{O}_2$ was recorded using a finger pulse oximeter (Wristox 3100 Nonin, Plymouth, Massachusetts, USA). Participants were instructed to relax their hands during the measurement of $\text{S}_\text{p}\text{O}_2$. RPE was obtained using a Borg scale (6-20). During all trials the participants wore only shorts and athletic shoes. The body mass was measured before and after each exercise testing on a weighing scale ($\pm 0.05\text{kg}$) (TPT 5N, Libela ELSI, Celje, Slovenia).

T_{re} was measured with a thermistor probe (MSR 145, Henggart, Switzerland) inserted 10 cm beyond the anal sphincter. Skin temperatures were measured at four sites (chest, arm, thigh and calf) using thermistors (MSR 147WD, Henggart, Switzerland) attached to the skin with a single piece of adhesive tape. The assessment of calf, thigh, chest and arm skin temperature enabled the calculation of T_{sk} (Ramanathan, 1964). The ventral fingertip and forearm temperatures were also measured. $\Delta T_{\text{f-f}}$ has been validated as an index of vasomotor tone during steady-state exercise (Keramidas et al., 2013). Minute mass flow of secreted sweat ($\dot{m}_{\text{sw}}$, $\text{mg} \cdot \text{cm}^{-2} \cdot \text{min}^{-1}$) was measured with a ventilated capsule placed on the forehead. Forced evaporation of sweat under the capsule (surface area 4.8 cm^2) was achieved by a constant flow of air ($1.0 \text{ L} \cdot \text{min}^{-1}$) through the capsule. Minute mass flow of secreted sweat was estimated each minute from the difference between the temperature and the humidity of inflowing and outflowing air. Air temperature was measured with thermistors (LM35, National Semiconductor Corp., Santa Clara, CA, USA) and RH with capacitance hygrometers (Valvo air humidity sensor, Valvo-Philips GmbH, Hamburg, Germany).

5.1.6. Steady-State and Incremental Exercise

Following a 2-min rest period, participants commenced pedaling on the cycle ergometer. During the first 2 min the load was set at 90 W (warm-up). Thereafter, they cycled for 30 min at a steady-state work rate equivalent to 40% of their NOR W_{peak} attained during the preliminary testing. The initial work rate was set at 100 W (2 min), thereafter increasing by 20 W every minute until volitional exhaustion. During each trial, $\text{S}_\text{p}\text{O}_2$ and sweat rate data were recorded at minute intervals whereas HR, CO and SV were measured every 10s. RPE values were recorded at 5-min and 2-min intervals during the steady-state and the incremental-load exercise, respectively. Attainment of $\dot{V}\text{O}_{2\text{max}}$, defined as the highest $\dot{V}\text{O}_2$ averaged over 30 s, was confirmed when participants met at least three of the following criteria, listed in order of priority: (a) severe fatigue or exhaustion leading to inability to maintain exercise at a given work rate (cycling cadence lower than 60 rpm), (b) a plateau in $\dot{V}\text{O}_2$, as indicated by the breath-by-breath values, despite an increase in power output (c) a subjective RPE near maximal (Borg scale rating greater than 17) and (d) respiratory quotient greater than 1.10. W_{peak} was calculated according to the following equation:

$$W_{\text{peak}} = \text{work rate of last stage completed} + [(\text{work rate increment}) * (\text{time into current stage}/60)]$$

5.1.7. Time to Exhaustion

Time to exhaustion was determined using a constant load test at 80% of the W_{peak} (274 ± 24 W). The test was held in hot (pre: $T_a = 34.4 \pm 0.5$ °C, $47 \pm 4\%$ RH, post: $T_a = 33.6 \pm 1.1$ °C, $54 \pm 3\%$ RH) hypoxic conditions on the same ergometer and chamber as the rest of the exercise tests. A metabolic cart (Quark CPET, Cosmed, Rome, Italy) was used to acquire the breath-by-breath respiratory responses during the time-to-exhaustion tests. After entering the climatic chamber, participants commenced breathing via a two-way respiratory valve (Model 2,700, T-Shape, Hans Rudolph, Shawnee, Kansas, USA). The inspiratory side of the valve was connected via corrugated tubing to a meteorological balloon containing a calibrated hypoxic gas mixture (pre: $F_{\text{I}}\text{O}_2 = 13.46 \pm 0.73\%$, post: $F_{\text{I}}\text{O}_2 = 13.06 \pm 0.39\%$). This gas mixture was decompressed from a high pressure cylinder, humidified by passing it through a water bath at room temperature, and collected in the meteorological balloon. The participants rested for 120 s while breathing the hypoxic gas mixture. The warm-up period included 165 s at 90 W followed by 15 s of rest. The test ended when participants were unable to maintain a cycling cadence above 60 revolutions per minute for more than 5 s. Constant verbal feedback was given to the participants by the researchers during the tests.

5.1.8. Hematological Data

Blood samples were obtained from an antecubital vein before, on days 1 and 10, and the first day post-acclimation. The participants rested in a seated position for ≥ 10 min before blood samples were obtained. Blood was taken using standard venopuncture technique at the hypoxic facility or at the laboratory where the criterion exercise tests were performed. Blood samples were allowed to clot for 1 hour at 2-8 °C and were then centrifuged at 2000 g for 15 minutes so that serum was completely separated from the cells. Serum was then stored in BD Vacutainers at -20 °C for further analysis. With regards to the hemogram analysis, an additional 3 mL of whole blood was collected into standard EDTA tubes (Vacutainer, Greiner BIO one, Austria) and the blood sample mixed to prevent clotting. Samples were analyzed for hematocrit, hemoglobin, ferritin and reticulocyte concentration and erythrocyte count on an automated hematology analyzer (ABX Micros ES 60, Horiba medical, Japan) twice within 3 hours after the sample was obtained. Since every sample was analyzed twice, the average value was computed and CV was less than 2%. Additional Erythropoietin (EPO) was determined from 200 μL samples of serum using a two-site ELISA (Enzyme-linked immunosorbent assay; IVD, Erythropoietin ELISA, IBL, Germany). The quantification of the optical density was performed on a microplate reader BioTek (BioTek instruments, Elx808TM, Winooski, Vermont, USA) set at 405 nm and 450 nm. Renin (active) was determined from 50 μL samples of serum using an ELISA based on the sandwich principle (IVD, Renin (active) ELISA, IBL, Hamburg, Germany). The quantification of the optical density was performed on a microplate reader BioTek (BioTek instruments, Elx808TM, Winooski, Vermont, USA) set at 450 nm. HSP70, HSP90 and VEGF concentrations were determined using ELISA (Enzo Life Sciences Inc., Farmingdale, NY, USA) in 50, 4 and 20 μL of plasma, respectively. Optical density was quantified on a microplate reader (Biotek Elx808TM; Biotek Instruments, Winooski, Vermont, USA) set at 450 nm and corrected at 630 nm for HSP70, HSP90 and VEGF. Two microplates were used for each participant's samples in order to avoid the possible variability between the plates. All techniques were performed in accordance with the protocol provided by the manufacturer. Samples and standards were assayed in duplicate. The sensitivity of the measurements was $0.09 \text{ ng} \cdot \text{mL}^{-1}$, $8 \text{ pg} \cdot \text{mL}^{-1}$, $0.05 \text{ ng} \cdot \text{mL}^{-1}$, $0.81 \text{ pg} \cdot \text{mL}^{-1}$, $1.1 \text{ mIU} \cdot \text{mL}^{-1}$ for HSP72, VEGF, HSP90, renin, EPO, respectively. Initial

resting vascular volumes (pre-acclimation) were calculated from lean body mass by the equations of Sawka et al. (1992) and post-acclimation volumes were calculated by correcting that initial values for the percent change in PV (Strauss et al., 1951) as well as blood and erythrocyte volume (Dill & Costill, 1974). The specific heat capacity of the blood was calculated by the equation of Blake, Petley, and Deakin (2000).

5.1.9. Data Analysis

Statistical analyses were performed using Statistica 5.0 (StatSoft, Tulsa, OK). Cardiorespiratory and temperature data collected during steady-state exercise were analyzed for the last twenty minutes of the steady-state exercise, unless stated otherwise. Cardiorespiratory and thermoregulatory data were analyzed using a two-way repeated-measures ANOVA (Environmental condition: NOR-HYP-HE; acclimation status: pre-post). Hematological variables were analyzed using one-way repeated-measures ANOVA (time: pre-D1-D10-post). Training data were also analyzed using one-way repeated-measures ANOVA (time: D1-D2-...-D10). When ANOVA revealed a significant F-ratio for interaction and/or main effect, pairwise comparisons were performed with Tukey honestly significant difference *post hoc* tests. A t-test for paired samples was performed to compare pre- to post-acclimation time to exhaustion in hot hypoxic conditions. RPE and gains of the sweating response (relative change in sweating as a function of the relative change in T_{re}) were analyzed using the non-parametric Friedman test. The sweat rate and temperature data were analyzed only for the steady-state exercise. Least square linear regression was used to determine the mean T_{re} threshold for initiation of sweating, as well as the gain of the sweating response. The reported values are averages of the individual thresholds and slopes derived from linear regression parameters. The anaerobic threshold was defined as the $\dot{V}O_2$ that corresponded to the deflection point of $P_{ET}CO_2$ confirmed by the nadir in the V_E/VCO_2 when plotted as functions of the workload during the incremental test (Binder et al., 2008). Data are presented as mean $\pm$ SD unless indicated otherwise. The alpha level of significance was set *a priori* at 0.05.

5.2. Results

5.2.1. Heat Acclimation

With the exception of one participant who developed gastrointestinal problems and a concomitant fever (resting T_{re} of $38.1\text{ }^{\circ}\text{C}$) during day 4 of the acclimation protocol, all participants completed the 10 heat-acclimation sessions. Table 5.1 demonstrates the progression of the adaptive response to heat-acclimation sessions.

There being no main effect of time on T_{re} after the 30th minute indicates that the desired thermal strain was successfully induced ($p = 0.51$). Specifically, participants' average T_{re} ranged between $38.4 \pm 0.1\text{ }^{\circ}\text{C}$ or $38.4 \pm 0.2\text{ }^{\circ}\text{C}$ (D1 and D8, respectively) and $38.6 \pm 0.1\text{ }^{\circ}\text{C}$ (D4 and D9) during the latter half of the HeA protocol. The cardiovascular strain imposed on the participants was also relatively constant as the observed main effect of time on exercise HR ($p = 0.011$) was only reflected in a higher HR at D9 compared to D1 (140 ± 9 vs 152 ± 8 bpm, $p = 0.007$). Resting T_{re} ($p = 0.43$) and ΔT_{re} ($p = 0.39$) remained unchanged as a result of acclimation. The time to reach the target T_{re} was also not affected by acclimation ($p = 0.23$). Pre-exercise body mass decreased by 1.9 ± 1.4 kg over the course of the acclimation protocol with the body mass loss reaching significance on D7 ($p = 0.041$). Due to technical reasons, we were unable to measure body weight for the first two days of the HeA protocol. However, a main effect for time ($p < 0.001$) suggests that participants were sweating more as adaptation to heat training was being established. Indeed, whole body sweat rate was higher on D7-10 ($p < 0.01$ for days 7, 9, 10 and $p = 0.06$ for day 8) compared to D4.

In contrast to the pre-exercise body mass, similar pre- to post- acclimation values were observed for the body composition variables as assessed using DEXA. More specifically, there was no change in either body fat percentage (17.3 ± 2.1 vs $17.5 \pm 1.9\%$, $p = 0.11$) or fat mass (12.8 ± 2.0 vs 13.0 ± 1.7 kg, $p = 0.24$). Similarly, no effect was observed in fat-free mass (61.6 ± 6.2 vs 61.2 ± 5.6 kg, $p = 0.18$). Consequently, participants completed the acclimation protocol with no change in body mass (pre: 74.4 ± 7.1 ; post: 74.2 ± 6.4 kg, $p = 0.31$).

5.2.2. Hematological Data

There was a main effect of time ($p < 0.001$) on hematocrit and hemoglobin concentration. Hematocrit was higher ($p = 0.021$) on D1 compared to baseline levels with mean values being 47.8 ± 2.3 and $45.3 \pm 2.5\%$, respectively. Hematocrit tended to be even higher during D10 ($49.8 \pm 2.7\%$, $p = 0.10$) compared to D1. The time interval of 24 hours after participants had returned to normoxia for the last sampling point did not seem to affect the acquired hematological adaptations since hematocrit values were similar to D10 values ($48.3 \pm 1.8\%$, $p = 0.27$), but differed from pre-acclimation values ($p = 0.006$). The same pattern was detected for hemoglobin concentration with a profound increase on D10 compared to baseline values (149 ± 9 vs $162 \pm 9\text{ g} \cdot \text{dL}^{-1}$, $p < 0.001$). Again, hemoglobin concentration remained higher than baseline after participants returned to normoxia ($156 \pm 6\text{ g} \cdot \text{dL}^{-1}$, $p = 0.032$), with a tendency, albeit not significant, of being lower than D10 ($p = 0.079$). Hemoglobin concentration on D1 ($154 \pm 8\text{ g} \cdot \text{dL}^{-1}$) remained unaltered compared to baseline ($p = 0.15$) but was lower than D10 ($p = 0.016$). Regarding the individual values, both indices were higher across all participants on D10 compared to pre-acclimation values.

Table 5.1: Average daily exercise responses during heat acclimation. Repeated measures one-way ANOVA was performed

	D1	D2	D3	D4	D5	D6	D7	D8	D9	p-value
T_{re} (°C) after 30 th min	38.4±0.2	38.5±0.1	38.5±0.1	38.6±0.1	38.5±0.1	38.5±0.1	38.5±0.1	38.4±0.1	38.6±0.1	0.51
HR (bpm)	140 ± 9	142 ± 9	144 ± 9	148 ± 8	145 ± 11	148 ± 8	146 ± 8	145 ± 9	152 ± 8	0.011
Resting T_{re} (°C)	37.1±0.3	37.3±0.2	37.2±0.3	37.3±0.3	37.3±0.3	37.3±0.3	37.4±0.3	37.2±0.3	37.3±0.3	0.43
ΔT_{re} (°C)	1.3 ± 0.3	1.2 ± 0.2	1.3 ± 0.3	1.4 ± 0.3	1.3 ± 0.3	1.2 ± 0.3	1.2 ± 0.2	1.3 ± 0.2	1.3 ± 0.3	0.39
Time to reach 38.5 °C (min)	51 ± 14	45 ± 13	46 ± 10	40 ± 9	37 ± 10	37 ± 6	39 ± 9	47 ± 14	42 ± 8	0.23
Pre-exercise mass (kg)	76.7±7.5	76.1±7.4	75.8±7.1	75.4±7.2	75.2±7.3	75.2±7.4	75.1±7.2	75.0±7.2	76.0±6.8	0.004
Whole-body sweat rate ($L \cdot hr^{-1}$)	-	-	1.7 ± 0.4	1.6 ± 0.3	1.5 ± 0.2	1.7 ± 0.3	1.8 ± 0.4	1.8 ± 0.4	1.9 ± 0.3	< 0.001

The estimated PV was already contracted by D1 ($-7.7 \pm 6.5\%$, $p = 0.023$) and was even lower by D10 ($-15.1 \pm 8.5\%$, $p = 0.043$). The decrease in blood volume only reached statistical significance by D10 ($-7.8 \pm 4.8\%$, $p < 0.001$), but not on D1 ($-3.3 \pm 3.5\%$, $p = 0.09$). No individual variation was noted since the fall in plasma and total blood volumes was consistent across all participants. The four different sampling timepoints also enabled us to investigate the EPO kinetics (Figure 5.1). Similar to previous hypoxic acclimation studies, plasma EPO increased from baseline ($17.8 \pm 7.0 \text{ mIU} \cdot \text{mL}^{-1}$) on D1 by $10.7 \pm 8.8 \text{ mIU} \cdot \text{mL}^{-1}$ ($p = 0.012$). By D10, plasma EPO had returned to baseline levels ($15.7 \pm 5.9 \text{ mIU} \cdot \text{mL}^{-1}$, $p = 0.90$) and tended to undershoot when participants exited the hypoxic environment ($10.6 \pm 5.9 \text{ mIU} \cdot \text{mL}^{-1}$, $p = 0.12$) (Figure 5.1). Another main effect of time was observed for extracellular HSP90 ($p = 0.05$), which was reflected only in a tendency for an increased concentration when participants exited the hypoxic environment compared to D10 (40.0 ± 10.1 vs $66.7 \pm 27.9 \text{ ng} \cdot \text{mL}^{-1}$, $p = 0.076$). The renin concentration was lower on D1 compared to the baseline value (48.5 ± 24.7 vs $34.0 \pm 24.9 \text{ pg} \cdot \text{mL}^{-1}$, $p = 0.048$). By D10, it had returned to baseline values ($39.0 \pm 22.7 \text{ pg} \cdot \text{mL}^{-1}$, $p = 0.29$), and it remained at this level on the first day post-acclimation ($42.1 \pm 22.6 \text{ pg} \cdot \text{mL}^{-1}$, $p = 0.61$). On the contrary, HSP70 and VEGF remained unaffected by the acclimation protocol ($p = 0.63$ and $p = 0.25$, respectively).

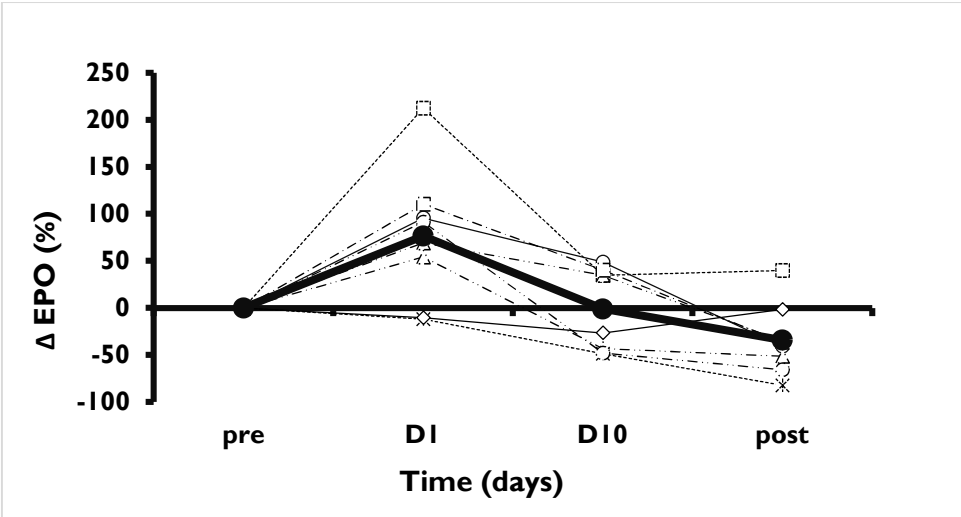


Figure 5.1: Individual and average (bold) percentage changes in plasma EPO concentration before (Pre), during (days 1 and 10), and after (D+1) the 10-day combined acclimation protocol (n=8).

5.2.3. Submaximal Exercise

Table 5.2 provides cardiorespiratory responses during the 30-min steady-state exercise in all environmental conditions. An interaction effect between environmental condition and acclimation indicated that the post-acclimation reductions in HR observed in NOR (-6 ± 13 bpm, $p = 0.035$) and HYP (-9 ± 12 bpm, $p = 0.004$) were not persistent in HE (-2 ± 10 bpm, $p = 0.96$). HR in NOR was lower than in HE and HYP across acclimation (main effect of environmental condition: $p < 0.001$). Submaximal CO remained unaffected by acclimation ($p = 0.14$), but there was a tendency for a main effect of environmental condition ($p = 0.052$). This may have resulted from the high pre-acclimation mean value in HYP ($18.4 \pm 2.5 \text{ L} \cdot \text{min}^{-1}$) which however was not statistically different than the respective values in NOR ($16.5 \pm 3.1 \text{ L} \cdot \text{min}^{-1}$, $p = 0.055$) or HE ($16.8 \pm 2.2 \text{ L} \cdot \text{min}^{-1}$, $p = 0.12$). Another main effect of environmental condition was observed for submaximal SV ($p = 0.024$) with the *post-hoc* tests suggesting a lower SV post-acclimation in HE compared to NOR (130 ± 22 vs $111 \pm 12 \text{ mL}$, $p = 0.014$). There was a main effect ($p = 0.019$) for acclimation on exercise economy, defined as the reciprocal of $\dot{V}O_2$ at 40% of the NOR W_{peak} . The *post-hoc* tests revealed that exercise economy was higher in NOR post-acclimation ($p = 0.010$).

Table 5.2: Cardiorespiratory responses and RPE in the last 20 of the 30 minutes of constant-work rate cycling before and after acclimation for the normoxic, the hypoxic and the heat trials. Heart rate, stroke volume and cardiac output values reflect the last minute of submaximal exercise (n = 8).

Pre-training			Post- training		
NOR	HYP	HE	NOR	HYP	HE

HR (bpm)	129 ± 17 ^{†#}	148 ± 12 [#]	145 ± 16	123 ± 11 ^{*†#}	140 ± 10 ^{*#}	144 ± 13
CO (L · min ⁻¹)	16.5 ± 3.1	18.4 ± 2.5	16.8 ± 2.2	16.1 ± 3.6	16.9 ± 2.9	15.9 ± 2.7
¹⁾						
SV (mL)	125 ± 20	123 ± 15	116 ± 12	130 ± 22 [#]	121 ± 17	111 ± 12
VO ₂	2212 ± 113	2190 ± 100	2150 ± 196	2070 ± 150 [*]	2122 ± 192	2048 ± 220
(mL · min ⁻¹)						
GME (%)	18.2 ± 1.4	18 ± 1.0	18.5 ± 0.8	19.3 ± 0.5	18.6 ± 1.4	19.4 ± 1.0
V _E (L · min ⁻¹)	49.8 ± 4.5 [†]	61.6 ± 5.5 [#]	52.8 ± 5.0	54.6 ± 5.5 ^{*†}	66.6 ± 8.6 ^{*#}	57.3 ± 6.6 [*]
¹⁾						
S _p O ₂ (%)	95.9 ± 1.3 [†]	79.1 ± 5.1 [#]	95.7 ± 0.6	96.1 ± 1.1 [†]	82.6 ± 4.1 ^{*#}	95.4 ± 1.0

Values are mean ± SD

* Significant difference to values before acclimation

Significant difference to heat values

† Significant difference to hypoxic values, p < 0.05

A main effect of acclimation on GME (p = 0.013) was subsequently observed. An interaction effect between environmental condition and acclimation status (p = 0.012) accompanied the increase in post-acclimation S_pO₂ in HYP (+3.5 ± 2.3%, p = 0.010) which however remained lower than NOR and HE (main effect of environmental condition: p < 0.001). This could be due to the ~5 L · min⁻¹ V_E post-acclimation across environmental conditions with the hypoxic values being persistently higher compared to NOR and HE (main effects of acclimation and time: p < 0.001).

5.2.4. Exercise Performance

Figure 5.2 represents the individual data for $\dot{V}O_{2\max}$ and W_{peak} in the three environmental conditions pre- and post-acclimation. The acclimation protocol did not increase $\dot{V}O_{2\max}$ in any environment (main effect for acclimation: p = 0.52) with mean percentage changes from pre-acclimation being -2.6 ± 1.8%, +0.3 ± 10.5% and -1.8 ± 7.2% in NOR, HYP and HE, respectively. On the contrary, there was a main effect of both acclimation (p < 0.001) and environment (p < 0.01) for W_{peak}. Indeed, W_{peak} was, or tended to be, higher post-acclimation in NOR (p < 0.01) and HE (p = 0.068), respectively. It is notable that in HYP, 5 participants increased and 3 participants maintained their W_{peak} rendering the increase not statistically significant (p = 0.21). Exercise performance in hot hypoxic conditions, as assessed by the time to exhaustion at 80% of the NOR W_{peak}, was improved post-acclimation by 12.9 ± 10.6% (249 ± 58 vs 286 ± 89 s, p = 0.010) with 7 participants

improving and one maintaining their pre-acclimation time to exhaustion. Interestingly, the participant that did not improve his exercise performance in the hot hypoxic conditions was the only one not to attain a higher W_{peak} post-acclimation in either NOR or HE, while his $\dot{V}O_{2\text{max}}$ was attenuated consistently across environments (-7.1%, -6.9% and -7.3% in NOR, HYP and HE, respectively).

An overview of aerobic performance measures is given in Table 5.3. Another main effect of environmental condition ($p < 0.001$) was observed for the anaerobic threshold. More specifically, the anaerobic threshold was lower in HYP compared to NOR pre-acclimation ($p = 0.022$). As expected, there was a main effect for environmental condition on maximal CO ($p = 0.012$) with post-acclimation values in HYP ($22.3 \pm 3.9 \text{ L} \cdot \text{min}^{-1}$, $p = 0.019$) and HE ($22.0 \pm 2.5 \text{ L} \cdot \text{min}^{-1}$, $p < 0.01$) being lower compared to NOR ($25.0 \pm 4.2 \text{ L} \cdot \text{min}^{-1}$). The substantial decreases in maximal CO in HE (compared to NOR) were also mirrored in a lower corresponding SV across acclimation status (141 ± 25 vs $123 \pm 15 \text{ mL}$ pre, $p = 0.034$; 138 ± 19 vs $118 \pm 11 \text{ mL}$ post, $p = 0.001$). On the contrary, there was no main effect for acclimation on either maximal CO ($p = 0.80$) or the corresponding SV ($p = 0.79$). Maximal V_E increased by $\sim 15 \text{ L}$ across environmental conditions (main effect of acclimation: $p = 0.006$). However, when V_E was normalized to W_{peak} , another main effect of condition emerged ($p = 0.025$) mirrored only in a difference between NOR and HYP post-acclimation ($p = 0.040$). In contrast to the submaximal exercise, S_pO_2 did not increase post-acclimation in HYP (78.5 ± 3.0 vs $81.3 \pm 4.6\%$, $p = 0.20$) and it remained lower than NOR and HE (main effect of environmental condition: $p < 0.001$).

5.2.5. Thermoregulatory Data

Thermoregulatory responses during submaximal exercise are shown in Table 5.4. Resting T_{re} remained unchanged by the acclimation protocol ($p = 0.39$) or the environmental condition ($p = 0.54$). As expected, there was a main effect of environmental condition ($p = 0.023$) on ΔT_{re} during submaximal exercise reflected on a lower ΔT_{re} in NOR compared to HYP and HE across acclimation. The acclimation protocol did not affect ΔT_{re} ($p = 0.42$).

T_{sk} was higher in HE as reflected in the main effect of environmental condition ($p < 0.001$). Furthermore, T_{sk} was higher post-acclimation in NOR compared to HYP (33.8 ± 0.5 vs 33.4 ± 0.6 , $p = 0.025$). Main effects of acclimation were also observed for H_{prod} expressed in absolute values ($p = 0.013$) or adjusted relative to body surface area ($p = 0.034$) implying lower values post-acclimation across environmental conditions. However, even the highest mean decrement observed in NOR did not reach statistical significance ($-6.59 \pm 7.61\%$, $p = 0.20$). H_{prod} normalized to body weight was affected by the environmental condition ($p = 0.041$), but not acclimation status ($p = 0.091$). Figure 5.3 shows the individual data for absolute sweating thresholds and peak sweat rate. Similar values for the absolute sweating threshold were reported across acclimation status and environmental conditions (main effects of acclimation and environmental condition: $p = 0.32$ and $p = 0.33$, respectively). On the contrary, there was a main effect of environmental condition ($p = 0.001$) accompanied by an interaction effect between environmental condition and acclimation ($p = 0.004$) on the peak sweat rate. Indeed, peak sweat rate was higher in HE ($1.54 \pm 0.35 \text{ mg} \cdot \text{cm}^{-2} \cdot \text{min}^{-1}$) compared to NOR ($1.07 \pm 0.17 \text{ mg} \cdot \text{cm}^{-2} \cdot \text{min}^{-1}$, $p = 0.004$) and HYP ($0.97 \pm 0.13 \text{ mg} \cdot \text{cm}^{-2} \cdot \text{min}^{-1}$, $p = 0.001$), but only post-acclimation. Peak sweat rate increased post-acclimation by $37 \pm 33\%$ in HE ($p = 0.018$). A similar tendency for an interaction effect between acclimation and environmental condition ($p = 0.067$) was noted for the gain of the sweating response. This interaction effect was reflected in the post-acclimation increase in the gain by $1.8 \pm 1.6 \text{ mg} \cdot \text{cm}^{-2} \cdot \text{min}^{-1} \cdot ^\circ\text{C}^{-1}$ again in HE ($p = 0.094$). As a result of the PV contraction, the specific heat capacity of the blood was lower even by day 1 (3553 ± 33 vs $3520 \pm 31 \text{ J} \cdot \text{kg}^{-1} \cdot ^\circ\text{C}^{-1}$, $p = 0.021$) and

remained in such levels by day 10 ($-1.6 \pm 1.0\%$, $p < 0.001$) but also the first day after participants exited the hypoxic environment ($-1.1 \pm 0.5\%$, $p = 0.006$).

Table 5.3: Maximal cardiorespiratory responses and peak power output during the incremental exercise to exhaustion pre- and post-acclimation in normoxic, hypoxic and heat trials. (n = 8)

	Pre-acclimation			Post-acclimation		
	NOR	HYP	HE	NOR	HYP	HE
$\dot{V}O_{2\max}$ ($\text{mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	$54.3 \pm 5.8^\dagger$	$43.8 \pm 4.7^\#$	50.1 ± 4.0	$52.4 \pm 4.9^\dagger$	$43.7 \pm 4.1^\#$	49.1 ± 5.0
$\dot{V}O_{2\max}$ ($\text{L} \cdot \text{min}^{-1}$)	$4.12 \pm 0.31^\dagger$	$3.26 \pm 0.36^\#$	3.83 ± 0.44	$3.96 \pm 0.40^\dagger$	$3.29 \pm 0.36^\#$	3.72 ± 0.53
W_{peak} (W)	$321 \pm 20^{\dagger\#}$	$268 \pm 30^\#$	297 ± 31	$343 \pm 3^{*\dagger\#}$	$282 \pm 27^\#$	310 ± 38
$\text{CO}_{\max}$ ($\text{L} \cdot \text{min}^{-1}$)	25.0 ± 4.2	24.1 ± 3.9	22.1 ± 2.9	$25.1 \pm 0.4^\dagger\#$	22.3 ± 3.9	22.0 ± 2.5
HR_{peak} (bpm)	177 ± 11	177 ± 7	180 ± 12	181 ± 13	$172 \pm 5^\#$	186 ± 8
SV_{peak} (mL)	$141 \pm 25^\#$	136 ± 23	123 ± 15	$138 \pm 19^\#$	130 ± 20	118 ± 11
SpO_2 (%)	$93.9 \pm 1.7^\dagger$	$78.5 \pm 3.0^\#$	93.6 ± 2.8	$94.6 \pm 1.6^\dagger$	$81.3 \pm 4.6^\#$	94.6 ± 1.7
Arterial Oxygen Content ($\text{mL} \cdot \text{L}^{-1}$)	$190 \pm 11^\dagger$	$157 \pm 14^\#$	187 ± 13	$198 \pm 10^{*\dagger}$	$170 \pm 13^{*\#}$	$198 \pm 6^*$
Anaerobic threshold ($\text{mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	$42 \pm 4.5^\dagger$	$35.2 \pm 3.8^\#$	40.3 ± 3.9	38.6 ± 3.2	33.7 ± 4.1	38.3 ± 2.3
$V_{E\text{ peak}}$ ($\text{L} \cdot \text{min}^{-1}$)	147 ± 33	136 ± 24	139 ± 23	162 ± 31	152 ± 19	152 ± 37
$V_{E\text{ peak}} / W_{\text{peak}}$ ($\text{L} \cdot \text{min}^{-1} \cdot \text{W}^{-1}$)	0.46 ± 0.07	0.51 ± 0.07	0.47 ± 0.04	0.47 ± 0.08	0.54 ± 0.05	0.49 ± 0.08

$\dot{V}O_{2max}$ maximal aerobic power, W_{peak} peak power output, CO_{max} maximal cardiac output.
 HR_{peak} heart rate corresponding to CO_{max} , SV_{peak} stroke volume corresponding to CO_{max} .
Values are mean $\pm$ SD

* Significant difference to pre-training values

Significant difference to heat values

† Significant difference to hypoxic values, $p < 0.05$

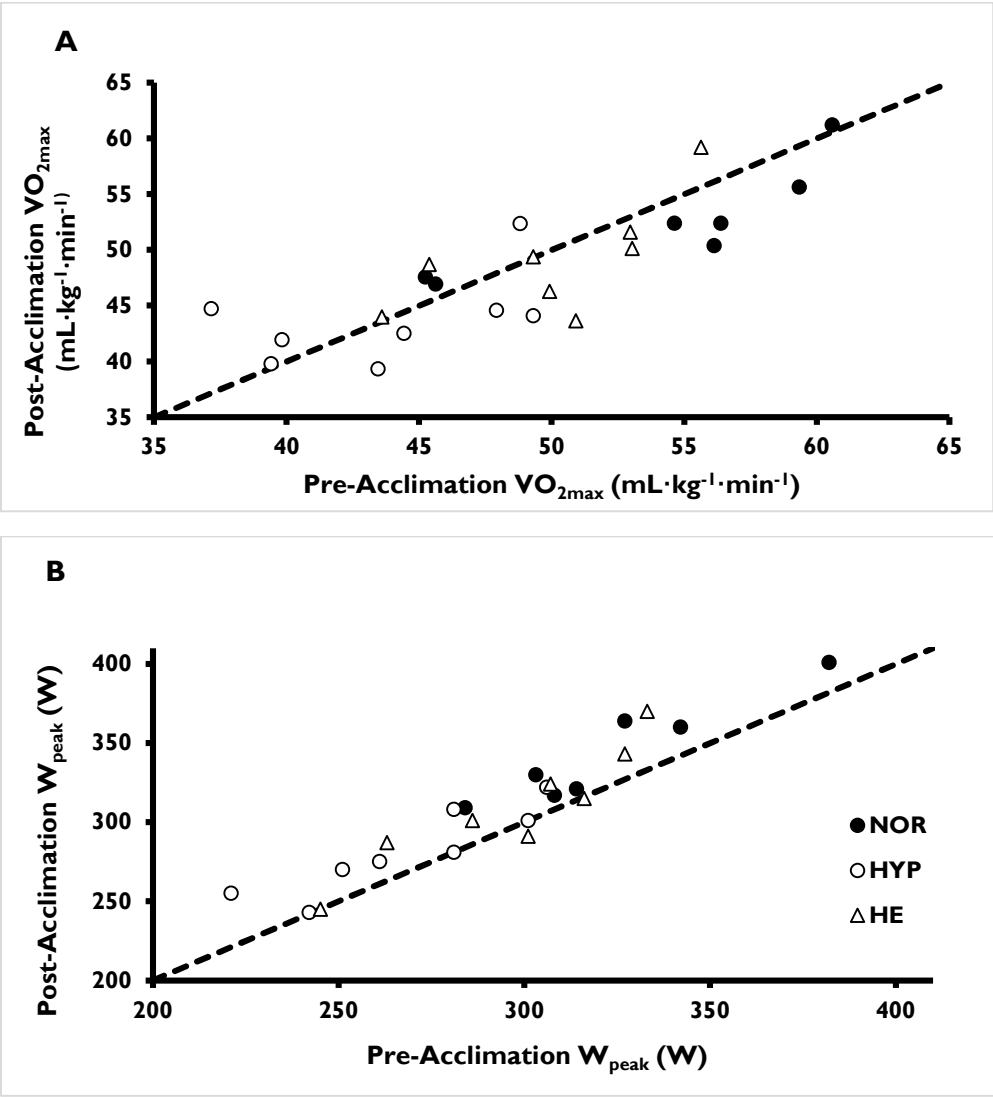


Figure 5.2: Pre- (horizontal axis) and post- (vertical axis) acclimation individual data for (A) maximal aerobic power and (B) peak power output in normoxia (closed circles), hypoxia (open circles), and heat (triangles).

Table 5.4: Thermoregulatory responses at rest and during the constant-work rate cycling before and after HA for the normoxic, the hypoxic and the heat trials (n = 8).

	Pre- acclimation						Post- acclimation					
	NOR		HYP		HE		NOR		HYP		HE	
$T_{\text{re rest}} (^{\circ}\text{C})$	37.36	±	37.29	±	37.34	±	37.28	±	37.24	±	37.36	±
	0.26		0.21		0.24		0.42		0.40		0.26	
$\Delta T_{\text{re}} (^{\circ}\text{C})$	0.42	±	0.57	±	0.60 ± 0.26		0.37 ± 0.21		0.50	±	0.56	±
	0.11		0.12						0.22		0.29	
$T_{\text{sk}} (^{\circ}\text{C})$	33.6	±	33.7	±	36.7 ± 0.3		33.8 ± 0.5 ^{†#}		33.4	±	36.6 ± 0.4	
	0.7 [#]		0.4 [#]						0.6 [#]			
$\Delta T_{\text{f-f}} (^{\circ}\text{C})$	-0.57	±	-0.37	±	-0.22 ± 0.25		0.22 ± 1.04		-0.71	±	-0.27	±
	1.49		1.60						1.52		0.37	
$H_{\text{prod}} (\text{W})$	617 ± 38		621 ± 30		601 ± 58		575 ± 44		597 ± 58		566 ± 58	
$H_{\text{prod}} (\text{W} \cdot \text{kg}^{-1})$	8.2 ± 1.0		8.2 ± 0.9		7.9 ± 0.8		7.6 ± 0.6		7.9 ± 0.9		7.5 ± 0.7	

H_{prod} ($\text{W} \cdot \text{m}^{-2}$)	316 ± 31	319 ± 26	307 ± 27	294 ± 20	307 ± 31	290 ± 24	
$\dot{m}_{\text{sw, peak}}$ ($\text{mg} \cdot \text{cm}^{-2} \cdot \text{min}^{-1}$)	1.17 ± 0.25	1.18 ± 0.27	1.13 ± 0.22	$1.07 \pm 0.17^{\#}$	$0.97 \pm 0.13^{\#}$	$1.54 \pm 0.35^*$	
$\dot{m}_{\text{sw gain}}$ ($\text{mg} \cdot \text{cm}^{-2} \cdot \text{min}^{-1} \cdot ^\circ\text{C}^{-1}$)	1.96 ± 0.73	1.66 ± 0.76	1.06 ± 0.85	1.98 ± 0.74	1.99 ± 1.43	2.93 ± 1.49	
Threshold T_{re} for sweating ($^\circ\text{C}$)	37.45 ± 0.24	37.30 ± 0.20	37.39 ± 0.29	37.37 ± 0.31	37.23 ± 0.40	37.39 ± 0.26	

T_{re} resting rectal temperature, ΔT_{re} difference from resting value in rectal temperature, T_{sk} weighted mean skin temperature, $\Delta T_{\text{f-f}}$ difference between forearm and fingertip skin temperatures, H_{prod} metabolic heat production, $\dot{m}_{\text{sw}}$ sweating rate. Values are mean $\pm$ SD

* Significant difference to values before HeA

[#] Significant difference to heat values

[†] Significant difference to hypoxic values, $p < 0.05$

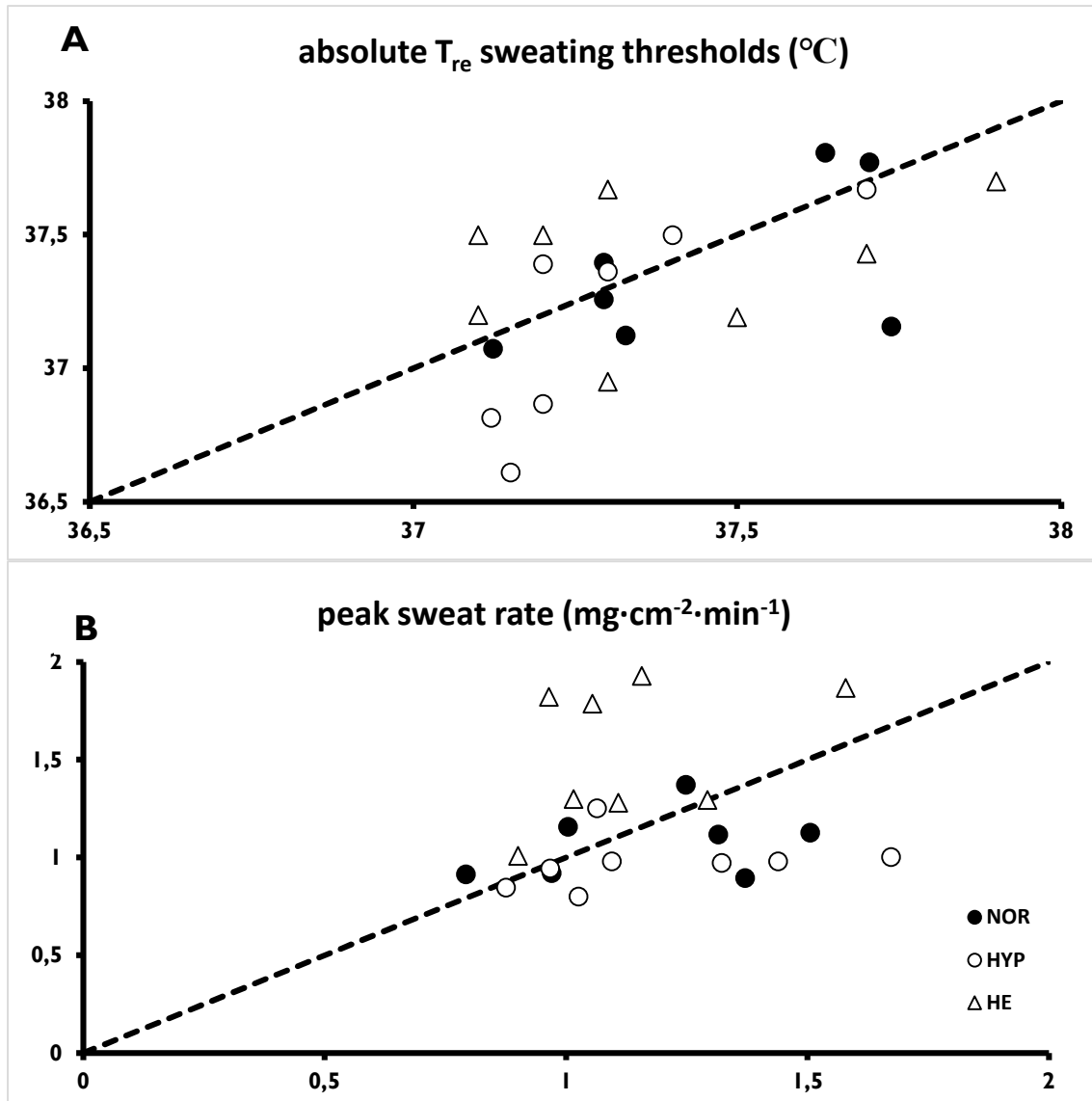


Figure 5.3: Pre- (horizontal axis) and post- (vertical axis) acclimation individual data for (A) absolute sweating thresholds and (B) peak sweat rate in normoxia (closed circles), hypoxia (open circles), and heat (triangles).

5.3. Discussion

Aerobic exercise performance has been shown to be enhanced by the independent application of heat or hypoxic acclimation. The current study examined the interactive effects of heat and hypoxic acclimation protocols on aerobic performance in normoxic thermoneutral or hot conditions. The main findings of the study were that 1) the combined acclimation protocol did not elicit substantial benefits in aerobic performance, and 2) heat acclimation-associated thermoregulatory benefits were partially negated by the superposition of the hypoxic confinement.

5.3.1. Exercise Performance

The first study to employ a combined stressor (heat & hypoxia) acclimation protocol was promising a “conditioning cocktail for team-sport athletes exercising at sea level in temperate conditions” (Buchheit et al., 2013). The fitness level of the participants in the present study could be deemed similar to that study. Considering the lack of effect on $\dot{V}O_{2\max}$, we cannot confirm that live-high train-low in the heat provides an ergogenic tool for aerobic performance in thermoneutral normoxic conditions. Rendell et al. (2017) have also reported unaltered $\dot{V}O_{2\max}$ values, whereas Buchheit et al. (2013) and McCleave et al. (2017) did not measure $\dot{V}O_{2\max}$ following a combined-stressor acclimation protocol. In the former study, the hypoxic stimulus (240 km · h) was probably not sufficient to elicit a substantial erythropoietic effect as verified by no evident changes in Hbmass. In contrast, higher hypoxic doses have potentiated increments of +3.8% and 2.6% in Hbmass in the latter studies, respectively. The differential results might be additionally explained by a baseline effect. Given that the individual Hbmass level is training-dependent (Heinicke et al., 2001), hypoxic acclimation protocols could increase Hbmass, especially in athletes of a lower fitness level (Robach & Lundby, 2012) such as the team-sport athletes recruited by Buchheit et al. (2013). The possibility that the interaction of the heat with the hypoxic stimulus negated erythropoietic adaptations can be safely excluded on the basis that a separate controlled-hyperthermia HeA has been shown not to influence Hbmass (Rendell et al., 2017).

Even though we were unable to measure Hbmass, we are quite confident that the applied hypoxic dose initiated the desired hematological adaptations. Firstly, in our previous study we observed an increase in $\dot{V}O_{2\max}$ following a similar hypoxic acclimation protocol ([Chapter 3](#)). The training stimulus was not of sufficient intensity to account for this increase in $\dot{V}O_{2\max}$, especially since training HR was set to 50% HYP W_{peak} . Secondly, according to a recent meta-analysis, an approximate increase in Hbmass of 4% is expected after the application of such a hypoxic dose (Garvican-Lewis et al., 2016). Finally, in a recent study that confined participants of a similar fitness level at a similar simulated altitude compared to the present study, an increase in Hbmass was noted in all, but one, participants by day 12 (Siebenmann, Cathomen, et al., 2015). Regarding the inability of the hypoxia-induced hematological changes to evoke a $\dot{V}O_{2\max}$ increase, it is notable that EPO concentration undershot baseline levels on the first day post-acclimation presumably permitting a selective destruction of young red blood cells (Alfrey, Rice, Udden, & Driscoll, 1997). Alternatively, the estimated PV contraction might have been accompanied by a reduction in maximal SV and CO mediated by the decreased venous return. Nevertheless, considering our participants performed the maximal tests between the second and fourth day post-acclimation, a restoration of blood volumes towards baseline values may have accounted for the observed unchanged values of peak SV and CO. In HE, as our participants were “training their sweat glands” (see Thermoregulatory data) and enhanced their sweating response (as reflected on peak sweat rate and the gain of the sweating

response in HE), an even lower amount of blood would return to the heart indicated by lower peak CO post-acclimation. In HYP, the failure of the CO to normalize towards baseline levels after hypoxic acclimation has been long considered as the limiting factor for $\dot{V}O_{2\max}$ (Calbet et al., 2003; Saltin et al., 1968).

With $\dot{V}O_{2\max}$ unaffected, an independent increase in GME could account for improvements in aerobic performance as reflected on W_{peak} . Exercise performance has been shown to improve after a combined-stressor acclimation protocol when assessed using a Yo-Yo intermittent recovery test in professional football players (Buchheit et al., 2013), but not a 3 km time-trial in trained runners (McCleave et al., 2017). In both studies, the respective improvement was not superior to the gains in exercise performance after an independent heat-acclimation protocol. These data are in agreement with our recent findings that higher increments in W_{peak} are observed after a controlled-hyperthermia heat-acclimation protocol ([Chapter 4](#)), but not a continuous hypoxic confinement ([Chapter 3](#)). Thus, the possibility that the hypoxic stressor adds significantly to the gains obtained by (heat) training is considered unlikely when exercise is conducted in normoxia. Regarding aerobic performance in hot hypoxic conditions, the employed acclimation protocol succeeded in alleviating the previously reported 51% decrement compared to thermoneutral normoxic conditions (Girard & Racinais, 2014). It is of note that all, but one, participants increased their time to exhaustion. Future research is needed to elucidate whether such a protocol might be recommended for professionals expected to perform long submaximal work, markedly elevating their T_c as a consequence, at high altitudes.

5.3.2. Thermoregulatory Data

Previous controlled-hyperthermia heat-acclimation protocols (Fox et al., 1963; Gibson, Mee, et al., 2015; Rendell et al., 2017) have confirmed the development of positive phenotypic adaptations when humans are acclimatized to heat with the main ones being: reduced T_{sk} and T_{re} , augmented sweating response, lower HR and an expansion of PV. The present combined-stressor approach that included a medium-term heat-acclimation protocol only partially potentiated these adaptive responses. In HE, HR was not reduced probably serving as a compensatory mechanism to the reduced SV compared to NOR during submaximal exercise. The pronounced PV contraction may have stimulated this reflex. Resting T_{re} and T_{sk} remained unaffected. Interestingly, participants were subjected to a higher thermal strain while exercising in HYP post-acclimation compared to NOR as suggested by a $\sim 0.13^\circ\text{C}$ difference (not significant) in ΔT_{re} during the submaximal exercise. This thermal challenge coincided with a lower T_{sk} in the same environmental condition (compared to NOR). The pronounced hypoxia-elicited PV contraction should have resulted in a lower skin blood flow during exercise (Fortney et al., 1981) as well as a lower specific heat capacity of the blood post-acclimation. On the contrary, the decrease in T_{sk} with a simultaneous increase in T_{re} augmented the $T_{\text{re}} - T_{\text{sk}}$ gradient and served to counteract the maladaptative effects of PV contraction in convective heat loss. This occurred at a cost of higher T_{re} which however did not exceed 38.1°C in any of the participants during submaximal exercise. Caution should be taken when extending these results to endurance-type athletes (e.g. mountaineers) or military personnel in which cases T_c could escalate after long bouts of physical activity.

Our participants demonstrated a higher peak sweat rate and gain of the forehead sweating response in HE. It was recently shown that trained humans possess a superior ability to dissipate heat when the total heat load exceeds a certain threshold (Lamarche et al., 2018). As our protocol failed to enhance aerobic fitness, this theory cannot adequately explain why our participants sweated more in HE post-acclimation. This observation together with the fact that participants did not start sweating at a lower T_{re}

might reinforce the idea of distinguishing central from peripheral heat adaptations. Specifically, the augmented sweat rate at any given T_c may be attributed to an effect of training the sweat glands, which is unrelated to aerobic fitness. Aerobic fitness most likely influences the threshold T_c at which sweating is initiated. Decreases in absolute sweating thresholds have been repeatedly related to decreases in resting T_{re} (Buono, Heaney, & Canine, 1998; Patterson et al., 2004). Conversely, as resting T_{re} remained unaltered in the present study, we did not expect any change in the sweating thresholds. For that type of analysis though, a more responsive index of T_c such as esophageal temperature might be warranted.

This is not the first study to demonstrate that adding normobaric hypoxic confinement to a heat-acclimation protocol will negate the expected thermoregulatory adaptations. Recently, McCleave et al. (2018) reported “no changes in T_c , sweat rate or PV” and reasoned that a low ratio of heat to hypoxic exposure, the intermittent nature of HeA together with the fact that training sessions were conducted using the traditional fixed-workload protocol rather than the controlled-hyperthermia technique could explain their findings. We do not agree with the authors that the stimuli should be quantified in a ratio of heat to hypoxic exposure. Both environmental stimuli should be applied as suggested by previous research if they are designed to mediate aerobic gains. Medium-term HeA protocols that have repeatedly induced cardiovascular as well as thermoregulatory adaptations rarely propose more than 15 h of total heat exposure. On the contrary, a hypoxic exposure should exceed 200 h to elicit hematological adaptations (i.e. 225 h in Hauser et al. (2017)). This is why the participants in the present study were confined for 230h at a simulated altitude of ~4000 m and were training everyday in the heat for 90 min. Furthermore, the intermittent nature of HeA did not hinder the adaptation process in the study by McCleave et al. (2018) given that the group that followed the same HeA protocol (training thrice per week for a time period of 3 weeks adding up to 13.5 h of heat exposure), but did not live in hypoxic conditions, displayed lower core and skin temperatures, a reduced HR and an augmented sweat rate. Despite the fact that a controlled-hyperthermia protocol exposes individuals to an ever-increasing forcing function that sustains the heat adaptation impulse (N. A. Taylor, 2014), Gibson, Mee, et al. (2015) elegantly showed that isothermic HeA is as potent as a fixed-work rate protocol in inducing heat adaptation. Consequently, it comes as no surprise that the phenotypic response of that group was identical to the results of our recent study that involved a 10-day controlled-hyperthermia heat-acclimation protocol ([Chapter 4](#)). Finally, the actual HeA-induced endogenous thermal load is usually underestimated as T_c remains high for the first 10-15 minutes after the completion of each acclimation session.

5.3.3. Hematological Data

Our hematological data support the idea that the observed changes were predominantly due to the hypoxic acclimation; increased concentrations of hemoglobin and hematocrit suggesting a pronounced PV contraction, EPO kinetics reaching peak concentration on day 1 of the protocol and returning towards baseline levels on day 10 and renin concentration following the decline in PV. A major limitation of the present study is that we did not measure blood volume compartments directly. By using the Strauss et al. (1951) and Dill and Costill (1974) equations, any erythropoietic effect could lead to a proportional overestimation of the magnitude of the PV contraction. Nevertheless, the PV contraction was similar to the one we reported after a 10-day continuous hypoxic acclimation protocol using the same simulated altitude at the same facility ([Chapter 3](#)). This persistent PV contraction is traditionally attributed to diuretic fluid loss over the course of the 10-day acclimation, a hypothesis that is further confirmed by virtue of the ever-decreasing daily pre-exercise body mass values. The combination of the acclimation protocols seems to have

also impacted the renin kinetics. In the Siebenmann, Cathomen, et al. (2015) study, renin activity decreased by day 3 and remained below baseline values for the remainder of the 28-day hypoxic confinement whereas Keramidas, Mekjavic, et al. (2016) reported a 25% decrease in renin on day 2 of their acclimation protocol, which was thereafter restored to baseline. In both studies, care was taken to ensure that individuals maintained their normal daily activity levels. In the present study, a heat training-mediated stimulation of the renin-aldosterone axis might have counteracted the hypoxia-induced renin suppression. This possibility, however, is excluded as HSP90 that acts to stabilize aldosterone was suppressed during acclimation as indicated by the overshooting the first day that participants returned to normoxia. The low sensitivity of the ELISA kits accompanied by the excessive interindividual variability did not enable us to observe changes in HSP70 and VEGF, respectively in agreement with McCleave et al. (2018).

Chapter 6

Autonomic Temperature Regulation in High Heat Loss Environments Following Heat Acclimation: No Evidence of Cross Adaptation

As endotherms, humans maintain their T_c at a metabolically favorable temperature zone. This is achieved by autonomic responses and behavioral actions. Of the autonomic thermoregulatory responses, vasomotor responses constitute the first line of defense against any undesirable changes in body heat storage, as a consequence of either excessive heat loss or heat gain, which would result in a change in T_c . Once vasomotor responses are incapable of limiting the changes in T_c , the metabolically costly autonomic responses of shivering and sweating are activated. As a result, the favorable zone is bound by the T_c thresholds for heat production (shivering) and heat loss (sweating) responses, and is defined as the “null-zone” (Mekjavic, Sundberg, & Linnarsson, 1991), or interthreshold zone (Mekjavic & Eiken, 2006). The autonomic thermoregulatory responses are characterized by the T_c at which they are initiated (threshold T_c), their gain (sensitivity; magnitude of the response as a function of the relative change in T_c), and their maximal capacity. Chronic or intermittent exposures to deviations of the thermal environment can initiate adaptive modifications to these characteristics of the autonomic thermoregulatory responses. Adaptation to a hot environment will result in a shift of only the T_c threshold for sweating onset to a lower T_c , thus augmenting heat loss. In contrast, exercise training has been reported to shift the T_c thresholds for both shivering and sweating to lower T_c (Baum *et al.* 1976, Dressendorf *et al.* 1977). This phenomenon of a response initiated during cold exposure (i.e. shivering) being modified as a result of adaptation to exercise training is defined as cross adaptation, and since this cross-adaptation can be beneficial for the performance of specific athletes, it is defined as positive cross adaptation (Hensel, 1981).

Brück and co-workers (Hessemer *et al.*, 1986; Schwennicke & Brück, 1976) investigated this cross-adaptation effect during passive HeA, thus omitting the potential effect of exercise training, as had previously been observed in long distance runners and swimmers (Baum *et al.* 1976, Dressendorf *et al.* 1977). They (Schwennicke & Brück, 1976) failed to show a cross adaptive effect of a HeA protocol involving men resting in 45 °C, on the shivering response, despite the expected reduction in the sweating threshold. They reasoned that this was due to the relatively mild heat exposure, eliciting peak esophageal temperatures not higher than 37.7 °C. In a subsequent study (Hessemer *et al.*, 1986), they

conducted a 5-day passive HeA protocol in which participants conducted daily 1-h exposures to an T_a of 55 °C, while resting. Lower mean body temperature shivering thresholds were reported for both shivering and sweating as well as cutaneous vasodilation, with the inter-threshold zone remaining unaffected.

HeA has been repeatedly shown to lower resting and exercise T_c and augment the sweating response in thermoneutral and/or hot conditions (Nadel et al., 1974; Patterson et al., 2004; Roberts et al., 1977); a lower threshold T_c as well as increased gain and maximal capacity have been commonly observed. However, a lower sweating threshold T_c would narrow the inter-threshold zone unless the shivering threshold T_c is simultaneously lowered. In turn, a narrower inter-threshold zone would result in a less economical temperature regulation with more water or energy expenditure despite a higher regulatory efficiency.

6.1. Methods

We took advantage of a larger study ([Chapter 4](#)) investigating the effects of HeA on cardiovascular, thermoregulatory and hormonal responses in normoxic and hypoxic conditions.

6.1.1. Participants

Twelve healthy males (age: 22.3 ± 2.8 years, $\dot{V}O_{2max}$: 52.4 ± 7.0 mL · kg⁻¹ · min⁻¹, body mass: 75.7 ± 7.1 kg, stature: 182.3 ± 6.7 cm and body fat: $15.4 \pm 2.2\%$) were recruited to participate the present study. All participants were not systemically exposed to $T_a > 30$ °C or cool water for the months preceding the experimental procedures. Moreover, they had to abstain from caffeine and alcohol consumption throughout the whole study. Prior to inclusion in the study, the participants gave written consent and were extensively informed regarding the study protocol as well as the possible risks involved.

6.1.2. Experimental Design

The study investigated the effect of HeA on autonomic and behavioural thermoregulatory mechanisms. The former was achieved by monitoring participants' heat production and heat loss during exposure to a high heat loss environment achieved by immersing them in a bath of well-stirred tepid water maintained at 28 °C (Mekjavic et al., 1991). Thermoregulatory behavior was reflected in participants' subjective determination of their thermal comfort zone during exposure to transient temperatures, achieved with a water-perfused suit (Ciuha & Mekjavic, 2016, 2017). Both of these thermoregulatory trials were scheduled not more than three days before and three days after the 10-d HeA. The training sessions were held at the same time of the day, as were the thermoregulatory trials (± 1 hour).

6.1.3. Heat Acclimation Protocol (HeA)

The HeA protocol employed has been described previously ([Chapter 4](#)). Briefly, it comprised 10 daily 90-min cycle ergometry training sessions. Participants performed their training in a climatic chamber (IZR d.o.o., Skofja Loka, Slovenia) maintained at 35°C, and 50% RH. The controlled-hyperthermia model was selected on the basis that the physiological thermal strain is sustained during HeA despite the ongoing adaptation (N. A. Taylor, 2014). In each training session, the participants commenced cycling at an absolute workload equal to 50% of their individual W_{peak} and reached a T_{re} of 38.5 °C

within 30-45 minutes (Gibson et al., 2017). Thereafter the workload was adjusted ($\pm 10\%$ PPO) at 5 min intervals, so that T_{re} was maintained at the target level. The participants were instructed to drink 500 mL of water two hours before each training session.

6.1.4. Autonomic Thermoregulation

Participants were instructed to consume the same meal 2 h before arriving at the laboratory for the days when the immersions were scheduled. Pre- and post- HeA, the participants underwent a maximal exercise test in thermoneutral conditions ($T_a = 23.0 \pm 0.04$ °C and $RH = 50.9 \pm 0.9\%$). Before conducting the exercise test, the participants emptied their bladder and their body mass was recorded. The test consisted of two stages; a steady-state exercise during which the participants were cycling at an absolute workload equal to 40% PPO. The steady-state exercise was followed by incremental exercise to exhaustion. The initial workload was set at 100 W thereafter increasing by $20 \text{ W} \cdot \text{min}^{-1}$. Upon completion of the performance test, the participants were immersed in a bath of well-stirred water maintained at 28°C. During the transition from the cycle ergometer to the water bath body mass was recorded. The duration of the immersions was 60 minutes.

Rectal as well as ventral forearm and fingertip temperatures were measured continuously during the performance test and the subsequent immersion using a rectal thermocouple probe inserted 10 cm beyond the anal sphincter and thermistors (MSR 12, Henggart, Switzerland) attached to the forearm and fingertip with a piece of adhesive tape, respectively. The forearm-fingertip skin temperature difference is considered as a valid index of cutaneous vasomotor tone (House & Tipton, 2002; Rubinstein & Sessler, 1990) and has been validated during water immersion (Mekjavic et al., 2012) with values of $\Delta T_{f-f} > 2$ °C indicating vasoconstriction. All testing sessions were held in the same laboratory as the training sessions. T_a was kept at 25 °C.

Breath-by-breath $\dot{V}O_2$ was measured with a portable metabolic system (K4, Cosmed, Rome, Italy). The gas analyzers and pneumotachograph of the metabolic system were calibrated before each immersion with two different gas mixtures and a 3-liter syringe, respectively. In addition to $\dot{V}O_2$, heart rate (HR) and S_pO_2 were recorded using a fingertip oximetry device (Nellcor, BCI 3301, Boulder, USA) at 5-min intervals.

Throughout the experiment, participants provided ratings of their thermal comfort and thermal sensation every 5 min. Thermal comfort was evaluated on a 7-point scale, with the assigned descriptors being: 0–0.5 = comfortable, 1–1.5 = slightly uncomfortable, 2–2.5 = uncomfortable and 3 = very uncomfortable. Thermal sensation was derived from a 7-point scale, with the following descriptors: 3 = hot, 2 = moderately hot, 1 = warm, 0 = neutral, -1 = cool, -2 = moderately cold and -3 = cold.

6.1.5. Data Analysis

Temperature and respiratory variables were analyzed for the first 45 minutes of the immersion. As one participant did not demonstrate any shivering response both before and after HeA, his data were excluded from the analysis of the shivering response ($n=11$). T_{re} data for another participant were excluded as a result of big minute-to-minute variations ($n=11$). The normality of the measured variables was checked using the Shapiro-Wilk test. The shivering threshold was defined as a sustained increase in the moving average of $\dot{V}O_2$ above baseline levels. Least square linear regression was used to determine the mean slope relating shivering to T_{re} . The reported values are averages of the individual thresholds and gains derived from linear regression parameters. Shivering thresholds and gains were analyzed using paired t-tests. Respiratory variables were averaged into 5-min bins. Temperature data were recorded into minute bins. A two-way ANOVA (acclimation status x time) with repeated measures was used to define the overall differences in temperature

and respiratory variables. Two-way repeated measures ANOVA (acclimation status x time) was used to compare T_{sk} measurements. If a significant F value was found ($p < 0.05$), critical differences were analyzed by Tukey's procedure to locate the significant mean differences. Subjective ratings of temperature perception and thermal comfort were analyzed with a Wilcoxon non-parametric test. Data are presented as means $\pm$ SD for parametric statistics or as medians (range) for nonparametric statistics. The alpha level of significance was set *a priori* at 0.05. Statistical analysis was performed using Statistica 5.0 (Statsoft Inc., Tulsa, OK) and SPSS (version 20, Chicago, IL, USA).

6.2. Results

6.2.1. Heat Acclimation

HeA induced the expected physiological adaptations: reduced resting and exercise T_{re} , increased $\dot{m}_{sw}$ as well as decreased exercise HR post-HeA ([Chapter 4](#)). End-exercise T_{re} was lower post-HeA (pre: 38.18 ± 0.38 vs post: 37.90 ± 0.35 ; $p = 0.032$) even though the PPO attained during the preceding maximal test was higher post-HeA (pre: 336 ± 40 vs post: 363 ± 38 ; $p < 0.001$).

6.2.2. Autonomic Thermoregulation Assessment

When the participants entered the tank, T_{re} was not different post-HeA ($p = 0.092$) (Table 6.1). There was a main effect for time ($p < 0.001$) but not for acclimation status ($p = 0.27$) on T_{re} . The rate of decrease in T_{re} was not affected by the acclimation status (pre: -0.029 ± 0.006 vs post: -0.030 ± 0.007 $^{\circ}\text{C} \cdot \text{min}^{-1}$; $p = 0.31$) (Figure 6.1).

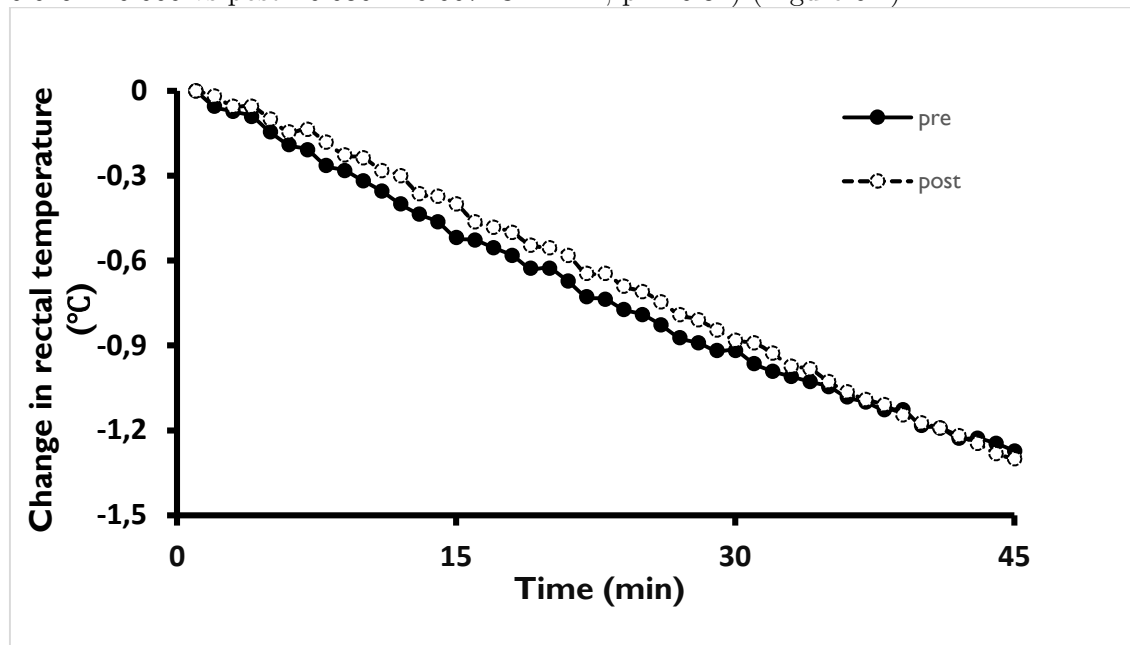


Figure 6.1: Relative changes in rectal temperature (ΔT_{re}) during the first 45 minutes of immersion before and after heat acclimation. Values are means $\pm$ SD.

Likewise, there was a main effect for time ($p < 0.001$), but not acclimation status ($p = 0.17$) on ΔT_{ef} . Figure 6.2 represents the index of vasomotor tone/peripheral perfusion as a function of T_{re} . Participants were immersed in the tank in a similar state of vasodilation (ΔT_{ef} pre = 0.73 ± 2.5 $^{\circ}\text{C}$ vs ΔT_{ef} post = 0.21 ± 1.19 $^{\circ}\text{C}$, $p = 0.21$). During immersion in both conditions, the index of vasomotor tone increased over time ($p < 0.001$) reaching values of 2.71 ± 2.33 $^{\circ}\text{C}$ and 2.75 ± 1.33 $^{\circ}\text{C}$ pre-HeA and post-HeA, respectively. The increase from baseline values became statistically significant ($p < 0.05$) at minutes 11 and 10 of pre-HeA and post-HeA immersions, respectively (Figure 6.2). The respective T_{re} declines were -0.35 ± 0.14 $^{\circ}\text{C}$ and -0.24 ± 0.07 $^{\circ}\text{C}$. Thus, T_c -induced vasoconstrictor response was higher post-HeA compared to pre-HeA.

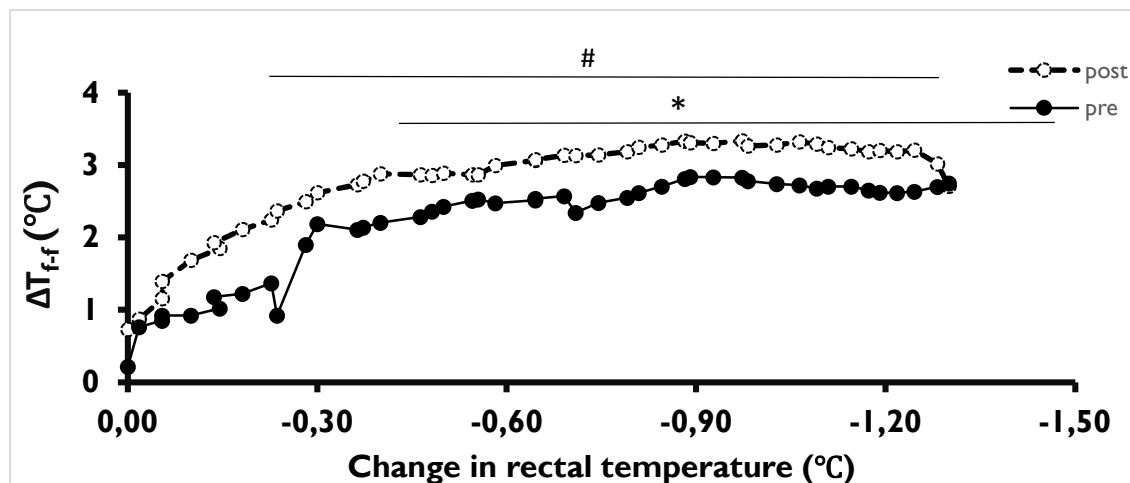


Figure 6.2: Changes in the forearm-fingertip temperature difference as a function of the relative decrease in rectal temperature for the pre- and post- heat acclimation immersions. *denotes values that are significantly different ($p < 0.05$) from 1st min values pre-heat acclimation. # denotes values that are significantly different ($p < 0.05$) from 1st min values post-heat acclimation.

The changes in the main cardiorespiratory parameters over time are depicted in Figure 6.3. There was no main effect of HeA on $\dot{V}O_2$ ($p = 0.84$) or V_E ($p = 0.89$), but there was a tendency for HR to be lower post-HeA ($p = 0.08$). However, there was a main effect for time on $\dot{V}O_2$, V_E and HR ($p < 0.001$). The time-acclimation interaction was also significant for $\dot{V}O_2$ ($p = 0.001$), but not V_E ($p = 0.21$) or HR ($p = 0.35$). Interestingly, $\dot{V}O_2$ was significantly higher ($p < 0.02$) in the 1st min (as an index of the cold shock response) only pre-HeA. $\dot{V}O_2$ was lower in the 10th min than after the 30th min ($p < 0.03$) pre-HeA and after 40th min post-HeA ($p = 0.02$). V_E was also higher ($p < 0.001$) in the 1st min across HeA status. However, post-hoc tests did not show any difference between the 10th min and the following time points ($p > 0.14$).

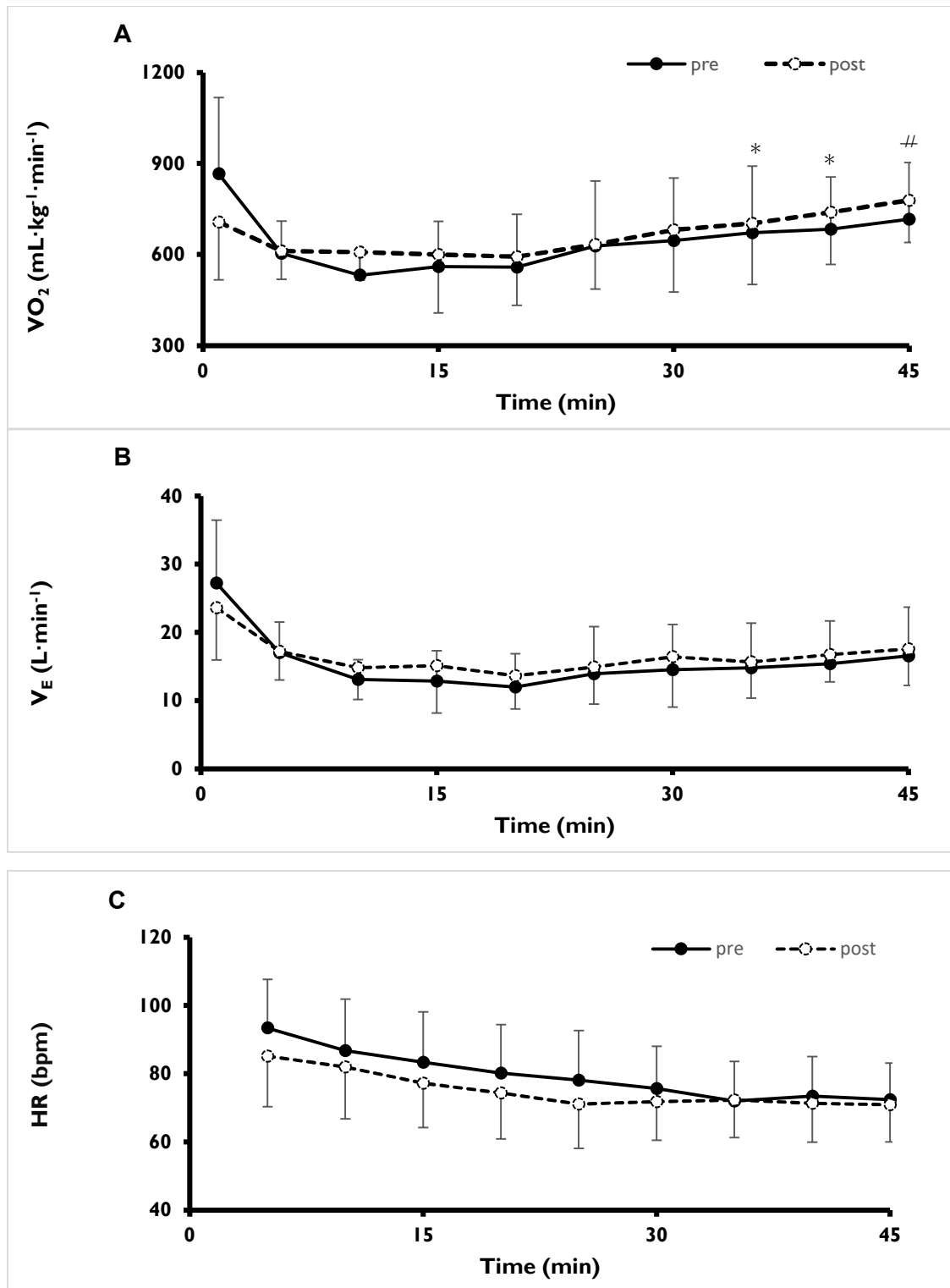


Figure 6.3: Change in the cardiorespiratory responses (oxygen uptake, minute ventilation, HR) as a function of time for the immersions pre- and post-heat acclimation. *denotes values significantly different from 10th min value pre-heat acclimation #denotes values significantly different from 10th min value both pre- and post-heat acclimation.

Shivering thresholds did not change post-HeA either expressed as absolute T_{re} s ($p = 0.27$) or changes in T_{re} ($p = 0.43$) (Table 6.1). There was a large inter-individual variability as thresholds (expressed as changes in T_{re}) ranged from -0.1 to -2.1 °C pre-HeA and -0.1

to -1.7°C post-HeA, respectively. No difference in the time elapsed between the immersion and the initiation of the shivering response was observed (Table 6.1) ($p = 0.69$).

Table 6.1: Selected average thermoregulatory responses calculated for the first 45 minutes of immersion before and after heat acclimation.

	Pre-heat acclimation	Post-heat acclimation
$T_{\text{re start}} (^{\circ}\text{C})$	38.33 ± 0.33	38.12 ± 0.36
$\Delta T_{\text{re}} (^{\circ}\text{C})$	-1.27 ± 0.31	-1.30 ± 0.27
Time to shivering (min)	27 (10-53)	26 (3-48)
Shivering gain ($\text{mL} \cdot \text{min}^{-1} \cdot ^{\circ}\text{C}^{-1}$)	-900 ± 349	-1102 ± 1053
Peak $\Delta \dot{V}\text{O}_2$ ($\text{mL} \cdot \text{min}^{-1}$)	271 ± 166	288 ± 159
Threshold ΔT_{re} for shivering ($^{\circ}\text{C}$)	-0.93 ± 0.62	-0.89 ± 0.51
Threshold T_{re} for shivering ($^{\circ}\text{C}$)	37.37 ± 0.76	37.20 ± 0.75

Values are mean $\pm$ SD except for Time to shivering median (range).

Similar to the thresholds, shivering gains remained unaffected post-HeA ($p = 0.61$). The individual values once again displayed a big variability ranging from -261 to $-1174 \text{ mL} \cdot \text{min}^{-1} \cdot ^{\circ}\text{C}^{-1}$ and -115 to $-4106 \text{ mL} \cdot \text{min}^{-1} \cdot ^{\circ}\text{C}^{-1}$ pre- and post-HeA, respectively. The peak average $\Delta \dot{V}\text{O}_2$ were identical between the two acclimation conditions ($p = 0.76$) (Table 6.1, Figure 6.4). Wilcoxon tests showed that 11 out of 12 participants felt significantly warmer ($p < 0.05$) post-HeA. Moreover, 9 out of 12 participants felt more comfortable ($p < 0.05$) post-HeA.

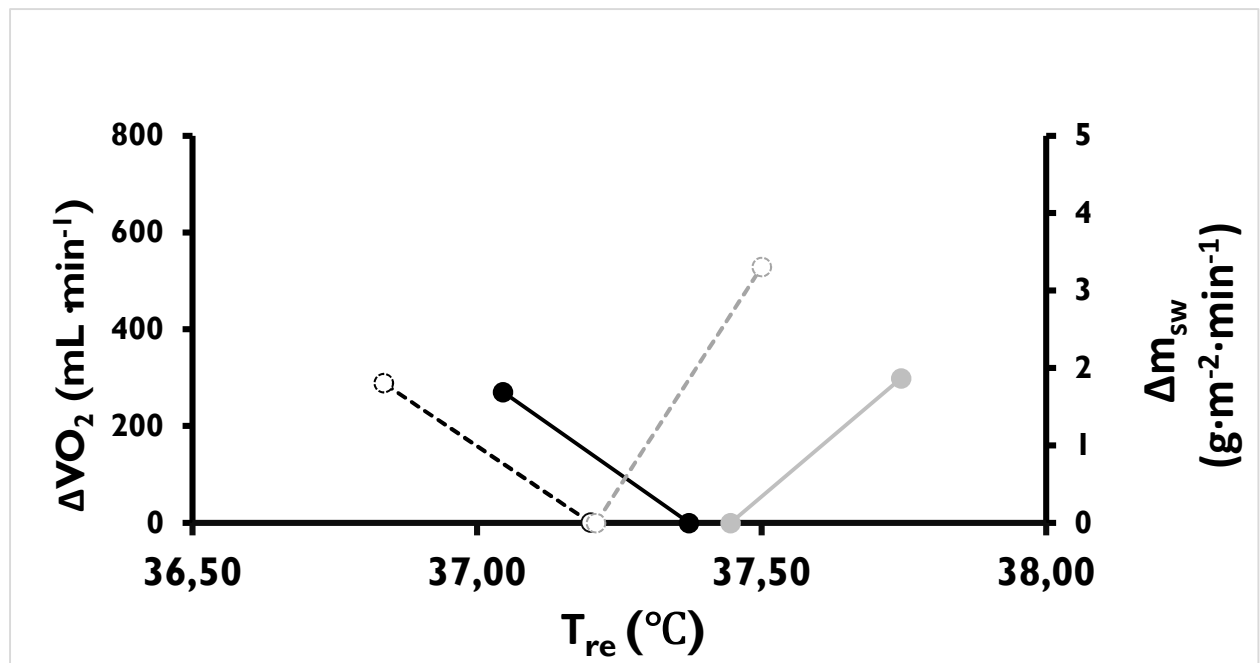


Figure 6.4: Average relative changes in the shivering and the sweating response (threshold, gain, maximal capacity) as a function of rectal temperature pre- and post-heat acclimation. Standard deviations omitted for clarity. Solid and dashed lines represent values pre- and post-heat acclimation, respectively. Gray and black circles represent the sweating and shivering responses, respectively.

6.3. Discussion

The rate of decrease in T_{re} during water immersion was identical pre- to post-heat acclimation. The change in T_{re} reflects the balance between heat production and heat loss responses. Since the shivering response (threshold, gain, peak value) was similar pre- to post-heat acclimation, and the difference in the pre- to post-heat acclimation vasoconstriction response was not of sufficient magnitude, T_{re} was not affected by heat acclimation status, rejecting the hypothesis that heat acclimation would decrease T_{re} in a high heat loss environment. Given that the sweating threshold was lowered post-heat acclimation ([Chapter 4](#)), the finding that the shivering threshold remained unaltered indicates a narrower and less economical interthreshold zone.

6.3.1. Core Temperature Threshold for Shivering

The only research group that has previously explored the effect of HeA on the shivering response reported equivocal results. Schwennicke and Brück (1976) demonstrated no effect of a passive HeA (T_a maintained at 45 °C) on the shivering response measured during a cold air test. The authors claimed that the HeA protocol employed was relatively mild to produce any physiological adaptations with the average esophageal temperature during HeA sessions not exceeding 37.7 °C. The same group also reported a 0.36 °C decrease in the body temperature threshold for shivering (increase in $\dot{V}O_2$) after a passive HeA that included five hourly sessions in 55 °C (Hessemer et al., 1986). A closer examination at the published data reveals that when the shivering threshold was expressed relative to esophageal temperature, a similar (minor) decrease, as in our study, was observed. More specifically, the esophageal temperature threshold fell from 36.58 ± 0.08 °C to 36.44 ± 0.09 °C whereas in our study T_{re} threshold fell from 37.37 ± 0.76 °C to 37.20 ± 0.75 °C. As our experiments involved whole-body immersions in tepid water, there was only a small change in skin temperature, which was then clamped at the same level as the water temperature. For this reason, we expressed the shivering threshold relative to T_{re} instead of body temperature.

6.3.2. Core Temperature Response and Peripheral Perfusion

Dynamic exercise preceding water immersion has been used in previous studies (Botonis, Kounalakis, Cherouveim, Koskolou, & Geladas, 2017; Mekjavic, Golja, Tipton, & Eiken, 2005) as a means to accelerate heat production and heat loss responses. However, a confounding factor in our attempt to compare our results with past studies is water temperature. Immersion in 28 °C is regarded as a thermoneutral stimulus albeit sufficient to initiate heat production responses (Castellani & Tipton, 2015; Mekjavic et al., 1991). Interestingly, a higher cooling rate (an average decrease in T_{re} of 1.3 °C in 45-min immersions) was noted in the present study compared to the same time point in most (0.8 °C in 24 °C water (Botonis et al., 2017), 0.75 °C in 28 °C water (Mekjavic et al., 2005)), but not all past studies (N. A. Taylor, Caldwell, Van den Heuvel, & Patterson, 2008). This is due to the exercise-induced elevation in T_c prior to the immersion, which then maintains a vasodilatory tone, initiating core cooling. Due to the thermoneutral water temperature, the T_c drive for vasodilatation predominates the peripheral skin temperature drive for vasoconstriction.

The decrease in T_{re} is mediated by the conductive/convective heat loss from the skin, the magnitude of the latter being dependent on skin perfusion, which in the present study is reflected in ΔT_{ef} values. Upon immersion ΔT_{ef} was below 1 °C, and thereafter increased in both trials attaining asymptotic values of ~3 °C in both conditions. $\Delta T_{ef} > 2$ °C is

indicative of vasoconstriction (House & Tipton, 2002), and this was achieved at lower decrements in T_{re} in the post-HeA trial compared to the pre-HeA trial. During immersion in cold water ΔT_{ef} may reach levels as high as 8 °C (Botonis et al., 2017), thus there was no effect of HeA on the level of vasoconstriction, only perhaps on its T_c -induced onset. The relative vasodilatation of our participants during immersion in 28 °C water augmented conductive/convective heat loss from the skin. Although the core-induced peripheral vasoconstrictor response was slightly greater post-HeA compared to pre-HeA, the resultant decrease in heat loss was most likely of insufficient magnitude to be reflected in the T_{re} response during immersion.

6.3.3. Immersion Response

The difference in the peripheral perfusion between the pre- and post-HeA immersions may explain, to a degree, the difference in $\dot{V}O_2$ responses observed upon immersion between the pre- and post-HeA trials. Namely, Mekjavic and Bligh (1989b) reported that the ventilatory and $\dot{V}O_2$ responses on immersion in water comprise a thermogenic and hydrostatic component, the latter causing a shift of venous blood to the thorax, and causing a transient increase in the uptake of oxygen, unrelated to any thermogenic needs. They reported transient elevations in V_E and $\dot{V}O_2$ during immersions in water ranging from 10 °C to 40 °C, with the magnitude of the transient elevations decreasing with water temperature. Thus, the significantly higher peripheral perfusion in the pre-HeA trial will have caused a higher volume of venous blood to be shifted towards the thorax, resulting in a transiently greater uptake of oxygen.

6.3.4. Neurophysiological Perspective

The theory of reciprocal cross-inhibition (RCI) attempts to link the thermoregulatory responses observed at the system level with the activity of the peripheral and core/central cold and warm receptors (Bligh, 2006). Specifically, this model investigates how the overlapping bell-shaped static firing characteristics of the cold and warm receptors are integrated centrally into the responses of shivering and sweating (Mekjavic & Eiken, 2006). Data from the present experiments clearly demonstrate that the sweating threshold occurred at a lower T_{re} ([Chapter 4](#)). According to the RCI model, HeA as a thermal factor may induce shifts in either the sweating threshold or both the sweating and shivering thresholds. If HeA only affects the sweating threshold and consequently narrows the interthreshold zone, it presumably acts on the pathway after the anatomical locus of cross-inhibition. Conversely, if HeA shifts the entire inter-threshold zone, it would act before the point of cross-inhibition (Mekjavic & Eiken, 2006). As shivering was initiated essentially at the same T_{re} , we could claim that HeA acts at a point of the sensor-to-effector neuraxis after the region of cross-inhibition, which means that it is mediated centrally. In this way, the conclusion made before that: “...there must be some adaptation between heat and cold” (Hessemer et al., 1986) is not supported by the present data.

6.3.5. Set-Point or Intertreshold Zone

Another interesting finding of the present study is that the shivering thresholds were observed to occur, paradoxically, at the same T_{re} as the sweating thresholds (pre-HeA: 37.37 ± 0.76 vs 37.45 ± 0.39 , post-HeA: 37.20 ± 0.75 vs 37.21 ± 0.38 °C). This seems to be direct evidence in favour of the set-point theory of human temperature regulation as there is no zone between the autonomic thresholds (see also Figure 6.4). A similar misinterpretation of such results was made by Cabanac and Massonnet (1977). However, in our experiments participants were cycling at a thermoneutral environment prior to being

immersed in the 28 °C water tank. Given that T_{sk} during exercise was ~34 °C ([Chapter 4](#)) and should have been rapidly clamped at water temperature during immersion, a decrease in T_{sk} may be postulated during the transfer from exercise to immersion. Alterations in skin temperature would result in T_c changes owing to the return of cooled venous blood from the cutaneous vasculature (Mekjavic & Bligh, 1989a). Thus, a protocol that will ensure uniform cooling of the core without fluctuations in T_{sk} should be applied to investigate the simultaneous changes in sweating and shivering threshold T_c (Mekjavic et al., 1991). Alternatively, the fact that shivering thresholds were measured following dynamic exercise argues against the set-point theory. Prior exercise (Kenny et al., 1998) or passive heating (Castellani, Young, Kain, Rouse, & Sawka, 1999)-induced increases in shivering thresholds demonstrate that the threshold is not held constant at an absolute level but is rather sensitive to acute changes in body temperature. Had we not scheduled water immersions following maximal exercise, a direct comparison between sweating and shivering thresholds would be feasible to establish the exact range of the inter-threshold zone.

6.3.6. Exercise Fatigue

We should acknowledge that residual fatigue (resulting from the 10-day exercise-induced HeA protocol preceded and followed by maximal tests) may have been another confounding factor in the post-HeA results. However, several days of exhaustive exercise do not seem to impair shivering, measured as metabolic heat production (Castellani et al., 2001). As acute fatigue does not influence either the shivering (Castellani et al., 1999) or the sweating (Kacin, Golja, Tipton, Eiken, & Mekjavic, 2008) response, the experimental design which included maximal exercise tests preceding water immersions should not be implicated in the study outcomes.

6.3.7. Limitations

The higher cooling rate observed in previous studies that utilized a similar temperate water temperature (26 °C) (N. A. Taylor et al., 2008) can be accredited to: a) the different site of T_c measurement (rectal vs esophageal), and b) the highest initial T_{re} – participants were exercising to reach a T_{re} of 39.5 °C as the authors were trying to mimic exertional heat stroke conditions. During exercise, gastrointestinal blood flow is immensely reduced. As a result, changes in T_{re} would be based on changes in local heat storage mainly via conduction. On the contrary, as esophagus lies closer to the left atrium and aorta, it responds faster to changes in the central blood volume temperature. Given that both studies involved dynamic changes in ambient conditions, using T_{re} as a surrogate marker for T_c constitutes a limitation in the present study.

Chapter 7

Combined Effects of Hypoxia and Heat: Importance of Hypoxic Dose

We congratulate Neal and colleagues (Neal et al., 2017) on a nicely executed cross-over study regarding the combined effects of heat and hypoxic acclimation on exercise performance in temperate conditions. They clearly demonstrate that during a HeA protocol the addition of a concomitant nocturnal hypoxic exposure does not provide any benefits to performance in normoxic environment. Whereas we concur with the authors' findings, we would advise caution regarding such a general conclusion. Namely, the hypoxia-induced increment in EPO concentration would suggest that the hypoxic acclimation protocol was either insufficient, or that the acclimation to hypoxia was perhaps confounded by the concomitant acclimation to heat. The authors interpreted their findings assuming that the hypoxic acclimation did not affect the acclimation to heat. However, on the basis of the observed EPO responses, one might also conclude that acclimation to hypoxia was impaired by the concomitant HeA.

The effects of hypoxic acclimation are predominantly modulated by the hypoxic dose (Wilber et al., 2007). While short-term hypoxic protocols might provoke a certain degree of ventilatory acclimation (Debevec & Mekjavic, 2012), a significantly greater hypoxic dose seems required for hematological benefits, namely Hbmass increase and subsequent performance enhancement. A robust increase in Hbmass requires at least two weeks of nocturnal (> 10 hrs/day) hypoxic exposures (Bonetti & Hopkins, 2009; Gore et al., 2013; Rasmussen, Siebenmann, Diaz, & Lundby, 2013). Thus, the duration and severity of hypoxia need to be considered when assessing the overall hypoxic dose (M. J. Tipton, Franks, Sage, & Redman, 1997). Based on a meta-analysis, of 30 recent studies (Garvican-Lewis et al., 2016), the hypoxic dose employed by Neal et al. (Neal et al., 2017) ($240 \text{ km} \cdot \text{h}$) could only result in $< 2\%$ increase in Hbmass. It is therefore, not surprising that Neal et al. (Neal et al., 2017) did not observe any significant increase of Hbmass, albeit a slight, but statistically significant, increase in plasma EPO. A recent investigation (Siebenmann, Cathomen, et al., 2015) did not find any correlation between the individual EPO and Hbmass responses during and following high altitude exposure, suggesting that plasma EPO could only serve as an indirect marker of the erythropoietic stimulus. These data indicate that the hypoxic dose employed by Neal et al. (Neal et al., 2017) was most likely insufficient to provoke a robust hematological acclimation response and would suggest that the hypoxic protocol was ineffective. The focus of their study was the simulation of the intermittent hypoxic (i.e. sleep high-train low) protocol incorporated by some endurance athletes in their training regimen. Thus, from an athletic performance aspect, the described limitation of the simulated protocol is important.

Due to the significant individual variability in responses to a hypoxic stimulus inherent in acclimation protocols (Chapman et al., 1998; Friedmann et al., 2005), and given the relatively small number of participants employed ($n=8$) and the even smaller number included in the hematological analysis (Neal et al., 2017), this variability might have considerably masked the potential effects of hypoxic acclimation. As speculated by the authors, the heat acclimation-induced increases in plasma (+3.5%) and blood (+5.9%) volumes would have been attenuated had the hypoxic dose been greater, as demonstrated previously (McCleave et al., 2017).

Prospective studies should perhaps ensure that a measurable degree of acclimation has occurred.

Chapter 8

Conclusions

Through a series of studies on human participants, this thesis has evaluated the concept of cross-adaptation, proposing that adaptation to one stressor was reflected in the physical performance during exposure to another stressor. Thus, cardiovascular and thermoregulatory responses to exercise in normoxic, hypoxic and hot ambient conditions were evaluated after adaptation to the stressors of exercise (Study 1, Chapter 2), hypoxia (Study 2, Chapter 3), and heat (Study 3, Chapter 4). The possibility that adaptation to the combined stressors of heat and hypoxia might act synergistically to provide a benefit to physical performance was also evaluated (Study 4, Chapter 5). Finally, focusing on the thermoregulatory system, the thesis has also evaluated whether adaptation to heat, in which heat loss mechanisms are augmented, is reflected in the responses during exposure to a high heat loss environment, in which the predominant autonomic thermoregulatory responses are those of heat conservation and heat production.

The main findings of the present thesis can be summarized as:

Study I. In any acclimation protocol incorporating a training regimen, the contribution of the training effect on aerobic gains will be dependent on an individual's initial fitness level, such that it is significant for individuals with low fitness level, and unlikely to be a significant contributing factor in individuals with a higher fitness level. This should be taken into account when comparing the efficiency of acclimation protocols. In contrast, thermoregulatory benefits were observed in the trained individuals owing to a higher thermal load sustained during the training sessions. The training effect may significantly contribute to the adaptations observed during acclimation to a hot or hypoxic environment.

Study II. A 10-day hypoxic acclimation protocol combined with daily moderate-intensity exercise enhanced maximal aerobic power, but not peak power output in both normoxic and hot conditions. No performance improvements were observed in hypoxia. The hypoxic protocol also did not affect thermoregulatory responses in any of the tested environmental conditions. The extent to which hypoxic living and/or training can improve performance measures, especially in the heat, should be explored further, as this was only the first study to examine this cross-adaptation potential.

Study III. A medium-term controlled-hyperthermia heat acclimation protocol can benefit thermoregulatory responses and peak power output, but not maximal aerobic power in both normoxic and hot conditions. Training in the heat did not facilitate maximal aerobic power, the sweating response or peak power output when participants exercised in acute hypoxia.

Study IV. A 10-day combined heat and hypoxic acclimation protocol does not improve aerobic performance in normoxic thermoneutral or hot conditions. Thermoregulatory gains known to be mediated by the controlled-hyperthermia heat-acclimation protocol employed in the present study are partially negated when superimposing a 10-day normobaric

hypoxic confinement in trained males. Thus, combining heat and hypoxic acclimation does not seem to be superior to traditional acclimation/training protocols.

Study V. Heat acclimation influenced heat dissipation, but not heat production during immersion in thermoneutral water. The observed increase in peripheral perfusion was not of sufficient magnitude to affect the decrease in core temperature during the immersion.

Based on the findings of the studies outlined above, the general conclusion that can be drawn regarding cross-adaptation is, that for acclimation protocols of 10-d in duration, the cross-adaptive responses observed at the cellular level are not reflected in the responses at the systems level.

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Publications Related to the Thesis

Journal Articles

- SOTIRIDIS, A.**, DEBEVEC, T., CIUHA, U., EIKEN, O., MEKJAVIĆ, I.B. (2018). Heat acclimation does not affect maximal aerobic power in thermoneutral normoxic or hypoxic conditions. *Experimental physiology*, [in press], doi: 10.1113/EP087268.
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- SOTIRIDIS, A.**, DEBEVEC, T., CIUHA, U., MCDONNELL, A., MLINAR T., ROYAL, J., Mekjavic, I.B. Maximal aerobic power is enhanced after 10 days of moderate-intensity training in untrained individuals. (In preparation)
- SOTIRIDIS, A.**, CIUHA, U., DEBEVEC, T., MEKJAVIC, I.B. Autonomic and behavioural temperature regulation in high heat loss environments following heat acclimation: no evidence of cross adaptation (In preparation)

Conference Papers

- SOTIRIDIS, A.**, DEBEVEC, T., CIUHA, U., MEKJAVIĆ, I.B. Heat acclimation augments peak power output and thermoregulatory responses in thermoneutral but not hypoxic conditions. Book of abstracts: Sport science at the cutting edge, 23rd Annual Congress of the European College of Sport Science, 4-7 July 2018, Dublin, Ireland. [S. l.]: European College of Sport Science. 2018, str. 19.
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Biography

Alexandros Sotiridis is a PhD candidate at the Jožef Stefan International Postgraduate School and a member of the Environmental Physiology Laboratory at the Jožef Stefan Institute. His research focuses on the independent and interactive effects of environmental stressors (heat, hypoxia, microgravity) on human physiological systems, with particular reference to cardiovascular, hematological and thermoregulatory functions. His supervisor for his doctoral dissertation was Professor [Igor B. Mekjavić](#) (co-supervisors: Dr. [Tadej Debevec](#) and Associate Professor [Maria Koskolou](#)).

In 2016, he completed his BSc (GPA: 9.19/10) at the Faculty of Physical Education and Sports Science, National and Kapodistrian University of Athens. The title of his undergraduate thesis, supervised by Professor [Nikolaos D. Geladas](#), was: “Effects of oral ingestion of nitrates on the arterial pressure during rest and static exercise”.

In 2012, Alexandros Sotiridis received his diploma in Electrical Engineering and Computer Science from the National and Technical University of Athens, Greece. His thesis was entitled “The time-optimal control problem: theory and applications” and was supervised by Professor [Ioannis Tsinias](#).

Appendix A



REPUBLIKA SLOVENIJA
MINISTRSTVO ZA ZDRAVJE

Komisija Republike Slovenije za medicinsko etiko

Prof. dr. Igor B. Mekjavič
Inštitut "Jožef Stefan"
Jamova cesta 39
1000 Ljubljana

igor.mekjavic@ijs.si

Številka: 0120-494/2018/9
Datum: 22. februar 2019

Zadeva: Ocena etičnosti predložene raziskave

Spoštovani gospod prof. dr. Igor Mekjavič

Komisija Republike Slovenije za medicinsko etiko (KME) je dne 22. 10. 2018 (datirano z datumom 19. 10. 2018) od vas prejela vlogo za oceno etičnosti raziskave z naslovom "Posamezni in skupni učinki aklimatizacije na hipoksično in vročino na vadbeno zmogljivost in termoregulacijsko funkcijo pri človeku X-Adapt".

V okviru ARRS projekta ARRS-RPROJ-JR-Prijava/2017-II/531 načrtujete poskuse, cilj katerih je ovrednotiti posamezne in skupna učinke aklimatizacije na večjo nadmorsko višino in vročino na vadbeno sposobnost pri človeku.

KME je na svoji 22. januarja 2019¹ ugotovila, da je vloga popolna ter raziskava etično sprejemljiva. S tem vam za izvedbo raziskave izdaja svoje soglasje.

P.S.: Pri morebitnih nadaljnjih dopisih v zvezi z raziskavo se obvezno sklicujte na številko tega dopisa.

S spoštovanjem.

Pripravila:
Maja Žejn

dr. Božidar Voljč, dr. med.,
predsednik KME

¹ Seznam članov KME, ki so podpisali o vlogi, in izjava, da KME deluje v skladu z zadevnimi zakoni in predpisi, sta na voljo na spletni strani KME (zavrniki "Meni", rubrika "Seje").

Appendix B

