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Altitude and Endurance Athletes

Effects of acute and chronic hypoxic exposure

DISSERTATION FROM THE NORWEGIAN SCHOOL OF SPORT SCIENCES · 2008

For Susanne, forever my best friend,
and for our most treasured gifts,
Nils Mathias and Lars Christian

Preface

This thesis is based on experimental work carried out at the Norwegian School of Sport Sciences, Department of Physical Performance in Oslo, Norway and at the Federal Institute of Sport, Section for Elite Sport in Magglingen, Switzerland. I want to express my sincere gratitude to everyone who has made this thesis possible.

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I also want to express a special thanks to my supervisor at the Federal Institute of Sport in Switzerland, Prof. Bernard Marti. You thought me to find compromises between strict scientific methodology and the elite sport relevance and practicability. Thank you very much for your confidence, patience and your support during the whole period.

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A special thank to all subjects, coaches and athletes participating in the different studies. You all did a tremendous job.

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Finally, I would like to thank my wife Susanne and our sons Nils Mathias and Lars Christian, for all the support and for giving me the greatest meaning of life.

Contents

List of papers

The present thesis is based on the following papers, which will be referred to by their Roman numerals:

I   Jon Peter Wehrlin, Jostein Hallén. **Linear decrease in $\dot{V}$ O$_{2max}$ and performance with increasing altitude in endurance athletes.** *Eur J Appl Physiol* 96(4):404-12, 2006

II   Jon Peter Wehrlin, Peter Zuest, Jostein Hallén, Bernard Marti. **Live high-train low for 24 days increases hemoglobin mass and red cell volume in elite endurance athletes.** *J Appl Physiol* 100(6):1938-1945, 2006

III   Jon Peter Wehrlin, Bernard Marti. **Live high-train low associated with increased hemoglobin mass as preparation for the 2003 World Championships in two native European world-class runners.** *Br J Sports Med* 40:e3, 2006

IV   Jon Peter Wehrlin, Jostein Hallén, German Clénin, Beat Villiger and Bernard Marti. **Effect of repeated altitude training during a five-month training period on hemoglobin mass, red cell volume and $\dot{V}$ O$_{2max}$ in elite cross country skiers.** *Manuscript submitted to High Alt Med Biol.*

Nomenclature and abbreviations

Abbreviation	Term	Paper
AG	Altitude group	II, IV
ATP	Altitude training phase	IV
BMI	Body mass index (kg/m^2)	III
BTPS	Body temperature and pressure, saturated with vapor	I
BV	Blood volume (ml)	II, III, IV
cm	Centimeter	I
CG	Control group	II, IV
CO	Carbon monoxide	I
CO_2	Carbon dioxide	I
2.3 DPG	2.3 Diphosphoglycerat	IV
EIH	Exercise-induced arterial hypoxemia	I
ETA	Endurance trained athletes	II
$FICO_2$	Fractional concentration of inspired CO_2	I
FIO_2	Fractional concentration of inspired O_2	I
Ftn	Ferritin (µg/L)	II, IV
h	Hour	II - IV
Hb	Hemoglobin (g/dl)	II, III, IV
HbCO	Carboxyhemoglobin (%)	II, IV
Hb_{mass}	Hemoglobin mass (g)	II, III, IV
Hematocrit	Percent of whole blood that is composed of red blood cells (%)	II, III, IV
hPa	Hecto Pascal	I
HR	Heart rate (beats $\cdot$ min^{-1})	I, II, IV
HR_{max}	Maximal heart rate (beats $\cdot$ min^{-1})	I, II, IV
LHTH	Live high - train high	II, IV
LHTL	Live high - train low	II, III, IV
LLTH	Live low - train high	IV
$[La^-]_b$	Blood lactate (mmol $\cdot$ L^{-1})	I, II, IV
$[La^-]_{b-max}$	Maximal blood lactate (mmol $\cdot$ L^{-1})	I, III, IV
min	Minute	I - IV
MCV	Mean corpuscular volume (fL)	IV

Abbreviation	Term	Paper
MCH	Mean corpuscular hemoglobin (pg)	IV
MCHC	Mean corpuscular hemoglobin concentration (g/dl)	IV
mm	Millimeter	I
PB	Personal Best (time)	III, IV
PV	Plasma volume (ml)	II, III, IV
R	Respiratory exchange ratio ($\dot{V}O_2 / \dot{V}CO_2$)	I
R_{max}	Respiratory exchange ratio at test cessation ($\dot{V}O_2 / \dot{V}CO_2$)	I
RBC	Red blood cell count (10e6/UL)	IV
Rct	Reticulocytes	II
RCV	Red cell volume (ml)	II, III, IV
RDW	Red cell distribution width (%)	IV
RPE	Rate of perceived exertion (6-20)	IV
s	Second	I - IV
sEpo	Serum Erythropoietin (mg/L)	II
SpO_2	Arterial hemoglobin oxygen saturation (%)	I
SpO_{2min}	Arterial hemoglobin oxygen saturation at test cessation (%)	I
STDP	Standard temperature and pressure (0°C, 760mmHg)	I
sTfR	Soluble Transferrin receptor (mg/L)	II
TF	Transferrin (g/L)	II
TTE	Time to exhaustion (s)	I, II, IV
$\dot{V}E$	Ventilation ($l \cdot min^{-1}$)	I, II, IV
$\dot{V}E_{max}$	Maximal ventilation ($l \cdot min^{-1}$)	I, II, IV
$\dot{V}O_2$	Oxygen consumption ($l \cdot min^{-1}$)	I, II, IV
$\dot{V}O_{2max}$	Maximal oxygen consumption ($l \cdot min^{-1}$)	I, II, IV
$v\dot{V}O_{2max}$	Velocity associated with 100% of $\dot{V}O_{2max}$ (km/h)	I
$\dot{Q}$	Cardiac output ($L \cdot min^{-1}$)	I
$\dot{Q}_{max}$	Maximal Cardiac output ($L \cdot min^{-1}$)	I

1. BACKGROUND AND AIMS OF THE THESIS

1.1. Introduction

"How does altitude affect performance"? This question has been asked by athletes, coaches and sport scientists for many years. Most of the current interest in altitude and altitude training can be traced back to the 1968 Summer Olympic Games held in Mexico City at an elevation of 2300m. At the 1968 Olympics, sprinters and jumpers in the sport of track and field set several world records in the "thin air" of Mexico City, whereas the distance runners ran markedly slower compared with 1968 world records. Interestingly, athletes from countries with moderate altitude such as Kenya and Ethiopia won a relatively high percentage of medals in the middle and long distance races (153). Since then, interest in altitude and altitude training has continued to grow. More recently, important endurance competitions, such as the Olympic Games (Salt Lake 2002 and Torino 2006) have taken place at low (1000 - 2000m) and moderate (2000 - 3000m) altitudes making it important to acclimatize in an optimal way for the target altitude. Furthermore, new altitude training concepts have been introduced with the goal of utilizing altitude in order to improve endurance performance not only at altitude, but at sea-level as well.

As altitude increases, partial pressure of O_2 in the inspired air (PIO_2) decreases. This condition is termed hypoxia (which literally means "deficient in oxygen"). This condition leads to oxygen deficiency in the blood and muscle, a condition that is called "hypoxemia". The athlete's response to hypoxia depends not only on its degree, but also on the period of exposure (144). We classify two types of hypoxia: acute (minutes to hours) and chronic (days to years) hypoxia. Acute hypoxic conditions lead to a reduction of maximal oxygen uptake ($\dot{V}O_{2max}$) with increasing altitude, with a corresponding increase in severity of hypoxia (144). With increasing acclimatization to the hypoxic conditions, the impairment of $\dot{V}O_{2max}$ decreases (123). There are even reports of improved $\dot{V}O_{2max}$ in athletes after chronic exposure to hypoxia, even when the effects are reported to be individual (10, 42, 52). For endurance athletes, then, the effects of altitude exposure can be considered on two different planes: On one side, it is important to understand athletes' response to acute altitude exposure in order to adapt training intensity and assess competition performance at altitude. On the other side, it is important to know how athletes respond to chronic altitude exposure and altitude training in order to improve sea-level performance. This thesis evaluates athletic-

related aspects of both acute and chronic hypoxia, which will be specified in the following chapters.

1.2. Acute hypoxia

Performance in endurance sport depends primarily on the capacity to deliver oxygen to active muscle tissue and the ability of the muscles to use oxygen. $\dot{V}O_{2max}$ is therefore an important parameter in endurance sport (7). As mentioned earlier, PIO_2 is reduced at altitude due to reduced air pressure. It was long believed that the sigmoid shape of the O_2 – hemoglobin dissociation curve and the increased ventilation ($\dot{V}E$) defend a reduction in arterial O_2 saturation (SaO_2) and $\dot{V}O_{2max}$ at altitudes below 1500m (154). Buskirk et al. (18) concluded in 1967 that up to an altitude of 1524m $\dot{V}O_{2max}$ is only reduced minimally, but thereafter the reduction is about 10.5% per additional 1000m. However, several more recent studies have shown that $\dot{V}O_{2max}$ can be reduced at altitudes even below 1000m (52, 54, 141) and that there is a substantial individual difference in the reduction of $\dot{V}O_{2max}$ with increasing altitude (79, 82).

Although the reasons for this individual response are not clear, it seems that fitness level may be important, as endurance-trained athletes (ETA; $\dot{V}O_{2max} > 60$ ml $\cdot$ kg^{-1} $\cdot$ min^{-1}) have demonstrated a larger decline in $\dot{V}O_{2max}$ with increasing altitude compared with untrained individuals (67, 79, 82). It has been suggested that this is due to the fact that ETA have developed exercise-induced desaturation already at sea-level (20, 52, 141) and operate at the steeper part of the oxygen equilibrium curve at low altitudes (33).

There are only few studies that have tested the reduction of $\dot{V}O_{2max}$ for ETA in acute hypoxia at altitudes relevant (0 - 3000m) for endurance disciplines in the laboratory (Figure 1) (21, 39, 52, 54, 82, 105-107, 134). Three studies showed that $\dot{V}O_{2max}$ declines even at altitudes as low as 750 - 900m (52, 54, 140) suggesting that the decrease is linear from sea-level to 3000m. However, none of these studies have tested $\dot{V}O_{2max}$ from sea-level (0 - 300m) to very low (300 - 1000m), low (1000 - 2000m) and moderate (2000 - 3000m) altitude in the same athletes. Another important factor is that the $\dot{V}O_{2max}$ tests used in prior studies were either an

incremental protocol to exhaustion (21, 51, 52, 82, 105, 107, 134, 141) or an all out test for a given distance (106) or time (54).
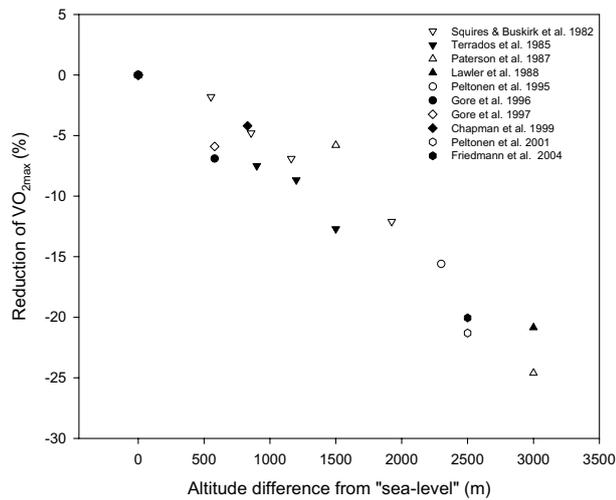
Figure 1. Percent decrease in $\dot{V}O_{2max}$ from sea-level values. "Sea-level" in these studies (20, 39, 52, 54, 82, 105-107, 134, 141) is set at 0m but varies originally from 0 to 362m. Only studies which tested male unacclimatized ETA with a mean $\dot{V}O_{2max} > 60$ ml $\cdot$ kg^{-1} $\cdot$ min^{-1} under laboratory conditions at acute hypoxia are included.

Under hypoxic conditions, these protocols result in reduced absolute exercise intensity. It has therefore been hypothesized that the decreased $\dot{V}O_{2max}$ in hypoxia is partially due to reduced maximal exercise intensity (104, 107). The objective in the "acute hypoxia" part of this thesis was to evaluate $\dot{V}O_{2max}$ and performance with a combined $\dot{V}O_{2max}$ and performance test using the same absolute exercise intensity, from sea-level to acute low and moderate altitude exposure in unacclimatized ETA.

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1.3. Chronic hypoxia

In the last 20 - 30 years, the world records in endurance performance have improved. Many Olympic and World Championship medals have been won by athletes living in altitude regions or athletes preparing for competitions with "chronic altitude exposure", hereafter called "altitude training" (121). Classic altitude training has been performed by living and training at altitude ("living high - training high"; LHTH). In the scientific literature it is unquestioned that this is an adequate method for preparing for competitions at altitude (1, 24, 27, 31, 32). In the last 30 years, scientists, coaches and athletes have debated whether LHTH increases endurance performance at sea-level. The scientific literature is equivocal as there are studies with improved (17, 24, 27, 50, 100), but also studies with no change (1, 8, 18, 31, 71, 73, 86, 138) in sea-level performance after LHTH. This has encouraged the search for alternative strategies for utilizing hypoxia as an additional stimulus for athletes (121).

1.3.1. The live high-train low altitude training concept

In 1992 (88), Levine and Stray-Gundersen introduced a new altitude training method termed "living high - training low" (LHTL). With this method the athletes live at altitude, but interrupt the time at altitude to train at low altitude. LHTL is therefore a sort of "intermittent hypoxic exposure". With living at moderate altitude athletes theoretically should acquire the beneficial effects of altitude acclimatization, particularly an increase in the hemoglobin mass (Hb_{mass}) and red cell volume (RCV) for maximizing the oxygen transport capacity. At the same time the low altitude or sea-level training would decrease the negative effects of reduced absolute training intensity caused by reduced $\dot{V}O_{2max}$ at altitude.

1.3.2. Does LHTL altitude training increase Hb_{mass} and RCV in elite endurance athletes?

The question of whether exposure to moderate altitude increases Hb_{mass} and RCV in elite endurance athletes is debated (5, 53, 85, 87). Some studies have shown increased Hb_{mass} and/or RCV after real (86) and artificial LHTL (81, 120) training camps while other studies reported no change after LHTL with real altitude (25), LHTL with artificial altitude (5, 6) and

LHTH at real altitude (38, 50, 51, 137, 139). However, a number of methodological differences like the chosen altitude for living and training, the different durations spent at altitude, different methods to measure changes in Hb_{mass} or RCV and different fitness levels of the athletes make it difficult to compare these studies. In the classic, carefully controlled, LHTL study conducted by Levine and Stray-Gundersen (86), RCV increased by ~ 5% in the LHTL group after living for four weeks at 2500m and training at 1250m. These results have been questioned (5, 59), however, because they measured RCV indirectly with the Evans blue dye method and there have been doubts about the adequacy of this method for estimating RCV after hypoxic exposure (89). In addition, they reported similar increases in RCV in the four-week sea-level training phase and a decrease in the control group (86). The results of studies using the carbon monoxide rebreathing (CO-rebreathing) method to directly measure Hb_{mass} give opposite results.

Interestingly most studies that show no increase in Hb_{mass} and RCV used a lower "hypoxic dose" (living and training altitude combined with the duration of altitude exposure) than the studies that reported increases in Hb_{mass} and RCV. Accordingly, the results of the studies in Figure 2 indicate that about 400h at an altitude of 2300 - 2600m are probably necessary to increase Hb_{mass} and RCV. However, no controlled LHTL studies have been published that use a hypoxic dose at real altitude similar to that used by Levine and Stray-Gundersen (86) and measured Hb_{mass} directly with the CO-rebreathing method. The first objective of the "chronic hypoxia" part of the thesis was, therefore, to measure the changes in Hb_{mass} and RCV with the CO-rebreathing method in a controlled LHTL study with an adequate "hypoxic dose" in elite national team level endurance athletes.
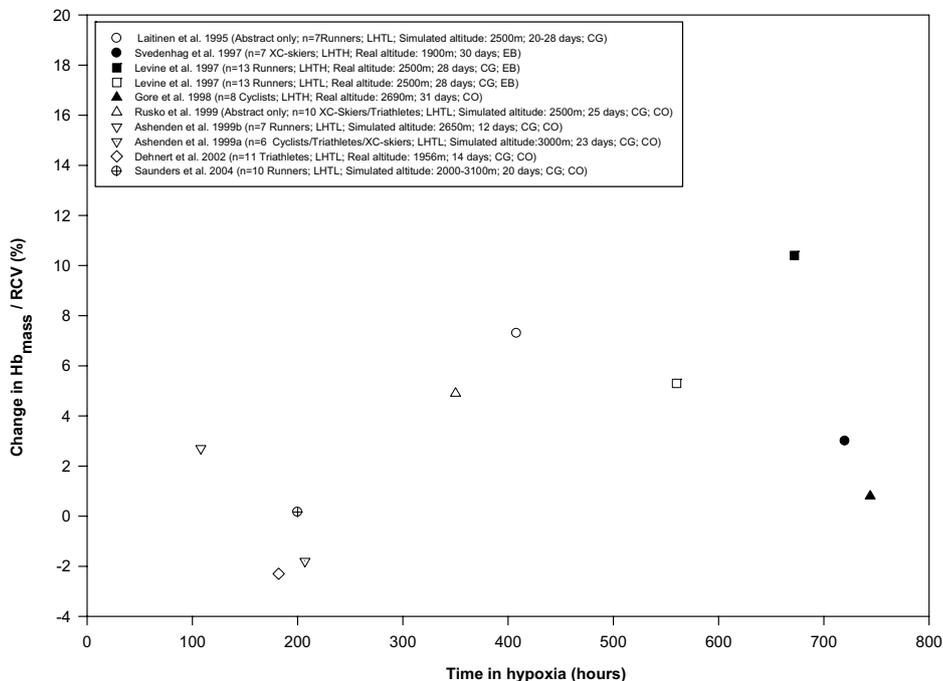
Figure 2. Change in hemoglobin mass (Hb_{mass}) or red cell volume (RCV) in relation to the time spent at altitude in studies with endurance-trained athletes (5, 6, 25, 50, 81, 86, 120, 125, 137). Reported values are: number of subjects (n), the sport, type of altitude training (LHTH = live high - train high; LHTL = live high - train low), the nature of the altitude (real or simulated), the living altitude, days spent at altitude, use of a control group (CG) and the technique used for the measurement of Hb_{mass} or RCV (EB = Evans blue dye; CO = carbon monoxide rebreathing).

1.3.3. Does LHTL increase Hb_{mass} and RCV even in world-class endurance athletes?

The question at hand -- whether altitude training based on the LHTL concept increases Hb_{mass} and RCV -- is discussed controversially. The magnitude of the "hypoxic dose" is probably the essential factor in this question. However, most athletes in the studies with increased Hb_{mass} and RCV (37, 81, 86, 120) were not reported to be world-class. In the only study with world-class endurance athletes (50), Hb_{mass} and RCV failed to increase despite an estimated adequate hypoxic dose. In addition, there is a lack of documented observations of endurance world-class athletes who successfully increase Hb_{mass} and RCV, especially when preparing for an important competition. Thus it is not clear whether it is possible to further increase an

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already high Hb_{mass} in world-class athletes. The second objective in the "chronic hypoxia" part of this thesis, was therefore to determine whether Hb_{mass} and RCV increase in world-class endurance athletes after a LHTL camp with a presumably high enough hypoxic stimulus in the immediate preparation for an important championship.

1.3.4. How does repeated LHTL during a long-term training period affect Hb_{mass} and performance?

Little is known about the effects of repeated altitude training during a long-term training period on Hb_{mass}, RCV and performance in elite endurance athletes, even though an early study showed promising results on sea-level performance parameters (24). In this study, the highly endurance-trained athletes lived alternately at 2300m and sea-level for 68 days, spending a total of 42 days at altitude using the LHTH concept. $\dot{V}O_{2max}$ increased by 5% but Hb_{mass} and RCV were unfortunately not measured. It is known that East German athletes performed some sort of repeated altitude training (41) but the results of this study are not published in western scientific journals, although we do know that Hb_{mass} and RCV were not reported. So, to my knowledge, there is no study with ETA that measured Hb_{mass} or RCV and performance after several altitude training camps over a longer period of time.

The third objective in the "chronic hypoxia" part of this thesis was to investigate whether Hb_{mass} and RCV increase in a cumulative way in world-class endurance athletes after several altitude training camps with a presumably adequate hypoxic dose and to evaluate if this preparation is associated with increased performance.

1.4. Aims of the thesis

The main purpose of the studies included in this thesis was to examine athletically-relevant effects of both acute and chronic hypoxia in endurance athletes. Specifically, the main objectives were:

1. To evaluate the changes in $\dot{V}O_{2max}$ and performance from sea-level to low and moderate altitudes in unacclimatized endurance trained athletes (Study I).

2. To investigate the effects of living 24 days at an altitude of 2500m and training at lower altitudes on Hb_{mass} and RCV in elite endurance athletes (Study II - IV).

3. To study the effects of living at an altitude of 2500m and training at lower altitudes for 26 days on Hb_{mass}, RCV and competition performance in the immediate preparation for the World Athletic Championships in two world-class runners (Study III).

4. To compare the effects of two different volumes of repeated altitude training during a five-month pre-season training period in Swiss national team cross-country skiers using the main outcome measurements of Hb_{mass}, RCV, PV, BV and $\dot{V}O_{2max}$ (Study IV).

2. METHODOLOGY

2.1. Subjects

Thirty-four athletes participated in the four studies. Subjects were Norwegian endurance-trained students (Study I; n = 8), members of the A and B Swiss orienteering (Study II; n = 10), Swiss athletic (Study II; n = 2) and Swiss cross-country (Study II; n = 7, study IV; n = 14) national teams. The same subjects served as control group in studies II and IV. Subjects were informed about all procedures before a written consent was obtained. The experimental protocols were approved by a Regional Norwegian Ethics Committee (Study I) and the institutional review board of the Swiss Federal Institute of Sports (Study II - IV). The studies were carried out according to the recommendations of the Helsinki Declaration. Table 1 gives an overview of the most important hematological and performance characteristics of the subjects. Further details are given in the relevant papers.

Table 1. Subjects' hematological and performance characteristics

	Subjects	Hb_{mass} (g $\cdot$ kg^{-1})	RCV (ml $\cdot$ kg^{-1})	$\dot{V}O_{2max}$ (ml $\cdot$ kg^{-1} $\cdot$ min^{-1})	Running Performance
Study I	8 male endurance-trained Students			66 ± 4	
Study II	AG (5 female and 5 male national team orienteers)	12.8 ± 2.0	37.4 ± 5.7	57 ± 7	18min 18s ± 1min 38s (5000m)
	CG (3 male and 4 female national team cross country skiers)	13.0 ± 1.6	36.3 ± 4.4	70 ± 6	
Study III	Elite 5000m runner	14.3	42.0		13min 12s
	Elite Marathon runner	15.7	43.0		2h 10min 54s
Study IV	AG7 (4 male and 3 female national team cross-country skiers)	12.9 ± 1.9	38.4 ± 5.0	68 ± 6	
	CG7 (3 male and 4 female national team cross-country skiers)	13.6 ± 2.0	39.8 ± 5.5	70 ± 6	

Altitude group (AG); Control group (CG); Hemoglobin mass (Hb_{mass}); Red cell volume (RCV); Maximal oxygen uptake ($\dot{V}O_{2max}$).

2.2.   Study design

The four studies were performed at different time points and with different altitude exposure durations. Study I examined the effects of acute altitude exposure, while studies II, III and IV analyzed several parameters during and after one three-week LHTL altitude training period (ATP). Study IV also investigated the effects of several ATP during a five-month pre-season training period (Figure 3). More detailed information about the different studies are given in the following subchapters.
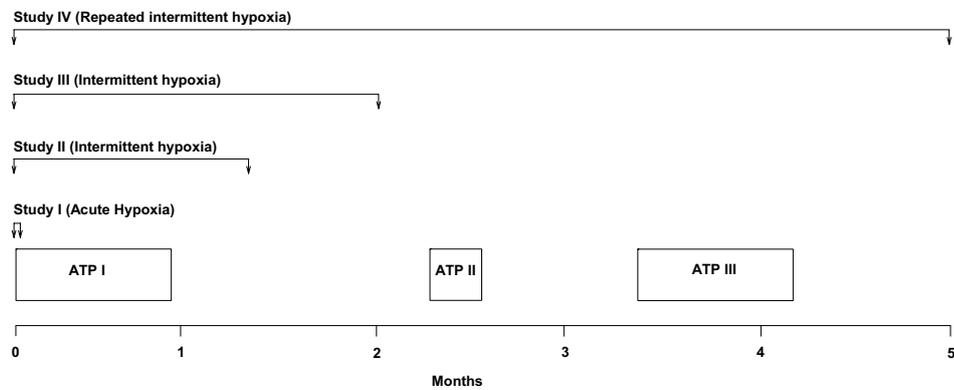
Figure 3. Overview of the various altitude exposures (ATP = altitude training period) during the four studies.

2.2.1. Study I

Study I evaluated the changes in $\dot{V}O_{2max}$ and performance from sea-level to low and moderate altitudes in unacclimatized endurance-trained athletes using a combined $\dot{V}O_{2max}$ and performance test, using one identical "maximal" and one identical "submaximal" absolute exercise intensity. An outline of the study design is shown in Figure 4. Subjects performed nine tests (T1 - T9) on a motor-driven treadmill in a hypobaric chamber over a period of four weeks with a minimum of 48h between tests.
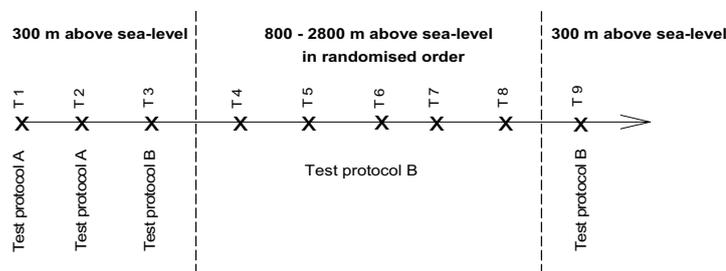
Figure 4. Design of Study I.

The first two exercise tests (T1 and T2) were pre-experimental tests and followed test protocol A. These tests were used to ensure that the subjects met the inclusion criteria ($\dot{V}O_{2max} > 60\ ml \cdot kg^{-1} \cdot min^{-1}$), to calculate the running speeds for test protocol B and to familiarize themselves with the test environment and equipment. All the other exercise tests at the different altitudes (T3 - T9) followed test protocol B. In these tests, the subjects ran at the same absolute constant submaximal and the same absolute "maximal" running speed at the different altitudes. The tests (T3 - T9) at the different altitudes were performed in randomized order except that the first (T3) and the last test (T9) were at "sea-level" (300m, 977hPa). The simulated altitudes were 800m (921 hPa), 1300m (868 hPa), 1800m (818 hPa), 2300m (772 hPa) and 2800m (727 hPa) above sea-level (T4 - T8).

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Test protocol A (T1 - T2).

Figure 5 shows an outline of test protocol A. After a 10min warm up, subjects ran at four different submaximal velocities for 5min each ①. At every stage, oxygen uptake was measured during the last minute to determine the individual velocity/$\dot{V}O_2$ relationship. After a 3min break, subjects performed an incremental $\dot{V}O_{2max}$ test ②. Subjects started with a running intensity approximating their estimated individual anaerobic threshold (14.3 $\pm$ 1.5km/h). The running speed was then increased every minute by 1km/h until exhaustion. For the estimated last minute of the test, the subjects could choose to continue with the same speed or to increase the running intensity by 0.5km/h rather than 1km/h. The mean maximal running speed was 17.3 $\pm$ 1.2km/h. The total test time until exhaustion was 308 $\pm$ 49s. The incline was set at 5.3% throughout test protocol A. From these data, the running speeds to reach 55, 60, 95 and 107% of $\dot{V}O_{2max}$ (velocity associated with the 100% of $\dot{V}O_{2max}$ = v$\dot{V}O_{2max}$) were inter- and extrapolated ③. These running speeds were used in test protocol B at the different simulated altitudes (T3 - T9).
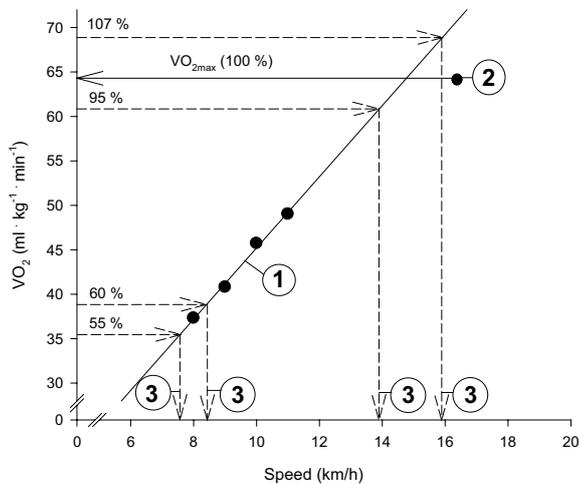

Figure 5. Outline of test protocol A, which was used to calculate the running velocities at 55, 60, 95 and 107% of $\dot{V}O_{2max}$. ①, ② and ③ refer to the text (for details, see text).

Test protocol B (T3 - T9). Test protocol B consisted of a submaximal and a maximal exercise test (Figure 6). *Submaximal exercise test.* Every test (T3 - T9) started with 15min submaximal running in normobaric conditions at 60% of $v\dot{V}O_{2max}$ ①. During a sea-level warm-up, oxygen uptake was measured to biologically control the reproducibility of the measurements. Thereafter, subjects continued to run at 55% of $v\dot{V}O_{2max}$ while the air pressure was gradually reduced over a period of about two to three min to the predetermined pressure ②. After the test altitude was reached, subjects continued to run for five min at this constant velocity ③. During the last two min of running, a Douglas bag was sampled for analysis of oxygen uptake ($\dot{V}O_2$), ventilation ($\dot{V}E$) and respiratory exchange ratio (R). In the same period, arterial hemoglobin oxygen saturation (SpO$_2$, estimated with pulse-oximetry) and heart rate (HR) were recorded. After a one-minute rest, a blood sample was drawn for blood lactate ([La⁻]$_b$) measurement.
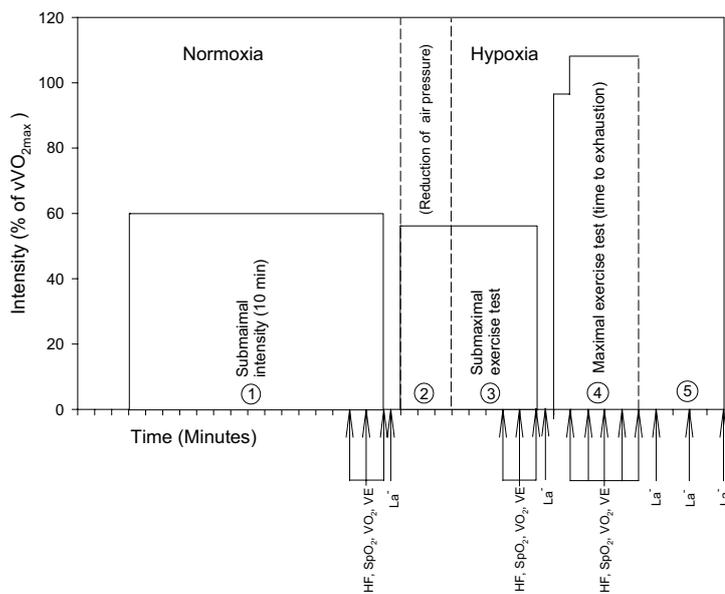
Figure 6. Course of test protocol B. ①, ②, ③, ④ and ⑤ refer to the text.

Maximal exercise test. Two min after cessation of the submaximal exercise test, the subjects began to run a combined $\dot{V}O_{2max}$ and performance test ④. They started at 95% of sea-level $v\dot{V}O_{2max}$ for one minute and continued at 107% until exhaustion. The subjects therefore ran at the same absolute velocity at all altitudes and performance was defined as time to exhaustion (TTE). Douglas bags were continuously sampled (30 - 40s per bag) from approximately 90s after the start of the test until exhaustion. SpO_2 and HR were registered every minute and at exhaustion. Blood lactate samples were taken at one, three and five minutes after cessation ⑤.

2.2.2. Study II

Study II investigated the effects of living at altitude and training at lower altitudes on erythropoiesis in elite endurance athletes. An outline of the study design is shown in Figure 7. The orienteering athletes were assigned to the "altitude group" (AG), and completed a 24-day LHTL phase, living 18h per day at 2456m and training at 1800 and 1000m above sea-level, in the Swiss alps. The cross-country skiers were assigned to the "control group" (CG), completing a normal training phase, which consisted of living and training between 500 and 1600m for 24 days. The study was carried out during the pre-season for both groups (different time of the year for orienteers and cross-country skiers).
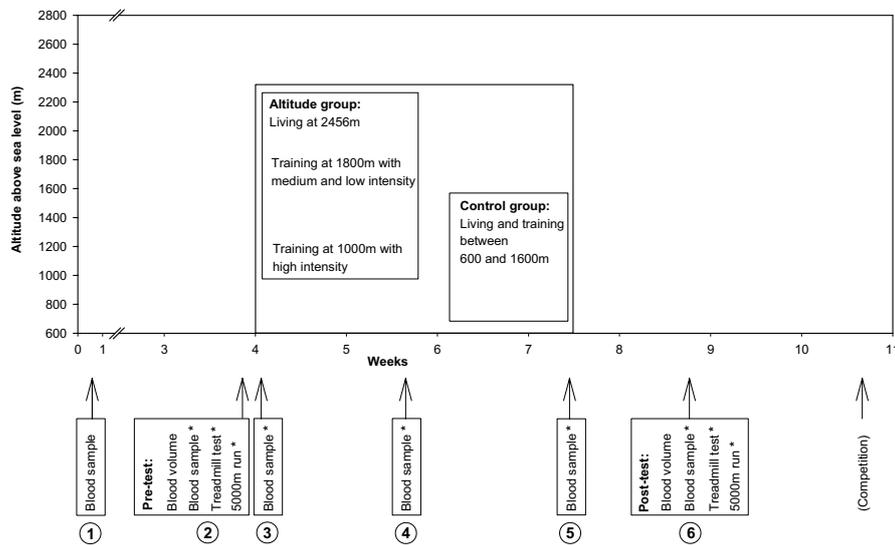
Figure 7. Design of Study II. ①, ②, ③, ④, ⑤ and ⑥ refer to the text.

① About four weeks prior to the LHTL phase (AG) and prior to the experimental phase (CG), blood samples were taken for measurement of serum ferritin in order to asses bone marrow iron stores. At the pre-test ②, one day before the LHTL phase began, a blood sample to determine hematocrit (Hct), hemoglobin (Hb), ferritin (Ftn), transferrin (TF), soluble transferrin receptor (sTfR), reticulocytes (Rct) and erythropoietin (sEpo), was taken and the athletes from both groups performed a treadmill test to determine $\dot{V}O_{2max}$ in the laboratory.

About 7 - 10h later on the same day, the AG ran a 5000m time trial on a 400m track. The blood volume parameters were measured the next day (AG and CG). Additional blood samples where taken from the AG athletes at day 1 ③, day 12 ④ and day 24 ⑤ of the LHTL phase. Eight days after the 24-day phase ⑥, the athletes performed the post-test with identical measurements as at the pre-test ② with the exception that the CG did not perform the $\dot{V}O_{2max}$ test.

2.2.3. Study III

Study III examined the effects of living at altitude and training at lower altitudes on Hb_{mass} and competition performance in the final preparation for the World Athletic championships in two world-class runners. An outline of the study design is shown in Figure 8. The athletes lived for 26 days at a natural altitude of 2456m and trained at an altitude of about 1800m.
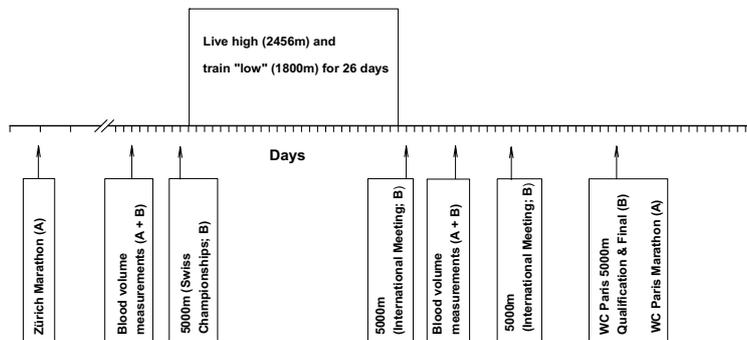
Figure 8. Design of Study III. A indicate tests for the marathon, B for the 5000m runner.

The marathon runner completed the Zürich Marathon three month before and the World Championships marathon 29 days after the end of the LHTL camp. The 5000m runner completed the 5000m at the Swiss championships one day before the LHTL camp. One and 14 days after LHTL, the 5000m runner competed in the 5000m at international track meets. At days 25 and 27 after the LHTL camp, the 5000m runner completed the 5000m qualification and final at the World Championships. Hb_{mass}, RCV, PV and BV of both athletes were measured one week before and one week after the LHTL camp.

2.2.4. Study IV

Study IV compared the effects of two different volumes of altitude training during a five-month pre-season training period in Swiss national team cross-country skiers using the main outcome measurements of Hb_{mass}, RCV and $\dot{V}O_{2max}$. An outline of the study design is shown in Figure 9.
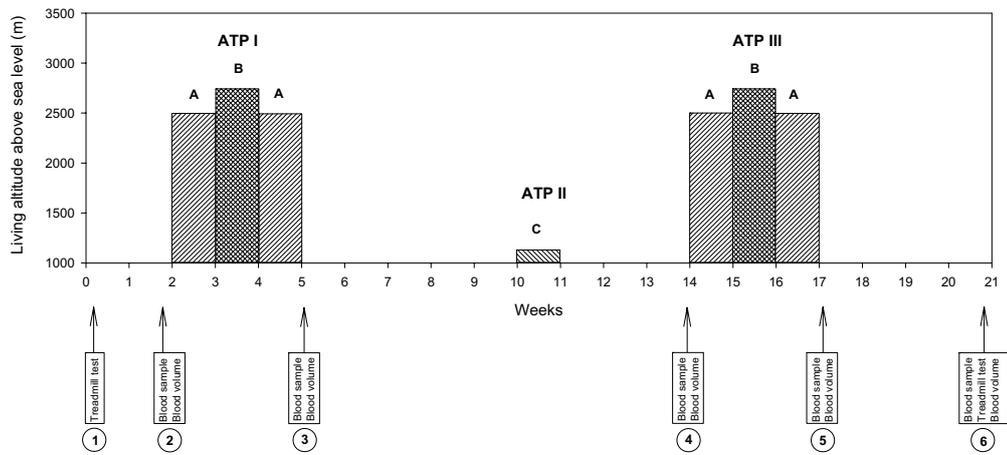
Figure 9. Design Study IV. ①, ②, ③, ④, ⑤, ⑥ and A, B, ATP I, ATP II and ATP III refer to the text.

The athletes were observed during a five-month pre-season training period starting in June and ending in October after the first World Cup sprint race. The Swiss national cross-country ski team was non-randomly divided into two different groups based on individual training preferences of athletes and coaches. The "control group" (CG7; four females and three males) completed the regular training program including two one-week periods (B) of LHTH altitude training living at 2760m (on the Stelvio pass) and training at 2760 - 3300m (on the Stelvio glacier) in the Italian alps. In addition, the CG7 underwent a one-week period (C) of LLTH altitude training living at 1150m in Les Diablerets and training at the Diablerets glacier located in the Swiss alps at approximately 3000m. The "altitude group" (AG7; five males and two females) completed the same training periods as CG7, but each of the two LHTH weeks were "sandwiched" between LHTL weeks, resulting in two 3-week altitude training periods (ATP I and ATP III). Specifically, one LHTL week before and one LHTL week after the

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LHTH week, the subjects lived on the Jakobshorn (2590m) or on the Gütsch (2344m) in the Swiss alps and trained in Davos (1550m) or Andermatt (1444m), respectively. Two weeks prior to ATP I ① and about 3 weeks after ATP III ⑥, all athletes performed a treadmill test at 400m above sea-level for determination of maximal oxygen uptake ($\dot{V}O_{2max}$) and time to exhaustion (TTE). One day prior ② and the first day after ATP I ③, venous blood was collected and blood volume parameters were determined. The measurements were repeated one day prior ④ (AG7), one ⑤ and about 25 days ⑥ after ATP III.

Three male AG7 athletes caught colds during ATP III and as a result spent only 10 days at altitude instead of 21. However, these athletes' training was not seriously affected. Because this lower hypoxic dose influenced the erythropoietic parameters during ATP III, we also present the results of the AG7 with these three athletes excluded. This subgroup was termed AG4.

2.3.   Testing procedures / Measurement methods

All testing during the acute hypoxia study was done at the Norwegian School of Sport Sciences, while the testing for the chronic hypoxia studies was done at the Institute of Sport Sciences, Federal Office of Sport in Switzerland and other locations in Switzerland. The testing protocols for each measurement are described in the respective papers. Table 2 gives a summary of the most important methods used. The most important methods, however, are introduced and described in detail in the following sections.

Table 2. Summary of the most important measurement methods and testing procedures

Parameter	Paper	Method
Altitude / Hypoxia	I	Hypobaric chamber
	II - IV	Real altitude (Alps)
$\dot{V}O_2$-measurement	I	Douglas Bag method
	II, IV	Automated method (Jaeger; Oxycon pro)
Blood volume parameters	II - IV	Carbon monoxide rebreathing method
Blood sample parameters	II	serum erythropoietin (chemiluminescence immunoassay)
		reticulocytes (flow cytometry)
		transferrin (immunoassay)
		soluble transferrin receptor (immunoturbimetric assay)
$\dot{V}O_{2max}$ and performance	I, II, IV	Time to exhaustion; $\dot{V}O_{2max}$ (treadmill test)
	II	5000m run
	III	Competitions (5000m run; marathon)

Maximal oxygen uptake ($\dot{V}O_{2max}$).

2.3.1. Acute altitude exposure (Study I)

All tests were conducted in a hypobaric chamber (Norwegian Universal Technology AS, Haugesund, Norway). In the chamber, air pressure, oxygen content, temperature, wind velocity were controlled and kept stable for each test at each simulated altitude. Carbon dioxide was removed by gas scrubbers. For exact calculations of $\dot{V}O_2$ during the tests and to control for the calculated altitudes, the fraction of inspired O_2 (FIO_2) and inspired CO_2 ($FICO_2$) were measured immediately before and after each test (see section 2.3.3 for details). For the calculation of the simulated altitudes, barometric pressures of 977, 921, 868, 772, 727hPa were used to simulate 300, 800, 1300, 1800, 2300 and 2800m. Chamber pressure stability indicated a variation < 1hPa from target barometric pressure.


Figures 10 and 11. The hypobaric chamber (left) and its control panel at the Norwegian School of Sport Sciences.

2.3.2. Chronic altitude exposure (Studies II - IV)

To study the chronic effects of hypoxia in endurance national team athletes, several locations in the Swiss and Italian alps were used. Table 3 gives an overview and Figures 12 - 14 show the different locations in the Swiss and Italian (Stelvio) alps.

Table 3. Overview of the altitude locations used in the chronic hypoxia studies (II - IV)

	Group	Days	Living altitude	Training altitude
Study II	AG	24	Muottas Muragl (2456m)	Engadin valley (1800m) and Posciavo (1000m)
	CG	24	Different places in Switzerland (< 1600m)	Different places in Switzerland (< 1600m)
Study III		26	Muottas Muragl (2456m)	Engadin valley (1800m)
Study IV	AG7	4 * 7	Gütsch (2344m) and/or Jakobshorn (2590m)	Andermatt (1444m) and/or Davos (1550m)
		2 * 7	Stelvio pass (2760m)	Stelvio glacier (2760 - 3300m)
		7	Les Diablerets (1150m)	Diablerets glacier (3000m)
	CG7	4 * 7	Different places in Switzerland (< 1600m)	Different places in Switzerland (< 1600m)
		2 * 7	Stelvio pass (2760m)	Stelvio glacier (2760m)
		7	Les Diablerets (1150m)	Diablerets glacier (3000m)
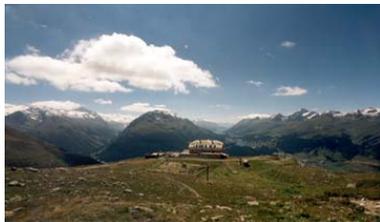
Figures 12, 13 and 14. The mountain hotel on Muottas Muragl (2456m, left), the meteorological station on the Gütsch (2344m, middle) and the mountain hotel on the Jakobshorn (2590m, right).

2.3.3. Measurement of oxygen uptake

Instruments and analytical procedures.

To ensure a high precision for measurements of oxygen uptake in the hypobaric chamber, we used the Douglas bag method in study I. During the tests, expired air was collected via a mouthpiece, a two-way respiratory valve (Type 2700 series, Hans Rudolph inc, Kansas, USA), a 180cm long tube (diameter) into 100L Douglas bags (Hans Rudolph inc.). Collection time of expired air for each Douglas bag was automatically measured using a watch (Excelsior, Switzerland). The volume (Flow turbine, Type S-430, Kl Engineering, Northridge, USA) and gas concentrations (Oxygen analyzer type S-3A/1 and carbon dioxide analyzer type CD-3A, Amatek inc, Pittsburg, USA) were measured for each bag (including room air bags) in the laboratory at 200m above sea-level (300 ± 77m when corrected for the barometric pressure in this period). Before and after each test in the chamber, room air was collected into Douglas bags and inspired fraction of O_2 (FIO_2) and CO_2 ($FICO_2$) were calculated. The mean values of the inspired air during the test were $20.85 \pm 0.03\%$ O_2 and $0.15 \pm 0.03\%$ CO_2. All Douglas bags where controlled for leakage before use and analyzed within 20min after the test. The $\dot{V}O_2$ and $\dot{V}CO_2$ uptake was calculated for STDP conditions.

Figures 15 and 16. The Douglas bag system (left) and how it was arranged inside the barometric chamber with the treadmill (right).

In the studies II and IV gas exchange was measured breath by breath with an open-circuit system (Oxycon Pro, Jaeber-Toennies, Hochberg, Germany).

2.3.4. Blood volume parameters

Carbon monoxide rebreathing method

We used the Carbon monoxide rebreathing method (CO-rebreathing method) to determine total mass of hemoglobin (Hb_{mass}). Red cell volume (RCV), plasma volume (PV) and blood volume (BV) were then calculated with the help of the Hct and Hb values. The CO-rebreathing method gives reliable and valid results with coefficients of variation equal to or lower than the other methods (16, 49).

CO-rebreathing protocol

Before starting the CO-rebreathing procedure, a blood sample was taken from a cubital vein under standardized conditions (between 07:00 and 08:00 AM before breakfast, in supine position after 15min of rest). Blood samples were analyzed for hemoglobin concentration (Hb; modified cyanomethemoglobin method, Coulter Gen S, Beckmann, Fullerton, USA) and hematocrit (Hct; Coulter Gen S, Beckmann, Fullerton, USA).

The CO-rebreathing procedure started with 15min rest in a sitting position. During this period, four capillary blood samples (30μL) were taken from an earlobe and analyzed for carboxyhemoglobin (HbCO) by a hemoximeter (ABL 520, Radiometer A/S, Copenhagen, Denmark). The mean of the four HbCO values was taken as the baseline HbCO value. Subjects were then connected to a Krogh Spirometer filled with a mixture of pure oxygen (>99.5%; Carbagas, Bern, Switzerland) and pure CO (>99.997%; Carbagas, Bern, Switzerland).

The volume of oxygen was 9.6L, 4.6L of which was the volume of the connecting tubes. During the rebreathing period, oxygen was refilled if necessary. The volume of inspired CO varied between 50 and 100ml depending on gender, barometric pressure, measured $\dot{V}O_{2max}$ and body mass, with the goal of reaching a ΔCOHb (difference between baseline values and plateau values) between 5 and 7%.

The athletes breathed the gas mixture in the closed system for 12min. Every 2min, earlobe blood samples were taken and immediately analyzed (<10s) for HbCO. The HbCO plateau was normally reached after 6 - 10min and the mean of the three HbCO values between the 8th and the 12th min was taken as the plateau value of HbCO. Hb_{mass} was then calculated as

described by Burge and Skinner (16) with minor modifications according to Heinicke et al. (66):

$$Hb_{mass} = K \cdot MCO \cdot 100/(\Delta HbCO\% \cdot 1.34)$$

K = current barometric pressure/(760 $\cdot$ [1 + (0.003661 current temperature)]); MCO = volume of added CO (ml); $\Delta HbCO\%$ = difference between baseline and the plateau value of HbCO (%); 1.34 Hüfner's number (1g Hb binds 1.34ml O_2, respectively CO).

Red cell volume (RCV), blood volume (BV) and plasma volume (PV) were calculated as follows:

$RCV = Hb_{mass}/MCHC \cdot 100$

$BV = RCV \cdot 100/Hct$

$PV = BV - RCV$

MCHC = mean corpuscular hemoglobin concentration, Hct = hematocrit corrected to whole body hematocrit by the cell factor 0.91 (35).

Figure 17. Carbon monoxide rebreathing method.

2.4. Statistical analysis

The particular statistical procedures used in this thesis are given in the respective papers. As a general principle the level of significance accepted was 5%. Commonly used statistical tests for the difference between groups or the effects of interventions are the independent samples t-test and ANOVA for repeated measures.

3. RESULTS

3.1. Acute hypoxia (Study I)

3.1.1. Effects on VO$_{2max}$ and performance

$\dot{V}$O$_{2max}$ decreased from 66.1 ± 4.3 ml $\cdot$ kg^{-1} $\cdot$ min^{-1} at 300m to 55.4 ± 3.6 ml $\cdot$ kg^{-1} $\cdot$ min^{-1} at 2800m, corresponding to a 6.3% decrease per 1000m ($p < 0.001$; Figure 18) with individual $\dot{V}$O$_{2max}$ decreases ranging from 4.6 to 7.5% per 1000m. At 800 m, $\dot{V}$O$_{2max}$ was lower than at 300m ($p < 0.01$) and the rate of decline between 300m and 1300m was not different from the rate of decline between 1800m and 2800m ($p = 0.91$).
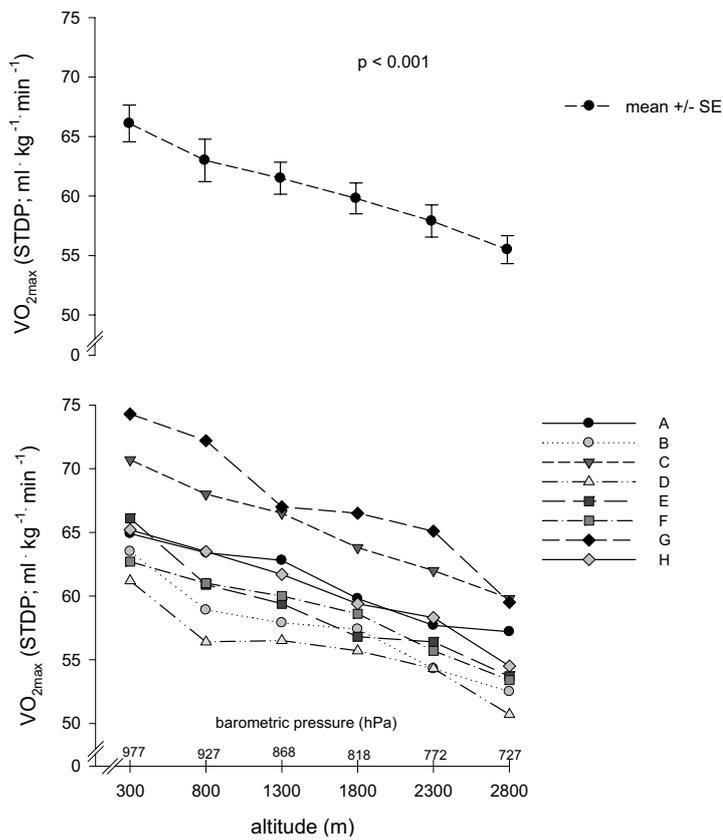
Figure 18. Effect of acute simulated altitude exposure on $\dot{V}$O$_{2max}$ in eight male, sea-level resident, endurance-trained athletes. The upper part of the figure shows means $\pm$ standard error (SE), the lower part of the figure shows the individual values of the subjects A – H.

At 800m, time to exhaustion (TTE) in the $\dot{V}O_{2max}$ test was shorter than at 300m (p<0.05) and TTE continued to decline up to 2800m altitude by 14.3% per 1000m altitude (p<0.01; range 10.3% to 18.1%). The rate of decline in TTE between 300m and 1300m was not different from the rate of decline between 1800m and 2800m. SpO_{2min} declined from $89.0 \pm 2.9\%$ at 300m to $76.5 \pm 4.0\%$ at 2800m (p<0.001). At 300m, all athletes had SpO_{2min} equal to or lower than 92%. The rate of decline in SpO_{2min} between 300 and 1300m was not different from the rate of decline between 1800 and 2800m (p=0.93).

3.2. Chronic hypoxia (Studies II-IV)

3.2.1. Effects of one altitude training period on erythropoiesis

Blood volume parameters.

Hb_{mass} and RCV were increased by about 4 - 6% (studies II III and IV) after a 3 - 4 week LHTL altitude training camp (ATP; Figure 19) whereas there was no change in the control groups (study II and IV). PV and BV were unchanged before and after one ATP.
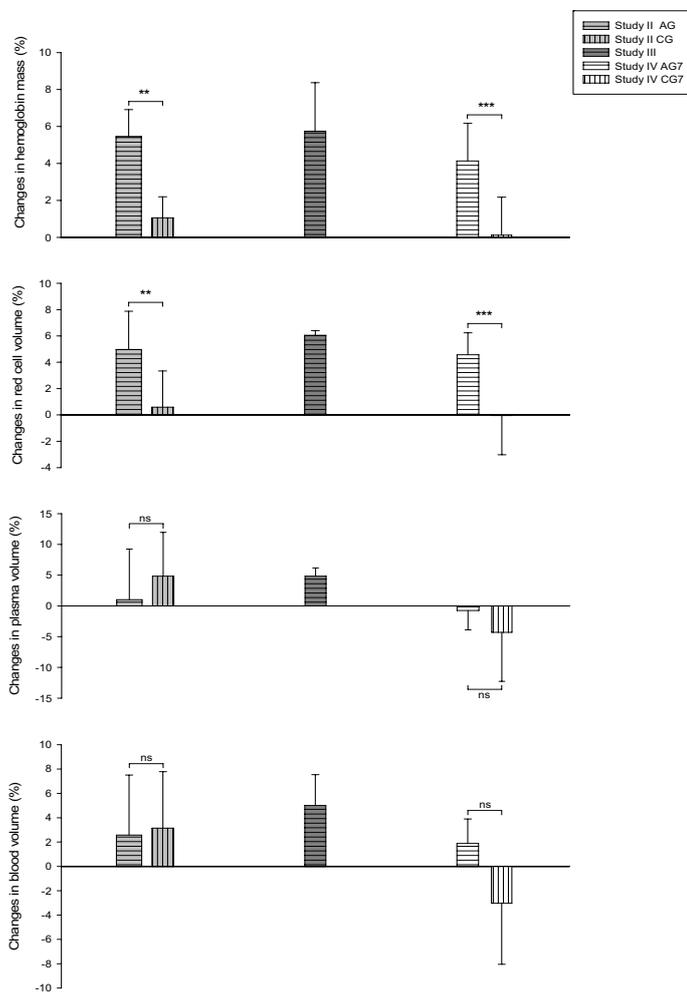

Figure 19. Effect of 3 - 4 weeks living at about 2500m and training at lower altitudes on hemoglobin mass, red cell volume, plasma volume and blood volume. AG7 = altitude group; CG7 = control group. * (p<0.05), ** (p<0.01) and *** (p<0.01) differences between pre and post altitude. NS indicates no difference.

Other blood and iron parameters (Study II).

The time courses of Hct, sEpo, Rct, Ftn, TF and sTfR before, during and after a 24 day LHTL-ATP are presented in Figure 20. Hct increased during the LHTL phase (p<0.01) and post hoc analysis indicated elevated values at day 24 (p<0.05). Values returned to pre-test (-1) level at post-test (+8). sEpo was affected by the LHTL phase (p<0.001) and post hoc analysis showed elevated sEpo values at day 1 (p<0.001; + 120%) and day 12 (p<0.05; + 34%) compared with pre-test (-1), whereas the values at day 24 and the post-test (+8) were not different from the pre-test (-1) values.
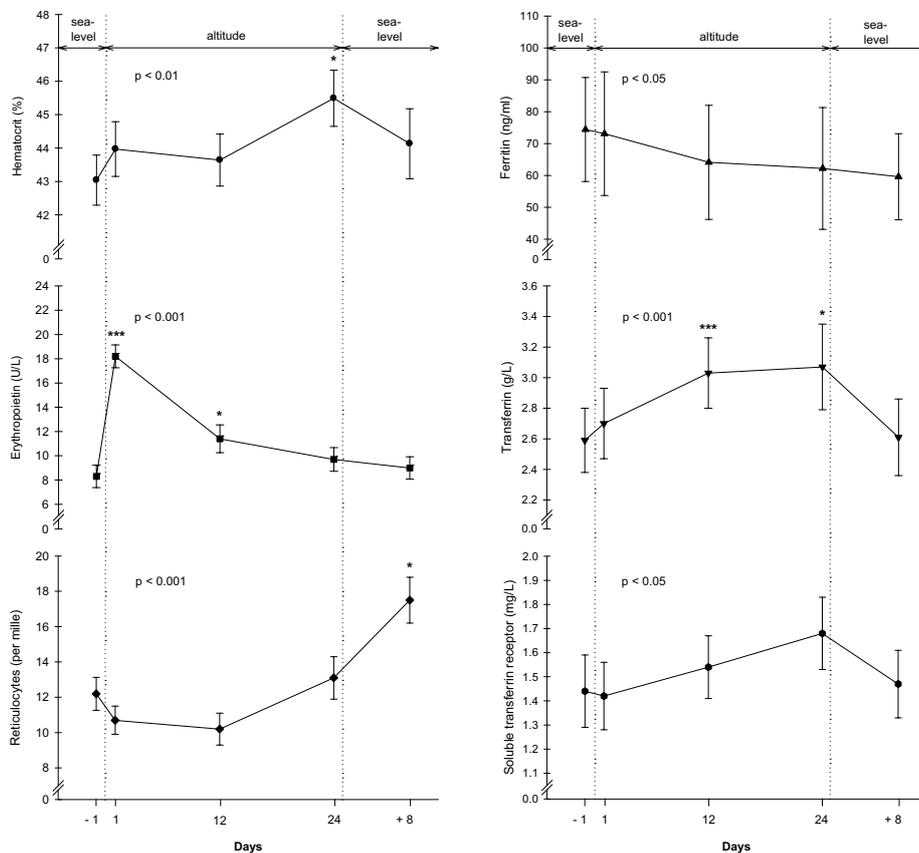
Figure 20. Time courses of hematocrit, erythropoietin, reticulocytes, ferritin, transferrin and the soluble transferrin receptor in the altitude group (five female and five male national team orienteers) measured before and after (day -1 and day +8) and during a "live high" (2456m) - "train low" (1800m and 1000m) altitude training camp (day 1, day 12 and day 24). Data are presented as mean ± standard error. The p-value indicates the effect of time on the parameter. * (p<0.05) and ** (p<0.01) indicate post-hoc differences from sea-level conditions (day -1).

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Rct values were affected by the LHTL phase ($p<0.001$) and post hoc analysis reported higher values at post-test (17.5 ± 4.2‰; $p<0.05$) than at pre-test (12.2 ± 2.9‰). Ftn decreased ($p<0.05$), TF increased ($p<0.001$) and sTfR increased ($p<0.05$) during the LHTL phase. For the CG, Hct (43.9 ± 3.7 vs 42.7 ± 3.5%), Hb (15.7 ± 1.2 vs 15.5 ± 1.1g/dl) and Ftn (65 ± 17 vs $62 \pm 19\mu$g/L) did not change during the experimental period (pre- and post-test values, respectively).

3.2.2. Effects of several altitude training periods on erythropoiesis (Study IV)

Blood volume parameters

Blood volume parameters are depicted in Figure 21. After ATP I ③, Hb_{mass} and RCV in the AG7 were increased by 4.3% ($p<0.001$) and 4.6% ($p<0.001$), respectively, while no changes were seen in PV and BV. No significant changes in these variables were seen for CG7. Prior to ATP III ④, Hb_{mass} and RCV were not statistically elevated in AG7 compared with pre-ATP I (baseline) ②. In the three-month training period between baseline ② and pre-ATP III ④, PV and BV increased in AG7 by 10.8% ($p<0.001$) and 6.4% ($p<0.01$), respectively.

After ATP III ⑤, Hb_{mass} in AG4 was elevated by 6.8% ($p<0.05$) above baseline ②, while there was no significant change for AG7. RCV remained unchanged in both AG7 and AG4 athletes after ATP III ⑤. In CG7, Hb_{mass} was not changed after ATP III ⑤, while RCV was 4.3% ($p<0.001$) lower after ATP III ⑤ than baseline ②.

Other blood parameters

After ATP I ③, Hb and Hct were increased ($p<0.01$) in AG7, while there was no significant change in CG7 compared with baseline ②. Prior to ATP III ④, Hb ($p<0.01$), Hct ($p<0.001$), RBC ($p<0.001$) and MCV ($p<0.01$) were lower in AG7 than at baseline ②. After ATP III ⑤, Hb values were unchanged in AG7, AG4 and CG7 when compared with baseline ②. Hct and MCV values were decreased after ATP III ⑤ in both AG7 ($p<0.05$; $p<0.01$) and CG7 ($p<0.001$; $p<0.01$) athletes compared with baseline ②. The absolute change in MCV in relation to the baseline ② (ΔMCV) tended to decrease more in AG7 athletes ($p=0.068$) after ATP III ⑤ than in CG7 athletes. Ftn did not change over the five-month training period for either AG7 or CG7. For details see Paper IV.
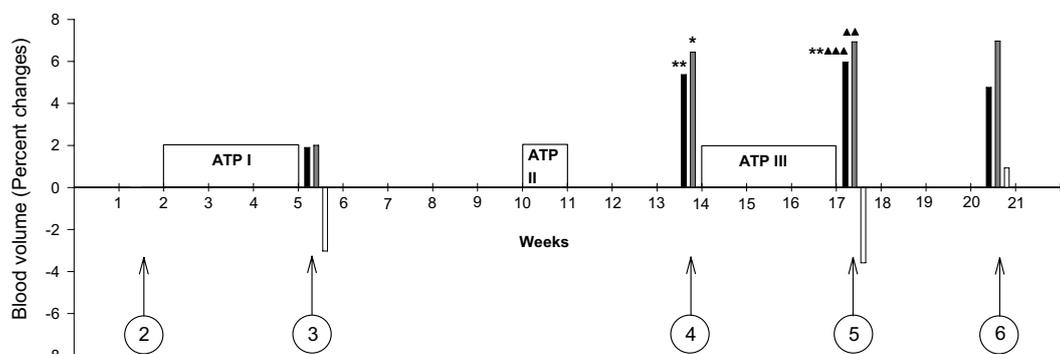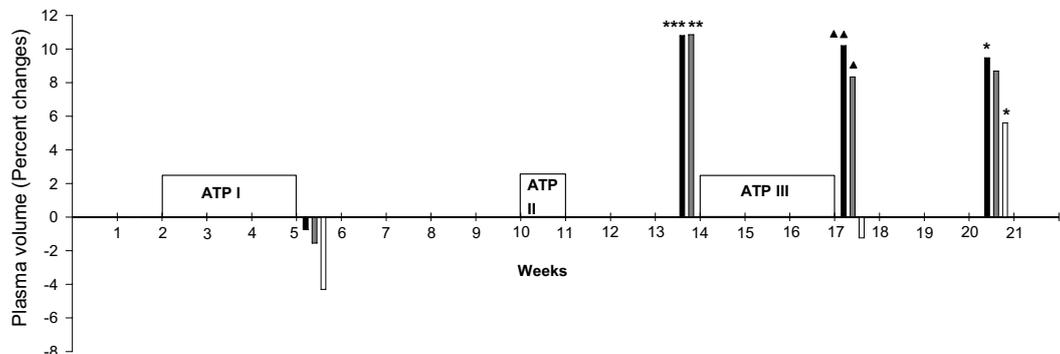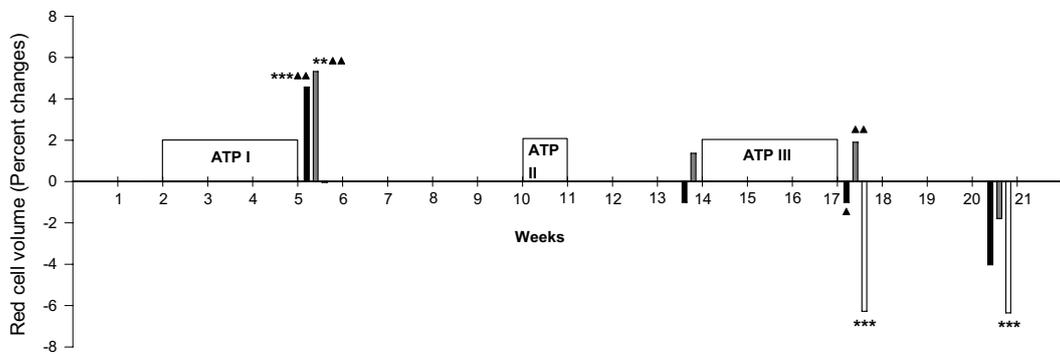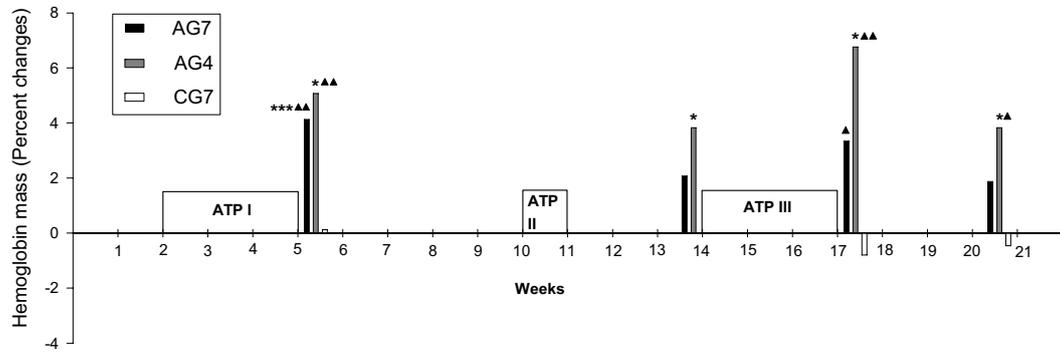
Figure 21. Hemoglobin mass, red cell volume, plasma volume and blood volume in Swiss national team cross country skiers measured at different time points during a five-month pre-season training period. ATP I - III = altitude training period I - III; ②, ③, ④, ⑤ and ⑥ refer to the study design in Figure 9. AG7 = altitude group (two females and five males). CG7 = control group (four females and three males). Three male athletes of the AG7 spent only 10 days at the third altitude camp (ATP III), we therefore also present all results of AG7 with these three athletes excluded. This subgroup was termed AG4. *** (p<0.001), ** (p<0.01) and * (p<0.05) indicate differences in relation to baseline values ②. ▲▲▲ (p<0.001), ▲▲ (p<0.01) and ▲ (p<0.001) indicate differences (t-test) in the relative change to the baseline value at that time in relation to the CG.

3.2.3. Reproducibility of the blood volume measurements

During studies II - IV, we determined the reproducibility of the blood volume parameters measured in our laboratory. Table 4 gives an overview of the coefficients of variance, Pearsons's correlations coefficients and the range for the blood volume parameters derived from two separate measurements taken 24h apart. These reproducibility measurements were done twice (i and ii).

Table 4. Reproducibility data of blood volume parameters derived from two measurements taken 24h apart. These reproducibility measurements were done in two separate experiments (i, ii) during the period when studies II - IV were performed.

		Hb_{mass}	RCV	PV	BV
Experiment i (n = 11)	CV	1.7%	2.2%	4.8%	3.4%
	R	0.991	0.974	0.903	0.946
	Range	608 - 1065g	1610 - 2703ml	2861 - 5154ml	4681 - 7856ml
Experiment ii (n = 7)	CV	1.4%	2.1%	4.5%	3.5%
	R	0.981	0.952	0.894	0.932
	Range	792 - 1002g	2210 - 2934ml	3117 - 5155ml	4744 - 8443ml

Hemoglobin mass (Hb_{mass}); Red cell volume (RCV); Plasma volume (PV); Blood volume (BV); Coefficient of variation (CV); Pearson's correlation coefficient (R).

3.2.4. Effects of one altitude training period on performance (Study II and III)

In study II, $\dot{V}O_{2max}$ increased by 4.1% from before to after a 24-day LHTL altitude training camp (females: 50.8 ± 2.1 vs 54.5 ± 2.8 ml $\cdot$ kg^{-1} $\cdot$ min^{-1}; males: 62.3 ± 5.2 vs 63.8 ± 5.5 ml $\cdot$ kg^{-1} $\cdot$ min^{-1}; $p<0.05$, females and males together). TTE in the $\dot{V}O_{2max}$ test increased by 41s ($p<0.05$), HR$_{max}$ decreased by 3b/min ($p<0.05$) whereas $[La^-]_{b\text{-}max}$ did not change. The athletes improved 5000m running time by about 18s (1.6%; $p<0.05$), with no difference in HR$_{max}$, $[La^-]_{b\text{-}max}$ or RPE.

In study III, the 5000m runner ran 40s faster (13:12:69) on the first day after the LTHL camp than on the day before the LHTL camp (Swiss Championships, Figure 22). In the final of the World Championships, he ran a time of 13:26:06. The marathon runner ran a time of 2:11:14 at the World Championships, compared with 2:11:05 three months earlier. The two athletes reached their best individual ranking at the respective World Championships.
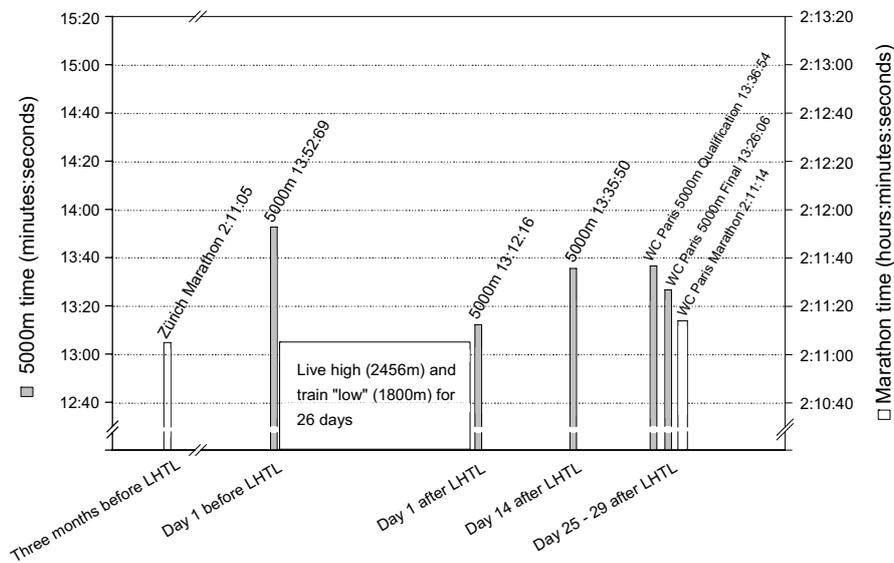
Figure 22. Competition results of one marathon runner and one 5000m runner before and after 26 days of living high and training low (LHTL) in preparation for the World Championships (WC; Paris 2003).

3.2.5. Effects of several altitude training periods on performance (Study IV)

$\dot{V}$O$_{2max}$ increased by 6.4% from pre-ATP I ② to post-ATP III ⑦ in AG7 and increased by 7.1% in AG4, while no change was seen in CG7 athletes. TTE improved ($p<0.05$) by 9% in CG7 and tended to increase in AG7 ($p=0.08$; + 7.3%). Performance at anaerobic threshold tended to increase in AG7 ($p=0.08$; +5.9%), while there was no change for AG4 and CG7 (Figure 23).
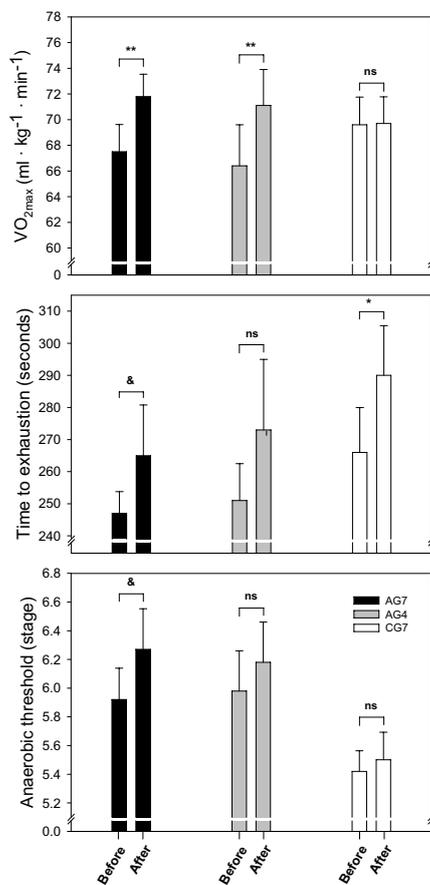
Figure 23. Mean ± SE values of maximal oxygen uptake ($\dot{V}$O$_{2max}$), time to exhaustion in the $\dot{V}$O$_{2max}$-test and performance at the anaerobic threshold measured in Swiss national team cross country skiers before and after a five-month pre-season training period. AG7 = altitude group (two females and five males). CG7 = control group (four females and three males). Since three male athletes spent only 10 days at ATP III, we also present all results of AG7 with these three athletes excluded. This subgroup was termed AG4. ** ($p<0.01$), * ($p<0.05$) and & ($p<0.1$) indicate differences after the training period. NS indicates no differerence.

4.   DISCUSSION

4.1. Impact of acute hypoxia on $\dot{V}O_{2max}$ and performance (Study I)

The main finding of study I was that both performance and $\dot{V}O_{2max}$ declined significantly from 300m to 800m and continued to decrease linearly to 2800m above sea-level. This is the first study to report a linear decrease in performance and $\dot{V}O_{2max}$ from sea-level (0 - 300m) to very low (300 - 1000m), low (1000 - 2000m) and moderate simulated altitudes (2000 - 3000m) during acute exposure in endurance-trained athletes.

4.1.1. Maximal oxygen uptake

Due to the sigmoidal shape of the oxyhemoglobin dissociation curve, it has been thought that very mild hypoxia corresponding to an altitude of less than 1500m will have only a minor effect on the O_2 content of the arterial blood and $\dot{V}O_{2max}$. However, in agreement with our results, other studies have reported a reduction in $\dot{V}O_{2max}$ at altitudes below 1000m in ETA (21, 52, 54, 141). The most important mechanism for this seems to be "exercise-induced arterial hypoxemia" (EIH), a phenomenon seen even at sea-level in endurance-trained athletes with high $\dot{V}O_{2max}$ (21, 52). EIH occurred already at sea-level in all our athletes, according to the definition of an EIH subject as one with SpO_2 less than 92% at maximal exercise (62, 109, 110, 145). In another study, mild hypoxia ($FiO_2 = 0.187$) caused a 4% reduction in $\dot{V}O_{2max}$ in EIH athletes, while non-EIH athletes experienced no reduction even if there was no difference in hypoxia-induced reduction in SpO_2 and therefore no correlation between ΔSpO_2 and $\Delta \dot{V}O_{2max}$ (20). Ferretti et al. (33) concluded that endurance-trained athletes, contrary to untrained subjects, work at the steep part of the oxygen hemoglobin dissociation curve even at low altitudes. In the present study, we found a difference in the pattern of decline in SpO_2 with altitude during submaximal compared with maximal exercise. Whereas SpO_{2min} declined in a linear manner during maximal exercise, it followed a curvilinear pattern during submaximal exercise (see Paper I). This conclusion was made based on visual analyses, curve fitting and the fact that the rate of decline was steeper between 1800 and 2800m than between 300 and 1300m during submaximal exercise, but not during maximal exercise. The curvilinear shape during submaximal exercise may reflect the sigmoidal shape of the oxyhemoglobin dissociation curve, but why is this not seen during maximal exercise? Our

hypothesis is that the linear relationship seen during maximal exercise is caused by the combined effect of exercise-induced hypoxemia and the right-shifted oxyhemoglobin curve due to acidification of the blood during maximal exercise (Bohr-effect). The Bohr-effect is elegantly demonstrated in both humans and horses, where an attenuation in the exercise-induced reduction in pH by bicarbonate infusion also attenuates the exercise-induced reduction SpO_2 with no change in arterial O_2-tension (93, 101). During submaximal exercise, SpO_2 is neither so low in normoxia nor affected by the Bohr-effect and the altitude-induced reduction follows a curvilinear pattern. Untrained subjects who do not experience EIH may be less susceptible to both the hypoxia-induced lowering of SpO_2 and the Bohr-effect during maximal exercise.

In the present study, $\dot{V}O_{2max}$ declined at the same rate between low altitudes (300m to 1300m) as between higher altitudes (1800 to 2800m) and together with the results from the studies presented in Figure 8, this supports the hypothesis that the $\dot{V}O_{2max}$ decreases linearly beginning at sea-level in endurance-trained athletes. Further, it supports the conclusions of Squires and Burkirk (134) and Gore et al. (52) who stated that the concepts of threshold altitudes for aerobic impairment, as suggested by others (18, 58, 118), are ambiguous in the case of unacclimatized ETA.

Rate of decline of $\dot{V}O_{2max}$ with altitude.

The mean decrease in $\dot{V}O_{2max}$ in the present study was 6.3% per 1000m, close to the 7.7% calculated from the studies in Figure 24. Individual decreases in $\dot{V}O_{2max}$ ranged between 4.7% and 7.5% per 1000m, a small variation compared with that found in ETA earlier by Gore et al. (52) (+1 to -12% change from 168m to 748m above sea-level) and Billat et al. (10) (–8 to -24% from sea-level to 2400m). Unfortunately, none of these studies or the studies included in Figure 24 reported test-retest reproducibility. It is therefore not clear how much of the reported variability is methodological variation and how much is biological variation between the subjects. In our study the test-retest reproducibility (coefficient of variation) at 300m was 1.4%. The decrease in SpO_2 is strongly associated with the decrease in $\dot{V}O_{2max}$ with altitude. According to Ferretti et al. (33), the decrease in SpO_2 accounts for about 86% of the decrease in $\dot{V}O_{2max,}$ which fits with the present study where approximately 70% of the

decrease in $\dot{V}O_{2max}$ can be explained by the decrease in SpO_2 at $\dot{V}O_{2max}$ (SpO_{2min}). Furthermore, the decrease in $\dot{V}O_{2max}$ of 6.3% per 1000m is close to the decrease in SpO_{2min} of 5.5% per 1000m and fits the conclusion of Powers et al. (110) that a reduction of 1% in SpO_2 below 92 - 93% causes a decrease of ~1% of $\dot{V}O_{2max}$. Hence, the main mechanism for the hypoxia-induced decrease in $\dot{V}O_{2max}$ at low and moderate altitude is the decrease in SpO_2. However, there was no correlation between the individual rate of decrease in SpO_2 at $\dot{V}O_{2max}$, and individual rate of decrease in $\dot{V}O_{2max}$, suggesting that there are other confounding mechanisms involved, such as a reduction in maximal cardiac output ($\dot{Q}_{max}$) (19). In the present study, HR increased during submaximal exercise (with no change in $\dot{V}O_2$) reflecting the reduced oxygen content of the arterial blood, while HR tended to decrease during maximal exercise, supporting the theory of a minor hypoxia-induced decrease in $\dot{Q}_{max}$.
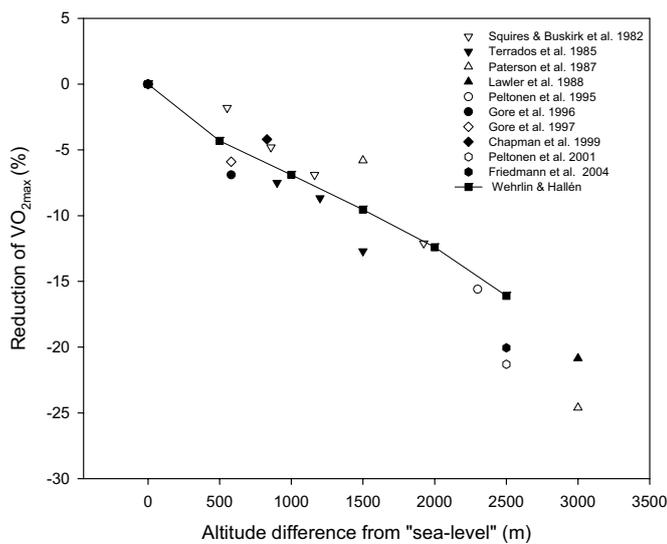
Figure 24. Decline in $\dot{V}O_{2max}$ with altitude from sea-level values. "Sea-level" in these studies (21, 39, 52, 54, 82, 105-107, 134, 141, 149) is set at 0m but varies originally from 0 to 362m. Only studies which tested male unacclimatized ETA with a mean $\dot{V}O_{2max} > 60\ ml \cdot kg^{-1} \cdot min^{-1}$ under laboratory conditions at acute hypoxia are included.

4.1.2. Decrease in performance with increasing altitude

Performance (time to exhaustion) decreased between 300m and 800m (- 9.4%) and continued to decrease by a mean of 14.3% per 1000m increasing altitude. Surprisingly, few studies have tested the change in exercise performance at acute exposure to altitudes below 3000m in ETA (52, 54, 82, 106, 108) and only two have tested ETA below 1000m (52, 54). In these studies, performance was determined either with an incremental (82, 108) or an all out test for a given distance (52, 54, 106). When performance declines during a graded exhaustive protocol or a self-selective workload, it can be argued that $\dot{V}O_{2max}$ decreases as a result of reduced workload and muscle recruitment (108). In the present study, the absolute exercise intensity was the same and constant at all altitudes (implying a similar muscle recruitment) (135), and the $\dot{V}O_2$ was different at all time points (Figure 25). This indicates that the reduced $\dot{V}O_{2max}$ in hypoxia is limited by O_2 availability and is not due to reduced absolute exercise intensity associated with reduced muscle recruitment (104). From a practical point of view, one has to point out that in highly trained endurance athletes, every increase in altitude leads to worse performance. This has to be taken into account when absolute performance (qualification time, etc.) is important.

Figure 25. Mean oxygen uptake of eight male sea-level resident athletes at different time points during the constant speed $\dot{V}O_{2max}$ tests at different altitudes.

4.2. Impact of chronic hypoxia on erythropoiesis and performance

4.2.1. Methodological aspects of blood volume measurement

Introduction.

Blood volume (BV) and its components of RCV and PV have been studied for more than 100 years (56, 60). By their nature, RCV, PV and BV can not be measu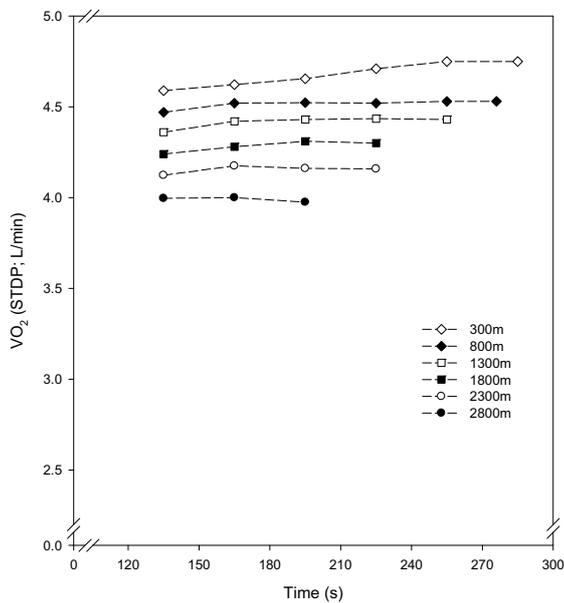red directly. The methods used to measure blood volumes are all indirect and based on dilution of tracers injected into the circulation (49). The tracers are red cells labelled with radioactive chromium (^{51}Cr) for measurement of RCV (55), albumin labelled with radioactive iodine (^{131}I or ^{125}I) for measurement of PV (130), and the dye Evans blue, which delineates PV by staining plasma proteins (46). For the last 25 years, the ^{51}Cr method for estimating RCV has been regarded as the criterion method by the International Committee for Standardization in Hematology (69), and the Iodine-labelled albumin is recommended for estimating PV (70). However, these radioactive methods are unsuitable for several measurements in athletes due to the long half-life period of the radioactive tracer as well as the associated health risks (49).

The Evans blue dye method, though not radioactive, is disadvantageous in that RCV is estimated "double indirect" because PV is first estimated and RCV then calculated with help of the hematocrit value. Furthermore, multiple blood samples must be taken over a period of 60min to identify the rapid disappearance phase. In is also unclear whether this method is appropriate for measuring RCV changes after hypoxic exposure (5, 49, 53), since there have been reports of increased albumin leakage after hypoxic exposure in the vascular space (89), which would result in an overestimation of RCV after hypoxic exposure.

Carbon monoxide rebreathing method

The CO-rebreathing method (labelling of hemoglobin (Hb) with CO) offers an alternative approach. The method has several advantages: it is minimally invasive (only 6 - 8 earlobe blood samples), has no negative health effects (half-time of HbCO is about 4h), and allows repeated measurements at frequent intervals. The most important advantage of the CO-rebreathing method is that the total mass of hemoglobin (Hb$_{mass}$) is measured "directly" with this method rather than being calculated indirectly. Hb$_{mass}$ is the most important parameter as it enables us to answer our research questions regarding changes in erythropoiesis due to altitude exposure. But this method also allowed us to calculate RCV, PV and BV from the Hct

and Hb values. We therefore chose the CO-rebreathing method to determine Hb_{mass}, RCV, PV and BV in our studies on chronic hypoxia.

Validity of the CO-rebreathing method

As aforementioned, has the ^{51}Cr method for estimating RCV been regarded as the criterion method by the International Committee for Standardization in Hematology (69) for the last 25 years. There have not been many comparisons between the CO-rebreathing method and the "gold standard" methods using radioactive tracers. However, Thomson et al. (142) showed good agreement in RCV values using the method of ^{99}Tc-labeled erythrocytes and therefore giving good external validity. The determination of Hb_{mass} with the CO-rebreathing method has been shown to give very high reproducibility. Burge and Skinner (16) reported a coefficient of variation of only 0.8% so that changes in Hb_{mass} as little as 1.5% are detectable. Gore et al. (49) showed in their recent meta-analysis about errors of measurement for blood volume parameters that mean error for the CO-rebreathing method in different studies was 2.2% with a 90% confidence interval ranging from 1.4 to 3.5%. This was better than the mean reproducibility for the ^{51}Cr method (2.8%; 2.4 - 3.2%) or the Evans blue dye method (6.7%; 3.4 - 14%). They concluded that the CO-rebreathing has error similar to the ^{51}Cr method, but the CO-rebreathing method has the advantages of easy handling, independence from biological variation in Hct and short half-life of HbCO.

Possible sources of error with the CO-rebreathing method are gas leak in the rebreathing apparatus or at mouthpiece by the volunteer, inadequate CO dose, and use of a rebreathing bag with excessive volume (16). Burge and Skinner (16) underscore that an adequate dose of CO becomes particularly important if estimating %HbCO levels with commercial CO-oximeters that display only to a single decimal place (usually $\pm$ 0.1%). Progressively lower doses of CO are associated with a substantial increase in measurement error.

For example 25ml and 75ml CO volumes result in errors of 4.1 and 1.3% for a woman with a Hb_{mass} of 600g, respectively (49). Burge and Skinner recommend using a volume of CO adequate to reach a delta %HbCO value of about 6%, which will lead to a coefficient of variation of 1.5% for Hb_{mass}.

Validity of blood volume results in our studies

We used between 70 to 100ml of CO in our studies, depending on gender and barometric pressure at the different altitudes. This led to delta %HbCO values ranging between 5 - 8%. During the period of each study, we determined the reproducibility of the blood volume measurements in separate experiments. As expected, the results (Table 4) agreed with the findings of Burge and Skinner (16) and Gore et al. (49). Further, there were no outliers in the reproducibility studies or in the altitude training studies that would indicate a gas leak in the system. We therefore conclude that the validity of our blood volume measurements is high.

4.2.2. Effect of one altitude training period on erythropoiesis (Studies II-IV)

The main findings of the present studies about the effects of one altitude training period on erythropoiesis in endurance athletes were that Hb_{mass} and RCV increased by about 4 - 6% after 3 - 4 weeks at altitude training camp living for about 400h at 2400 - 2600m (Figure 19). Several factors support the validity of the observation that Hb_{mass} and RCV increased in our studies. We found a significant increase in both Hb_{mass} and RCV in all altitude training groups (study II - IV) with no increases in the control groups ($p<0.05$ between groups; Study II + IV). The increases in Hb_{mass} and RCV (about 4 - 6%) were 2 - 3 times higher than the CV of the methods. The increases in Hb_{mass} and RCV were further supported by the changes in several blood parameters (increase in sEpo and Rct) and iron (decrease in Ftn and increase in TF and sTfR) parameters (study II; figure 20):

Erythropoietin. Our finding that sEpo was doubled after day 1 at altitude is in line with other studies where sEpo increased, with considerable individual variation, by about 50 - 150% after 24h of altitude (2500m) exposure (4, 22, 25, 45, 86). However, increased sEpo is not necessarily associated with an increase in Rct and Hb_{mass}. Ashenden (4) reported no increase in Rct and Hb_{mass} after three 5-day LHTL exposures at 2650m (simulated altitude) despite an increase in sEpo.

Reticlulocytes. Rct are not only changed by altitude exposure, but are also affected by normal endurance training (128). The absolute variation in Rct during normal training in athletes has been reported to be 2 - 4‰ during periods of 30 (5) and 70 (6) days (5, 6, 25). Thus, the effect size of an absolute change in the Rct of around 2 - 4‰ may in a way reflect normal changes due to training including measurement error. In our study II, the mean absolute change in ‰

Rct was within 3‰ during the first 12 days, but was increased 7‰ (10.2 to 17.5‰) from day 12 to the post-test. Interestingly, this increase occurred at the end of and even after the LHTL-ATP. We do not know how much of this increase in Rct is due to the altitude-induced increase in erythropoiesis and how much may be attributed to training intensity or measurement error. However, these results suggest that duration of the LHTL-ATP is important. *Ferritin*. Ftn decreased during the LHTL camp despite oral iron supplementation (study II). Ftn levels reflect the magnitude of iron stores (92), but the Ftn level can be masked by various infections and inflammatory states, which cause increased values (2, 9, 68). C-reactive protein levels, an indicator for the inflammatory status (111) were not increased in study II and did not indicate false high Ftn values. A decrease in Ftn despite iron supplementation during altitude training has been associated with an elevated production of red blood cells (136), Birkeland et al. (11) reported a decrease in Ftn levels during an artificial low-dose erythropoietin treatment from 110μg/L to 50μg/L during three weeks. T*ransferrin and soluble transferrin receptor*. TF and sTfR were elevated during the LHTL-ATP in the AG (study II). TF (23) and especially sTfR (92, 133) were reported to significantly increase in the case of stimulated erythopoiesis by, for example, artificial erythropoietin treatment (11, 92). Birkeland et al. (11) suggest that serum levels of sTfR may be used as an indirect marker of supranormal erythropoiesis up to 1week after the administration of rhEPO.

Taken together, these changes in the blood parameters and iron metabolism can be interpreted to occur with increased erythropoiesis (11) and therefore support our data regarding increased Hb$_{mass}$ and RCV after 3 - 4 weeks of LHTL-ATP.

Whether LHTH or LHTL altitude training camps increase Hb$_{mass}$ and RCV is debated (5, 53, 84, 87). Figure 26 includes the results of all studies (to our knowledge) in which endurance-trained athletes participated in either a LHTH or LHTL altitude training camp and measured Hb$_{mass}$ and/or RCV (5, 6, 15, 25, 37, 50, 64, 81, 86, 116, 117, 120, 125, 137, 150-152). The results of these studies range from an increase in Hb$_{mass}$ and RCV of about 10% to a decrease of 2%. We have grouped the studies according to hypoxic doses (Figure 26). Group A (5, 6, 25, 116, 125) includes the studies where the athletes spent about 100 - 200h at altitude and reported no change in Hb$_{mass}$ or RCV. The studies in group B (37, 64, 81, 86, 120, 150-152) include athletes who spent between 350 and 550h at altitude and whose Hb$_{mass}$ or RCV

increased about 4 - 7%. Group C is the LHTH group from the classic Levine and Stray-Gundersen study (86) in which athletes spent about 700h at altitude and RCV increased by 10%. In group D the athletes spent about 750h at altitude, but Hb_{mass} remained unchanged (50, 137). In group E, the athletes spent only about 200 - 250h at altitude, but Hb_{mass} was increased by 8 - 10% (15, 117). Based on the fact that Hb_{mass} in lifelong residents (65) of moderate altitude (2600 - 3550m), including athletes (13, 129), is elevated, it has been suggested (84, 121, 122, 146) that moderate altitude increases Hb_{mass} and RCV and that the "hypoxic dose" (living altitude combined with the time spent at altitude) used in altitude training plays a major role in whether or not Hb_{mass} and RCV are increased. Rusko et al. (121) concluded that the minimum dose necessary to attain a hematological acclimatization is > 12h per day for at least three weeks (about 250h) at an altitude of 2100 - 2500m. There is a clear dose - response relationship between the groups A, B and C in Figure 26. In group A, the hypoxic dose was probably too low, whereas the hypoxic dose (350 - 550h at 2100 - 2600m) in group B was high enough to increase Hb_{mass} or RCV by about 5%. Group C's results indicate that Hb_{mass} and RCV can be increased further with a higher hypoxic exposure, as shown by Heinicke et al. (65) where Hb_{mass} was increased by 11% after a six-month exposure to 3550m.

In group D, Hb_{mass} was unchanged despite the fact that the athletes in the two studies (50, 137) spent more than 700h at altitude. In the first study (137), the athletes spent 30 days LHTH at an altitude of 1900m, an altitude which might be too low to cause an increase Hb_{mass} and RCV. In the second study by Gore et al. (50) reported no increase in absolute Hb_{mass} after 31 days LHTH at 2690m, though the authors pointed out that all athletes succumbed to illness during the period, which can have depressive effects on the erythropoiesis (40).

Finally, group E showed an Hb_{mass} increase of 8 - 10% with a relatively low hypoxic dose. The nine AG athletes in the study by Robach et al. (117) lived at simulated altitudes between 2500 and 3000m for only 13 nights (16h per day). However, the reproducibility of the method used to determine Hb_{mass} was not investigated and one athlete increased Hb_{mass} by 31%, which seems to be an unnaturally high increase in after only 13 days at altitude. The mean increase would have been reduced to about 4.7% when excluding the result of this athlete. In the study by Brugniaux et al. (15) five athletes from the AG lived for 18 days at simulated altitudes between 2500 and 3000m. Hb_{mass} increased by 10.1% and RCV was elevated by 9.2% though the latter result was not statistically significant. Visual analysis of the individual RCV data

showed that two of five athletes increased RCV by 20 - 30%, which also seems to be unnaturally high.
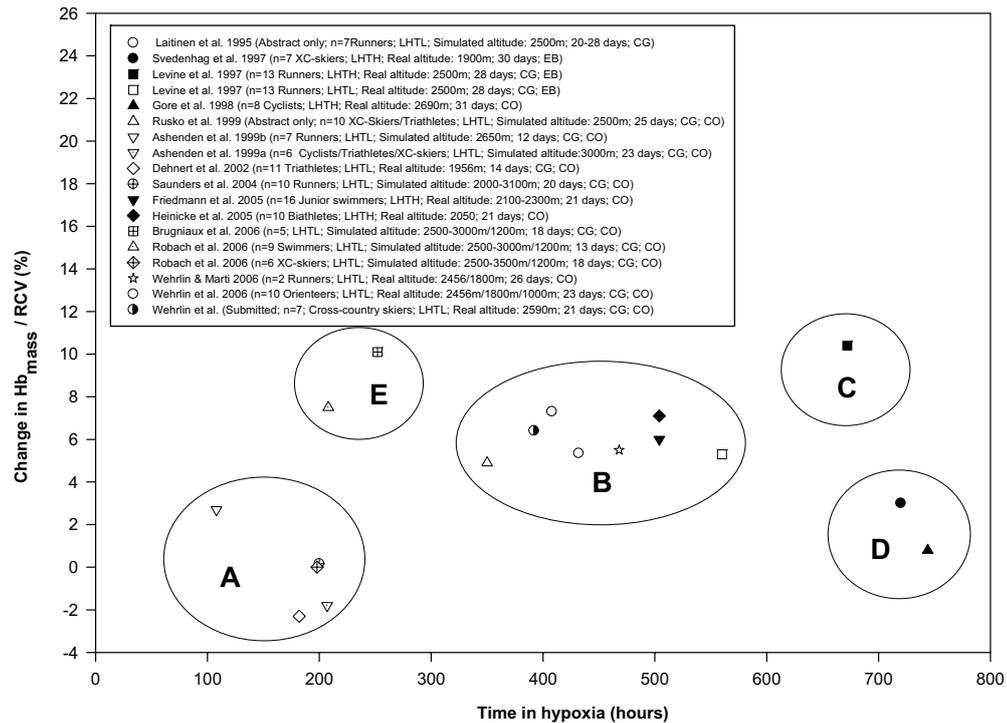
Figure 26. Change in hemoglobin mass (Hb$_{mass}$) or red cell volume (RCV) in relation to time spent at altitude in studies with endurance-trained athletes (5, 6, 15, 25, 37, 50, 64, 81, 86, 116, 117, 120, 125, 137, 150-152). Reported are: number of subjects (n), the sport, the type of altitude training (LHTH = live high - train high; LHTL = live high - train low), the nature of the altitude (real or simulated), the living altitude, days spent at altitude, use of a control group (CG) and technique used for measurement of Hb$_{mass}$ or RCV (EB = Evans blue dye; CO = carbon monoxide rebreathing). A, B, C, D and E refer to the text.

In both studies, a low amount of CO (44 and 49ml) was used. In endurance athletes with high absolute Hb$_{mass}$ and RCV this will lead to a very low ΔCOHb and low reproducibility of measurement (16). However, other studies have also used a small amount of CO (37, 64) and, except for our studies II – IV, these other studies did not measure the reproducibility of their Hb$_{mass}$ and RCV measurements. In addition, two studies used the "Evans blue dye" (T-1824) or ^{125}I-albumin method to determine PV and then calculate RCV (86, 137). This method has been criticized (5) since it may show spurious increases in RCV due to increased albumin

leakage as a consequence of altitude exposure (61, 89). Other possible confounding factors are the extent of the athlete's relative Hb_{mass} and RCV used, or possible insufficient iron stores or illness. These aspects emphasize the importance of precise measurements and that one should be careful with interpretations.

In summary, we conclude that one altitude training period (LHTL) with a hypoxic dose like the one we used in studies II - IV (living about 400h at an altitude of about 2500m) can increase Hb_{mass} and RCV. A lower hypoxic dose may have little or no effect on erythropoiesis. Study III further indicates that Hb_{mass} and RCV can even be increased in world class athletes with already high Hb_{mass} and RCV levels.

4.2.3. Effects of several altitude training periods on erythropoiesis (Study IV)

The main findings of the effects of several altitude training periods on erythropoiesis were that Hb_{mass} and RCV changed in a different way: whereas both Hb_{mass} and RCV increased during the first ATP (Study II - IV) Hb_{mass} and RCV remained unchanged in AG7. In CG7, Hb_{mass} remained unchanged and RCV even decreased during the five-month training period. This was due to a decrease in MCV for all athletes. When excluding the three AG7 athletes who completed only 10 of the 21 days at ATP III, Hb_{mass} was still elevated after ATP III, but RCV remained unchanged. The long-term training combined with altitude training increased PV in all athletes. No similar study exists that measures Hb_{mass} and RCV over a several-month training period containing several altitude training camps.

Hemoglobin mass

Hb_{mass} was increased by 4.1% after ATP I in AG7, while no change was seen in CG7. This finding is, as previously mentioned, in accordance with several LHTL and LHTH studies that used a similar hypoxic dose and measured Hb_{mass} with the CO-rebreathing method. After ATP II, during two months of endurance training and only one week of LLTH altitude training, Hb_{mass} was decreased by approximately 2% and did not increase further with ATP III in AG7. One plausible reason for this was that three of seven athletes spent only 10 days at altitude due to a cold. When the results of these three subjects were excluded (AG4), Hb_{mass} was elevated by 6.8% after ATP III compared with pre-ATP I. These results indicate that Hb_{mass} in athletes can be further increased after one altitude training camp. Heinicke et al. (65) showed 11% higher Hb_{mass} in a group of soldiers serving for six months alternating between 3550m at altitude (11-day periods) and sea-level (three-day periods) compared with a soldier control group living at sea-level. This may reflect the potential effect size to increase Hb_{mass} in athletes. Hb_{mass} was also measured three weeks after the ATP III because this time period is often used for competitions (151-153). During this period, Hb_{mass} returned to the value prior to ATP III in AG4 and AG7 athletes, but was still 3.8% higher than prior to ATP I in AG4. There was no change in Hb_{mass} in CG7 athletes throughout this period, which is in line with the findings of Gore et al. (51) that endurance training over a longer period does not change Hb_{mass} in already highly trained endurance athletes.

Red cell volume

RCV was increased by 4.6% in AG7 after ATP I. This finding is also in accordance with several LHTL and LHTH studies using a similar hypoxic dose. On completion of two months endurance training, RCV was not increased after ATP III even in the athletes that spent the entire ATP III and ATP II as planned at altitude (AG4). RCV was reduced in CG7 by approximately 6% while Hb_{mass} remained unchanged. We initially assumed that RCV would increase to a similar extent as Hb_{mass}.

In an attempt to find possible explanations for this unexpected result, methodological aspects will be discussed prior to physiological aspects: First, with the CO-rebreathing method, Hb_{mass} is measured "directly" whereas RCV is calculated indirectly based on Hb (g/dl) concentration and Hct values (RCV = Hb_{mass}/MCHC $\cdot$ 100 where MCHC = Hb/Hct). The RCV is, therefore, more susceptible to error. Interestingly, Hb concentration values remained relatively stable during the five-month period, whereas Hct decreased from about 45% in June to approximately 41% in October. This different course of Hb and Hct is thus the key factor in the different results in Hb_{mass} and RCV. So did the hematologic analyzer measure correctly during this period?

In spite of Hb being measured by photometry, Hct is calculated as a product of RBC and MCV (flowzytometry). Therefore, theoretically there could be a drift in the measurement of Hb and/or Hct during that five-month period. MCV was reduced by approximately 3 - 4% in the athletes which, in combination with lower RBC, resulted in lower Hct and a lower RCV. Critical inspection of quality control data (from quality controls performed every test day) did not indicate a relevant drift in the hematologic analyzer for the mentioned parameters during that five-month period. Nevertheless, it appears that the indirectly measured RCV is more susceptible to measurement error than the directly measured Hb_{mass} (49). In addition, only small drifts in Hb and Hct measurements could significantly influence RCV. This was also illustrated by the higher CV for RCV compared with Hb_{mass}.

When considering possible physiological explanations for the unexpected results, it should be emphasized that MCV of the athletes was within the normal range even after the training period and the Ftn values were normal during the whole period (97). As reported, RCV decreased and Hb_{mass} remained stable in the CG7 whereas Hb_{mass} increased and RCV remained stable in the AG4. Consequently, MCV decreased in both groups suggesting that the

erythrocytes were smaller but had a higher Hb content after the five-month training period. A reduction in MCV after continuous hypoxic exposure has been reported elsewhere (132). Similar to our findings, several studies have reported no change in MCV after one altitude training period (75, 78). However, to our knowledge, no study exists reporting the course of MCV during a several-month long endurance training period in elite athletes. However, a tendency for decreased MCV was reported after an intensified training period with a very high training load (115). Decreased MCV could also be explained by seasonal variations (80) or the influence of altitude-induced increases in 2.3 DPG (91).

Another possible mechanism could be the negative regulation of the red cell volume by neocytolysis. This mechanism has recently been recognized (114) and describes the physiological process that negatively regulates red cell volume by selectively hemolyzing young circulating erythrocytes (113). Neocytolysis is initiated by a fall in erythropoietin levels and has been observed in high altitude dwellers transported to sea-level (114). In our study, neocytolysis could at least partly explain the decrease in MCV and RCV during the five-month training period, since older erythrocytes are smaller and have more hemoglobin than the younger ones. However, these speculations give no answer as to the mechanism and to our knowledge no other studies exist that measured the influence of a long-term altitude training period on Hb_{mass}, RCV and MCV in elite athletes. The different changes in RCV in AG7 during the five month period (increase during ATP I and no change during ATP III) further indicate that the time selected for a LHTL ATP during the year can influence the changes in RCV. It is recommended to measure Hb_{mass} and not only RCV in future studies.

4.2.4. Effects of one altitude training period on endurance performance (Studies II - IV)

Because the primary aim of study II was to investigate the effect of the LHTL-ATP on the hematological parameters, we had no control group for the changes in the performance parameters. However, we report the changes in performance, because it cannot be taken for granted that our athletes improved performance after 3 - 4 weeks of LHTL training only. $\dot{V}O_{2max}$ (+ 4.1%) and TTE (+ 11.6%) were increased after the LHTL-ATP and the performance in the 5000m time trials was improved (-1.6%). Although optimal performance is shown to be achieved 14 – 21 days following altitude training (86, 153) we measured performance parameters 8 days following the LHTL-ATP since the athletes participated in the European Championships 21 days following ATP. If performance measurements had been taken at 3 weeks following the ATP, the altitude training effect on performance may have been larger. In order to measure increased Hb_{mass} and RCV, the post-test date should not extend too far from the end of the LHTL-ATP. That the study had a small number of subjects must be considered when examining the correlation between the increase in Hb_{mass} and the increase in $\dot{V}O_{2max}$. However, there are reports about the positive effect of increased Hb_{mass} and RCV on performance (83). In addition, the decrease in 5000m time and the increase in $\dot{V}O_{2max}$ were very similar to the findings of Levine and Stray-Gundersen (86). However, our study was uncontrolled and we cannot therefore conclude that the LTHL camp improved performance more than normal sea-level training. As previously mentioned, this aspect has recently been part of a Point:Counterpoint debate in the JAP (53, 87) and has widely been commented (3, 12, 14, 26, 28-30, 34, 36, 43, 44, 47, 48, 57, 63, 72, 74, 76, 77, 90, 94-96, 98, 99, 102, 103, 112, 119, 124, 126, 127, 131, 143, 147, 155-157).

Study III was scheduled to coincide with preparation for the 5000m and the marathon competitions at the 2003 World Championships taking place 27 - 29 days after the end of the LHTL camp. The 5000m runner ran 40s faster (13:12:69) on the first day after the LHTL camp than before (Figure 22), and improved his personal best by 24s (previous PB: 13:36:54). In the World Championships 5000m final, he ran in a time of 13:26:06, about 10s faster than his previous PB. The marathoner performed 2:11:14 in Paris and this could be interpreted to be an improvement to the performance reached three months earlier (2:11:05) because of the more hilly course and irregular pace in Paris. The absence of a control group and the

anecdotal nature of the report are weaknesses of this study. These weaknesses are mainly due to the limited number of world class runners available. However, it can be argued that it is very difficult to substantially improve performance in world class athletes and our observations do match findings from a well-controlled study of elite runners (136). That study seems to be one of the first documented observations of increased Hb_{mass} and RCV after a LHTL camp, followed by a successful endurance performance at an important championships event that included native European world-class endurance athletes approaching their 30th year of life.

Taken together, in both studies either $\dot{V}O_{2max}$ and/or endurance performance was improved after LHTL. However, the studies were not controlled and we do not know how $\dot{V}O_{2max}$ and performance would have been affected following normal sea-level training.

4.2.5. Effects of several altitude training periods on performance

After several altitude training periods, $\dot{V}O_{2max}$ increased in the AG7 group with seven weeks of altitude training (six weeks for three athletes), but not in the control group with three weeks altitude training. On the other hand, time to exhaustion in the $\dot{V}O_{2max}$ test improved significantly in the CG7 group (+ 9.0%; p=0.04) and the change was similar (but not significant) in the AG7 (+ 7.3%; p=0.08). Hence, CG7 athletes improved TTE without an increase in $\dot{V}O_{2max}$. Therefore, our results may suggest that the effect of spending more time living at altitude enhances $\dot{V}O_{2max}$ to a greater extent than anaerobic performance in endurance athletes.

The result of increased $\dot{V}O_{2max}$ in our study after repeated long-term altitude training is in line with the results of Daniels & Oldrige (24), in which six world-class runners increased $\dot{V}O_{2max}$ by approximately 5% after alternating living and training at 2300m and living and training at sea-level for 68 days, spending a total of 42 days at altitude. This "hypoxic dose" is comparable to our study, in which the AG athletes lived for 42 days at altitudes between 2344 and 2760m for a 120-day period. To our knowledge, no other study exists that investigated the effect of alternate altitude training on performance with a similar hypoxic dose during a similar training period.

However, it is very difficult to control for all the possible confounding factors during such a long training period. We therefore want to be cautious with possible interpretations.

5. CONCLUSIONS

The present thesis showed the following:

1. In endurance trained athletes, $\dot{V}O_{2max}$ and performance can decrease in acute hypoxia already from 300 to 800m above sea-level and continue to decrease linearly by 6.3 and 14.3% respectively, per 1000m altitude, up to 2800m (Paper I).

2. In national team endurance-trained athletes, Hb_{mass} and RCV can increased by about 5% after living at 2344 - 2590m (for about 430 - 470h) and training at 1800 and 1000m for 24 - 26 days (Paper II - IV).

3. Living at 2456m and training at 1800m for 26 days in preparation for the World Athletic Championships can lead to improved running performance in world-class runners (Paper III).

4 a). A five-month training period containing several altitude training camps (3 + 1 +3 weeks) in national team cross-country skiers (Paper IV) can lead to:
    - increased Hb_{mass}
    - decreased MCV of the erythrocytes
    - unchanged RCV
    - increased $\dot{V}O_{2max}$

4 b). A five-month training period containing several altitude training camps (1 + 1 + 1 weeks) in cross country skiers (Paper IV) can lead to:
    - unchanged Hb_{mass}
    - decreased MCV of the erythrocytes
    - decreased RCV
    - unchanged $\dot{V}O_{2max}$

6. FURTHER STUDIES

Experiments may answer some questions, but usually create plenty of new once. Blood volume and its components have been studied for more than 100 years (56, 60), but the methods used are all indirect and based on dilution of tracers injected into circulation (49). Because most of these techniques are invasive and/or use radioactive tracers (which may not be adequate for repeated measurements), little is known about changes over time of blood volume and its components in athletes. The technique that allows measurements with high enough precision with athletes has, as presented in 1995 (16) and has now been rated as the most accurate technique (49).

We realized during Study II that there is a lack of reference values for female athletes. We have therefore started a project to determine reference values for Swiss female endurance athletes.

Little is known about the long-term effects (years) of training on blood volume (148), and we began to study these effects in a several-year follow-up with endurance athletes of different disciplines aged 16 to 24 years.

It is popular to discuss the individual response to altitude training with athletes and coaches and there have been studies that divide athletes into "responders" or "non-responders" (22, 37). Since studies only have measured once before and once after an altitude training camp, it is not possible to discriminate between the individual variation and measurement error. In future studies, several measurements should be taken before and after an intervention like altitude training One can hypothesize that this study would show that, due to measurement error, it is only roughly possible to detect an individual response.

In study IV, we had the unexpected finding of decreased RCV and MCV. It would be interesting to study the reasons for this decrease in a follow-up study.

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Paper I

Jon Peter Wehrlin, Jostein Hallén.

Linear decrease in $\dot{V}O_{2max}$ and performance with increasing altitude

in endurance athletes

Paper II

Jon Peter Wehrlin, Peter Zuest, Jostein Hallén, Bernard Marti.

Live high-train low for 24 days increases hemoglobin mass and red cell volume

in elite endurance athletes

Live high-train low for 24 days increases hemoglobin mass and red cell volume in elite endurance athletes

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Wehrlin, Jon Peter, Peter Zuest, Jostein Hallén, and Bernard Marti. Live high-train low for 24 days increases hemoglobin mass and red cell volume in elite endurance athletes. *J Appl Physiol* 100: 1938–1945, 2006. First published February 23, 2006; doi:10.1152/japplphysiol.01284.2005.—The effect of live high-train low on hemoglobin mass (Hb_{mass}) and red cell volume (RCV) in elite endurance athletes is still controversial. We expected that Hb_{mass} and RCV would increase, when using a presumably adequate hypoxic dose. An altitude group (AG) of 10 Swiss national team orienteers (5 men and 5 women) lived at 2,500 m (18 h per day) and trained at 1,800 and 1,000 m above sea level for 24 days. Before and after altitude, Hb_{mass}, RCV (carbon monoxide rebreathing method), blood, iron, and performance parameters were determined. Seven Swiss national team cross-country skiers (3 men and 4 women) served as "sea level" (500–1,600 m) control group (CG) for the changes in Hb_{mass} and RCV. The AG increased Hb_{mass} (805 ± 209 vs. 848 ± 225 g; $P < 0.01$) and RCV (2,353 ± 611 vs. 2,470 ± 653 ml; $P < 0.01$), whereas there was no change for the CG (Hb_{mass}: 849 ± 197 vs. 858 ± 205 g; RCV: 2,373 ± 536 vs. 2,387 ± 551 ml). Serum erythropoietin ($P < 0.001$), reticulocytes ($P < 0.001$), transferrin ($P < 0.001$), soluble transferrin receptor ($P < 0.05$), and hematocrit ($P < 0.01$) increased, whereas ferritin ($P < 0.05$) decreased in the AG. These changes were associated with an increased maximal oxygen uptake (3,515 ± 837 vs. 3,660 ± 770 ml/min; $P < 0.05$) and improved 5,000-m running times (1,098 ± 104 vs. 1,080 ± 98 s; $P < 0.01$) from pre- to postaltitude. Living at 2,500 m and training at lower altitudes for 24 days increases Hb_{mass} and RCV. These changes may contribute to enhance performance of elite endurance athletes.

altitude training; hypoxia; blood volume; erythropoietin; maximal oxygen uptake

THE CONCEPT OF LIVING AT "high" altitude and training at "low" altitude ("live high-train low," LHTL) has been increasingly used in recent years by endurance athletes with the expectation that sea-level performance may as a consequence be improved (36). The LHTL strategy combines living at moderate altitude, to increase hemoglobin mass (Hb_{mass}) and red cell volume (RCV), with training at low altitude to maintain a high absolute training intensity (26). This concept has been shown to be superior to normal sea-level training or classical live high-train high (LHTH) altitude training for improving sea-level performance in elite endurance athletes (24). However, studies of whether exposure to moderate altitude increases Hb_{mass} and RCV in elite endurance athletes have given controversial results (2, 16, 25). Results from the only published LHTL study that reported increase in RCV (24) have been discussed (2, 17), because RCV was measured indirectly with the Evans blue dye method, for which the adequacy for estimating RCV after hypoxic exposure has been questioned (13, 16, 27). LHTL

studies that directly measured Hb_{mass} with the carbon monoxide (CO)-rebreathing method did not report increased Hb_{mass} and RCV (2, 3, 8).

However, two LHTH studies, in which subjects generally spend more time at altitude, have recently reported increased Hb_{mass} after exposure to moderate altitude (9, 18). Thus it has been hypothesized that the hypoxic dose (living altitude combined with the duration of the altitude exposure) is the key factor (23, 28). To our knowledge, no controlled LHTL study has been published that has used a presumably adequate hypoxic dose at real altitude similar to the study by Levine and Stray-Gundersen (24) and measured Hb_{mass} directly with the CO-rebreathing technique. Therefore, the purpose of our study was to investigate the effects of living at an altitude of ~2,500 m and training at lower altitudes for 24 days on erythropoiesis in elite endurance athletes by using direct measurement of Hb_{mass}.

MATERIAL AND METHODS

Subjects

Ten athletes (5 women and 5 men) from the Swiss national orienteering team, aged 23 ± 4 yr, and seven athletes from the Swiss national cross-country team (4 women and 3 men), aged 21 ± 1 yr, gave written, informed consent to participate in the study, which was approved by the institutional review board of the Swiss Federal Institute of Sport and was carried out according to the recommendations of the Helsinki Declaration.

Study Design

The orienteering athletes were assigned to the altitude group (AG) and completed a 24-day LHTL phase, living 18 h per day at 2,456 m and training at 1,800 and 1,000 m above sea level, in the Swiss Alps. The cross-country skiers were assigned to the control group (CG), completing a normal training phase, which consisted of living and training between 500 and 1,600 m for 24 days. The study was carried out during the preseason for both groups (different time of the year for orienteers and cross-country skiers). An outline of the study design is presented in Fig. 1. Approximately 4 wk before the LHTL phase (AG) and before the experimental phase (CG) (A), blood samples were taken for measurement of serum ferritin (Ftn) to assess bone marrow iron stores. At the pretest (B), 1 day before the LHTL phase began, a blood sample was taken and the athletes from both groups performed a maximal oxygen uptake ($\dot{V}o_{2\,max}$) test in the laboratory. About 7–10 h later on the same day, the AG ran a 5,000-m time trial on a 400-m track. The blood volume parameters were measured the next day (AG and CG). Additional blood samples where taken from the AG athletes at *day 1* (C), *day 12* (D), and *day 24* (E) of the LHTL phase. Eight days after the 24-day phase (F), the athletes performed the posttest with identical measurements as at the pretest B with the exception that the CG did not perform the $\dot{V}o_{2\,max}$ test.
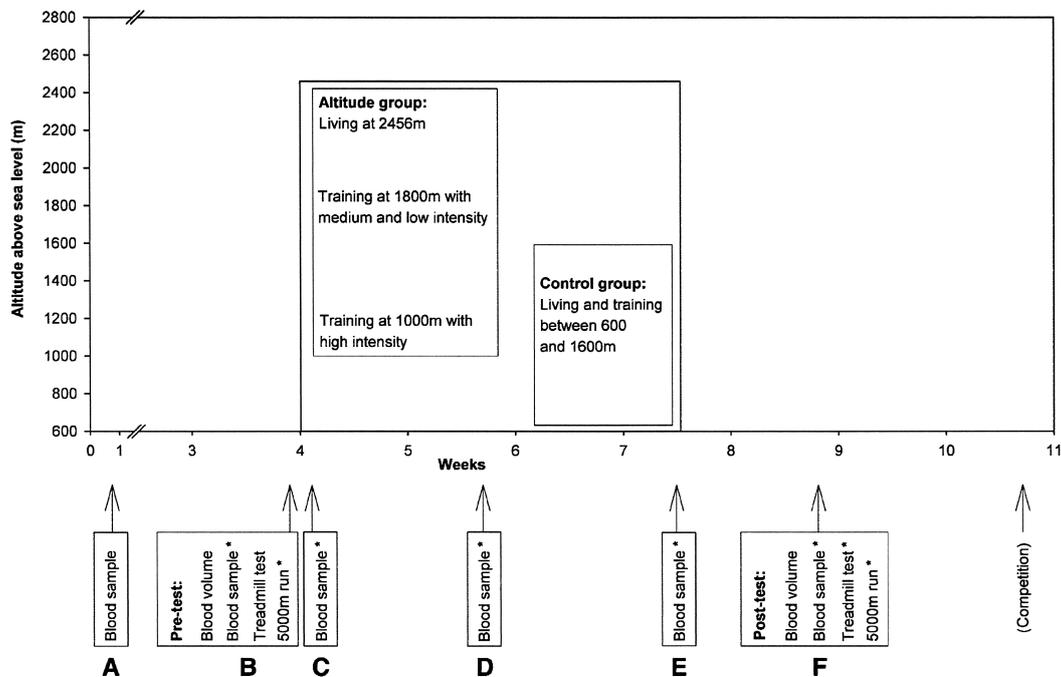
Fig. 1. Study design. A–F refer to the text. *Measurements in the altitude group only.

Evaluation of Blood Volume Parameters

Hb$_{mass}$, RCV, plasma volume (PV), and blood volume (BV) were determined by the CO-rebreathing method according to Burge and Skinner (6) with minor modifications (20). The method is briefly described here. After 15 min in a sitting position, four capillary blood samples (30 μl) were taken from an earlobe and analyzed for carboxyhemoglobin (HbCO) by a hemoximeter (ABL 520, Radiometer A/S, Copenhagen, Denmark). The mean of the four HbCO values was taken as the baseline HbCO value. Subjects were then connected to a Krogh spirometer filled with a mixture of oxygen (5 liters) and CO. The volume of inspired CO varied between 50 and 100 ml depending on gender, barometric pressure, measured $\dot{V}O_2$ max, and body mass with the goal of reaching a ΔHbCO (difference between baseline values and plateau values) between 5 and 7%. The athletes breathed the gas mixture in the closed system for 12 min. If necessary, oxygen was refilled. Every 2 min, earlobe blood samples were taken for assessment of HbCO. All blood samples for measurement of HbCO were immediately analyzed (<10 s). The HbCO plateau was normally seen after 6–10 min and the mean of three adjacent HbCO values between the 6th and the 12th minute was taken as the plateau value of HbCO. Hb$_{mass}$, RCV, BV, and PV were then calculated as described elsewhere (20). For the RCV, PV, and BV calculations, the hemoglobin (Hb) and hematocrit (Hct) values from a venous blood sample taken on the same day were used. The same equipment was used by the same investigators for all tests. The reproducibility of the blood volume parameters (coefficient of variance; CV) was determined in a separate experiment, in which Hb$_{mass}$, RCV, PV, and BV were measured in other subjects two times separated by 24 h. We did this twice, once during the LHTL phase of the AG ($n = 11$) and once during the experimental phase of the CG ($n = 7$). The mean measured

CV during the AG and CG were Hb$_{mass}$ = 1.7 and 1.4%, RCV = 2.2 and 2.1%, PV = 4.8 and 4.5%, BV = 3.4 and 3.5%, respectively.

Blood Sample

All blood samples were drawn from a cubital vein under standardized conditions (between 7:00 and 8:00 AM before breakfast, in supine position after 15 min of rest). Blood samples from both groups were analyzed for Hb concentration (modified cyanomethemoglobin method, Coulter Gen S, Beckmann), Hct (Coulter Gen S, Beckmann), and serum Ftn (photochemiluminescence; Advia Centaur, Bayer, Leverkusen, Germany). Blood samples from the AG were also analyzed for serum erythropoietin (sEpo; chemiluminescence immunoassay; Advantage, Nichols Institute Diagnostics, San Juan Capistrano, CA), reticulocytes (Rct; flow cytometry; Epics XL, Beckmann), transferrin (TF; immunoassay; Cobas Integra 800; Roche Diagnostics, Basel, Switzerland), and soluble transferrin receptor (sTfR; immunoturbidimetric assay; Cobas Integra 800; Roche Diagnostics). At the pretest, all blood samples were analyzed twice and the CV for the different parameters was calculated: Hb = 0.3%, Hct = 0.6%, Ftn = 4.5%, sEpo = 9.8%, Rct = 6.7%, TF = 0.64% and sTfR = 2.0%.

Evaluation of Performance

5,000-m time trial (AG). The pre- and posttest 5,000-m time trials were conducted on a 400-m track at 400 m above sea level at 7 PM under similar conditions (temperature 19.8 and 20.3°C, humidity 79 and 67%; air pressure 969 and 972 hPa, for pre- and posttest, respectively). Heart rate was monitored throughout the run, and rating of perceived exertion (RPE) was recorded immediately after the run by using the category scale of Borg (5). A capillary blood sample was taken from the earlobe to measure blood lactate concentration.

$\dot{V}_{O_2\ max}$ *tests.* The AG performed $\dot{V}_{O_2\ max}$ tests at pre- and posttest to determine $\dot{V}_{O_2\ max}$, maximal ventilation, maximal heart rate (HR_{max}), maximal blood lactate ($[La^-]_{b-max}$), and time to exhaustion (TTE). These tests were conducted on a treadmill in the laboratory at the Swiss Olympic Medical Center (SOMC) in Magglingen, located 900 m above sea level. After a 10-min warm-up jog, the athletes began running at their individual anaerobic threshold intensity (previously determined). The speed was increased by 1 km/h every minute until the subjects reported having ~90 s left until exhaustion. The treadmill incline was set at 0% throughout the test. Identical "individual" tests were used for the pre- and the posttest. Gas exchange was measured breath by breath with an open-circuit system (Oxycon Pro, Jaeger-Toennies, Hochberg, Germany), heart rate was monitored with Polar Accurex plus (Polar Electro, Kempele, Finland), and blood lactate was analyzed with Ebbio-Plus (Eppendorf, Germany). The CG performed a $\dot{V}_{O_2\ max}$ test at pretest only, at the SOMC Bad Ragaz located 400 m above sea level, using identical equipment, but another protocol: After a warm-up jog, the male athletes began running at 13 km/h. The speed was increased by 1 km/h every minute for the first 3 min of the test and thereafter by 0.5 km/h every 30 s until exhaustion. The female athletes followed the same protocol but started at 11 km/h. The treadmill incline was set at 7% throughout the test. During the $\dot{V}_{O_2\ max}$ tests, both athletes and experimenters were blinded for any result.

Training regimen. The AG completed low- and moderate-intensity training at an altitude of 1,800 m (1–2 training sessions per day), whereas the high-intensity training was performed at 1,000 m above sea level (twice per week). The CG completed all training (1–2 training sessions per day) at altitudes between 400 and 1,600 m. For both groups, ~85% of the training completed was at low and moderate intensity and 15% was at high intensity.

Supplementation (AG). The AG athletes started a combined iron (Ferrum Hausmann, 100 mg Fe^{2+}/day orally; Astellas Pharma, Leiderdorp, Netherlands), multivitamin (Burgerstein multivitamin-mineral ABC 25; Burgerstein Nährstoffe; Rapperswil, Switzerland), and vitamin C (Burgerstein vitamin C; Burgerstein Nährstoffe) supplementation when the LHTL phase began. Despite preliminary testing and oral supplementation, two female and one male athlete had Ftn levels below 20 µg/l at the start of the LHTL phase. The low Ftn values must be seen in the light of high PV, and we assume that these athletes actually had no relevant iron deficiency under normal sea-level training conditions. Because we wanted to be on the safe side for the novel circumstances at altitude, these athletes received venous iron supplementation (Venofer; Novartis, Basel, Switzerland) in the first week. The iron status results of these three subjects were therefore excluded from the data. The changes in Hb_{mass} and performance values during LHTL in these subjects did not differ from those observed in the other athletes. We therefore did not exclude other data from these three athletes.

Statistics

Data are presented as means ± SD in tables and as means ± SE in figures. The effect of time on several blood parameters measured before, during, and after the LHTL phase was evaluated with one-factor analysis of variance for repeated measures. When the F value was considered statistically significant ($P < 0.05$), the Bonferroni correction was used to evaluate differences at the different time points in relation to the pretest value at sea level. Differences between pre- and posttest within the groups were evaluated by paired Student's t-tests, and differences between the two groups were analyzed by comparing the absolute group differences between pre- and posttest with unpaired t-tests. The relationship between increase in Hb_{mass} and the increase in $\dot{V}_{O_2\ max}$ was compared by linear regression and Pearson's coefficient. All statistical tests were done with the SPSS statistical package 13.0 (SPSS, Chicago, IL). Significance was set at $P < 0.05$; $P < 0.1$ was called a trend.

RESULTS

There was no difference in height, weight, body-mass index, Hb_{mass}, RCV, PV, and BV between the groups, but the cross-country skiers (CG) had higher $\dot{V}_{O_2\ max}$ values than the orienteers (AG) (Table 1 and Fig. 2).

Blood Volume Parameters

Hb_{mass} increased by 5.3% and RCV increased by 5% from pre- to posttest ($P < 0.01$) in the AG, whereas there was no change in Hb_{mass} or RCV in the CG (Table 2 in absolute values and Fig. 2 in individual body weight-adjusted values). The changes in Hb_{mass} and RCV were different between the groups ($P < 0.01$). Neither BV nor PV changed for either group.

Blood Samples

The time course of Hct, sEpo, Rct, Ftn, TF, and sTfR are presented in Fig. 3. Hct increased during the LHTL phase ($P < 0.01$), and post hoc analysis indicated elevated values at *day 24* ($P < 0.05$), but values returned to pretest level at posttest. sEpo was affected by the LHTL phase ($P < 0.001$), and post hoc analysis showed higher sEpo values at *day 1* ($P < 0.001$; +120%) and *day 12* ($P < 0.05$; +34%) than at pretest, whereas the values at *day 24* and the posttest were not different from the pretest values. Rct values were affected by the LHTL phase ($P < 0.001$), and post hoc analysis reported higher values at posttest (17.5 ± 4.2‰; $P < 0.05$) than at pretest (12.2 ± 2.9‰). Ftn decreased ($P < 0.05$), TF increased ($P < 0.001$), and sTfR increased ($P < 0.05$) during the LHTL phase. For the CG, Hct (43.9 ± 3.7 vs. 42.7 ± 3.5%), Hb (15.7 ± 1.2 vs. 15.5 ± 1.1 g/dl), and Ftn (65 ± 17 vs. 62 ± 19 µg/l) did not change during the experimental period (pre- and posttest values, respectively).

Table 1. *Anthropometric data and maximal oxygen uptake of the altitude group and the control group*

	Altitude Group				Control Group			
	Height, cm	Weight, kg	BMI, kg/m^2	$\dot{V}_{O_2max}$, ml·kg^{-1}·min^{-1}	Height, cm	Weight, kg	BMI, kg/m^2	$\dot{V}_{O_2max}$, ml·kg^{-1}·min^{-1}
Men	179±5	69±4	21.5±0.8	62±3	181±56	74±3	22.7±0.4	73±2*
Women	168±5	55±2	19.6±0.6	51±2	169±2	59±4	20.5±0.7	66±6*
All	174±8	62±8	20.5±0.6	57±7	175±7	66±8	21.6±1.4	70±6†

Values are means ± SD. The altitude group consisted of 5 female and 5 male national team orienteers, and the control group consisted of 4 female and 3 male national team cross-country skiers. BMI, body mass index; $\dot{V}_{O_2max}$, maximal oxygen uptake. *$P < 0.05$ and †$P < 0.01$, differences between the groups.

Performance Parameters (AG Only)

$\dot{V}_{O_2\ max}$ *test.* $\dot{V}_{O_2\ max}$ increased by 4.1% from pre- to posttest (women: 50.8 ± 2.1 vs. 54.5 ± 2.8 ml·kg^{-1}·min^{-1}; men: 62.3 ± 5.2 vs. 63.8 ± 5.5 ml·kg^{-1}·min^{-1}; $P < 0.05$, women

and men together). TTE increased by 41 s ($P < 0.05$), HR$_{max}$ decreased by 3 beats/min ($P < 0.05$) whereas [La$^-$]$_{b\text{-max}}$ did not change (Table 3). Maximal ventilation increased from 129 ± 33 to 133 ± 32 l/min; ($P < 0.05$) during the LHTL phase. Spearman's correlation coefficient for the change in
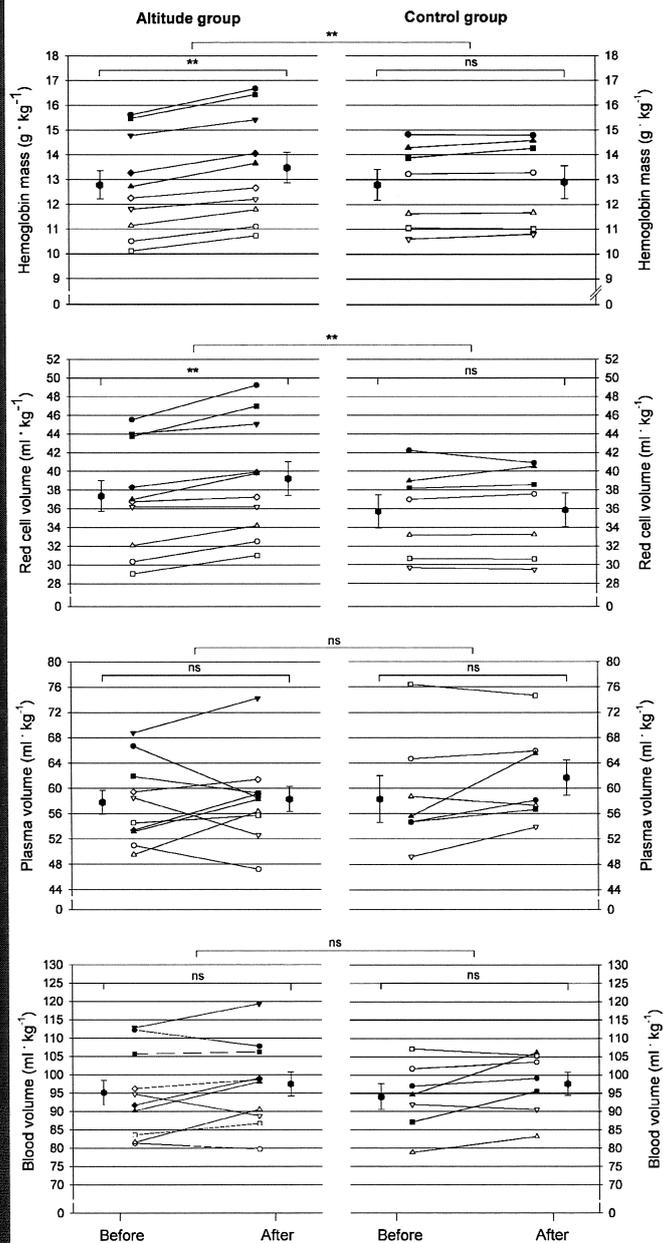
Fig. 2. Effects of 24 days of either "live high-train low" altitude training (altitude group) or normal "sea-level" training (control group) on body mass-related hemoglobin mass, red cell volume, plasma volume, and blood volume. Open symbols represent female athletes, solid symbols male athletes, and hexagon represents mean values. **Differences ($P < 0.01$) before and after the altitude training camp for 1 group (women and men together) or differences between the groups; ns, no difference.

Table 2. *Blood volume parameters measured before and after the 24-day training period in the altitude group and the control group*

	Altitude Group				Control Group			
	Hb_{mass}, g	RCV, ml	PV, ml	BV, ml	Hb_{mass}, g	RCV, ml	PV, ml	BV, ml
Pre	805±210	2,353±611	3,616±771	5,969±1,367	849±197	2,373±536	3,620±351	5,993±854
Post	849±226*	2,470±65*	3,653±834	6,123±1,434	858±205	2,387±551	3,795±587	6,182±1,119

Values are means ± SD. The attitude group consisted of 5 female and 5 male national team orienteers; the control group was 4 female and 3 male national team cross-country skiers. Pre, before training; Post, after training; Hb_{mass}, hemoglobin mass; RCV, red cell volume; PV, plasma volume; BV, blood volume BV. *$P < 0.01$, differences between pre- and posttest.
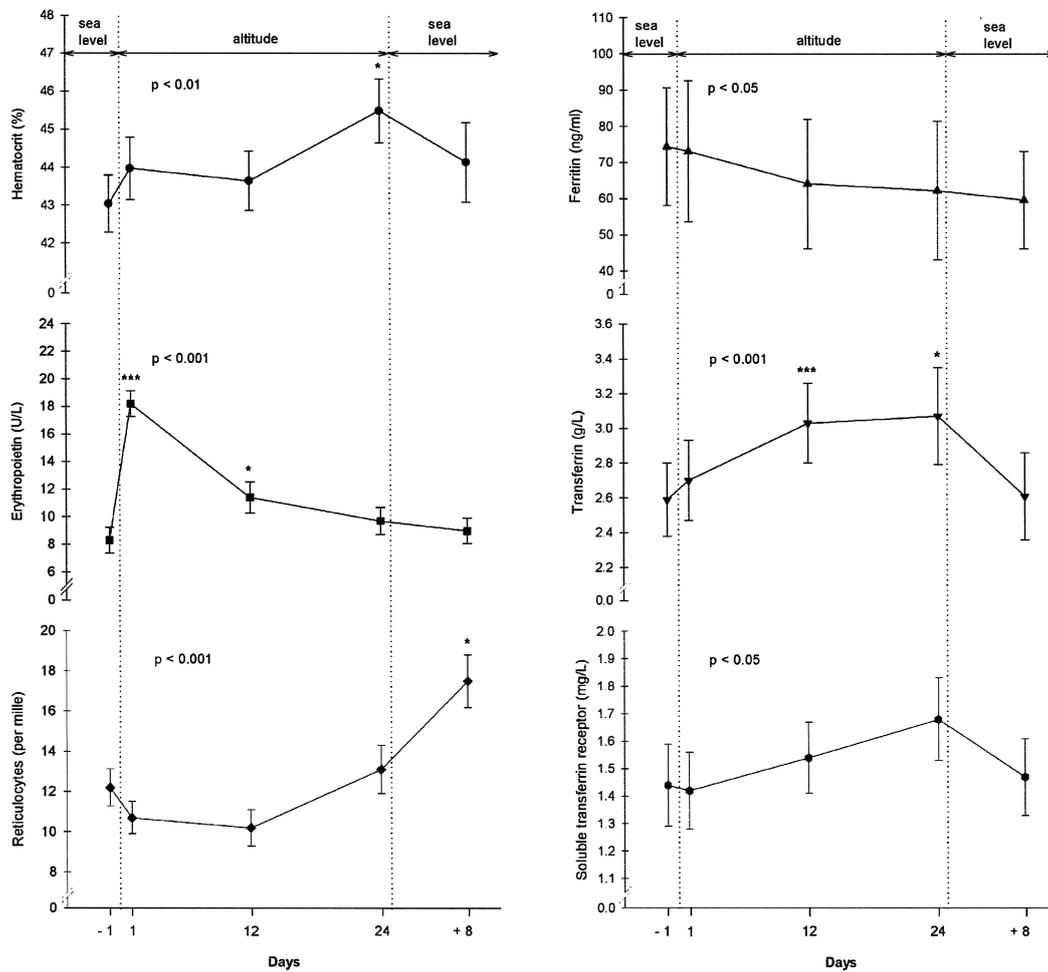
Fig. 3. Time course of hematocrit, erythropoietin, reticulocytes, ferritin, transferrin, and the soluble transferrin receptor in the altitude group (5 female and 5 male national team orienteers) measured before and after (*day −1* and *day +8*) and during a "live high" (2,456 m)-"train low" (1,800 and 1,000 m) altitude training camp (*day 1, day 12,* and *day 24*). Data are means ± SE. *P* value indicates the effect of time on the parameter. *$P < 0.05$ and ***$P < 0.001$, post hoc differences from sea-level conditions (*day −1*).

Hb_{mass} (ΔHb_{mass}, in %) and the change in $\dot{V}_{O_2\,max}$ ($\Delta\dot{V}_{O_2\,max}$, in %) were $r = 0.68$ ($P = 0.21$) for the men, $r = 0.75$ ($P = 0.15$) for the women, and $r = 0.35$ ($P = 0.29$) for men and women together.

5,000-m time trials. The athletes improved 5,000-m running time by ~18 s (1.6%; $P < 0.05$), with no difference in HR_{max}, $[La^-]_{b\text{-}max}$ (Table 3), or RPE (18.7 ± 0.7 vs. 19.0 ± 1.0).

DISCUSSION

The main results of the present study show that Hb_{mass} and RCV increased by ~5% after living at 2,456 m while training at lower altitudes (1,800 and 1,000 m) for 24 days. There was no change in Hb_{mass} and RCV in a control group living and training at altitudes between 500 and 1,600 m for 24 days. The improvements in Hb_{mass} and RCV in the AG were associated with increased sEpo, Rct, TF, sTfR, and decreased Ftn values as well as improved $\dot{V}_{O_2\,max}$ and 5,000-m running times.

Limitations of the Study

Any research conducted with elite athletes will encounter the challenge to have an appropriate control group, ideally with a randomized design. To have a sufficient number of elite endurance athletes for one altitude group and one control group, we recruited national team member athletes of two different endurance disciplines. It was not possible to randomly assign athletes to either altitude or normal training, because the athletes and coaches in each discipline preferred to train together. Thus the allocation to the AG or CG was based on the specific endurance discipline. This nonrandomized classification raises the question of whether the athletes in both groups have similar endurance characteristics. Indeed, the cross-country skiers had higher $\dot{V}_{O_2\,max}$ values than the orienteers, but their $\dot{V}_{O_2\,max}$ test was conducted at a lower altitude, which may partly explain the higher results (35). However, both groups consisted of elite athletes who have trained seriously for many years, suggesting that the differences in aerobic capacity were more a genetic predisposition than a difference in training status. Importantly, both Hb_{mass} and RCV were not different between the groups at pretest. We did not perform specific doping tests during the study, but it must be noted that Switzerland has one of the toughest anti-doping programs, which includes numerous unannounced doping controls. In addition, the reasonable increases in Hb_{mass} and RCV as well as the small variation between the subjects do not support the suspicion of blood doping during this study. It was not possible to measure sEpo, Ret, TF, and sTfR in the CG. However, these parameters have been measured in previous controlled studies with a sea-level control group (1, 8), so our results may be compared with these. With the above considerations, we feel that the design adaptations made in this study to evaluate the effect of LHTL training on Hb_{mass} and RCV did not compromise the validity of the results.

Increased Hb_{mass} and RCV After the LHTL

The influence of 3- to 4-wk altitude exposure at 2,100–2,500 m on Hb_{mass} and RCV in endurance athletes is controversial (2, 16, 23, 25, 27) and has recently been part of a point (25)-counterpoint (16) debate in this journal. Within this debate, it is important to differentiate between a number of methodological distinctions, such as different altitudes chosen for living and training, different methods of measuring changes in Hb_{mass} or RCV (16), and the different performance levels of the athletes. In the classical well-controlled LHTL study conducted by Levine and Stray-Gundersen (24), RCV increased by ~5% in the LHTL group after a 4-wk period of living at 2,500 m and training at 1,250 m. These results have been debated (2, 17), because they measured RCV indirectly with the Evans blue dye method, for which the adequacy for estimating RCV after hypoxic exposure has been questioned (16, 27). Studies using the CO-rebreathing method to directly measure Hb_{mass} have shown contradictory results. Most of these studies failed to show increased Hb_{mass} and RCV after altitude training (either LHTL or LHTH) (2, 3, 8, 15, 34). However, as we previously pointed out, it seems obvious that the hypoxic dose is a key factor in this debate. There is no doubt about elevated Hb_{mass} in lifelong residents (19) of moderate altitude (2,600–3,550 m), including athletes (30). It has therefore been suggested that the hypoxic dose in these studies (2, 3, 8, 15, 34) was too low to significantly increase Hb_{mass} and RCV (23, 28). It is likely that either the living altitudes were too low (8, 15, 34) and/or the durations of altitude exposure were too short (2, 3), compared with regimens that increased Hb_{mass} or RCV after LHTL or LHTH (28). The hypoxic dose used in our study was very similar to the one used in the LHTL study conducted by Levine and Stray-Gundersen (24), as the athletes lived at the same altitude (2,456 m in our study, 2,500 m in the Levine and Stray-Gundersen study) for a similar duration (24 vs. 28 days) and trained at a similar altitude (1,800 and 1,000 m vs. 1,250 m). RCV results were also very similar, with RCV increasing by 5% and Hb_{mass} by 5.3% in our study and RCV increasing by 5.3% in the Levine and Stray-Gundersen study. In addition, the increases in Hb_{mass} and RCV in our study related well (21) to the measurement reproducibility (the increase was 3.1 times higher than the CV for the Hb_{mass} and 2.3 times higher than the CV for RCV). To our knowledge, no other study has been published that used the LHTL protocol at real altitude and found an increased Hb_{mass} and RCV. Two recently published studies that used the LHTH protocol also found increased

Table 3. $\dot{V}_{O_2max}$ *test and 5,000-m time trial results measured before and after the 24-day "live high-train low" altitude training camp in the altitude group*

	$\dot{V}_{O_2max}$ Test				5,000-m Time Trial		
	$\dot{V}_{O_2max}$, ml/min	TTE, s	HR_{max}, beats/min	$[La^-]_{b\text{-}max}$, mM	Time, s	HR_{max}, beats/min	$[La^-]_{b\text{-}max}$, mM
Pre	3,515±837	355±57	189±10	6.6±1.3	1,099±104	190±10	5.9±1.5
Post	3,660±770*	396±60†	186±8*	7.0±1.5	1,081±98†	190±10	6.3±1.4

Values are means ± SD. TTE, time to exhaustion; HR_{max}, maximal heart rate; $[La^-]_{b\text{-}max}$, maximal blood lactate; Time, 5,000-m time trial time. *$P < 0.05$ and †$P < 0.01$, differences between pre- and posttest.

Hb_{mass} in endurance athletes after a 3-wk altitude training camp at real altitude (9, 18). Hb_{mass} increased 6% after 3 wk of LHTH at 2,100–2,300 m in junior swimmers (9) and 9% in elite biathlon athletes living and training for 3 wk at 2,050 m (18). The higher increase in Hb_{mass} in the biathlete study was mainly due to the result of one athlete, whose Hb_{mass} increased by 18%, whereas Hb_{mass} increased by 7% for the rest of the group (31). Unfortunately, neither LHTH study included a control group, reported the reproducibility of Hb_{mass} measurement, or controlled for blood doping (33). In addition, they used a relatively small amount of CO in the CO-rebreathing method in relation to the barometric pressure and the estimated magnitude of the athletes' Hb_{mass}, which may lead to a high measurement error (6). There is only one study (LHTH protocol, no study with LHTL protocol) that used an estimated adequate hypoxic dose and found no increase in Hb_{mass}: Gore et al. (14) reported no increase in absolute Hb_{mass} after 31 days LHTH at 2,690 m. However, the authors pointed out that all athletes in the study succumbed to illness during the period, a condition that can have depressive effects on the erythropoiesis (11).

Changes in Blood and Iron Parameters After LHTL

The increases in Hb_{mass} and RCV in this study were in line with changes in several iron and blood parameters during the LHTL phase. sEpo was elevated 120% at *day 1* at altitude compared with the pretest. Such an increase has also been seen in other studies, in which sEpo increased, with considerable individual variation, from sea level to ~2,500 m by 50–150%, measured after ~24 h of altitude exposure (1, 8, 12, 24). The increase in sEpo in the AG in our study was even higher than the 60% reported in the study by Chapman et al. (7) after 30-h altitude exposure for the "responder" group of their athletes. Our results are also supported by Ge et al. (12), who measured the sEpo responses at different altitudes and concluded that the threshold altitude for a robust increase in sEpo is ~2,100–2,500 m. In our study, sEpo was still elevated at *day 12* at altitude, which has been determined to be an important factor for a relevant increase in RCV (7). However, an increased sEpo is not necessarily associated with an increase in Rct and Hb_{mass}. Ashenden et al. (1) showed no different changes in Rct between an altitude group with three 5-day LHTL exposures at 2,650 m in an altitude house and a sea-level control group. It must be noted that Rct is affected not only by altitude exposure but also by normal endurance training (29). The absolute changes in Rct in controlled altitude training studies that did not show a change in Hb_{mass} were within 2–4‰ in the sea-level control groups (2, 3, 8) during controlled periods as long as 30 (2) and 70 (3) days. Thus an absolute change in the reticulocytes of ~2–4‰ reflect normal changes due to training. In our study, the mean absolute change in Rct was within 3‰ during the first 12 days of the LHTL phase but was increased by 7‰ (from 10.2 to 17.5‰) from *day 12* to the posttest. It is interesting that this increase occurred at the end of and even after the LHTL phase. We do not know how much of this increase in Rct is due to the altitude-induced increase in erythropoiesis and how much can be attributed to changes in training intensity. However, these results may suggest the importance of spending a sufficient amount of time at altitude. TF and sTfR also increased in the AG, whereas Ftn values

decreased, even if the absolute changes were smaller than in the study by Levine and Stray-Gundersen (24). Such changes in iron metabolism have been interpreted to occur with increased erythropoiesis (4) and therefore support our findings of increased Hb_{mass} and RCV.

Performance Parameters

Because the aim of our study was primarily to investigate the effect of the LHTL phase on hematological parameters, performance parameters were only measured in the AG and we do not know what performance changes may have taken place in the CG. Therefore, the improvement in performance in the AG should be interpreted with caution, because it could have been influenced by training and their own expectation of improved performance after the LHTL camp. Changes in the AG performance are reported, however, because it cannot be taken for granted that our athletes improved performance after 4 wk of LHTL training only. Both $\dot{V}O_{2\,max}$ (+4.1%) and TTE (+11.6%) were increased after the LHTL phase. The almost identical $[La^-]_{b\text{-max}}$ values at the pre- and posttest support that the athletes ran with a similar volitional exhaustion in the tests. The 5,000-m time trial performance improved (−1.6%), and measurement of HR_{max}, $[La^-]_{b\text{-max}}$, and RPE indicated that volitional exhaustion was similar for both trials. Considering the small number of subjects, the correlation between the increase in Hb_{mass} and the increase in $\dot{V}O_{2\,max}$ must be put into perspective. However, the decrease in 5,000-m time and the increase in $\dot{V}O_{2\,max}$ were very similar to those of Levine and Stray-Gundersen (24, 32), and it is known that increased Hb_{mass} and RCV is associated with increased endurance performance (22). Therefore, the improved performance parameters may be supported by the increases in the hematological parameters.

We conclude that Hb_{mass} and RCV in elite endurance athletes are increased by ~5% after living at 2,500 m and training at 1,800 and 1,000 m for 24 days.

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Paper III

Jon Peter Wehrlin, Bernard Marti

Live high-train low associated with increased haemogobin mass as preparation for the

2003 World Championships in two native European world-class runners

Live high-train low associated with increased haemoglobin mass as preparation for the 2003 World Championships in two native European world class runners

J P Wehrlin, B Marti

Background: It is unclear whether world class endurance athletes, in contrast with less well trained subjects, increase their haemoglobin mass on a regimen of living high and training low (LHTL).

Objective: To assess whether haemoglobin mass increases in world class athletes on LHTL and whether this increase is associated with peak performance at a subsequent important competition.

Methods: Two Swiss world class runners (one 5000 m and one marathon) lived for 26 days (18 hours a day) at an altitude of 2456 m and trained at 1800 m. This LHTL camp was the preparation for the World Athletic Championships taking place 27–29 days after the end of the camp. Haemoglobin mass and other haematological variables were measured before and after the LHTL camp. The performance parameter was the race times during that period.

Results: Haemoglobin mass increased by 3.9% and 7.6%, and erythrocyte volume by 5.8% and 6.3%. The race times, as well as the ranking at the World Championships, indicated clearly improved performance after the LHTL camp.

Conclusions: The results suggest that LHTL with an adequate dose of hypoxia can increase haemoglobin mass even in world class athletes, which may translate into improved performance at important competitions at sea level.

I n the last few years, the concept of living high and training low (LHTL) has become very popular with endurance athletes. By living at altitude, the athlete should theoretically benefit from an increased haemoglobin mass (Hb_{mass}) and erythrocyte volume (EV), whereas training at low altitude should minimise the disadvantage of reduced absolute training intensity achieved when training at moderate altitude.[1] Together, this should lead to an improved performance at sea level.[2] Apparently at variance with this concept, several studies have shown unchanged Hb_{mass} and/or EV after LHTL or living high and training high,[3–10] but critical inspection of their data indicates that either the selected altitude for "living high" was probably[11] too low,[6 8–10] and/or the duration was too short.[3–5] Rusko *et al*[12] indicated that the hypoxic stimulus to increase Hb_{mass} and EV should be least 400 hours at 2300–2500 m. Several studies[2 13–15] have shown increased Hb_{mass} or EV after LHTL with this stimulus. However, the athletes in these studies were not reported to be world class. In the only study with world class athletes[7] and an appropriate hypoxic stimulus, Hb_{mass} and EV failed to increase. Thus it is not clear whether it is possible to increase further an already high Hb_{mass} in world class athletes. There is a lack of documented observations of world class athletes who successfully increased Hb_{mass} (or EV), especially when preparing for an important competition. The aim of our "double case" study was therefore to measure Hb_{mass} and performance before and after a LHTL camp, with a presumably high enough hypoxic stimulus, in the immediate preparations for the World Athletic Championships (Paris 2003) in two world class Swiss runners.

METHODS

The best Swiss 5000 m runner (personal best (PB) = 13:36:54) and the best Swiss marathon runner (PB = 2:10:56) participated in this study. Both are native Swiss citizens, were 29 years old at time of the study, and had participated in the European Championships in 2002. The subjects gave their written informed consent to the study, which was approved by the institutional review board of the Swiss Federal Office of Sport. The two runners had participated since winter 2002 in the project "Doping-free top-class sport", conducted by the Swiss Federal Institute of Sports and the WADA accredited Swiss laboratory of anti-doping. Besides a public written anti-doping statement from the 20 best Swiss athletes in endurance disciplines, this project included seven additional unannounced doping tests per year (in which these athletes among others were checked for erythropoietin abuse). The two iron supplemented athletes had no injuries or ill health during the study period. They lived for 26 days (~18 hours a day) at a natural altitude of 2456 m and trained at an altitude of about 1800 m. The 5000 m and marathon competitions at the World Championships were due to take place 27–29 days after the end of the LHTL camp. One week before and one week after the LHTL camp, Hb_{mass}, EV, plasma volume, and blood volume were determined by the CO rebreathing method described by Burge and Skinner[16] with minor modifications.[17] Carboxyhaemoglobin (COHb) was determined with an ABL 520 (Radiometer, Copenhagen, Denmark). The amount of CO during the rebreathing period was 80 ml in both subjects, leading to a ΔCOHb of 5–6%. In a separate study, we determined the accuracy of our method by performing two tests separated by 24 hours (n = 12). Coefficients of variation of 1.6% (Hb_{mass}), 2.2% (EV), 4.1% (plasma volume), and 3.1% (blood volume) were obtained. For the measurement of packed cell volume and haemoglobin concentration, a whole blood sample (2.7 ml) was drawn from the antecubital vein after the subject had lain supine for 15 minutes. The blood was immediately analysed with a haematology analyser (CELL-DYN 3200, Abbott Laboratories, Abbott Park, Illinois, USA). The performances of the two world class athletes were evaluated from their competition results.

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Abbreviations: EV, erythrocyte volume; Hb_{mass}, haemoglobin mass; LHTL, live high-train low; PB, personal best

Table 1 Haematological variables before and after 26 days of living high and training low in two world class runners

	5000 m runner			Marathon runner		
	Before	After	% change	Before	After	% change
Packed cell volume	0.388	0.386	−0.5	0.427	0.438	+2.5
Haemoglobin (g/l)	132	133	−0.8	156	157	+0.6
Haemoglobin mass (g)	878	945	+7.6	952	988	+3.9
Erythrocyte volume (ml)	2581	2742	+6.3	2605	2757	+5.8
Plasma volume (ml)	4728	5064	+5.8	4099	4160	+3.9
Blood volume (ml)	7309	7807	+6.8	6704	6917	+3.2

RESULTS

The absolute blood values (Hb_{mass}, EV, plasma and blood volume) had increased after the LHTL camp, whereas there was no change in packed cell volume and haemoglobin concentration (table 1).

Figure 1 presents the results of the marathon and 5000 m competitions before and after the LHTL camp. Both the marathon runner (14th place) and the 5000 m runner (13th place, best European runner) achieved their best individual results at an important championship.

DISCUSSION
Blood variables

The increase in Hb_{mass} (+7.6% and +3.9%) and EV (+6.3% and +5.8%) in relation to the reproducibility of the measurement (the increase was about 3–4 times the typical error of measurement for both Hb_{mass} and EV), the fact that the magnitude of these changes was in line with previous findings,[2] the fact that the use of erythropoietin was unlikely, and the fact that there were no changes in the packed cell volume and haemoglobin concentration lead us to the interpretation that there was a real change in Hb_{mass} and EV. Gore et al[7] questioned whether their failure to increase Hb_{mass} in world class cyclists after 31 days of living and

training at 2690 m could be due to the Hb_{mass} (14.3 g/kg) already being near to the natural physiological limits. In our study, the 5000 m runner started with a Hb_{mass} of 14.3 g/kg and increased it to 15.4 g/kg. The relative Hb_{mass} of the marathon runner increased from 15.7 to 16.3 g/kg. These high values are in line with other world class athletes[17] measured with the same technique. Interestingly, the high Hb_{mass} (and EV) of the 5000 m runner was associated with a very low packed cell volume (below 0.4), which is also explained by the high plasma volume.

Performance

The 5000 m runner ran 40 seconds faster (13:12:69) on the first day after the LHTL camp than on the first day (Swiss Championships) before the LHTL camp. Even related to his PB (13:36:54), he had improved by about 24 seconds. Also in the final of the World Championships, he ran in a time of 13:26:06, about 10 seconds faster than his previous PB. When comparing the marathon times, one has to take into account that the course in Paris had several inclines and was run at a somewhat irregular pace, which was not conducive to PB times. With this in mind, the marathon time reached in Paris (2:11:14) could be interpreted as an improvement on the performance achieved three months before (2:11:05) in a race that was also prepared for with altitude training. The absence
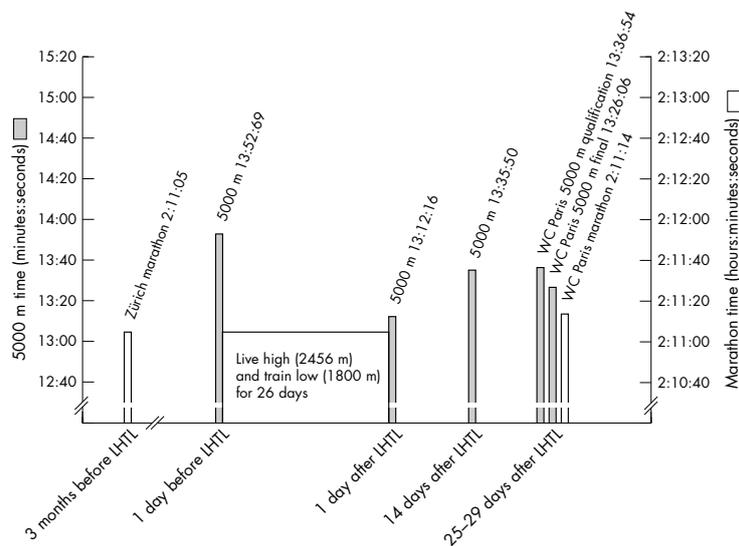

Figure 1 Competition results of one marathon runner and one 5000 m runner before and after 26 days of living high and training low (LHTL) in preparation for the World Championships (Paris 2003). WC, World Championships.

of a control group and the anecdotal nature of the report are weaknesses of the study. This is mainly due to the limited number of world class runners available. However, it can be argued that it is very difficult to substantially improve the performance of world class athletes, especially when they are already approaching 30 years of age. In addition, our performance observations agree with previous findings in a well controlled study of elite runners.[18] Taken together, this seems to be one of the first documented observations of increased Hb_{mass} and EV after an LHTL camp, followed by a successful endurance performance at an important championship of native European world class endurance athletes approaching their thirties.

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. . . . **COMMENTARY** . . . .

The absence of a control group in this study does not allow the conclusion that the measured effects are purely the result of living high and training low. In addition, in all similar studies it is difficult to be certain that extraneous factors such as the covert use of erythropoietin (EPO) by the athletes was also not a factor contributing to their increased exercise performance. However, this would seem unlikely in this case as elite Swiss athletes are exposed to one of the toughest anti-doping controls in the world. In addition, the athletes did not show an increase in packed cell volume—an expected effect of EPO use. One strength of the study is that the athletes' performances were measured in real competition so that they probably tried equally hard in the performance tests before and after training. An obvious criticism of similar studies is: how do you ensure that athletes perform maximally in both the performance trials, before and after an intervention? For the subconscious tendency would surely be to try less hard before the intervention and harder after the intervention. Hence the need for adequate control groups. The balance of probability is that the large measured performance effects were indeed the result of training low and living high, although an effect simply of training in a novel environment cannot be totally excluded. The study invites similar well conducted studies that include an appropriate control group. It is appreciated that such studies are probably prohibitively expensive so that we are left to interpret findings from the less definitive uncontrolled trials.

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Paper IV

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Effect of repeated altitude training during a five month training period on hemoglobin mass, red cell volume and VO$_{2max}$ in elite cross country skiers