Dissertation

Influence of Exercise on Circulating Human Hematopoietic Stem and Progenitor Cells

submitted by

Mag.rer.nat. BSc

Julia Maria KRÖPFL

For the Academic Degree of

Doktorin der medizinischen Wissenschaft

(Dr. scient. med.)

at the

Medical University of Graz

Institute of Biophysics

under supervision of

Univ.-Prof. Mag. Dr. Wolfram MÜLLER

O.Univ.-Prof. Dr.med.univ. Gottfried DOHR

Assoz. Prof. Priv.-Doz. Mag. Dr.rer.nat. Sandra WALLNER-LIEBMANN

2013

Summary (English)

Objective: Adult human hematopoietic stem and progenitor cells (HSPCs) mobilized from the bone marrow into the peripheral blood show great potential for regeneration over the whole lifetime of a human. This thesis deals with the influence of physical exercise at both normoxia and normobaric hypoxia on the number and functionality of adult circulating hematopoietic progenitor cells (CPCs) which represent the part of HSPCs defined as *CD34+/CD45dim side scatter low* in the adult human blood circulation system. Two studies were designed to investigate CPC kinetics and functionality in the peripheral blood after defined exercise test protocols as well as the involvement of different exercise-induced blood parameters as possibly influencing agents. **Methods:** In study design I, ten healthy male subjects (25.3 ± 4.4 yrs) underwent a standardized cycle incremental exercise test protocol (40 W + 20 W/min) under either normoxic (FiO_2 ~ 0.21) or hypoxic conditions (FiO_2 < 0.15, equals 3,500 m, 3 h exposure). Blood was drawn from the cubital vein before and 10, 30, 60 and 120 min after exercise. Study design II involved the testing of seven patients (63.4 ± 7.0 yrs) undergoing cardiac rehabilitation. All subjects performed 2-3 different exercise tests (randomly chosen out of four) on a cycle ergometer; each exercise test was expected to trigger a different blood lactate concentration. Venous blood was drawn from the cubital vein before and immediately after each intervention. **Results:** Data of study I showed a significant increase of CPC release under normoxic as well as hypoxic conditions after 10 min of recovery. Most interestingly, although CD34+/CD45dim cells increased in number, the proliferative capacity/functionality of CPCs decreased significantly 10 min after cessation of exercise. Blood parameters of oxidative stress and cortisol levels significantly correlated with CPC count. The pro-inflammatory cytokine interleukin-6 as well as norepinephrine showed a significant increase after cessation of exercise. Hypoxia corresponding to 3,500 m altitude did not provoke an additional effect. In addition, exercise-induced norepinephrine concentrations seen *in vivo* also had a significant effect on CPC functionality tested *in vitro*. Study design II revealed a significant relationship between maximum exercise-induced blood lactate concentration and CPC count, independent of the exercise mode. **Conclusion:** Physical exercise stress influences CPCs in a complex way; therefore effects of physical exercise on regeneration and repair processes are to be expected.

Zusammenfassung (German)

Zielsetzung: Aus dem Knochenmark mobilisierte adulte humane hämatopoetische Stamm- und Progenitorzellen (HSPZ) spielen für körperliche Regenerationsprozesse lebenslang eine wichtige Rolle. Diese Dissertation behandelt den Einfluss von körperlicher Belastung unter Normoxie sowie auch Hypoxie auf Anzahl und Funktionalität adulter zirkulierender hämatopoetischer Progenitorzellen (ZPZ), die *CD34+/CD45dim side scatter low* HSPZ im peripheren Blut darstellen. Zwei Studien Designs wurden konzipiert um die ZPZ Kinetik und Funktionalität im peripheren Blut einerseits nach definierten Bewegungsprotokollen zu erfassen sowie auch die Beteiligung von bewegungsinduzierten Blutparametern als mögliche Einflussfaktoren zu untersuchen. **Methoden:** In Studie I wurden zehn gesunde, männliche Probanden (25.3 ± 4.4 Jahre) einer standardisierten Rad-Ergometrie (40 W + 20 W/Min) unter normoxischen (FiO_2 ~ 0.21) als auch hypoxischen Bedingungen (FiO_2 < 0.15, entspricht 3500 m, für 3 h) unterzogen. Blut wurde sowohl vor als auch 10, 30, 60 und 120 Minuten nach Belastung aus der Ellenbeuge entnommen. Studie II testete sieben Patienten (63.4 ± 7.0 Jahre) in der kardiologischen Rehabilitation. Alle Probanden führten 2-3 unterschiedliche Belastungstests (zufällig ausgewählt aus vier) auf einem Rad-Ergometer durch; jedes Belastungsprotokoll sollte eine andere Blutlaktat-Konzentration hervorrufen. **Ergebnisse:** Studie I zeigte eine signifikante Erhöhung der ZPZ Ausschüttung 10 min nach Ende der Belastung unter normoxischen als auch hypoxischen Bedingungen. Interessanterweise verringerte sich die Funktionalität der ZPZ 10 Minuten nach Ende der Belastung signifikant, obwohl sich die Anzahl an *CD34+/CD45dim* Zellen erhöhte. Oxidative Stressparameter und Cortisol korrelierten signifikant mit dem ZPZ Level. Interleukin-6 als auch Noradrenalin (NA) zeigten einen signifikanten Anstieg nach Ende der Belastung. Hypoxie auf einer Höhe von 3500 m ergab keinen zusätzlichen Einfluss. Die *in vivo* ermittelte bewegungsinduzierte NA-Konzentration zeigte ebenfalls einen signifikanten Effekt auf die ZPZ Funktionalität *in vitro*. Studie II enthüllte eine signifikante Korrelation zwischen der maximal akkumulierten Blutlaktat-Konzentration und der Anzahl an ZPZ unabhängig vom Bewegungsprotokoll. **Conclusio:** Der Stress körperlicher Belastung beeinflusst die ZPZ Anzahl und Funktionalität in sehr komplexer Weise und könnte somit bei Regeneration und Reparaturmechanismen eine wichtige Rolle spielen.

Table of contents

1 Introduction

1.1 The fate of adult hematopoietic stem and progenitor cells in the bone marrow: Hematopoiesis

Hematopoietic stem and progenitor cells (HSCs) are responsible for substituting a person's blood cells during the whole lifetime. Chute et al. (2010) summarized the current knowledge regarding hematopoiesis in one of their articles in 2010[1] (Fig. 1). HSCs can be subdivided into long- (LTHSC) and short-term (STHSC) repopulating stem cells within the bone marrow, depending on how long their self-renewal ability lasts. A multipotent progenitor cell (MPP) can evolve in both leucocyte and erythrocyte lineages by a common myeloid progenitor cell (CMP) or can also develop into the lymphoid lineage by a common lymphoid progenitor cell (CLP). The leucocyte lineage is characterized by granulocyte monocyte progenitor cells (GMP) that develop to monocytes, and eosinophil, basophil or neutrophil granulocytes, whereas the origin of the erythrocyte lineage is the megakaryocyte-erythroid progenitor cell (MEP) that subsequently forms red blood cells (RBCs) and megakaryocytes. The CLP development ends either in B- or T-cells. Self-renewal ability and therefore functionality of these progenitor cells can be proofed by different types of repopulating assays (CRU, competitive repopulating unit assay; CFU-Spleen, colony forming unit-spleen assay; CFC-D 14, colony-forming cell assay, incubation for 14 days).

Each hematopoietic stem cell pool has its own characteristics. The main criteria defining HSCs are specific surface proteins that can be useful in separating HSCs from other stem cell types[2]. In general you can subdivide into three different kinds of stem cells that can be isolated from the bone marrow: hematopoietic (HSCs), endothelial (ESCs) and mesenchymal (MSCs) stem cells for blood, vascular and structural development and repair. According to Lancrin et al. (2009) endothelial stem cells have the same origin as hematopoietic stem cells called the hemangioblast[3]. This is why it is very important to exactly define the surface profile of target cells in order to separate these two populations. Both populations are marked with the CD34 surface protein, but ESCs are CD45 negative. Therefore it is possible to derive a more or less pure population of hematopoietic stem and progenitor cells (HSPCs) by using a

CD34+/CD45dim side scatter low (CD34+/CD45dim SSClow) antibody combination in a cell sorting analysis (flow cytometry) that will be described in detail in the methods section of this thesis.

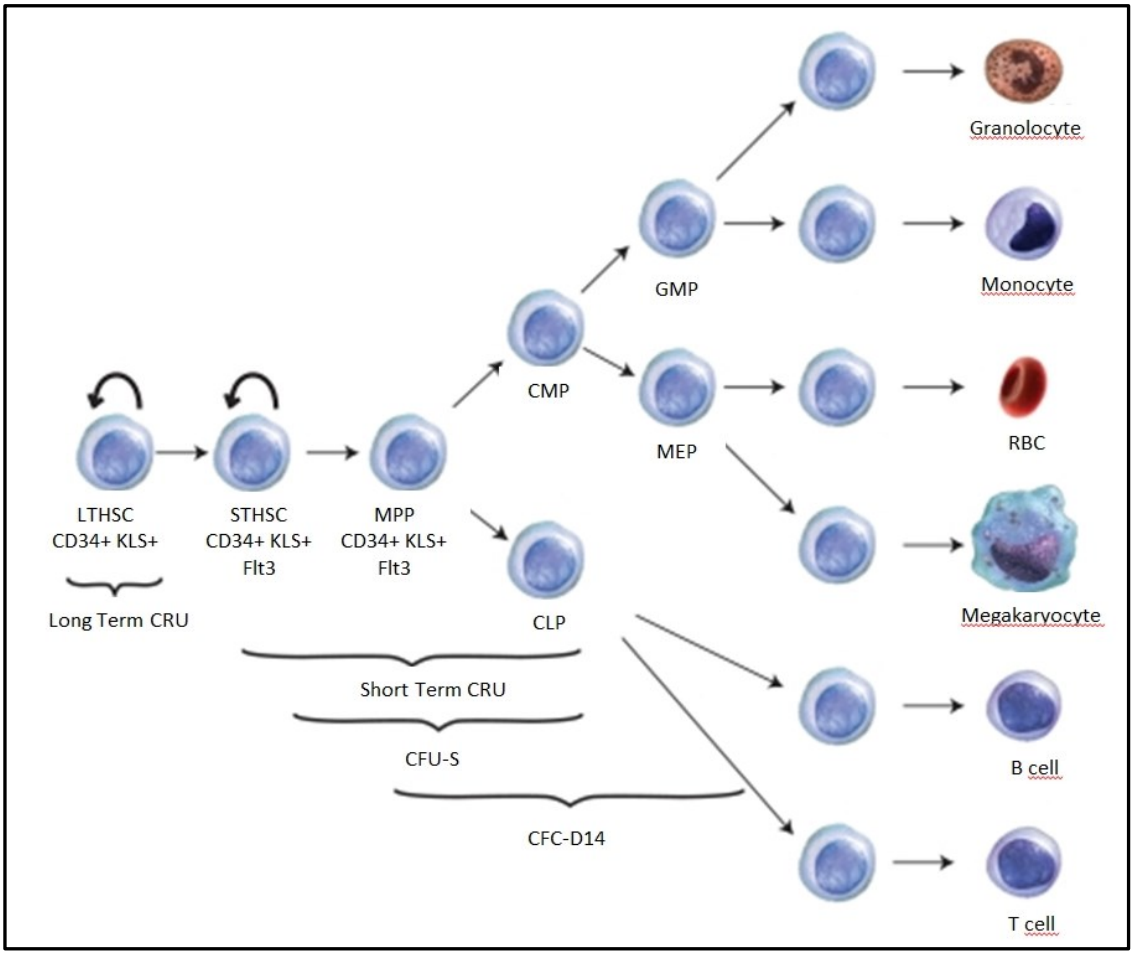

Figure 1: The fate of hematopoietic stem cells during hematopoiesis, reproduced with permission from Chute et al. (2010)[1]. Phenotypes and functional assays used to characterize each of the individual stem- and progenitor cell pools are shown. Erythrocytes (RBC) are the carriers of oxygen in the blood; leucocytes (monocytes, granulocytes, lymphocytes) are responsible for the immune system and thrombocytes, build from megacaryocytes in the bone marrow, are important for blood coagulation. LTHSC, Long-term repopulating hematopoietic stem cell; STHSC, short-term repopulating hematopoietic stem cell; MPP, multipotent progenitor cell; CMP, common myeloid progenitor cell; MEP, megakaryocyte-erythroid progenitor cell; GMP, granulocyte monocyte progenitor cell; CLP, common lymphoid progenitor cell; RBC, red blood cell; 34-KLS, CD34-c-*kit*+lineage-sca-1+ cells; CRU, competitive repopulating unit assay; CFU-Spleen, colony forming unit-spleen assay; CFC, colony-forming cell assay, day 14;

1.2 The environment makes the rule

In the bone marrow HSCs are sitting in a microenvironment called the stem cell niche[4]. This niche is made up of supportive non-hematopoietic cell populations, such as mesenchymal stromal cells, adipocytes, endothelial cells and an extracellular matrix consisting of fibronectin, collagen, proteoglycans, heparins and binding sites for signaling molecules. These micro-environmental cellular and matrix components supply cytokines and cell-cell signals important for maintenance of steady-state hematopoiesis and a rapid response to hematopoietic demand involving mobilization processes[5]. Depending on their surrounding influences HSCs are driven into different development stages. Li summarized in 2011 the possible stages of HSCs during their development[6]: 1.relative quiescence, 2. self-renewal and 3. ability to differentiate into multiple lineages. Usually most HSCs present in the bone marrow remain quiescent and only few of them enter the cell cycle towards differentiation. Self-renewal and differentiation are the most important features of stem cells, which enable them to give rise to themselves and at the same time differentiate into more mature cells, which is called asymmetric cell division. Stem cells, however, can also do symmetric cell division by only increasing the existing number of their kind, which is needed after injuries, chemotherapy or engraftment after hematopoietic stem cell transplantation in order to have enough cells to participate in regeneration processes. A defining feature of stem cells is their ability to periodically divide in a symmetric as well as an asymmetric way. The molecular switch between symmetric and asymmetric cell division, however, still remains elusive[7].

The strict regulation of these two different division processes is very important in order to maintain tissue homoeostasis and a functioning stem cell pool in the bone marrow. Extrinsic (such as bone-lining osteoblast cells and stem cell factor signaling, Wnt or hedgehog signaling pathways) and intrinsic regulators (such as different transcription factors e.g. p53 or Lnk) make the decision between relative quiescence, self-renewal and differentiation[6].Once on the path of differentiation, HSPCs can be mobilized to leave the bone marrow and enter circulation[8] (Fig. 2), triggered by different factors.

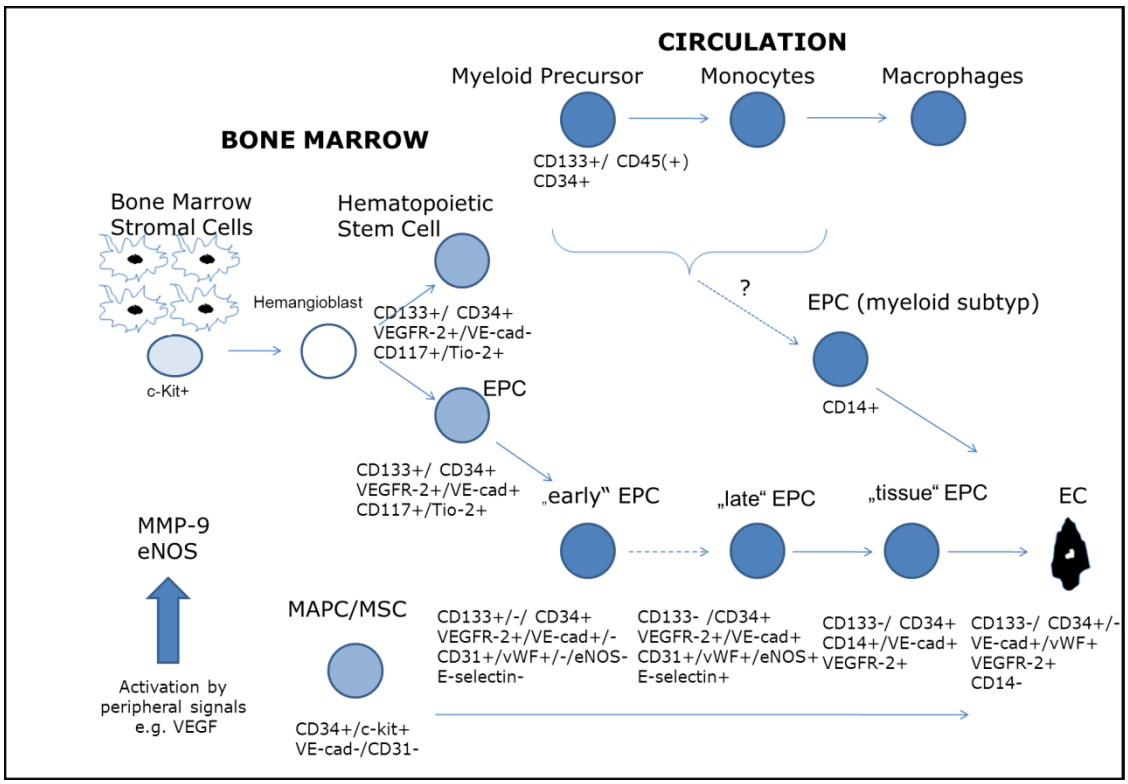

Figure 2: Development of hematopoietic and endothelial stem and progenitor cells in the bone marrow, reproduced with permission from Wahl et al. (2007)[8]. HSCs that stay quiescent in the bone marrow are attached to stromal cells. Only if there is a specific trigger mechanism, they are mobilized to the peripheral blood (see Section 1.3).

1.3 Possible trigger mechanisms for HSPCs from the bone marrow to the peripheral blood

Under normal conditions, there are few HSPCs in the peripheral blood, approximately one of 10 000 cells (0.01 % of blood cells). There are various trigger mechanisms that are responsible for mobilizing HSPCs from the bone marrow to the circulation. Lanza et al. (2004) summarized some experimental models for stem cell mobilization[5]: Very well documented ways of forcing HSPCs to leave the bone marrow are the injections of cytokines, signaling molecules such as stem cell factor 1 (SCF-1), colony stimulating factors (CSFs), different Interleukins (IL-3, IL-5, IL-6, IL-7) or growth factors (such as erythropoietin). These procedures also find broad clinical applications and are highly researched especially in leukemia settings, whereas HSPC mobilization by granulocyte colony-stimulating factor is seen as the gold standard today[9, 10]. Other mechanisms to release HSPCs from the bone marrow of humans is to externally stress the body through either e.g. a hypoxic environment[11] or physical exercise[12, 13].

1.4 Possible signaling cascades triggered by exercise forcing HSPCs to the peripheral blood

Exercise has an effect on the bone marrow and triggers HSPCs to the circulation, ideally to places of tissue defects, where regeneration is needed. Wahl et al. (2007) hypothesizes two possible signaling cascades triggered by exercise for endothelial progenitor cell (EPC) and HSPC mobilization[8]: Exercise-induced elevated oxygen consumption forces tissue hypoxia and ischemia in the blood, which in turn elevates transcription factors and growth hormones that could trigger stem and progenitor cells. Another signaling cascade could be the exercise-induced elevated blood flow increasing shear stress in blood vessels and therefore also raising needed growth hormones for stem and progenitor mobilization (Fig. 3). And it is even possible that there are other triggers that are not yet identified, such as the exercise-induced increase in reactive oxygen species, measured by blood parameters of oxidative stress (such as malondialdehyd or myeloperoxidase), increased pro-inflammatory cytokines (such as interleukin-6), elevated stress hormones (such as cortisol or norepinephrine) or physical strain (indicated by a raised blood lactate concentration).

It is very likely that the effect of exercise on hematopoietic stem and progenitor cells is a combination of the possible trigger mechanisms named above. One goal of this thesis was to evaluate the importance of different exercise-induced factors for hematopoietic stem and progenitor cell mobilization as well as cell number and functionality in the peripheral blood.

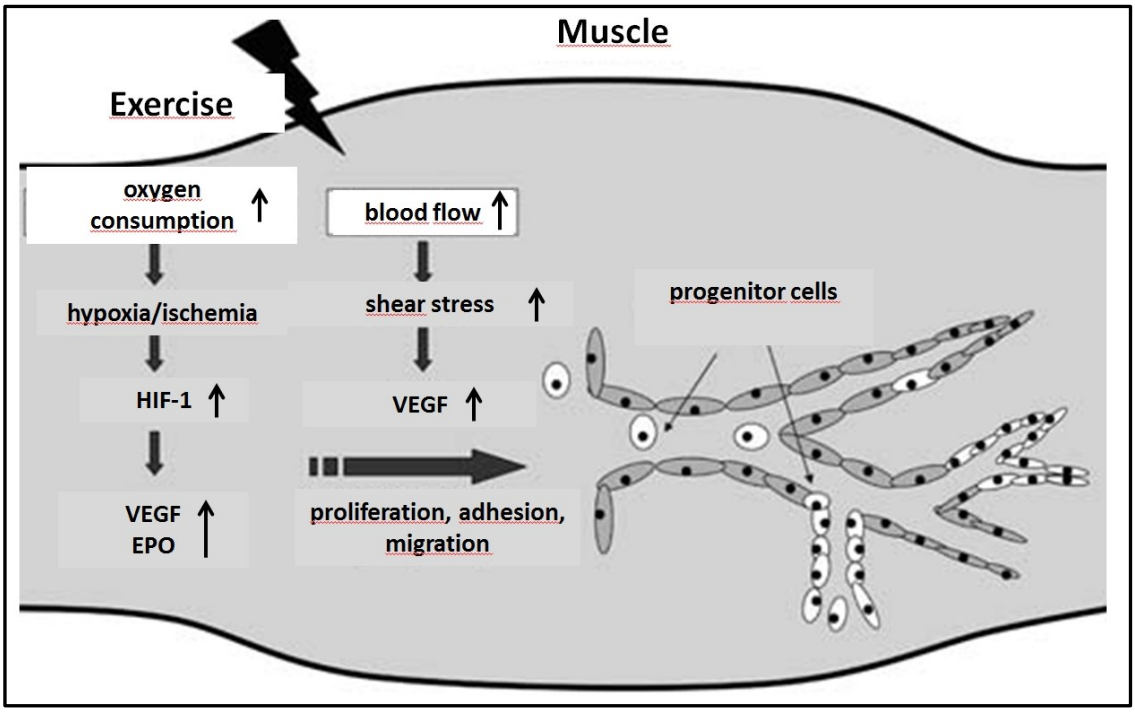

Figure 3: Possible signaling cascades triggered by exercise, reproduced and modified with permission from Wahl et al. (2007)[8]. Exercise increases body functions such as oxygen consumption or blood flow through the vascular system which in-turn raise transcription factors and growth hormones needed for mobilization.

1.5 Hematopoietic stem and progenitor cells in the peripheral blood: number and functionality

The positive influence of exercise on circulating hematopoietic progenitor cell (CPC) number has well been documented. Morici et al. (2005) showed an increase of hematopoietic progenitor levels after 1,000 m all-out rowing exercise[14], whereas Bonsignore et al (2002) found an increase of circulating hematopoietic progenitor cells after a marathon[12].Muscle electrostimulation or hypobaric hypoxia on their own did not force progenitor mobilization, but the combination of both stimuli achieved this[15]. Thijssen et al. (2006) suggested that baseline and exercise-induced numbers of HSCs and EPCs were not dependent on a person's training status[13]. Laufs et al. (2005) found a maximal increase of progenitors 10-30 min after intensive running[16], whereas Moebius-Winkler et al. found the peak-point of CD34+ cells 210 min after the onset of 4 h constant-load exercise[17]. One main goal of this thesis was to identify the kinetics of CPCs in the peripheral blood after a standardized cycle incremental step test protocol (ergometry).

When talking about exercise, it is also important to think about the involvement of stem and progenitor cells in regeneration processes that are occurring with physical activity[18-20]. Since not only the number of CPCs in the peripheral blood but also their functionality/proliferative capacity is very important for recovery and tissue repair, the main goal of this thesis was to address CPC functionality. What does functionality of a stem and progenitor cell mean? One special characteristic of stem cells compared to other cell types is their potential for self-renewal. Even after multiple generations, they still show the ability to form colonies[21, 22]. In the context of physical strain, circulating hematopoietic progenitor cell functionality has not yet been investigated extensively. "Only a limited number of studies addressed the effect of exercise on CPC colony formation in human subjects[23], where only the different types of CPCs were studied by colony forming unit assays but not for their functionality. Animal based data are also sparse, but some results were given by Stelzer et al. (2010). The influence of voluntary life-long exercise on the decline of bone marrow derived hematopoietic progenitor cell quality was investigated during aging in rats[24], where a higher proliferative capacity of myeloid progenitor cells in a voluntary life-long exercising group of rats was found. This raises the question whether the exposure to acute stressors, such as high intensity

physical exercise, also forces hematopoietic stem cell renewal and regeneration in the human body, supported by the release of CPCs into the peripheral blood."[25]

1.6 Aim of the thesis and research questions

The aim of this thesis was to evaluate the influence of a standardized cycle incremental step test protocol (ergometry) on hematopoietic stem and progenitor cell (HSPC) number and functionality in the peripheral blood in health and disease. Two study designs were formulated where the *first study design* was used to investigate the *following research questions* in healthy subjects:

A. HSPC kinetics in peripheral blood
B. Influence of hypoxia on HSPC mobilization
C. HSPC functionality before/after exercise
D. Influence of oxidative stress (MDA, MPO) on HSPC mobilization
E. Influence of inflammatory parameters (IL-6) on HSPC mobilization
F. Influence of stress hormones (cortisol, norepinephrine) on HSPC mobilization
G. Influence of norepinephrine and lactate on HSPC functionality

The *second study design* investigated the *following*:

H. Influence of blood lactate concentration on HSPC mobilization in heart disease patients

2 Methods

2.1 Subjects and study designs

Study design I (Fig. 4)

"Ten healthy athletic male subjects (age: 25.3 ± 4.4 yrs, BMI: 22.9 ± 1.7 kg/m^2) were recruited for the study. They all met the inclusion criteria of no medication intake, no previous or current health problems, being non-smokers and no intake of dietary supplements. Informed consent was obtained from all participants. The study protocol was approved by the local ethics committee of the Medical University of Graz, Austria, (decision number 21-126 ex 09/10). All subjects performed a standardized cycle incremental ergometry test protocol (3 min resting phase, 40 W starting load, increasing 20 W/min) until exhaustion. The hypoxic interventions were done in a sealed normobaric hypoxia chamber (altitude above sea level: Graz, Austria: 383 m), where a simulated altitude of 3,500 m (F_iO_2 < 0.15) was generated (Hypoxico, Everest Summit II) for the time of test duration (~ 3 h). CO_2 content was held constant by continuous absorption."[25].Venous blood was collected from the cubital vein and plasma prepared at rest, 10, 30, 60 and 120 min postexercise.

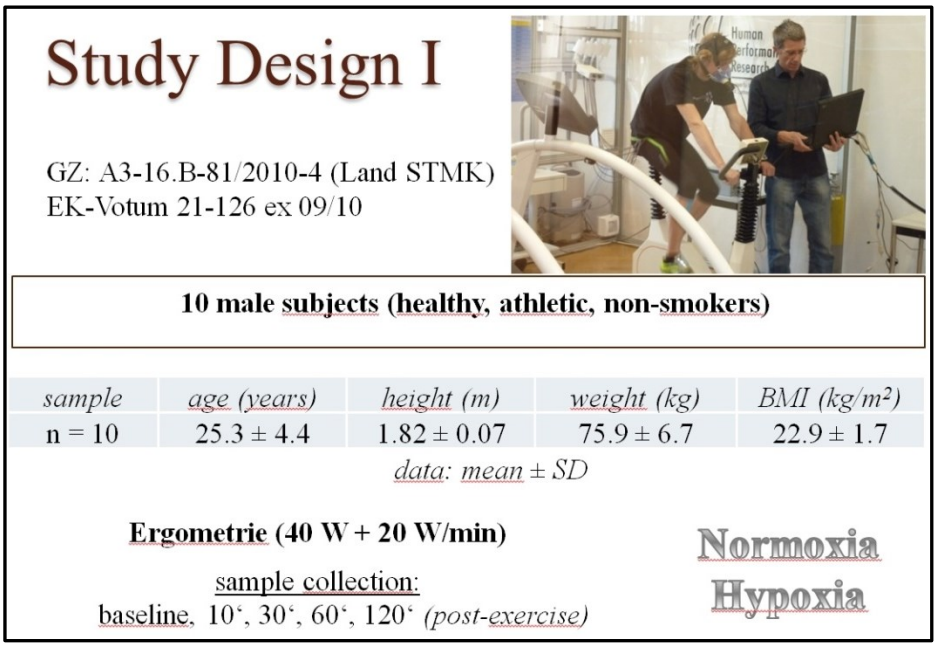

Figure 4: Study design overview for investigating hematopoietic stem and progenitor cell behavior in healthy subjects

Study design II (Fig. 5)

Seven patients (age: 63.4 ± 7.0 yrs, BMI: 28.0 ± 3.0 kg/m^2) were included in the study. They all met the inclusion criteria of being part of the recovery program for coronary incidences phase-III at ZARG (Centre for Ambulant Rehabilitation Graz, Graz, Austria). Informed consent was obtained from all participants. The study protocol was approved by the local ethics committee of the Medical University of Graz, Austria, (decision number 23-397 ex 10/11). All subjects performed 2-3 different randomized exercise test protocols (n = 18) out of 4 possibilities (ergometry, constant-load, short-interval or long-interval tests) on an electronically braked cycle ergometer (Ergoline Reha System, Ergoline, Germany), where each exercise test protocol would trigger a different blood lactate concentration. Venous blood was drawn from the cubital vein before and immediately after each intervention.

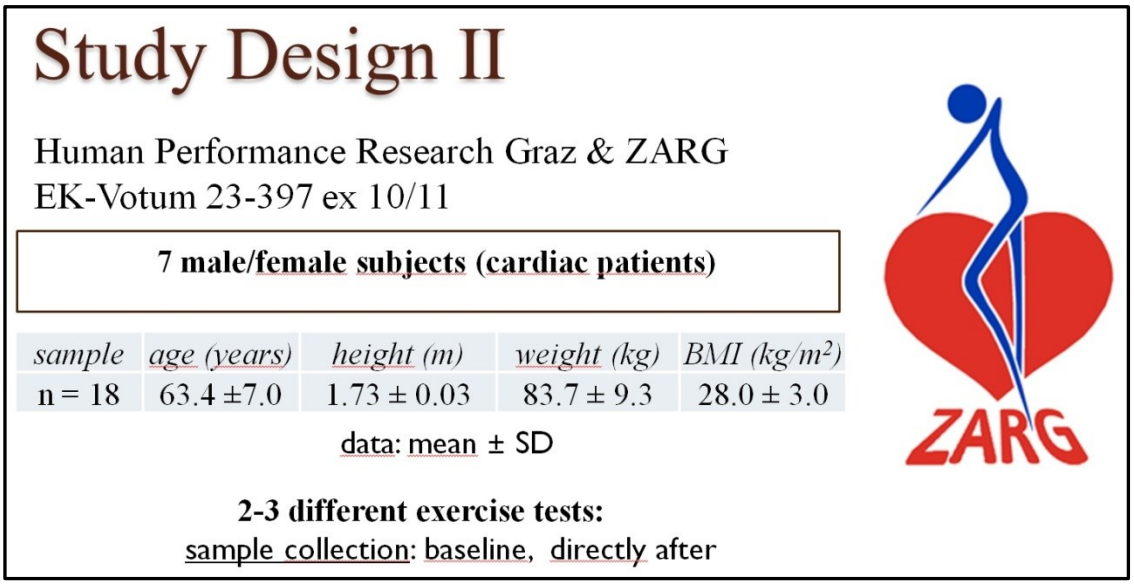

Figure 5: Study design overview for investigating hematopoietic stem and progenitor cell behavior in patients recovering from coronary incidences.

2.2 Used exercise test protocols

Study design I

- Ergometry: cycle incremental step test protocol

  Work load started at 40 W and was increased by 20 W every minute until exhaustion (Fig. 4)[26]. "The subjects ECG was monitored by a physician and the heart rate (HR, PE 4000, Polar Electro), blood lactate concentration (lactate, Biosen S-line, EKF-Diagnostic) and gas exchange variables (ZAN 800, ZAN) were measured throughout the tests as well as during 3 minute of active and 3 minute of passive recovery. Maximal oxygen uptake (VO_{2max}) and maximal power output (P_{max}) were determined as markers of exercise performance. Test duration was depending on the individual physical condition (min-max: 16-23 minutes under normoxia, 16-20 minutes under hypoxia, respectively)."[25]

Study design II

Exercise intensity prescription was done by means of percentage of output power of lactate-turn-points 1 and 2 (LTP_1, LTP_2) according to Hofmann and Tschakert (2011) [27]and Mezzani et al. (2012)[28]. Blood lactate concentration was evaluated by means of ear-capillary method (lactate, Biosen S-line, EKF-Diagnostic). Additionally, electrocardiogram (Cardiosoft v6.51 GE Healthcare, GE Healthcare, UK), heart rate (PE 4000, Polar Electro, Finland) as well as gas exchange variables (MetaMax 3B, Cortex, Germany) were monitored.

- Ergometry: symptom-limited cycle incremental step test protocol

  Power increments were 10 W per minute starting out from 10 W[26].

  Cycle constant-load test:

  Warm up phase consisted of 3 work load steps (one work load step per minute), depending on the individual target work load (P_{target}), which was defined as 20 % below output power at lactate-turn-point 2 (P_{LTP2}) from ergometry and was maintained for 28 minutes. Cool down phase lasted for 5 min (passive recovery).

- Cycle short- and long high-intensity interval-tests:

In general, interval-tests are determined by peak work load (P_{peak}), recovery work load (P_{rec}), peak work load duration (t_{peak}), and recovery work load duration (t_{rec}). By these four parameters the mean work load (P_{mean}) can be calculated as such $P_{mean} = \frac{P_{peak} \cdot t_{peak} + P_{rec} \cdot t_{rec}}{t_{peak} + t_{rec}}$ (*formula 2.2)*[29]

or any other individual parameter can be calculated by knowing the other three ones and P_{mean}.

In addition, the number of intervals is of importance. Interval tests were maintained for 28 min (no warm up or cool down phase included).

Long intervals (4 x 4 min):

Warm up phase was the same as for constant load test. P_{mean} was defined as 20 % below P_{LTP2} from ergometry, P_{peak} as output power at 85 % of the maximal heart rate (HR_{max}) from ergometry, t_{peak} as 4 min, t_{rec} as 3 min, and P_{rec} was subsequently calculated according to formula 2.2.Cool down phase was the same as for constant load test adopted and modified according to Wisloff et al. (2007)[30].

Short intervals (20 sec):

Warm up phase was the same as for constant load test. P_{mean} was defined as 20% below P_{LTP2} from ergometry, P_{peak} as P_{max} from ergometry, t_{peak} as 20 sec, P_{rec} as 10 % below P_{LTP1} from ergometry, and t_{rec} was subsequently calculated according to formula 2.2. Cool down phase was the same as for constant load test adopted and modified according to Tschakert et al. (2011)[31].

2.3 Nutritious anamnesis: 24 h-recall

Nutritious status of subjects was addressed before each exercise test by means of a 24 h-recall[32] and evaluated with a specialized program (nut.s science, Vienna, Austria).

2.4 Blood sample collection and post-processing

"Blood from the cubital vein was collected in EDTA tubes for oxidative stress measurements as well as stem and progenitor cell counts and in ammonium-heparin tubes for stem and progenitor cell isolation and functionality tests."…"They were kept at room temperature until analysis for counting of total blood cells (Hematology Analyzer KX-21N, Sysmex) and estimation of circulating progenitor cell (CPC) numbers by flow cytometry (FACS Calibur, BD Biosciences)."…" Blood samples were centrifuged (~1156 g, 10 min) and plasma was stored at - 80°C until analysis."[25]. For study I, blood samples were obtained at rest, 10 min, 30 min, 60 min and 120 min after the intervention. "All blood preparations for flow cytometry analysis for all 5 different time points (per subject and test) were done at the same time and day. Oxidative stress markers and CPC counts were obtained from all 10 subjects; progenitor cell functionality tests (secondary CFU-GM assays) were done for a subgroup of 6 subjects for normoxic as well as hypoxic conditions. To avoid the influence of diurnal variation, the incremental test was always performed at the same time of day (starting at 8:00 am)."[25]. For study design 2, blood samples were only collected before and directly after each exercise test.

2.5 Analysis of circulating hematopoietic progenitor cells

"The gating strategy followed the two-platform ISHAGE guidelines based on light scatter characteristics and presence of CD34+/CD45dim SSClow HSCs/HPCs[33]" (Fig. 6)…"Gates were established using umbilical cord blood. CPCs in the peripheral blood were analyzed by flow cytometry using the antibody combination CD34-PE/CD45-FITC (BD Biosciences, Vienna, Austria) following the instructions of the manufacturer. Flow cytometry, using a FACSCalibur (BD Biosciences; Cell Quest Pro Software) was performed with correct fluorescent parameters (compensation and light scatter gating), excluding dead cells. In total 500,000 leukocytes were acquired and the percentage of CD34+/CD45dim CPCs within the leukocyte fraction was determined. The total CD34+/CD45dim cell number per ml peripheral blood was calculated based on the counts of CPCs related to the leukocyte fraction."[25]

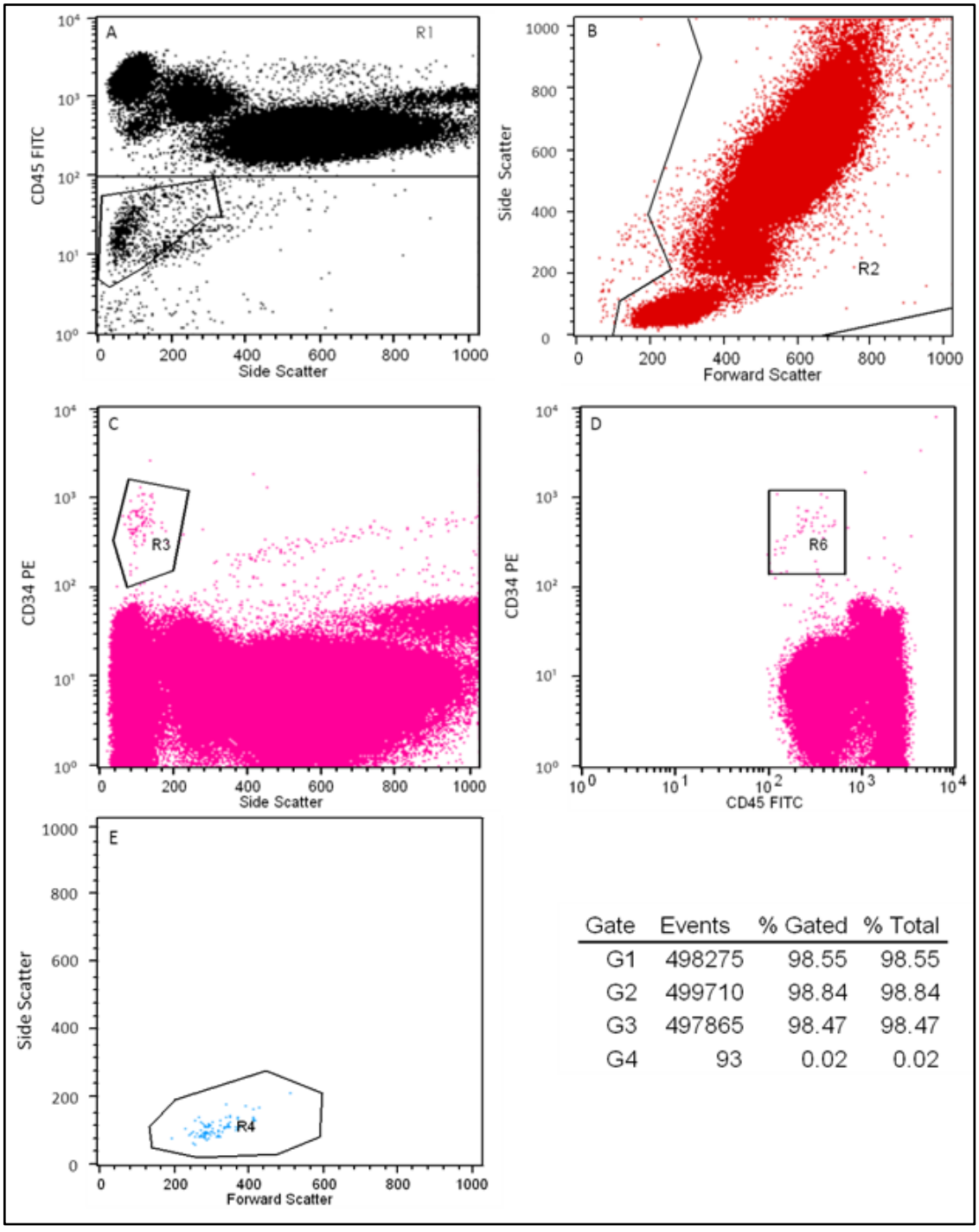

Figure 6: "FACS plots of a representative analysis of circulating hematopoietic progenitor cells (CPCs) in the peripheral blood are shown (subject 1, before exercise, normoxia). Total leukocytes were stained with the antibody combination CD34 PE/CD45 FITC and analyzed by flow cytometry. CPCs were defined according to the ISHAGE protocol. In order to exclude debris and erythrocytes, we selected CD45+ cells (A) which are shown in (B) as dot plot SSC versus FSC. CPCs were defined by high CD34 expression and low side scatter signal (C) as well as dim CD45 expression (D). The finally targeted CD34+/CD45dim SSClow CPCs are a subgroup of the lymphocyte fraction of the peripheral blood (E)."[25]; SSC, side scatter; FSC, forward scatter;

2.5.1 FACS Calibur baseline

The flow cytometry sampling procedure was evaluated pooling mean values (n = 3) out of 6 different sample preparations (Table 1). The intra-assay coefficient of variation (CV) for the used FACS Calibur (BD Biosciences) was 9.2 % for total CD34+/CD45 dim cells. This is within the range of already published results[12, 23].

Table 1. ***Precision of the used measurement method (FACS Calibur)***

	Sample 1	*Sample 2*	*Sample 3*	*Sample 4*	*Sample 5*	*Sample 6*
mean CPC/ml	1432.2	1836.7	2136.1	1711.0	786.0	1078.1
SD CPC/ml	164.2	201.6	123.8	143.5	43.4	143.3
CV, %	11	11	6	8	6	13

Table 1

2.6 Primary and secondary colony forming unit (CFU) assays

Analyzing the acquired data of study 1, CFU assays were performed for a subgroup of 6 subjects. "Peripheral mononuclear blood cells (PMNC) were isolated from heparin-anticoagulated blood by standard Ficoll density gradient centrifugation (Histopaque®, Sigma-Aldrich, Vienna, Austria) according to the manufacturer's instructions using seven to nine ml of heparin-anticoagulated blood. Cells for CFU-assays were collected before and 10 min after ergometry. The harvested cell pellet was resuspended in DMEM/F-12 culture medium (Gibco®, Invitrogen, Lofer, Austria) and cell counts were estimated using a CASY®cell counter (Roche Diagnostics, Risch, Switzerland). For the CFU assay with erythropoietin (EPO), mononuclear cells were plated at a concentration of $1.5*10^5$ cells/ml in 500 µl methylcellulose culture medium (MethoCult® H4434, StemCell Technologies, Vancouver, Canada) in 12-well flat-bottom suspension culture plates (Greiner Bio One, Kremsmünster, Austria) and incubated at 37°C in a humidified atmosphere containing 5 % CO_2 for 14 days. Colonies consisting of more than 40 cells were scored and gave information on the frequency of myeloid progenitor cells (BFU-E and CFU-GM).

For the secondary CFU-GM assay, mononuclear cells were plated at a concentration of $1.5*10^5$ cells/ml in 500 µl methylcellulose culture medium without EPO (MethoCult® H4534, StemCell Technologies) in 12-well flat-bottom suspension culture plates (Greiner Bio One, Kremsmünster, Austria) and incubated at 37°C in a humidified atmosphere containing 5 % CO_2 for 8 days. After counting, 90 primary CFU-GM colonies consisting of more than 40 cells were individually plucked from the methylcellulose culture medium; each single colony was transferred to a separate well of a 48-well flat-bottom microtitre plate, dispersed in alpha medium (Gibco®) supplemented with 15 % FBS and thoroughly mixed with methylcellulose culture medium to obtain a single cell suspension [21]. After 14-16 days each well was again scored for the presence and number of CFU-GM colonies consisting of more than 40 cells (= secondary CFU-GM). The secondary replating capacity correlates with the proliferative capacity of myeloid progenitor cells[21, 22]." (Fig. 7)[25]

2.7 Analysis of secondary colony forming unit (CFU) assays

"For analysis of secondary CFUs, the number of secondary CFU-GM produced by each primary CFU-GM was used as raw data. Counts exceeding a number of 100 were truncated. The secondary replating capacity of an individual was defined as the mean log 2 of the number of colonies plus one for the following reasons: The log 2 scale is natural as the distribution of the number of secondary colony-forming cells is skewed to the right. One was added as the log 2 of zero cannot be calculated and the log 2 of this number is a continuous measure of number of duplications of a primary CFU-GM. This measure has properties similar to the measure used by Gordon et al. (1998)[21]; as counts of zero are adequately taken into account the log scale reduces the skewness as well. Cumulative percentages are expressed as Area Under the Curve, AUC[24, 34, 35]."[25]

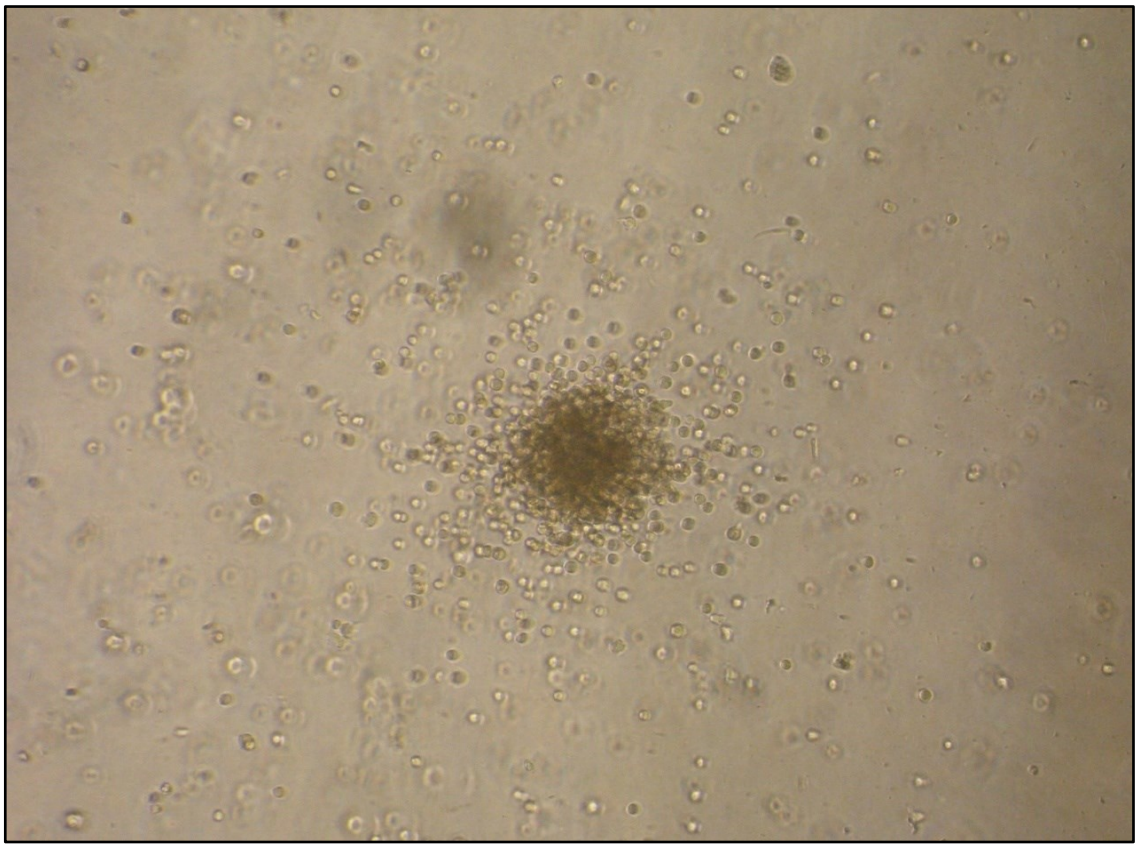

Figure 7: CFU-GM colony under 400 x magnification[36]

2.8 Evaluation of oxidative stress parameters (MDA, MPO)

"Aliquots of plasma were prepared and stored at -80°C until analysis. Malondialdehyde was determined with a high-performance liquid chromatographic (HPLC) method with spectrofluorimetric detection as described by Khoschsorur et al.[37]. Myeloperoxidase concentration was measured by the Architect-i-System (Architect MPO assay, Abbott Laboratories Diagnostics, Abbott Park, IL, USA), using the automated chemiluminescent microparticle immunoassay (CMIA) technology[38]. Detection limits were 0.2 µmol/l for MDA and 2.9 µg/l for MPO."[25]

2.9 Evaluation of inflammatory parameters (IL-6)

Interleukin-6 (IL-6) was analyzed by means of electrochemiluminescence immunoassay (Roche Diagnostics, South San Francisco, Calif).

2.10 Determination of stress hormones (cortisol, norepinephrine)

Serum cortisol was determined by luminescence immunoassay (Bayer, Leverkusen, Germany)[39]. Intra-assay and interassay variation coefficients for ELISA were below 10 %.

For catecholamine determination blood samples were collected in ammonium-heparin coated vials, containing 1.25 mg of glutathione per ml blood. After immediate centrifugation at 1,300 g for 10 min, plasma samples were treated with ClinRepR complete kit (RECIPE, Munich, Germany) as follows: 1.0 ml plasma was spiked with an internal standard and was transferred into the sample preparation column. Catecholamines were adsorbed at aluminium oxide and isolated from the sample matrix. The plasma supernatant free of catecholamines was removed by centrifugation. Interfering substances, being co-adsorbed at aluminium oxide, were removed by three consecutive washing steps. Afterwards catecholamines were eluted from the sample preparation column and were stable for injection (40µl) into the high-performance liquid chromatography (HPLC) system. Conjugated catecholamines were determined by a sulfatase/glucuronidase mixture (Sigma, Munich, Germany)after hydrolysis. Free and

conjugated catecholamines were assayed by HPLC using an amperometrical detector (RECIPE, Munich, Germany) and specialized software (ClarityTM, DataApex, Prague, Czech Republic).

2.11 Reevaluation of in vivo study results in vitro (study I)

As a comparison to the already accomplished results, a timely separated single blood withdrawal was performed in a randomized subgroup of 6 subjects. In order to reevaluate the influence of exercise-induced stress parameters norepinephrine and blood lactate concentration determined *in vivo*, their impact on CPC functionality was also analyzed in cell culture *in vitro.* Circulating hematopoietic progenitor cells were incubated with the detected concentrations at rest, and after exhausting physical strain (free NE: $5*10^{-9}$ ng/ml baseline, $5*10^{-8}$ ng/ml directly after ergometry; La: 0.01 mmol/l baseline, 12 mmol/l directly after ergometry).

2.12 Statistics

Data are given as means ± SEM, except for physiological exercise/nutritious variables and subject specifications (age, BMI), which are reported as means ± SD, and primary CFU assay statistics, which are presented as median, minimum, maximum. “Statistical analysis was done with SPSS (IBM SPSS Statistics 19). All used variables were tested for normal distribution with the Kolmogorov-Smirnov test ($p > 0.05$)”.[25] Changes in blood cell counts, CD34+/CD45dim cells, MDA, MPO, IL-6, norepinephrine and cortisol levels during both normoxic and hypoxic interventions were tested by repeated-measures ANOVAs with Fisher’s-LSD (Least Significant Difference), whereas changes in secondary CFUs before/10 min after the incremental test and secondary CFUs incubated with NE (concentration after ergometry vs. baseline), La (concentration of baseline vs. control) or both (concentration of NE after ergometry vs. concentration of NE + La after ergometry) as well as differences between normoxia and hypoxia were assessed by paired t-tests (one- or two-tailed tests, depending on the presence/absence of previous hypothesis). Pearson’s product-moment correlation coefficient[40] was used for calculating the relationship between blood parameters and absolute as well as delta CD34+/CD45dim counts. "For pooling values, Friedman’s test was used to verify if samples came from the same distribution.”…”A p-value < 0.05 was considered as significant.”[25]

3 Results

3.1 Results of study I

Study I revealed the following results:

3.1.1 *Exercise performance evaluation*

"Subjects showed the following physical parameters recorded under both conditions (mean ± SD): maximal heart rate (normoxia: 189.5 ± 7.7/min, hypoxia: 185.3 ± 7.3/min), maximal work load (normoxia: 314 ± 49.9 W, hypoxia: 274 ± 29.9 W), maximal blood lactate concentration (normoxia: 12.6 ± 2.1 mmol/l, hypoxia: 12.0 ± 2.0 mmol/l) and maximal oxygen uptake (normoxia: 51.8 ± 7.4 ml/kg/min, hypoxia: 43.1 ± 8.3 ml/kg/min)."[25]

3.1.2 *Nutritious status*

During the study-time,subjects showed a satisfying nutritious status (listed in g, mean ± SD), where proteins (102.6 ± 29.6), carbohydrates (273.5 ± 92.6) and fat (86.4 ± 23.3) were within acceptable ranges.

3.1.3 *Blood cell counts*

"Red blood cells (RBCs), white blood cells (WBCs), platelets, hematocrit (Hct), and neutrophils showed a significant rise 10 min after the intervention (10 min post) under normoxic conditions. Similarly, all these variables as well as lymphocytes increased significantly under hypoxic conditions. All values went back to baseline after another 20 min (30 min post) and RBCs, Hct, and lymphocytes dropped significantly under baseline values after another 30 min (60 min post) under hypoxic conditions (not apparent under normoxia). At the last blood collection (120 min post) the level of WBCs and neutrophils increased significantly again under normoxia and hypoxia

respectively, whereas RBCs, Hct and lymphocytes stayed significantly below baseline values under hypoxic conditions."[25] (Table 2a, 2b)

Table 2a. ***Pre and post exerciseblood parameters under normoxic conditions***

Blood Collection	*baseline*	*10 min post*	*30 min post*	*60 min post*	*120 min post*
RBC, $10^6/\mu l$	5.13 ± 0.10	5.47 ± 0.11***	5.20± 0.11	5.08 ± 0.10	5.08 ± 0.10
Hct, %	45.1 ± 0.7	48.7 ± 0.9***	45.7 ± 0.9	44.5 ± 0.7	44.3 ± 0.7
Platelets, $10^3/\mu l$	185 ± 10	222 ± 7**	189 ± 9	180 ± 9	186 ± 9
WBC, $10^3/\mu l$	4.84 ± 0.30	8.32 ±0.95**	5.79 ±0.71	5.63 ± 0.74	8.17 ± 0.98**
Neutrophils, $10^3/\mu l$	2.64 ± 0.16	4.37 ± 0.31*	3.62 ± 0.15	3.89 ± 0.14	6.20 ± 0.13**
Lymphocytes, $10^3/\mu l$	1.62 ± 0.14	3.08 ± 0.39	1.59 ± 0.20	1.15 ± 0.14	1.22 ± 0.14

Values are means ± SEM, n = 10. Significant differences between time points are indicated as follows: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

Table 2b. ***Pre and post exerciseblood parameters under hypoxic conditions***

Blood Collection	*baseline*	*10 min post*	*30 min post*	*60 min post*	*120 min post*
RBC, $10^6/\mu l$	5.19 ± 0.10	5.42 ± 0.1***	5.12 ± 0.11	4.93 ± 0.12***	5.05 ± 0.07**
Hct, %	45.3 ± 0.6	47.9 ± 0.7***	44.8 ± 0.7	43.0 ± 0.9***	44.0 ± 0.5**
Platelets, $10^3/\mu l$	192 ± 10	223 ± 8***	188 ± 10	177 ± 10	187 ± 8
WBC, $10^3/\mu l$	5.15 ± 0.37	8.13 ± 0.60***	5.57 ± 0.57	5.34 ± 0.55	7.01 ± 0.76*
Neutrophils, $10^3/\mu l$	2.84 ± 0.26	4.04 ± 0.47**	3.40 ± 0.54	3.53 ± 0.50	4.99 ± 0.72*
Lymphocytes, $10^3/\mu l$	1.75 ± 0.15	3.20 ± 0.3***	1.62 ± 0.14	1.30 ± 0.12**	1.44 ± 0.12**

Values are means ± SEM, n = 10. Significant differences between time points are indicated as follows: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

Table 2a, 2b

3.1.4 Changes of CD34+/CD45dim cells

"A significant twofold rise ($p < 0.01$) of circulating hematopoietic progenitor cells was observed 10 min following the exercise intervention under both test conditions" (normoxia, hypoxia, Fig. 8, Table 3a, 3b)…"A significant decrease below baseline ($p < 0.01$) values was only observed under normoxic conditions 120 min post exercise. There was a significant decrease 30 min, 60 min and 120 min post exercise in comparison to the peak value 10 min post exercise ($p < 0.01$).

There was no significant difference between normoxic and hypoxic conditions regarding the number of CD34+/CD45dim cells released at all-time points of blood collection ($p > 0.05$)."[25]

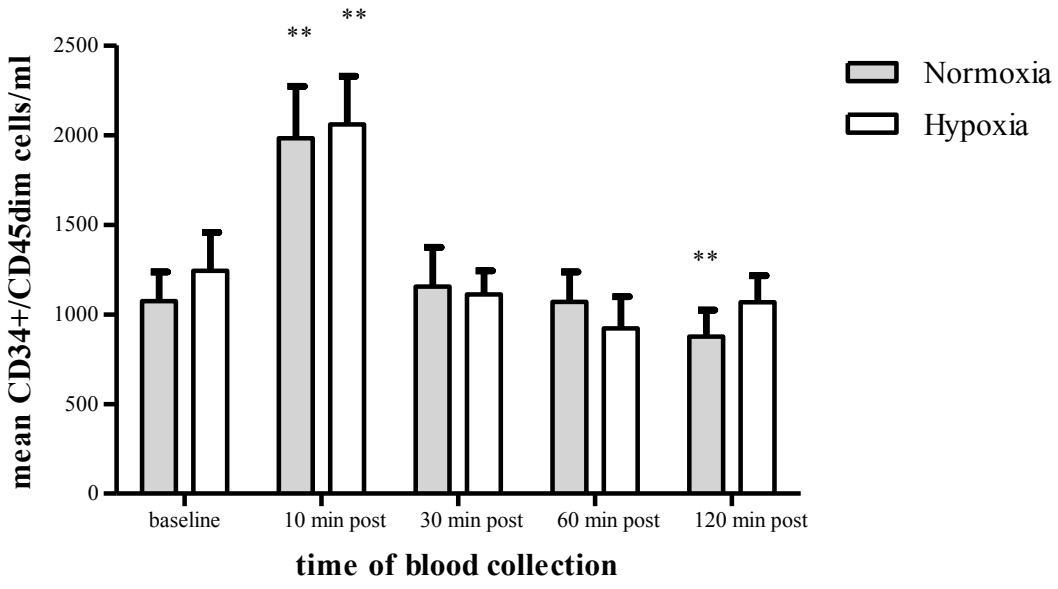

Figure 8: "CPC (CD34+/CD45dim) kinetics triggered by a maximal incremental exercise intervention under normoxic and hypoxic conditions. Time of blood collection: baseline (1), 10 min (2), 30 min (3), 60 min (4) and 120 min (5) after the intervention. Data are reported as means ± SEM. There was a significant increase in CD34+/CD45dim cell number 10 min after cessation of exercise (2) and also a significant decrease to below baseline values 120 min post exercise (5) during normoxia (** $p < 0.01$). During hypoxia, the CD34+/CD45dim cell level returned to baseline values 120 min postexercise (5). There was no significant difference between normoxia and hypoxia ($p > 0.05$) for all time points of blood collection."[25]

3.1.5 Changes of CD34+/CD45bright cells

"CD34+/CD45bright cells did not show any significance regarding all time points in comparison to baseline value under normoxic conditions." (Table 3a)…"Under hypoxia, there was a significant increase ($p < 0.05$) 10 min post exercise (Table 3b). There was no significant difference between normoxia and hypoxia regarding released CD34+/CD45bright cells between time points ($p > 0.05$)."[25]

Table 3a. *CD34+/CD45dim and CD34+/CD45bright cell counts*

Normoxia	*baseline*	*10 min post*
CPCs/ml	1073.5 ± 162.3	1982.2 ± 290.4**
CD34+/CD45bright cells/ml	661.0 ± 228.0	1410.2 ± 704.6

Values are means ± SEM, n = 10. Significant differences between time points (ANOVA with Fisher's LSD) are indicated as follows: $^{**}p < 0.01$, $^{*}p < 0.05$

Table 3b. *CD34+/CD45dim and CD34+/CD45bright cell counts*

Hypoxia	*baseline*	*10 min post*
CPCs/ml	1242.7 ± 214.8	2059.3 ± 268.0**
CD34+/CD45bright cells/ml	320.1 ± 30.6	489.0 ± 65.0*

Values are means ± SEM, n = 10. Significant differences between time points (ANOVA with Fisher's LSD) are indicated as follows: $^{**}p < 0.01$, $^{*}p < 0.05$

Table 3a, 3b

3.1.6 Primary CFU assays with EPO

"Primary CFU assays showed no significant difference (two-tailed paired t-test, $p > 0.05$) between baseline and 10 min post exercise values under both test conditions ($n = 6$)."…"Pooled values (Friedman's test, $p > 0.05$) did also not turn out to be significant ($n = 12$, $p > 0.05$)."[25]. Descriptive statistics of BFU-E and CFU-GM data is summarized in Table 4a, 4b.

Table 4a. ***Primary CFU assay results: BFU-E descriptive statistics (n = 6)***

BFU-E	*Baseline (normoxia)*	*10min post(normoxia)*	*Baseline (hypoxia)*	*10 min post (hypoxia)*
Median	144	145.5	170	165
Minimum	69	78	91	62
Maximum	247	303	381	360

Table 4b. ***Primary CFU assay results: CFU-GM descriptive statistics (n = 6)***

CFU-GM	*Baseline (normoxia)*	*10min post(normoxia)*	*Baseline (hypoxia)*	*10 min post (hypoxia)*
Median	18	24.5	17	17
Minimum	10	7	12	10
Maximum	72	35	24	23

Table 4a, 4b

3.1.7 Secondary CFU assays

"Under normoxic conditions, secondary colony forming units showed a significant decline in proliferation capacity 10 min after physical exertion" (one-tailed paired t-test, $p < 0.05$, 1-β (power) = 0.85, Fig. 9)…"Under hypoxic conditions only a trend was seen ($p = 0.07$). As hypoxia had no additional effect (Friedman's test, $p > 0.05$), values were pooled (n = 12), which also resulted in a significant decline of proliferative capacity 10 min post exercise."[25] ($p < 0.05$, Fig. 9).

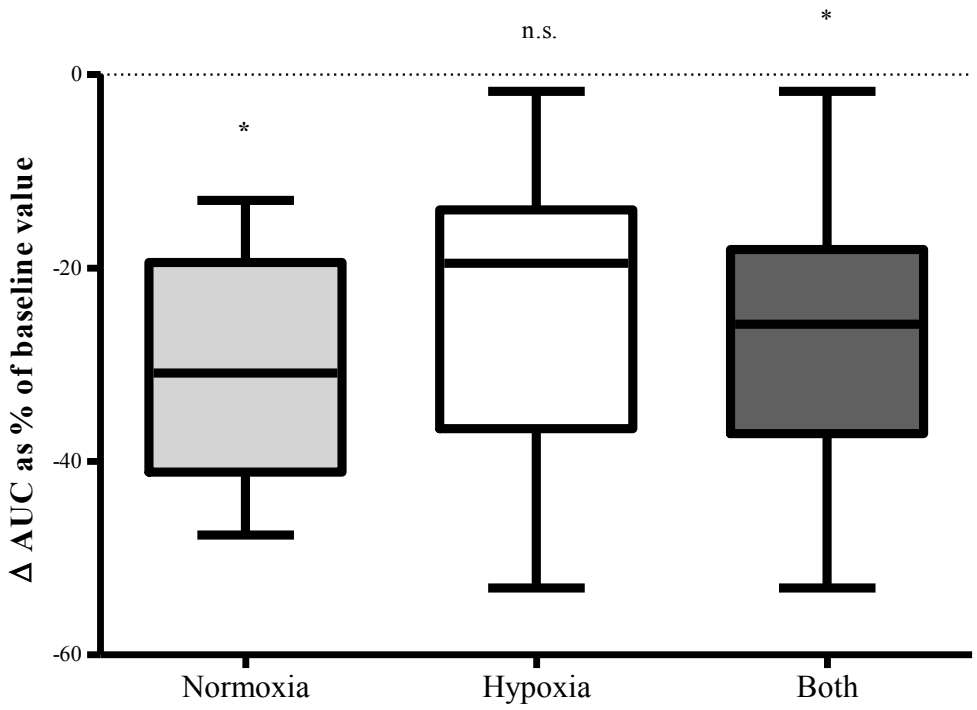

Figure 9: "Box-plot statistics of the change in functionality/proliferative capacity 10 min after cessation of exercise for normoxia, hypoxia and all values pooled; data are expressed as Area Under the Curve (AUC). Secondary colony forming units were significantly decreased 10 min post exercise under normoxic conditions (* $p < 0.05$), a trend was seen under hypoxic conditions ($p = 0.07$), and significantly decreased as pooled values (both, * $p < 0.05$). "[25]. Normoxia, Hypoxia: n = 6, Both: n = 12

3.1.8 Kinetics of oxidative stress parameters

"Parameters of oxidative stress showed a dynamic pattern similar to that of circulating hematopoietic progenitor cells" (Fig. 8, 10, 11)…"Under normoxia, MDA rose significantly above baseline values 10 min after the intervention ($p < 0.05$) and dropped significantly below baseline 60 and 120 min post exercise." (Fig. 10, $p < 0.01$)…"There was a significant decrease 30 min, 60 min and 120 min post exercise in comparison to the peak value 10 min post exercise ($p < 0.01$). Exercise under hypoxic conditions showed the same results, except with no significant increase 10 min after the intervention due to relatively high baseline values. Sixty and 120 minutes post exercise there was a significant difference from the peak value 10 min post exercise ($p < 0.05$)."[25]. MPO only showed a significant difference from baseline values 10 min after physical exertion under hypoxic conditions (Fig. 11, $p < 0.01$) as well as a significant decrease 30 min post exercise in comparison to the peak value 10 min postexercise ($p < 0.01$).

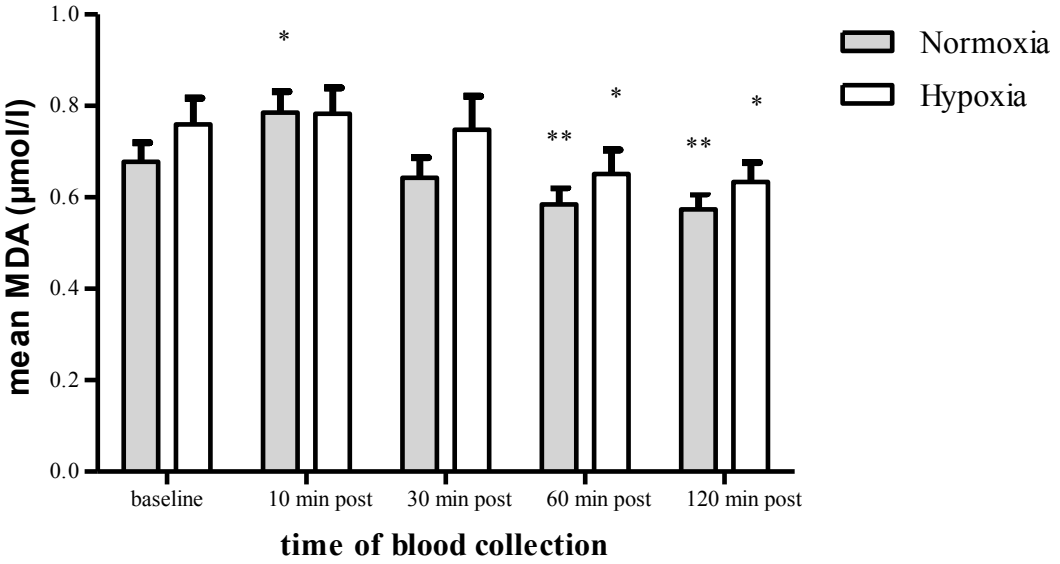

Figure 10: "Malondialdehyde (MDA) kinetics before and after the maximal incremental exercise intervention for normoxia and hypoxia; time of blood collection: baseline (1), 10 min (2), 30 min (3), 60 min (4) and 120 min (5) after the intervention. Data are reported as means ± SEM. Malondialdehyde kinetics are similar to those of CPCs, which suggests a contemporaneous trigger effect of Malondialdehyde levels on the release of CPCs. There were significant differences for both normoxia and hypoxia at different time points in comparison to baseline values (* $p < 0.05$, ** $p < 0.01$)."[25]

"There was no significant difference between the releases of MDA/MPO levels under normoxic vs. hypoxic conditions at all times of blood collection ($p > 0.05$)."[25]

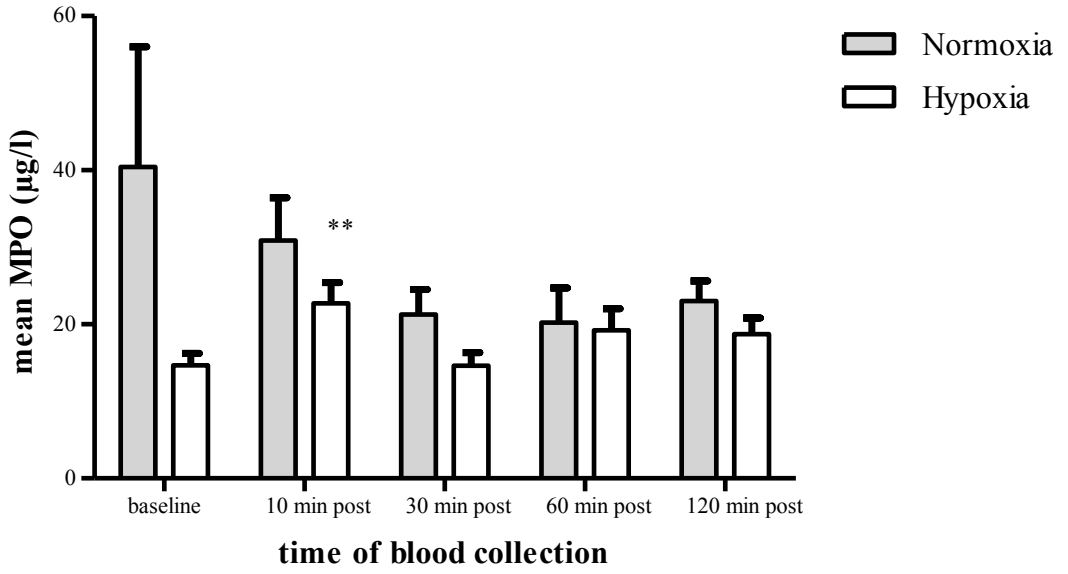

Figure 11: "Myeloperoxidase (MPO) kinetics before and after the maximal incremental exercise intervention for normoxia and hypoxia; time of blood collection: baseline (1), 10 min (2), 30 min (3), 60 min (4) and 120 min (5) after the intervention. Data are reported as means ± SEM. The high SEM of the baseline mean under normoxic conditions can be traced back to two very high sampling values at this time point, probably due to underlying short-term inflammatory processes. Myeloperoxidase kinetics are similar to those of circulating hematopoietic progenitor cells (CPCs), which also suggests a contemporaneous trigger effect of Myeloperoxidase levels on the release of CPCs. There was a significant difference 10 min post exercise compared to baseline values (** $p < 0.01$) for hypoxic conditions."[25]

3.1.9 Relationship of CPC release to oxidative stress values

"A correlation of released CPCs for all 10 subjects between sampling time points and the respective MDA/MPO differences resulted in significant outcomes for normoxia ($r = 0.389/0.484$; $p < 0.05/p < 0.01$) and hypoxia ($r = 0.326/0.427$; $p < 0.05/p < 0.01$)"[25], ($n = 40$: 10 subjects, 4 time-differences, Fig. 12-15).

Normoxia:

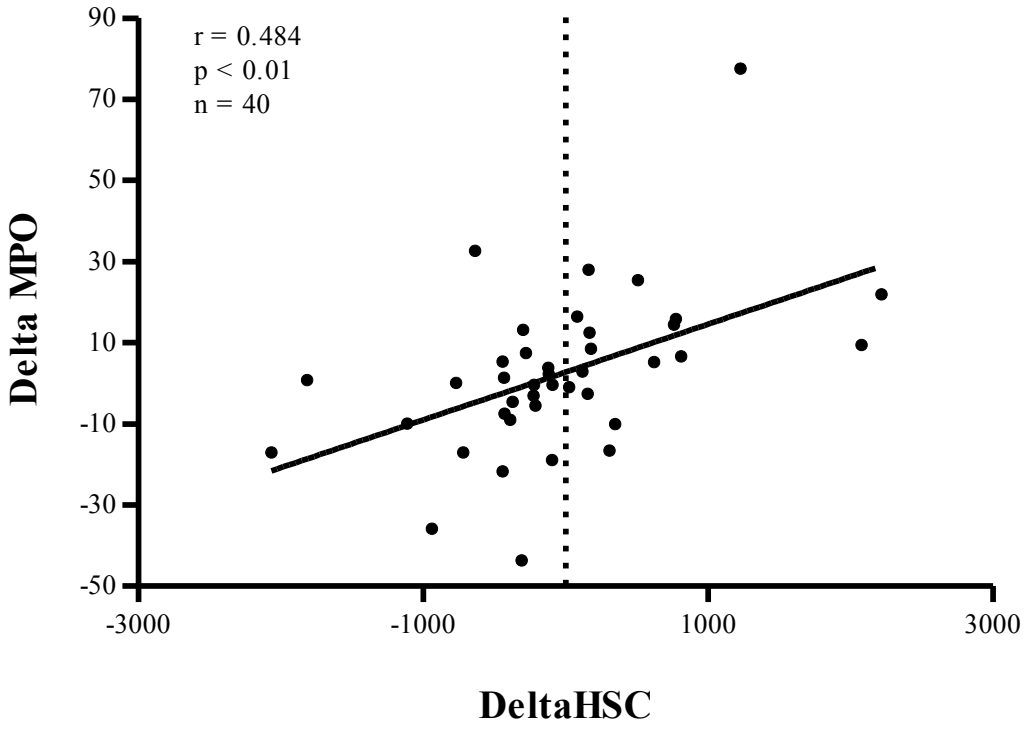

Figure 12: Correlations of released circulating hematopoietic progenitor cells (CPCs) and respective differences in myeloperoxidase (MPO) are shown under normoxic conditions. Data are represented in a cumulative way, where correlations are done for all 10 subjects at all 4 time differences ($n = 40$). There were significant positive correlations of released CPCs and the respective MPO differences for both normoxic and hypoxic interventions, modified from Kroepfl and Pekovits et al. (2012)[25].

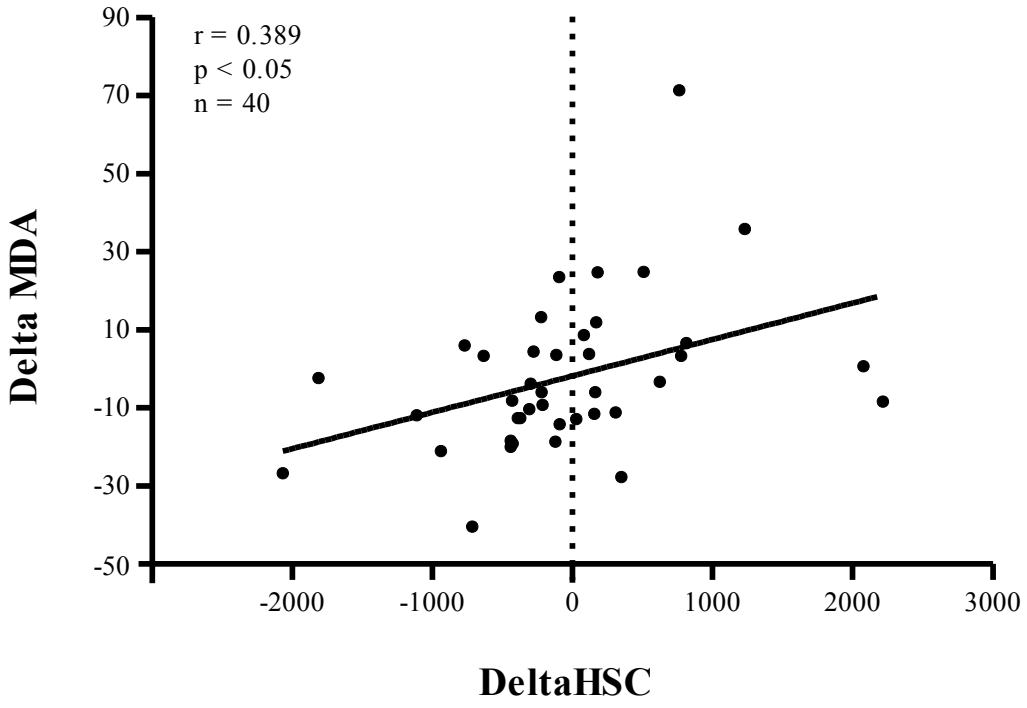

Figure 13: Correlations of released circulating hematopoietic progenitor cells (CPCs) and respective differences in malondialdehyd (MDA) are shown under normoxic conditions. Data are represented in a cumulative way, where correlations are done for all 10 subjects at all 4 time differences (n = 40). There were significant positive correlations of released CPCs and the respective MDA differences for both normoxic and hypoxic interventions, modified from Kroepfl and Pekovits et al. (2012)[25].

Hypoxia:

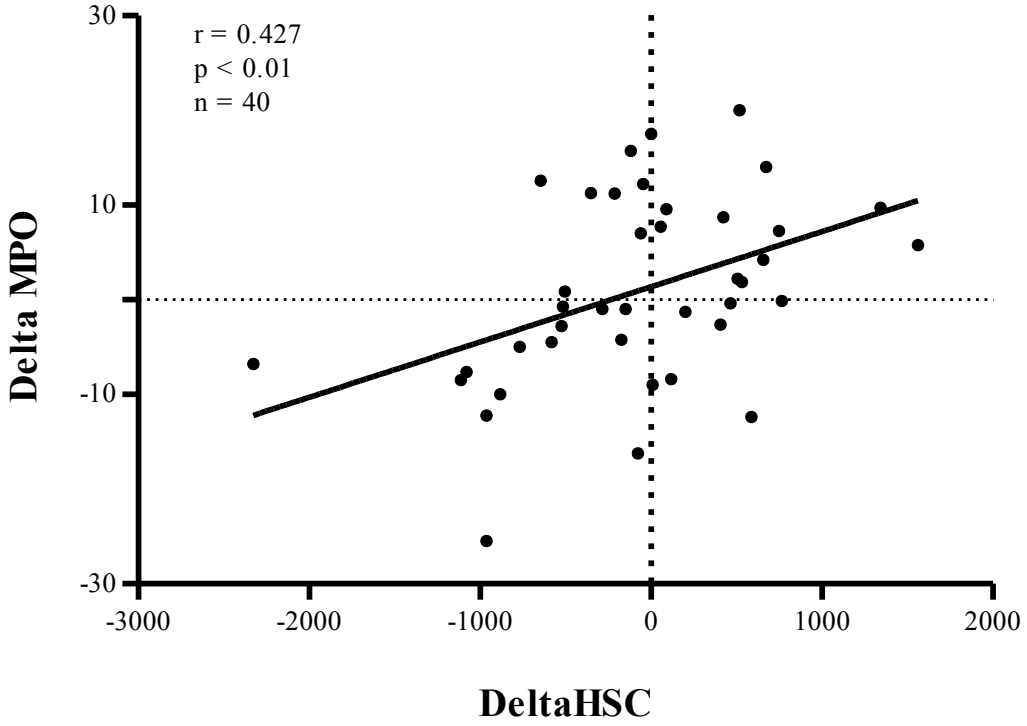

Figure 14: Correlations of released circulating hematopoietic progenitor cells (CPCs) and respective differences in myeloperoxidase (MPO) are shown under hypoxic conditions. Data are represented in a cumulative way, where correlations are done for all 10 subjects at all 4 time differences (n = 40). There were significant positive correlations of released CPCs and the respective MPO differences for both normoxic and hypoxic interventions, modified from Kroepfl and Pekovits et al. (2012)[25].

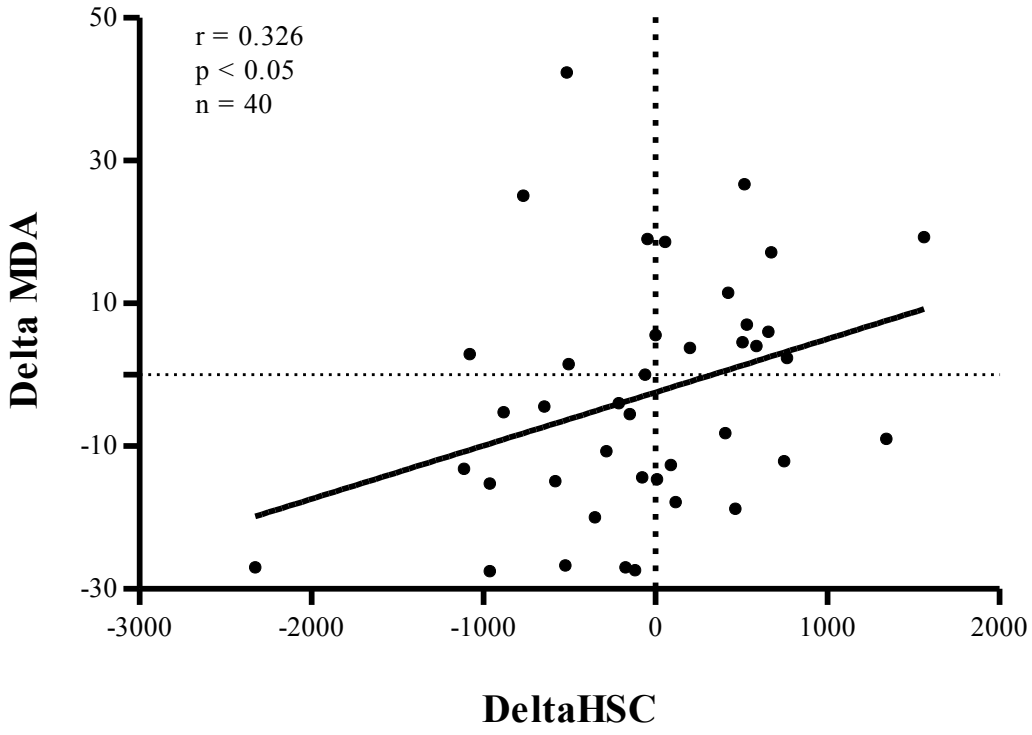

Figure 15: Correlations of released circulating hematopoietic progenitor cells (CPCs) and respective differences in malondialdehyd (MDA) are shown under hypoxic conditions. Data are represented in a cumulative way, where correlations are done for all 10 subjects at all 4 time differences (n = 40). There were significant positive correlations of released CPCs and the respective MDA differences for both normoxic and hypoxic interventions, modified from Kroepfl and Pekovits et al. (2012)[25].

3.1.10 Inflammatory parameter kinetics (interleukin-6)

Interleukin-6 (IL-6) levels showed different kinetics as observed by Moebius-Winkler et al. (2009) under normoxic conditions[17] (Fig.16). In our study, after a time delayed onset (until 10 min postexercise), IL-6 levels started to rise significantly above baseline values after 30 min postexercise ($p < 0.01$) reaching its peak-point at 120 min postexercise ($p < 0.05$). In the Moebius-Winkler study 120 min postexercise IL-6 levels had already begun to drop and returned to baseline 24 h postexercise. Significant correlations were detected for IL-6 levels with platelets ($r = 0.328$, $p < 0.05$) and neutrophils ($r = 0.365$, $p < 0.01$). Hypoxic conditions revealed a significant rise of IL-6 levels already 10 min postexercise ($p < 0.01$), also reaching its peak-point after 120 min postexercise (Fig. 16, not significant). Correlations were similar as under normoxia (platelets: $r = 0.345$, $p < 0.05$; neutrophils: $r = 0.487$, $p < 0.01$), except for lymphocytes ($r = - 0.301$, $p < 0.05$). There was no significant difference between the releases of IL-6 levels under normoxic vs. hypoxic conditions at all times of blood collection ($p > 0.05$).

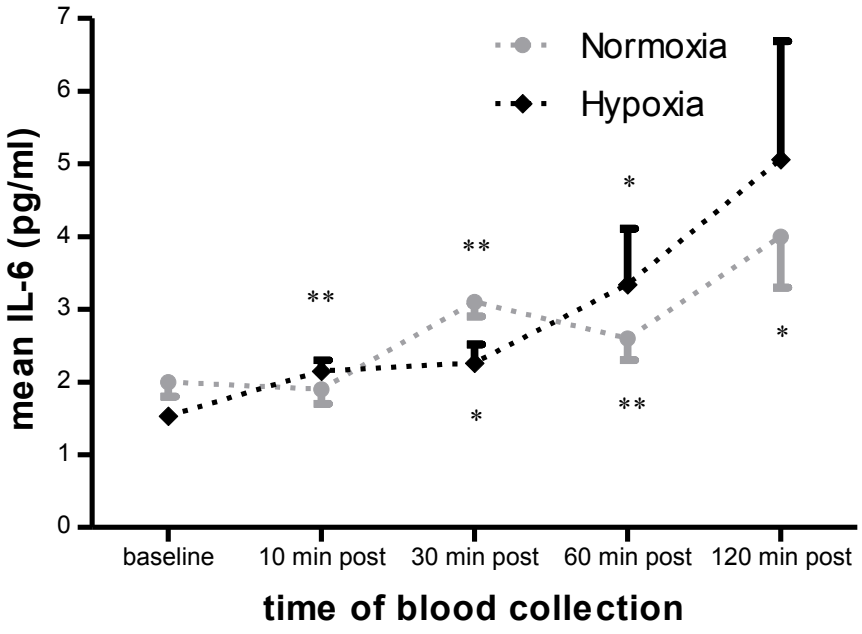

Figure 16: IL-6 kinetics in the peripheral blood before and after an ergometry: time of blood collection: baseline (1), 10 min (2), 30 min (3), 60 min (4) and 120 min (5) after the intervention. Data are reported as means ± SEM. The increase in IL-6 shows time-delayed kinetics as the one of CPCs. There were significant differences at all time points postexercise compared to baseline values (* $p < 0.05$, ** $p < 0.01$).

3.1.11 Cortisol and norepinephrine kinetics

Under normoxic as well as hypoxic conditions plasma cortisol levels showed a similar pattern to the kinetics of CPCs, increasing significantly 10 min postexercise and dropping significantly below baseline values 120 min postexercise ($p < 0.01$, Fig.17). Significant correlations were visible for plasma cortisol and red blood cells ($r = 0.474$, $p < 0.01$) and platelets ($r = 0.344$, $p < 0.05$) under normoxia. There was also a significant relationship between released cortisol levels and the difference in CPCs between time-points for normoxic conditions ($r = 0.374$, $p < 0.05$). Under hypoxia cortisol levels significantly correlated with white blood cells ($r = 0.312$, $p < 0.05$), red blood cells ($r = 0.473$, $p < 0.01$) and lymphocytes ($r = 0.420$, $p < 0.01$). There was no significant difference between the releases of cortisol levels under normoxic vs. hypoxic conditions at all times of blood collection ($p > 0.05$)

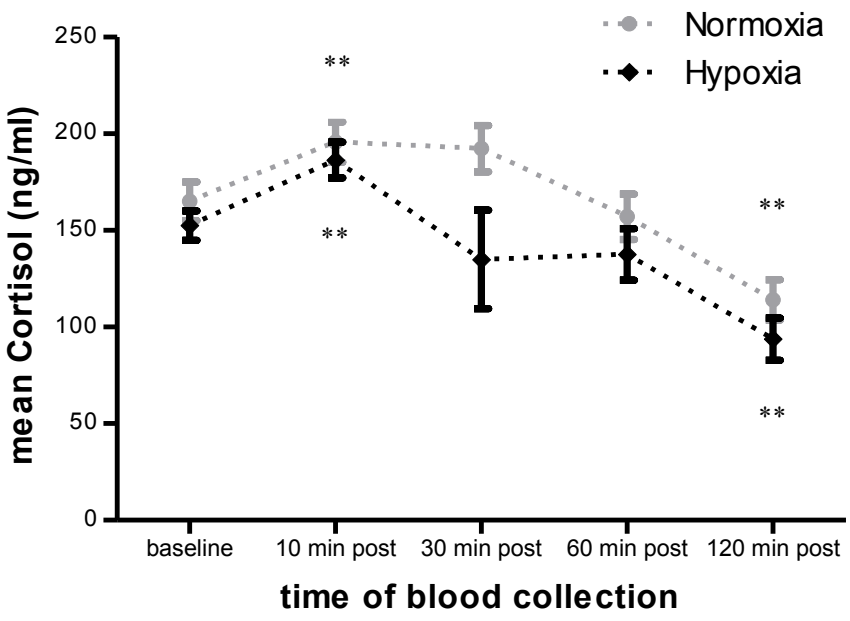

Figure 17: Cortisol kinetics in the peripheral blood before and after an ergometry; time of blood collection: baseline (1), 10 min (2), 30 min (3), 60 min (4) and 120 min (5) after the intervention. Data are reported as means ± SEM. Plasma cortisol follows the same patterns as CPC kinetics in the circulation which may suggest a contemporaneous trigger effect on CPC mobilization.There were significant differences 10 min and 120 min postexercise compared to baseline values (** $p < 0.01$).

Free norepinephrine (free NE) levels showed a significant 10-fold rise directly after cessation of exercise under normoxic as well as hypoxic conditions, whereas 10 min

postexercise levels were only 2.5 fold increased under normoxia ($p < 0.05$) and almost disappeared under hypoxia ($p < 0.001$). Bound norepinephrine (bound NE) levels significantly increased directly after the intervention under normoxic conditions ($p < 0.05$) and did not significantly change under hypoxic conditions (Table 5a, 5b).

Table 5a. ***Free norepinephrine levels***

Free NE	*Baseline (normoxia)*	*Directly after (normoxia)*	*10 min post (normoxia)*
Mean	800.42	8198.71 ***	2006.27 *
± SEM	± 94.81	± 1095.17	± 399.60
N	10	10	8
	Baseline (hypoxia)	*Directly after (hypoxia)*	*10 min post (hypoxia)*
Mean	850.33	7794.90 ***	27.38 ***
± SEM	± 80.53	± 887.08	± 7.30
N	10	10	8

Significant differences between time points (ANOVA with Fisher's LSD) are indicated as follows: $^{***}p < 0.001$, $^{*}p < 0.05$

Table 5b.*Bound norepinephrine levels*

Bound NE	*Baseline (normoxia)*	*Directly after (normoxia)*	*10 min post (normoxia)*
Mean	2225.61	2631.93 *	3020.85
± SEM	± 667.50	± 688.19	± 899.49
N	10	10	8
	Baseline (hypoxia)	*Directly after (hypoxia)*	*10 min post (hypoxia)*
Mean	2363.82	2323.20	2195.23
± SEM	± 562.20	± 663.32	± 526.72
N	10	10	8

Significant differences between time points (ANOVA with Fisher's LSD) are indicated as follows: $^{***}p < 0.001$, $^{*}p < 0.05$

Table5a, 5b: NE kinetics

Significant correlations between free norepinephrine levels to CPC absolute counts were observed (NE sampled directly after cessation of exercise, CPCs sampled 10 min postexercise; normoxia/hypoxia: $r = 0.663$/ $r = 0.592$, $p < 0.01$, $n = 20$). There was no significant difference between the releases of neither free nor bound NE levels under normoxic vs. hypoxic conditions at all times of blood collection ($p > 0.05$).

3.1.12 Influence of norepinephrine and lactate on HSPC functionality

The influence of the exercise-induced stress parameters norepinephrine (NE) and maximal blood lactate concentration (La_{max}) were evaluated *in vitro*. The *in vivo* study design was modeled by incubating isolated mononuclear cells from a randomly chosen subgroup of six subjects with both stress parameters separately and together. Stress parameter concentrations were taken from the *in vivo* study results (free NE: $5*10^{-9}$ ng/ml baseline, $5*10^{-8}$ ng/ml directly after an ergometry; La: 0.01 mmol/l baseline, 12 mmol/l directly after an ergometry). Circulating hematopoietic progenitor cells incubated with a NE concentration of 5×10^{-8} mol/l showed a significant decrease in CPC functionality *in vitro* ($p < 0.05$) compared to baseline. A La concentration simulating baseline conditions did not show any significant influence on the functionality of CPCs. Simultaneously adding both substances at an ergometry-induced concentration to the cell culture, a trend was visible ($p = 0.08$, Fig. 18).

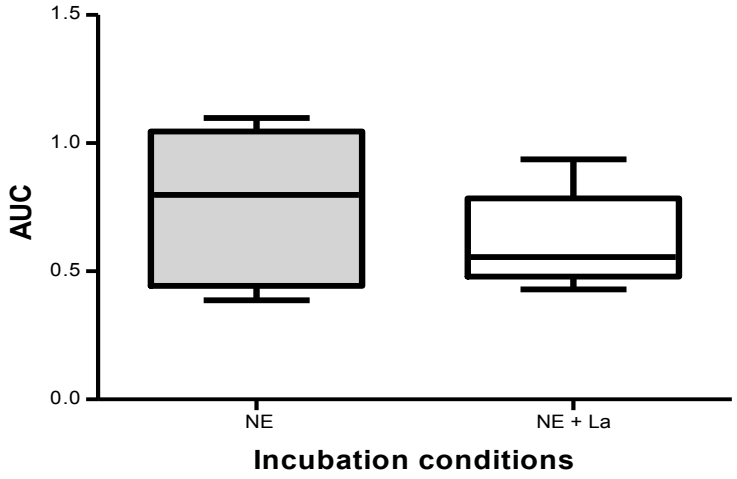

Figure 18: Box-plot statistics (n = 6) of the influence of norepinephrine and blood lactate concentration on CPC functionality of the *in vitro* study results; data are expressed as Area Under the Curve (AUC). Secondary colony forming units were not significantly decreased in cell culture under the influence of both stress parameters at the same time, but a definite decreasing trend could be detected ($p = 0.08$).

3.2 Results of study II

Study II revealed the following result:

3.2.1 Influence of blood lactate on HSPC mobilization

The results of study II showed a significant positive correlation ($r = 0.63$, $p < 0.01$) between the difference in HSPCs in the peripheral blood and the maximal blood lactate concentration (La_{max}) accumulated during each test independent of the exercise mode (Fig. 19). This suggests a direct influence of La_{max} on HSPC release from the bone marrow.

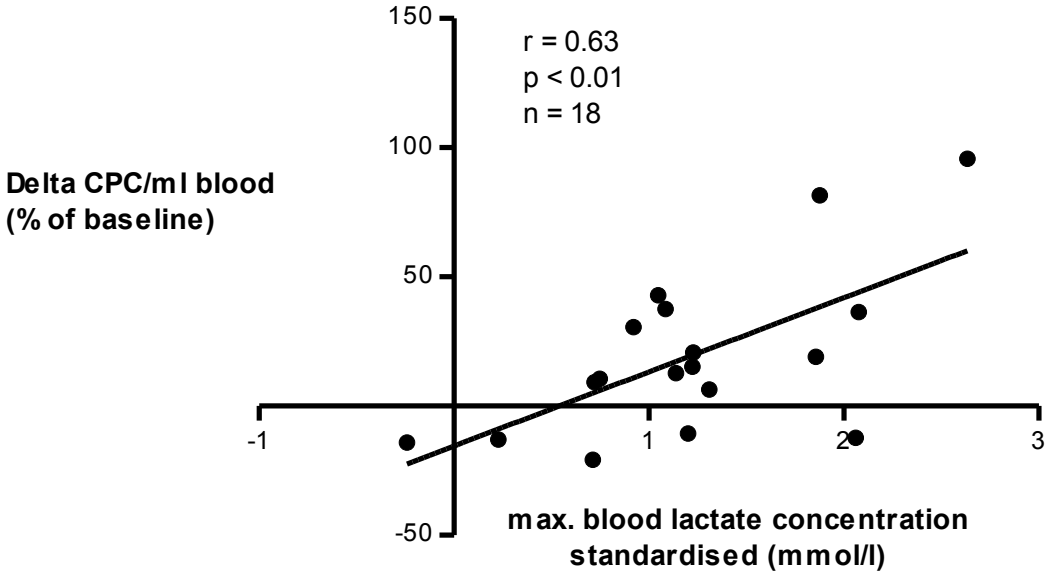

Figure 19: Relationship of the release of circulating hematopoietic progenitor cells in the peripheral blood and the maximal accumulated blood lactate concentration. Maximal blood lactate was standardized in order to get rid of the interaction of the subject variable; modified according to Kroepfl et al. (2012)[41].

4 Discussion

The main result of study I is that ergometry increases circulating progenitor cell frequency twofold 10 min post exercise, but at the same time reduces hematopoietic colony forming capacity.

A. HSPC kinetics before/after exercise

"Little information on kinetics of circulating progenitor cell release can be found in the literature. Moebius-Winkler et al.[17] stated in 2009 that endurance exercise of 4 hours cycling led to significant changes in circulating progenitor cells during exercise with a maximum at 210 min after the onset. The pattern of progenitor cell kinetics after the intervention differed from our results. In the Moebius-Winkler study, blood was not collected 10 min post exercise, and 30 min post exercise the stem cell level had already diminished, but values did not return to baseline. In contrast, our results showed a peak release of CD34+/CD45dim cells at 10 min after short-term incremental exercise, where values are already back to baseline 30 min after the cessation of exercise (at this time point there was also a significant decrease to the peak value 10 min post exercise). Furthermore, 120 min post exercise under normoxic conditions, we saw a significant decrease in the progenitor cell level, unlike the Moebius-Winkler group. These findings indicate a different fate of circulating progenitor cells during endurance type and short-term high intensity exercise. Similar to our findings, Bonsignore et al.[23] reported a different response of hematopoietic progenitors to endurance and maximal exercise."[25]

B. Influence of hypoxia on HSPC mobilization

Under hypoxic conditions there was also a significant twofold rise of CPCs visible 10 min post exercise and values returned back to baseline 120 min after cessation of exercise. "Hypoxia at moderate altitude does not seem to have a significantly different effect on progenitor cell release than exercise under normoxic conditions until 60 min post exercise. Regarding the last time point of blood collection (120 min post exercise), it can be hypothesized that homing dynamics might be different under normoxic and hypoxic conditions, as suggested by Lekli et al. in 2009[42]. Progenitors could also migrate from the peripheral blood into surrounding tissues, possibly for repair processes[43, 44]. Moreover, the CD34+/CD45bright cell fraction, which shows light-scatter properties that are consistent with monocytes[45], significantly increased under hypoxia 10 min post exercise (Table 3b)."…"Within the study-specific observation time, our findings also suggest that short-term hypoxia at moderate altitude does not have any additional forcing effect on CPC release."[25] In addition, one has to consider the time-delayed effect of hypoxia at moderate altitudes on EPO production and hematopoiesis. "According to Bärtsch et al. 2008[46] even high altitudes show a delayed effect on erythropoiesis. In addition, intermittent hypoxia at 12-10 % O_2 did not show any effect on EPO production[47]. Katayama et al. (2004)[48] reports that even 3h at 12.3% O_2 daily over 14 days did not show any effect on EPO in plasma."[25]

C. HSPC functionality before/after exercise

"The most interesting finding is the significant decrease of the proliferative capacity expressed as AUC values in the secondary colony forming unit (CFU-GM) assay. Although the frequency of CD34+/CD45dim cells in the flow cytometry analysis increases significantly after ergometry, the functionality/proliferative capacity of CPCs in the peripheral blood decreases. Moreover, the frequency of colonies in the CFU assay with EPO showed no significant increase, which also suggests that, the functionality/proliferative capacity of CPCs decreased significantly 10 min after cessation of exercise. Different outcomes, however, were detected after an endurance exercise model in mice[49], where BFU-E and CFU-GM counts significantly increased in exercise-trained animals vs. sedentary controls. Our group also found a significant increase in the proliferative capacity of bone marrow derived HSCs/HPCs after a life-long voluntary exercise versus sedentary housing condition in a rat model[24]. This additionally suggests a different response of HSC/HPC behavior to endurance and maximal exercise. As far as we know, this is the first study to turn attention to the importance of the functionality/proliferative capacity of CPCs in association with cell frequency and exercise. Our results, as described above, lead to multiple hypotheses. Obviously, the proliferative capacity of hematopoietic progenitor cells in the peripheral blood 10 min post exercise is significantly reduced compared to baseline values. On the one hand this suggests that the cells present are more differentiated progenitors[50] and have already been decreased in their proliferative capacity (shown by secondary CFU-GM expressed as AUC)[24, 34]. The measure of CPC functionality by a secondary CFU-GM assay gives only information of the impairment of white progenitor cells. On the other hand, this raises the question whether progenitor release is triggered by increased shear stress in the vascular intraepithelial layer in blood vessels outside of the bone marrow, where CPCs could just be detached from the vessel walls due to the increased blood flow and not directly come from the bone marrow itself[51]. Since the CD34+CD45dim cell fraction also contains circulating endothelial progenitor cells[52], it is to discuss whether the obvious discrepancy between the increase in CPCs and obtained colony counts is due to exaggerated mobilization of endothelial but not hematopoietic progenitors. According to the manufacturer, the MethoCult® culture medium does not support the growth of endothelial progenitor cells. Another hypothesis

might be that only cells near to the sinusoids in the bone marrow are mobilized; unlike the ones in the far end of the bone marrow cavity, these do not have very efficient repopulating activity, as summarized by Cross et al. in 2009[53].”[25]

D. Influence of oxidative stress parameters (MDA, MPO) on HSPC mobilization

“Exercise-induced regeneration might home younger progenitors from the peripheral blood to surrounding tissues where they are needed for repair and substitution processes[54]. In this context, oxidative stress might also play an important role, because it has already been linked to disrupted signal transduction pathways[55]. A rise of oxidative stress markers, as seen in our study, may also lead to a decrease of secondary colony forming units in murine HSCs/HPCs[34]. In 2010 Wang and Lin[56] showed that systemic hypoxia can promote lymphocyte apoptosis induced by oxidative stress during moderate exercise. This raises the question whether apoptosis of circulating CPCs could also be induced by oxidative stress triggered by incremental exercise. In this case, our results may suggest that exercise on its own has an influence on apoptotic processes due to oxidative stress (significantly reduced proliferative capacity 10 min post under normoxia). The results of our study also provide evidence that hematopoietic progenitor cell mobilization might be triggered by a contemporaneous induction of oxidative stress as indicated by elevated malondialdehyde (MDA) or myeloperoxidase (MPO) levels due to physical exercise. There was a significant positive correlation between the differences in CPCs in the peripheral blood and releases in MDA/MPO plasma levels for both normoxia and hypoxia. It is well known that ROS act as second messenger molecules but also have the potential to damage cellular structures by oxidation[57, 58]. In the case of HSCs/HPCs the influence of ROS resulted in a dose-dependent decrease of colony formation due to a p38/mitogen-activated protein kinase (MAPK) dependent pathway[34]. This pathway is of important physiological relevance, because ROS have a great influence on apoptosis, self-renewal, senescence, proliferation and differentiation of hematopoietic and endothelial progenitor cells, as documented in the work of Case et al. (2008)[59]. Furthermore, a restriction of the self-renewal capacity of HSCs due to an accumulation of oxidative DNA damage was reported in the work of Yahata et al. (2011)[60]. Regarding the HSC/HPC release from the bone marrow, Hosokawa et al. (2007) found that the N-cadherin-mediated cell adhesion is suppressed by ROS, which

results in an exit of HSCs from the bone marrow niche[61]. This finding supports our fairly weak but significant correlations between the difference in MDA/MPO levels and CPC counts."[25]

E. Influence of inflammatory parameters (IL-6) on HSPC mobilization

The importance of Interleukin-6 (IL-6) in context of hematopoietic progenitor cell mobilization has not yet been very well researched. IL-6 is usually associated with endothelial progenitor cell number modulation, playing a role in acute inflammatory processes as well as angiogenesis and vascular remodeling[62, 63]. Studies investigating the influence of exercise on IL-6 plasma levelsand hematopoietic stem and progenitor cell mobilization are discordant regarding their results. A 1000m all-out rowing intervention did not significantly change IL-6 plasma levels[14], whereas 4h cycling at 70 % of the individual anaerobic threshold increased IL-6 levels 16.5 fold[17]. A marathon revealed a significant rise of IL-6 levels at the end[12]. CD34+ cell number, however, increased after all the named interventions. These results show that different exercise protocols have unequal impact on the pro-inflammatory marker IL-6 with a simultaneous increase in hematopoietic progenitors. This suggests an indirect effect of IL-6 on the fate of hematopoietic progenitors, possibly triggered by a neural activation of bone marrow stromal cells, which in turn actives ERK and p38-MAPK signaling as suggested by Rezaee et al. in 2010[64]. The activation of MAPK signaling was also found by Schraml et al. (2009) in a mouse model[34], where an influence of a noradrenergic stimulus on HSPC functionality was seen. It is also suggested that an elevation of IL-6 is regulated by central and peripheral catecholamines[65, 66] and could therefore also have an impact on HSPC mobilization (see Section F). Furthermore our study also showed the significant involvement of IL-6 in known neutrophil activation (under normoxic as well as hypoxic conditions) and T-lymphocyte regulation (only under additional hypoxic stress).

F. Influence of stress hormones (cortisol, norepinephrine) on HSPC mobilization

An exhaustive ergometric strain significantly increased cortisol levels 10 min after cessation of exercise and significantly decreased them below baseline values 120 min post exercise under normoxic as well as hypoxic conditions. Cortisol kinetics followed CPC kinetics in the peripheral blood, both reaching their peak-points 10 min post exercise (Fig. 8, Fig. 17). This suggested a contemporaneous trigger effect of cortisol on hematopoietic stem and progenitor cells, which was supported by the significant positive relationship between time-points of the two parameters. According to Dimitrov et al. (2009)[67], CXCR4 up-regulation on T-cell subsets followed cortisol infusion in young, healthy, male subjects. It is to discuss if cortisol might also induce the expression of CXCR4 on hematopoietic stem and progenitor cellsand in turn might lead to homing processes.

Catecholamines, in contrast to cortisol, act by recruiting immune cells to the peripheral circulation[68]. In our study, free norepinephrine (free NE) levels were 10-fold elevated directly after the exercise intervention and highly significantly correlated with CPC number in the peripheral blood before and after exercise (NE sampled directly after cessation of exercise, CPCs sampled 10 min postexercise), which indicated a noradrenergic effect on CPCs under normoxic as well as hypoxic conditions. A postexercise rise of NE has already been described in the literature[69], but it has not yet been frequently connected to hematopoietic progenitor cell stimulation. According to Benschop et al. (1996) and Dar et al. (2011) increased catecholamines resulted in elevated leucocytes[68] and hematopoietic stem and progenitor cells[70] in the peripheral blood. Furthermore, mice showing a reduced production of NE failed to respond to HSPC mobilization by G-CSF[71]. As recently shown, circadian HSPC release from the bone marrow follows rhythmic secretion of norepinephrine from nerve terminals which can be seen as a contributing mechanism to HSPC egress[72]. The impact of catecholamines on cells present in the bone marrow, progenitor mobilization, and erythropoiesis may be regulated by the dose and duration of the neurotransmitter stimulus[71, 73]. All these findings support our hypothesis that hematopietic stem and progenitor cell modulation could be triggered by catecholamines.

Since exercise activates the sympathetic nervous system and the hypothalamic-pituiary-adrenal (HPA) axis, exercise can be seen as a stress model (Fig. 22). Taken the effect of NE and cortisol together, it would result in an elevated mobilization of hematopoietic stem and progenitor cells to the circulation by elevated NE levels, where at the same time elevated cortisol levels may affect the hematopoietic component of the bone marrow microenvironment through Notch signaling[74, 75]. It is left to discuss if this mechanism might lead to CPC homing processes and could explain the increased CPC number and reduced progenitor cell functionality found in our study.

G. Influence of norepinephrine and lactate on HSPC functionality

In a pilot study[76] as well as after an ultra-distance race[77], we found a significant relationship between HSPC release and maximal blood lactate values. This result formed the hypothesis that both exercise-induced physical stress parameters could have an influence on progenitor cell number and functionality. In study I the influence of NE and/or lactate on cell functionality was determined *in vitro*.

NE levels equivalent to exhausting exercise significantly reduced CPC functionality *in vitro*, besides an already suggested influence on CPC mobilization (see Section H). To evaluate a possible direct effect, La + NE were added to CPCs in cell culture *in vitro*. Norepinephrine alone showed a significantly reduced CPC functionality with concentrations found after exercise, whereas a physiological dose didnot have a significant impact on CPC functionality. This underlines a possible direct influence on CPC functionality besides known mobilization effects. A possible indirect trigger function on CPC modulation, however, cannot be excluded. Ye et al. (1998) described lactate uptake into a cell using a rat hindlimb model[78]. The same authors found that lactate uptake by the skeletal muscle cell was inhibited by arterial noradrenaline (norepinephrine) possibly related to its vasoconstrictive action. Besides glucose, lactate represents one of a cell's important energy sources. When lactate uptake into a cell is inhibited, the cell could react by up-regulating the enzyme lactate-dehydrogenase (LDH) in order to produce lactate itself. Kumar et al. had the same association in 1980[79]: In the norepinephrine-treated C6 rat glial tumor cell line the synthesis rate of two different lactate dehydrogenase types (LDH-1, LDH-5) significantly increased.

Norepinephrine regulated the expression of both genes for LDH in these cells and the NE induction of LDH was entirely due to an increase in the synthesis of new molecules. Passaquin et al. (1986)[80] also suggested the same hypothesis. In 1987 Kremer et al.[81] described that in homozygous lactate dehydrogenase deficient mice the hemolysis was compensated by anincrease in the total number of late erythroid progenitors. This leads to the question if an enhanced activity of LDH enzymes in a cell has consequences regarding cell status and (stem cell) mobilization patterns. A recent study[82]suggested a significant correlation between LDH activity and peripheral blood CD34+ cell count and Menekay et al. (2002)[83] linked an elevation of LDH to the differentiation of myeloid progenitors. Summarizing these literature results, the following hypothesis regarding the combined influence of norepinephrine and/or blood lactate concentration on CPC functionality arises (Fig. 20):

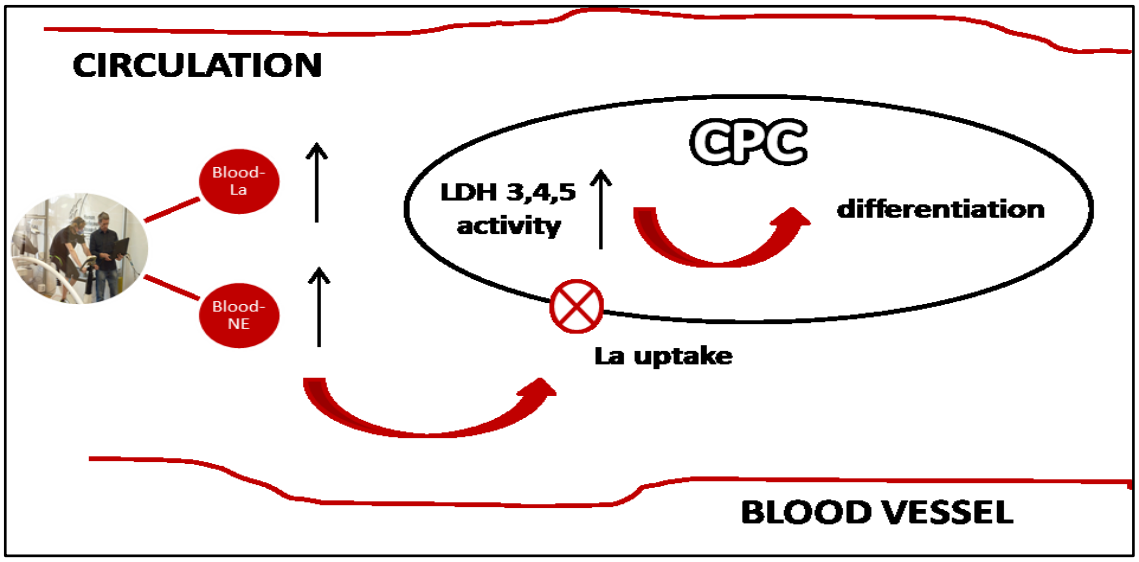

Figure 20: Hypothesis of the underlying biological mechanism of the influence of NE and La on CPC functionality.

H. Influence of blood lactate on HSPC mobilization

The influence of blood lactate concentration on stem cell mobilization has still not been very well investigated so far, especially in heart disease patients. There are only a few studies that link lactate (La) to hematopoietic stem and progenitor mobilization. In the work of Milovanova et al. (2008) the recruitment and differentiation of circulating stem/progenitor cells in subcutaneous Matrigel in mice was assessed. Including a polymer to elevate the lactate concentration in the Matrigel the number of stem and progenitor cells increased by 3.6 fold[84]. Egan et al. (2007) described a strong positive correlation between serum lactate dehydrogenase and stem cell mobilization in patients with hematologic malignancies[85]. Lactate seems to be an important parameter in deciding when to start apharesis and could also be a trigger mechanism for hematopoietic stem and progenitor cells to leave the bone marrow.

4.1 Limitations to study design I

"Only healthy athletic male subjects were used in this study design, which limits the results to a very specific group of people.

Determining cell functionality:

Limiting dilution transplant experiments are the gold-standard regarding the analysis of stem cell quality, but impracticable for this experimental design due to the following simple reason: The frequency of circulating hematopoietic progenitor cells in the human peripheral blood is very low. To reach the required number of $1*10^5$ CD34+ cells for transplantation in a NOD/SCID mouse model[60, 86], a blood withdrawal of 200 ml would be necessary at each time point, which is infeasible and not justifiable.The secondary CFU assay, however, is a measurement of the clonal myeloid proliferative capacity and therefore function of CPCs[21, 24, 34] and only requires a reasonable amount of peripheral blood for the procedure.*"*[25]

4.2 Limitations to study design II

The biggest drawback of this study design is that only the relationship of the number of CPCs and blood lactate concentration in the peripheral blood was analyzed. Underlying pathophysiological mechanisms are still to be elucidated in further studies. Furthermore, it has to be mentioned that the subject group was inhomogeneous regarding the variables sex (1 female participant) and age (range: 54-72 yrs).

5 Conclusion and Outlook

5.1 Summary of the effect of exercise on HSPCs

The underlying biological mechanisms of how exercise acts on circulating hematopoietic stem and progenitor cells are complex and not easy to summarize. All possibly involved mechanisms discussed in this thesis are important when speaking of an ergometry-induced effect on CPCs. High intensity exercise acts as physical stress on the body and elevates stress hormones such as cortisol and norepinephrine (NE) in the peripheral blood. Furthermore, exercise produces oxidative stress and raises parameters associated with free radical oxygen species (ROS) in blood plasma, such as Malondialdehyd (MDA) and Myeloperoxydase (MPO). At the same time an ergometry also triggers a short-term inflammation in the body, which is confirmed by elevated plasma levels of the cytokine interleukin-6 (IL-6). Ergometry-induced physical strain also leads to elevated levels of blood lactate concentration (Fig. 21).

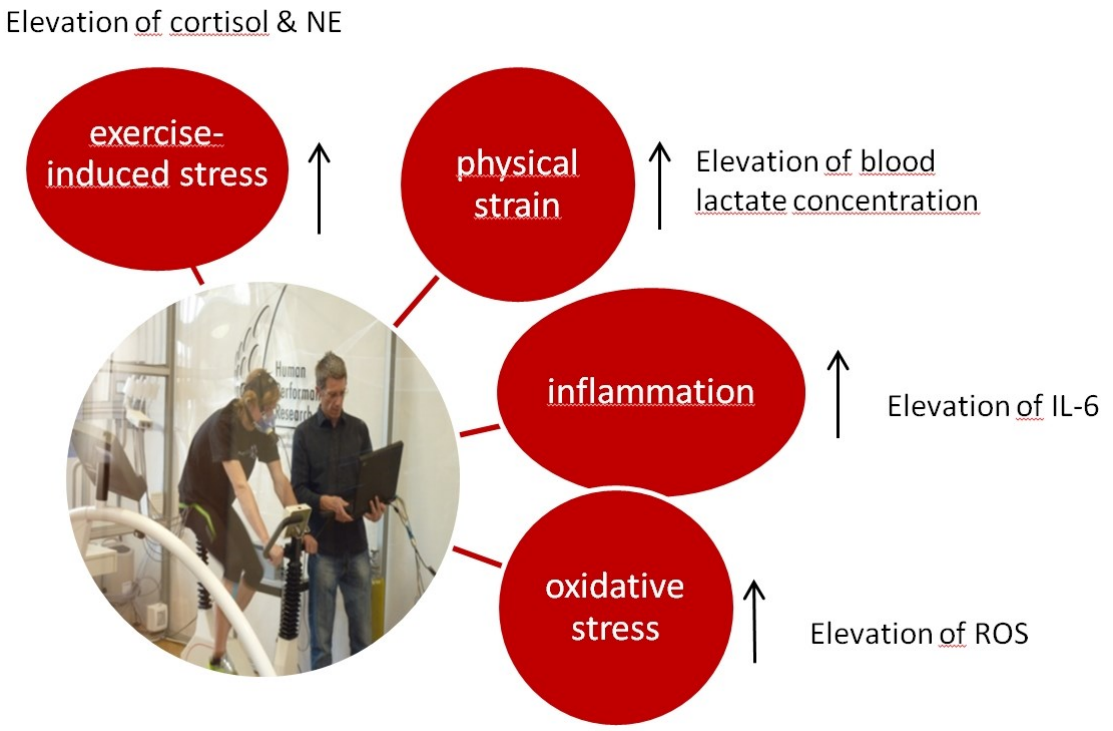

Figure 21: Complex influence of exercise on the body.

All these effects have a contemporaneous impact on the dynamics and functionality of stem and progenitor cells present in the bone marrow and in the circulation. Exercise-induced NE levels triggered from the locus ceruleus/norepinephrine (LC/NE) sympathetic system[69], act on the bone marrow and trigger HSPC mobilization to the peripheral blood. Contrary, elevated cortisol levels triggered from the hypothalamic-pituiary-adrenal axis (HPA), support homing processes from the circulation into the tissue. All these effects taken together could justify the increased mobilization mechanisms of hematopoietic stem and progenitor cellsto the circulation 10 min postexercise, since triggering processes predominate, and homing by cortisol is possibly time-delayed (Fig. 22). In addition, both elevated oxidative stress (ROS) and inflammatory parameters (IL-6) support HSPC mobilization from the bone marrow.

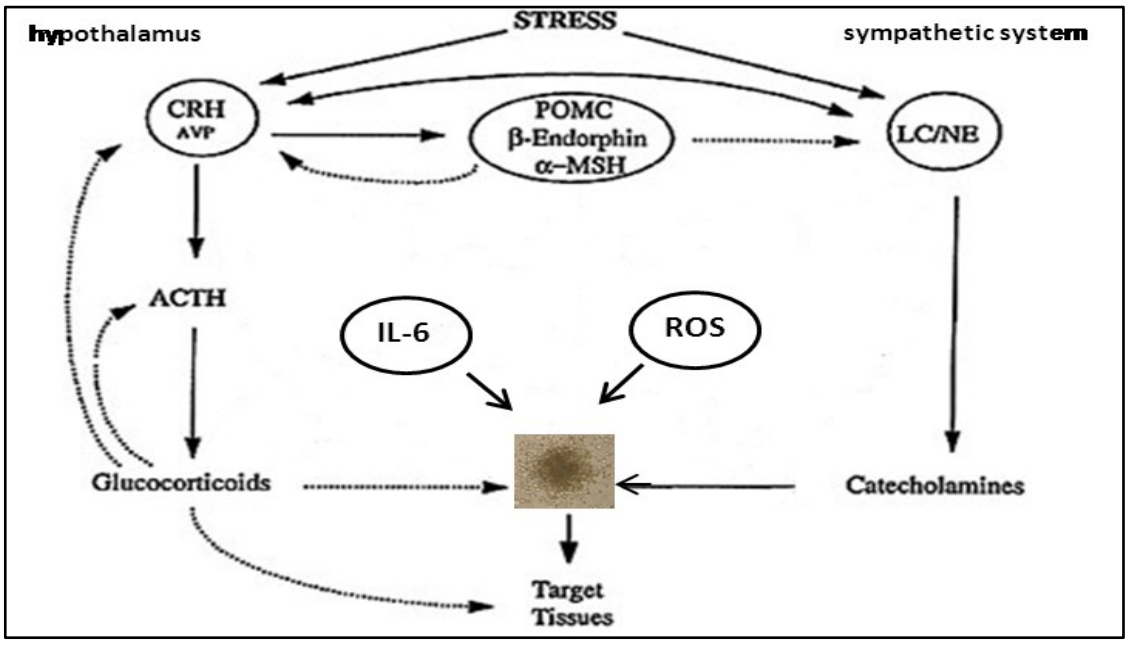

Figure 22: Summary of exercise-induced changes of blood parameters in humans and their influence on CPC number and functionality; modified with permission from Mastorakos et al. (2005)[69]. Exercise-induced stress has an impact on the HPA (hypothalamic-pituiary-adrenal axis) as well as the sympathetic nervous system, which are interrelated by different hormones (POMC, Proopiomelanocortin; β-Endorphin; α-MSH, α-melanozyt stimulating hormone). The end-products glucocorticoids (cortisol) and catecholamines (norepinephrine) act on HSPC mobilization and functionality, as well as does exercise-induced IL-6 and oxidative stress (ROS).

The same parameters that act on HSPC mobilization from the bone marrow could have an impact on cell functionality in the peripheral blood. In this thesis also the impact of NE and La on cell functionality was evaluated and the following hypothesis stated:
An increased level of NE in the peripheral blood inhibits La uptake into the circulating stem or progenitor cell that induces an elevation of the cell's production of lactate dehydrogenase, which in turn drives the cell into differentiation. This is one possible mechanism to explain the reduced cell functionality found 10 min after cessation of ergometry.

5.2 Importance for clinical applications

The certainty that exercise has an impact on circulating hematopoietic stem and progenitor cells in the peripheral blood leads to multiple applications in the fields of regenerative and preventive medicine. First, it seems obvious that an elevated level of circulating hematopoietic stem and progenitor cells in the circulation may be beneficial to support tissue renewal and patient recovery[43, 87]. Exercise has an impact on the bone marrow and releases hematopoietic progenitors to the circulation, ideally to places of tissue defects, where regeneration and cell substitution is needed (Fig. 23). Second, exercise represents a non-invasive method to elevate the amount of CPCs and could therefore be easily integrated in rehabilitation programs. Patients would also profit from the positive side effects of an additional exercise therapy, such as psychological improvement, muscle strengthening or a higher cardiopulmonary capacity. Third, it could be a possibility to ease apheresis procedures for healthy donors by combining the application of stem cell triggering growth factors such as G-CSF with a standardized exercise test protocol, which could possibly reduce the amount of the injected growth factor. It still has to be discussed, however, if stem cells triggered by exercise are "potent" enough to be used in transplantation protocols and the perfect timing for apharesis would also have to be evaluated.

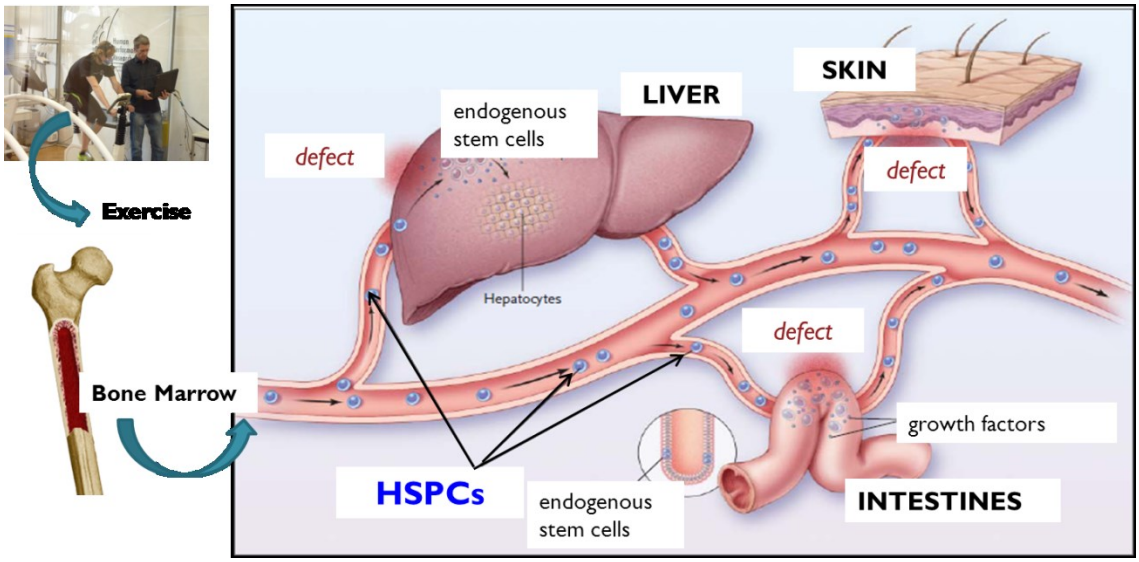

Figure 23: Exercise-induced released HSPCs are led to places of tissue defects to support regeneration processes, modified and reproduced with permission from Koerbling an Estrov et al. (2003)[43], Copyright Massachusetts Medical Society.

Last, but not less important would be the application of CPCs in preventive medicine. Healthy people could keep their stem cell pool in the bone marrow through the right amount of exercise in balance and would benefit from the multiple functions of CPCs for a lifetime[24].

6 References

1. Chute JP, Ross JR, McDonnell DP. Minireview: Nuclear receptors, hematopoiesis, and stem cells. *Mol Endocrinol.* Jan 2010;24(1):1-10.

2. Wognum AW, Eaves AC, Thomas TE. Identification and isolation of hematopoietic stem cells. *Arch Med Res.* Nov-Dec 2003;34(6):461-475.

3. Lancrin C, Sroczynska P, Stephenson C, Allen T, Kouskoff V, Lacaud G. The haemangioblast generates haematopoietic cells through a haemogenic endothelium stage. *Nature.* Feb 12 2009;457(7231):892-895.

4. Mendez-Ferrer S, Michurina TV, Ferraro F, et al. Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. *Nature.* Aug 12 2010;466(7308):829-834.

5. Lanza R, Weissman I, Thomson J, et al. *Handbook of Stem Cells, Two-Volume Set: Volume 1-Embryonic Stem Cells; Volume 2-Adult & Fetal Stem Cells (v. 1)*2004.

6. Li J. Quiescence regulators for hematopoietic stem cell. *Exp Hematol.* May 2011;39(5):511-520.

7. Morrison SJ, Kimble J. Asymmetric and symmetric stem-cell divisions in development and cancer. *Nature.* Jun 29 2006;441(7097):1068-1074.

8. Wahl P, Bloch W, Schmidt A. Exercise has a positive effect on endothelial progenitor cells, which could be necessary for vascular adaptation processes. *Int J Sports Med.* May 2007;28(5):374-380.

9. Pelus LM. Peripheral blood stem cell mobilization: new regimens, new cells, where do we stand. *Curr Opin Hematol.* Jul 2008;15(4):285-292.

10. Vose JM, Ho AD, Coiffier B, et al. Advances in mobilization for the optimization of autologous stem cell transplantation. *Leuk Lymphoma.* Sep 2009;50(9):1412-1421.

11. Theiss HD, Adam M, Greie S, Schobersberger W, Humpeler E, Franz WM. Increased levels of circulating progenitor cells after 1- week sojourn at moderate altitude (Austrian Moderate Altitude Study II, AMAS II). *Resp Physiol Neurobi.* 2008;160:232-238.

12. Bonsignore MR, Morici G, Santoro A, et al. Circulating hematopoietic progenitor cells in runners. *J Appl Physiol.* Nov 2002;93(5):1691-1697.

13. Thijssen DH, Vos JB, Verseyden C, et al. Haematopoietic stem cells and endothelial progenitor cells in healthy men: effect of aging and training. *Aging Cell.* Dec 2006;5(6):495-503.

14. Morici G, Zangla D, Santoro A, et al. Supramaximal exercise mobilizes hematopoietic progenitors and reticulocytes in athletes. *Am J Physiol Regul Integr Comp Physiol.* Nov 2005;289(5):R1496-1503.

15. Viscor G, Javierre C, Pages T, et al. Combined intermittent hypoxia and surface muscle electrostimulation as a method to increase peripheral blood progenitor cell concentration. *J Transl Med.* 2009;7:91.

16. Laufs U, Urhausen A, Werner N, et al. Running exercise of different duration and intensity: effect on endothelial progenitor cells in healthy subjects. *Eur J Cardiovasc Prev Rehabil.* Aug 2005;12(4):407-414.

17. Mobius-Winkler S, Hilberg T, Menzel K, et al. Time-dependent mobilization of circulating progenitor cells during strenuous exercise in healthy individuals. *J Appl Physiol.* Dec 2009;107(6):1943-1950.

18. Volaklis KA, Tokmakidis SP, Halle M. Acute and chronic effects of exercise on circulating endothelial progenitor cells in healthy and diseased patients. *Clin Res Cardiol.* Nov 2 2012 [Epub ahead of print 2012/11/03].

19. Moreno PR, Sanz J, Fuster V. Promoting mechanisms of vascular health: circulating progenitor cells, angiogenesis, and reverse cholesterol transport. *J Am Coll Cardiol.* Jun 23 2009;53(25):2315-2323.

20. Paulsen G, Mikkelsen UR, Raastad T, Peake JM. Leucocytes, cytokines and satellite cells: what role do they play in muscle damage and regeneration following eccentric exercise? *Exerc Immunol Rev.* 2012;18:42-97.

21. Gordon MY, Marley SB, Lewis JL, et al. Treatment with interferon-alpha preferentially reduces the capacity for amplification of granulocyte-macrophage progenitors (CFU-GM) from patients with chronic myeloid leukemia but spares normal CFU-GM. *J Clin Invest.* Aug 15 1998;102(4):710-715.

22. Withey JM, Marley SB, Kaeda J, Harvey AJ, Crompton MR, Gordon MY. Targeting primary human leukaemia cells with RNA interference: Bcr-Abl targeting inhibits myeloid progenitor self-renewal in chronic myeloid leukaemia cells. *Br J Haematol.* May 2005;129(3):377-380.

23. Bonsignore MR, Morici G, Riccioni R, et al. Hemopoietic and angiogenetic progenitors in healthy athletes: different responses to endurance and maximal exercise. *J Appl Physiol.* Jul 2010;109(1):60-67.

24. Stelzer I, Fuchs R, Schraml E, et al. Decline of bone marrow-derived hematopoietic progenitor cell quality during aging in the rat. *Exp Aging Res.* Jul 2010;36(3):359-370.

25. Kroepfl JM, Pekovits K, Stelzer I, et al. Exercise increases the frequency of circulating hematopoietic progenitor cells but reduces hematopoietic colony forming capacity. *Stem Cells Dev* May 22 2012 21(16):2915-2925.

26. Wonisch M, Berent R, Klicpera M, et al. Praxisleitlinien Ergometrie. *Journal für Kardiologie - Austrian Journal of Cardiology.* 2008;15 [Suppl A]:3-17.

27. Hofmann P, Tschakert G. Special needs to prescribe exercise intensity for scientific studies. *Cardiol Res Pract.*2011:209-302.

28. Mezzani A, Hamm LF, Jones AM, et al. Aerobic exercise intensity assessment and prescription in cardiac rehabilitation: a joint position statement of the European Association for Cardiovascular Prevention and Rehabilitation, the American Association of Cardiovascular and Pulmonary Rehabilitation and the Canadian Association of Cardiac Rehabilitation. *Eur J Prev Cardiol.* Oct 26 2012.

29. Tschakert G, Groeschl W, Schwaberger G, von Duvillard SP, Hofmann P. Prescription for aerobic high-intensity interval training by means of incremental exercise tests markers. *Med Sci Sports Exerc.* 2009;41((5, Suppl.)):430.

30. Wisloff U, Stoylen A, Loennechen JP, et al. Superior cardiovascular effect of aerobic interval training versus moderate continuous training in heart failure patients: a randomized study. *Circulation.* Jun 19 2007;115(24):3086-3094.

31. Tschakert G, Gröschl W, Hofmann P. Physiological responses during short and long high-intensity interval and continuous exercise. Paper presented at: Annual Meeting of the American College of Sports Medicine2011 (Abstract); Denver, Colorado, USA.

32. Gibson RS. *Principles of Nutritional Assessment*. 2nd ed: Oxford University Press; 2005.

33. Gajkowska A, Oldak T, Jastrzewska M, et al. Flow cytometric enumeration of CD34+ hematopoietic stem and progenitor cells in leukapheresis product and bone marrow for clinical transplantation: a comparison of three methods. *Folia Histochem Cytobiol.* 2006;44(1):53-60.

34. Schraml E, Fuchs R, Kotzbeck P, Grillari J, Schauenstein K. Acute adrenergic stress inhibits proliferation of murine hematopoietic progenitor cells via p38/MAPK signaling. *Stem Cells Dev.* Mar 2009;18(2):215-227.

35. Schraml E, Voglauer R, Fortschegger K, et al. Haploinsufficiency of senescence evasion factor causes defects of hematopoietic stem cells functions. *Stem Cells Dev.* Apr 2008;17(2):355-366.

36. Kroepfl JM, Pekovits K, Stelzer I, et al. Bewegungseinfluss auf zirkulierende hämatopoetische Stamm- und Progenitorzellen unter Normoxie und Hypoxie (Influence of exercise on circulating hematopoietic stem and progenitor cells under normoxic and hypoxic conditions). *ÖGAHM Jahrbuch.* 2012 (*in print*).

37. Khoschsorur GA, Winklhofer-Roob BM, Rabl H, Auer T, Peng Z, Schaur RJ. Evaluation of a Sensitive HPLC Method for the Determination of Malondialdehyd, and Application of the Method to Different Biological Materials. *Chromatographia.* 2000;52:181-184.

38. Zelzer S, Khoschsorur G, Stettin M, Weihrauch G, Truschnig-Wilders M. Determination of myeloperoxidase in EDTA plasma: comparison of an enzyme-linked immunosorbent assay with a chemiluminescent automated immunoassay. *Clin Chim Acta.* Aug 2009;406(1-2):62-65.

39. Graupp M, Wehr E, Schweighofer N, Pieber TR, Obermayer-Pietsch B. Association of genetic variants in the two isoforms of 5alpha-reductase, SRD5A1 and SRD5A2, in lean patients with polycystic ovary syndrome. *Eur J Obstet Gynecol Reprod Biol.* Aug 2011;157(2):175-179.

40. Zar JH. *Biostatistical Analysis*. 4th ed. London: Pearson Education Inc.; 2006.

41. Kroepfl JM, Tschakert G, Pekovits K, et al. Relationship between exercise-induced blood lactate concentration and hematopoietic stem cell mobilization in heart disease patients. *J Tissue Eng Regen M.* 2012(6 (Suppl.1)):105 (Abstract).

42. Lekli I, Gurusamy N, Ray D, Tosaki A, Das DK. Redox regulation of stem cell mobilization. *Can J Physiol Pharmacol.* Dec 2009;87(12):989-995.

43. Koerbling M, Estrov Z. Adult Stem Cells for Tissue Repair- A New Therapeutic Concept? *N Engl J Med.* 2003;349(6):570-582.

44. Sandri M, Adams V, Gielen S, et al. Effects of exercise and ischemia on mobilization and functional activation of blood-derived progenitor cells in patients with ischemic syndromes: results of 3 randomized studies. *Circulation.* Jun 28 2005;111(25):3391-3399.

45. Zerbini G, Lorenzi M, Palini A. Tumor angiogenesis. *N Engl J Med.* Aug 14 2008;359(7):763; author reply 764.

46. Bartsch P, Saltin B. General introduction to altitude adaptation and mountain sickness. *Scand J Med Sci Sports.* Aug 2008;18 Suppl 1:1-10.

47. Julian CG, Gore CJ, Wilber RL, et al. Intermittent normobaric hypoxia does not alter performance or erythropoietic markers in highly trained distance runners. *J Appl Physiol.* May 2004;96(5):1800-1807.

48. Katayama K, Sato K, Matsuo H, Ishida K, Iwasaki K, Miyamura M. Effect of intermittent hypoxia on oxygen uptake during submaximal exercise in endurance athletes. *Eur J Appl Physiol.* Jun 2004;92(1-2):75-83.

49. Baker JM, De Lisio M, Parise G. Endurance exercise training promotes medullary hematopoiesis. *FASEB J.* Dec 2011;25(12):4348-4357.

50. Wardyn GG, Rennard SI, Brusnahan SK, et al. Effects of exercise on hematological parameters, circulating side population cells, and cytokines. *Exp Hematol.* Feb 2008;36(2):216-223.

51. Wojciechowski JC, Narasipura SD, Charles N, et al. Capture and enrichment of CD34-positive haematopoietic stem and progenitor cells from blood circulation using P-selectin in an implantable device. *Br J Haematol.* Mar 2008;140(6):673-681.

52. Bellows CF, Zhang Y, Simmons PJ, Khalsa AS, Kolonin MG. Influence of BMI on level of circulating progenitor cells. *Obesity (Silver Spring).* Aug 2011;19(8):1722-1726.

53. Cross M, Alt R, Niederwieser D. The case for a metabolic stem cell niche *Cells Tissues Organs.* 2008;188:150-159.

54. Spiegel A, Shivtiel S, Kalinkovich A, et al. Catecholaminergic neurotransmitters regulate migration and repopulation of immature human CD34+ cells through Wnt signaling. *Nat Immunol.* Oct 2007;8(10):1123-1131.

55. Haddad JJ. Redox and oxidant-mediated regulation of apoptosis signaling pathways: immune-pharmaco-redox conception of oxidative siege versus cell death commitment *Int Immunopharmacol.* 2004;4(4):475-493.

56. Wang JS, Lin CT. Systemic hypoxia promotes lymphocyte apoptosis induced by oxidative stress during moderate exercise. *Eur J Appl Physiol.* Jan 2010;108(2):371-382.

57. Adler V, Yin Z, Tew KD, Ronai Z. Role of redox potential and reactive oxygen species in stress signaling. *Oncogene.* Nov 1 1999;18(45):6104-6111.

58. Camello-Almaraz C, Gomez-Pinilla PJ, Pozo MJ, Camello PJ. Mitochondrial reactive oxygen species and Ca2+ signaling. *Am J Physiol Cell Physiol.* Nov 2006;291(5):C1082-1088.

59. Case J, Ingram DA, Haneline LS. Oxidative stress impairs endothelial progenitor cell function. *Antioxid Redox Signal.* Nov 2008;10(11):1895-1907.

60. Yahata T, Takanashi T, Muguruma Y, et al. Accumulation of oxidative DNA damage restricts the self-renewal capacity of human hematopoietic stem cells. *Blood.* Sep 15 2011;118(11):2941-2950.

61. Hosokawa K, Arai F, Yoshihara H, et al. Function of oxidative stress in the regulation of hematopoietic stem cell-niche interaction. *Biochem Biophys Res Commun.* Nov 23 2007;363(3):578-583.

62. Cesari F, Caporale R, Marcucci R, et al. NT-proBNP and the anti-inflammatory cytokines are correlated with endothelial progenitor cells' response to cardiac surgery. *Atherosclerosis.* Jul 2008;199(1):138-146.

63. Fan Y, Ye J, Shen F, et al. Interleukin-6 stimulates circulating blood-derived endothelial progenitor cell angiogenesis in vitro. *J Cereb Blood Flow Metab.* Jan 2008;28(1):90-98.

64. Rezaee F, Rellick SL, Piedimonte G, et al. Neurotrophins regulate bone marrow stromal cell IL-6 expression through the MAPK pathway. *PLoS One.* 2010;5(3):e9690.

65. Yu CX, Zhu B, Xu SF, Cao XD, Wu GC. The analgesic effects of peripheral and central administration of melatonin in rats. *Eur J Pharmacol.* Sep 1 2000;403(1-2):49-53.

66. DeRijk RH, Boelen A, Tilders FJ, Berkenbosch F. Induction of plasma interleukin-6 by circulating adrenaline in the rat. *Psychoneuroendocrino.* 1994;19(2):155-163.

67. Dimitrov S, Benedict C, Heutling D, Westermann J, Born J, Lange T. Cortisol and epinephrine control opposing circadian rhythms in T cell subsets. *Blood.* May 21 2009;113(21):5134-5143.

68. Benschop RJ, Rodriguez-Feuerhahn M, Schedlowski M. Catecholamine-induced leukocytosis: early observations, current research, and future directions. *Brain Behav Immun.* Jun 1996;10(2):77-91.

69. Mastorakos G, Pavlatou M, Diamanti-Kandarakis E, Chrousos GP. Exercise and the stress system. *Hormones.* Apr-Jun 2005;4(2):73-89.

70. Dar A, Schajnovitz A, Lapid K, et al. Rapid mobilization of hematopoietic progenitors by AMD3100 and catecholamines is mediated by CXCR4-dependent SDF-1 release from bone marrow stromal cells. *Leukemia.* Aug 2011;25(8):1286-1296.

71. Katayama Y, Battista M, Kao WM, et al. Signals from the sympathetic nervous system regulate hematopoietic stem cell egress from bone marrow. *Cell.* Jan 27 2006;124(2):407-421.

72. Mendez-Ferrer S, Battista M, Frenette PS. Cooperation of beta(2)- and beta(3)-adrenergic receptors in hematopoietic progenitor cell mobilization. *Ann N Y Acad Sci.* Mar 2010;1192:139-144.

73. Penn A, Mohr AM, Shah SG, et al. Dose-response relationship between norepinephrine and erythropoiesis: evidence for a critical threshold. *J Surg Res.* Oct 2010;163(2):e85-90.

74. Porter RL, Calvi LM. Communications between bone cells and hematopoietic stem cells. *Arch Biochem Biophys.* May 15 2008;473(2):193-200.

75. Pereira RM, Delany AM, Durant D, Canalis E. Cortisol regulates the expression of Notch in osteoblasts. *J Cell Biochem.* 2002;85:252-258.

76. Kroepfl J, Pekovits K, Stelzer I, et al. Are hematopoietic stem cell kinetics linked to different exercise modes? *Med Sci Sports Exerc.* 2010;42(5):365-366 (Abstract).

77. Stelzer I, Kroepfl JM, Pekovits K, et al. High-volume exercise induced stress leads to a decline of hematopoetic cell functionality in an ultra-distance exercise model. *Clin Chem Lab Med.* 2012;50(4):A83-A84 (Abstract).

78. Ye JM, Steen JT, Matthias A, Clark MG, Colquhoun EQ. Effects of noradrenaline and flow on lactate uptake in the perfused rat hindlimb. *Acta Physiol Scand.* May 1998;163(1):49-57.

79. Kumar S, McGinnis JF, de Vellis J. Catecholamine regulation of lactate dehydrogenase in rat brain cell culture. Norepinephrine differentially increases the rate of synthesis of the individual subunits in the C6 glial tumor cell line. *J Biol Chem.* Mar 25 1980;255(6):2315-2321.

80. Passaquin AC, Coupin G, Poindron P. Rat interferon inhibits catecholamine-inducible synthesis of lactic dehydrogenase in rat glial tumoral C6 cells. *J Interferon Res.* Apr 1986;6(2):71-77.

81. Kremer JP, Datta T, Pretsch W, Charles DJ, Dormer P. Mechanisms of compensation of hemolytic anemia in a lactate dehydrogenase mouse mutant. *Exp Hematol.* Jul 1987;15(6):664-670.

82. Donmez A, Kabaroglu C, Gokmen N, Tombuloglu M. The difference in lactate dehydrogenase activity between pre- and post-mobilization periods is a good indicator of successful stem cell mobilization. *Transfus Apher Sci.* Jun 16 2012 [Epub ahead of print 2012/06/20].

83. Menekay S, Ozsan GH, Demirkan F, et al. Effect of granulocyte-colony-stimulating factor on serum lactate dehydrogenase levels and isoenzymes in a rabbit model. *Acta Haematol.* 2002;107(1):18-22.

84. Milovanova TN, Bhopale VM, Sorokina EM, et al. Lactate stimulates vasculogenic stem cells via the thioredoxin system and engages an autocrine activation loop involving hypoxia-inducible factor 1. *Mol Cell Biol.* Oct 2008;28(20):6248-6261.

85. Egan K, Singh V, Gidron A, Mehta J. Correlation between serum lactate dehydrogenase and stem cell mobilization. *Bone Marrow Transplant.* Nov 2007;40(10):931-934.

86. Ko KH, Holmes T, Palladinetti P, et al. GSK-3beta inhibition promotes engraftment of ex vivo-expanded hematopoietic stem cells and modulates gene expression. *Stem Cells.* Jan 2011;29(1):108-118.

87. Brunner S, Engelmann MG, Franz WM. Stem cell mobilisation for myocardial repair. *Expert Opin Biol Ther.* Nov 2008;8(11):1675-1690.

7 Abbreviations

HSC	hematopoietic stem cell
EPC	endothelial stem cell
EC	endothelial cell
MSC	mesenchymal stem cells
LTHSC	long-term repopulating HSC
STHSC	short-term repopulating HSC
MPP	multipotent progenitor cell
CMP	common myeloid progenitor cell
MEP	megakaryocyte-erythroid progenitor cell
GMP	granulocyte monocyte progenitor cell
CLP	common lymphoid progenitor cell
RBC	red blood cell (erythrocyte)
34-KLS CD34-	c-*kit*+lineage- sca-1+ cell
CRU	competitive repopulating unit assay
CFU-Spleen	colony forming unit-spleen assay
CFC	colony-forming cell assay, d 14
HSPC	hematopoietic stem and progenitor cell
CPC	circulating hematopoietic progenitor cell
SSClow	side scatter low
FSC	forward scatter
RBC	red blood cells
WBC	white blood cells
Hct	hematocrit
VEGF	vascular endothelial growth factor
VEGF-2	vascular endothelial growth factor-2
p53	tumor protein 53
Lnk	SH2B adaptor protein 3
VE-cad = CD144	vascular endothelial-cadherin
MMP-9	matrixmetallopeptidase-9
eNOS	endothelial nitric oxide synthase
E-selectin	endothelial-leukocyte adhesion molecule 1

vWF	von Willebrand factor
CD 14, 31, 34, 45, 117, 133	cluster of differentiation
SCF-1	stem cell factor-1
IL-3, 5, 6,7	interleukin
VO_{2max}	maximal oxygen uptake
P_{max}	maximal output power
P_{mean}	mean output power
P_{peak}	peak work load
P_{target}	individual target work load
P_{LTP1}	work load at LTP_1
P_{LTP2}	work load at LTP_2
P_{rec}	recovery work load
t_{rec}	recovery work load duration
t_{peak}	peak work load duration
La_{max}	maximal blood lactate concentration
NE	norepinephrine
MDA	malondialdehyde
MPO	myeloperoxidase
HIF-1	hypoxia-inducible-factor-1
EPO	erythropoietin
CFU	colony forming unit
CFU-GM	CFU-granulocyte-macrophage
BFU-E	burst forming unit-erytroid

8 Annex: additional publications and awards

Kröpfl, Julia; Karin, Pekovits; Stelzer, Ingeborg; Sedlmayr, Peter; Gröschl, Werner; Hofmann, Peter; Domej, Wolfgang; Dohr, Gottfried; Müller, Wolfram: *Are hematopoietic stem cell kinetics linked to different exercise modes?*, in: Medicine and Science in Sports and Exercise 42,5 (2010), 365 - 366. (Abstract)

ARE HEMATOPOIETIC STEM CELL KINETICS LINKED TO DIFFERENT EXERCISE MODES?

Hematopoieticstem- and progenitor cell release from the bone marrow to the peripheral circulation is known to be enforced by physical work load and hypoxia. The exact stem cell kinetics induced by these stressors, however, has not yet been identified. **PURPOSE:** The aim of the study was to determine the effect of endurance exercise, anaerobic exercise, and hypoxia on the number of hematopoietic stem cells (HSC) in the peripheral circulation. **METHODS:** A single subject (25yrs, male, 1.88 m, 79 kg, VO_2max = 57.6 $ml.kg^{-1}.min^{-1}$, non-smoker, healthy) performed different exercise tests in this pilot study. Incremental (I, P_{max} = 360 W, P_{LTP2} = 243 W), constant-load all-out (CLA, 220 W for 31 min), CL at 70% of all-out duration (CL70), and transition power (TP30, 30s all-out at 9,7 % body weight ~ 770 W) tests as well as a hypoxia test (HY, gradual O_2-saturation decrease from 97 % to 75 % within 23 min) without physical work load were carried out between 8:00 AM and 11:00 AM. Peripheral blood was collected before as well as 10, 30, 60 and 120 min after the intervention. HSCs (CD 34/ CD 45 positive) were analysed by means of flow cytometry analysis. **RESULTS:** I, CLA, CL70 and TP30 showed an increasing stem cell release 10 to 30 min after the intervention, whereas hypoxia alone did not. I and TP30 were associated with the highest stem cell increase (Δ_I = 788 $CD34^+/CD45^+$ cells/ml, Δ_{TP30} = 701 $CD34^+/CD45^+$ cells/ml respectively) and also with the highest maximal lactate values ($La_{max,I}$ = 11.97 $mmol.l^{-1}$, $La_{max,TP30}$ = 13.77 $mmol.l^{-1}$ respectively). Hypoxia was linked to a decrease in HSC counts in the peripheral blood (Δ_{HY} = -253 $CD34^+/CD45^+$ cells/ml) and was also linked to very low lactate values ($La_{max,HY}$ = 0.80 $mmol.l^{-1}$). There was a significant correlation between HSC counts and lactate concentration (r = 0.95, $p<0.05$). **CONCLUSIONS:** Results of this pilot study suggest that HSC release from the bone marrow is increased by exercise modes that provide high lactate concentrations, such as I or TP30. Accumulated blood lactate concentration may support HSC mobilization.

Kröpfl, Julia; Pekovits, Karin; Stelzer, Ingeborg; Sedlmayr, Peter; Hofmann, Peter; Dohr, Gottfried; Müller, Wolfram: *Stem cell kinetics induced by different high intensive exercise bouts*, in: European International Society for Cellular Therapy (ISCT-Europe) (Ed): Final Programm - European International Society for Cellular Therapy (ISCT-Europe) (2010), 72 (Abstract)

STEM CELL KINETICS INDUCED BY DIFFERENT HIGH INTENSIVE EXERCISE BOUTS

The release of hematopoietic stem- and progenitor cells (HSCs) from the bone marrow to the peripheral blood can be modulated by different triggers such as hypoxia or physical work load. PURPOSE: The aim of the study was to determine the effect of different high intensive exercise bouts (endurance and anaerobic) on the number of HSCs in the peripheral blood. METHODS: A single subject (25yrs, male, 1.88 m, 79 kg, VO2max = 57.6 ml/min/kg, non-smoker, healthy) performed different cycle ergometer exercise tests in this pilot study. Incremental (I, P_{max} = 360W, PLTP2 = 243W), constant-load all-out (CLA, 220W for 31 min) just below LTP2, CL at 70 % of all-out duration (CL70), sprint power series (SP10, 10 s 7 x all-out at 4-18 % body weight ~ 318W-1431W, 5 min rest in-between) and transition power (TP30, 1 x 30 s all-out at 9,7% body weight ~ 770W) tests were carried out between 8am and 11am on different days in a randomized order. Peripheral blood was drawn before as well as 10, 30, 60 and 120 minutes after the intervention. HSCs (CD34/CD45 positive) were analyzed by means of flow cytometry analysis. RESULTS: I, CLA, CL70 and TP30 showed an increasing stem cell release 10 to 30 min after the intervention, whereas SP10 did not. I and TP30 were associated with the highest stem cell increase. CLA and CL70 mobilized approximately the same amount of HSCs. SP10, however, was linked to a decrease of HSCs in the peripheral blood (Table 1). CONCLUSIONS: We suggest that high intensive exercise bouts (at or beyond LTP2) need at least a time duration of 30 s or more to mobilize HSCs from the bone marrow to the peripheral blood. In addition, accumulated lactate might support HSC release.

exercise bouts	Δ $CD34^+/CD45^+$ cells/ml
incremental test	788
constant load all-out	353
constant load 70%	374
transition power test	701
sprint power test	-310

Table 1

Kröpfl, Julia; Pekovits, Karin; Stelzer, Ingeborg; Zelzer, Sieglinde; Hofmann, Peter; Dohr, Gottfried; Müller, Wolfram; Domej, Wolfgang: *24 h Overnight-Trial: stem cell mobilization at moderate altitudes*, in: Österreichische Gesellschaft für Alpin- und Höhenmedizin (ÖGAHM) (Ed): ÖGAHM Jahrbuch (2011), 115-124

24 h OVERNIGHT-TRIAL: STEM CELL MOBILIZATION AT MODERATE ALTITUDES

Little information on the influence of hypoxia at moderate altitudes on adult *hematopoietic stem and progenitor cell (HSC/HPC)* mobilization can be found in the literature. Regarding the effect of hypoxia alone or hypoxia in combination with physical exercise on *HSC/HPC* release data are sparse. This makes it necessary to separately evaluate the two possible stem cell triggers hypoxia and/or exercise. The aim of the current pilot study was to investigate the influence of hypoxia at a simulated moderate altitude of 3,500 m on hematopoietic stem cell release to the peripheral blood in inactive subjects. *Methods:* Three healthy, male subjects were studied in a 24 h overnight trial in a hypoxia chamber. They were supervised all the time with respect to their activity level and their food and water uptake. Venous blood samples were collected every four hours. *HSC/HPC* counts and plasma levels of *malondialdehyd* (*MDA*), a standard oxidative stress marker, were analyzed at each blood collection. Blood lactate concentration and heart rate were monitored as additional parameters. *Results:* Subjects remained inactive throughout all 24 h (no increase in blood lactate concentration and heart rate). *HSC/HPC* counts and *MDA* plasma levels did not show any increasing tendency during the 24 h period in the hypoxia chamber, although all subjects clearly responded to hypoxia (decreased oxygen saturation). *Conclusions:* 24 h of hypoxia at a simulated moderate altitude of 3,500 m did not have any mobilizing influence on hematopoietic stem cell release from the bone marrow to the peripheral blood in inactive subjects, although data are limited due to the low number of subjects.

Awards

- Young Investigator Grant of the Karl-Franzens University of Graz (2010/2012)
- ÖGAHM Science Award 2010
- 1st Poster Award at the Congress for Cardiac Rehabilitation (Vienna, Austria, October 2012)

Acknowledgements

This dissertation was an interdisciplinary project, where a lot of different people supported this work. First of all, I am very grateful to Wolfram Müller for the possibility of becoming a member of his research group and consecutively being involved in this interesting project. It was a pleasure to work with you and to learn from you! I want to thank Gottfried Dohr and Sandra Wallner-Liebmann for being great supervisors and project coordinators. Without their ideas and support this project would not have come to life. In addition, I want to express my appreciation to Gottfried Dohr to have been able to do all the lab work at the Institute of Cell Biology, Histology and Embryology.

Furthermore, I am immensely thankful to Ingeborg Stelzer for sharing her huge knowledge and skills and for her moral as well as work support. My sincere thanks also go to Karin Pekovits for her involvement in optimizing the cell culture procedure and the FACS analysis in the beginning, her valuable help during the study and for having been a good friend.

I want to thank the whole team of the former *HPR*^{Graz} named Alfred Fürhapter-Rieger, Gerhard Tschakert, Claus Cagran, Philipp Huber, Werner Gröschl, Evelyn Schneider, Alex Müller, Tanja Zarfl, Patrick Rossmann, Peter Rohrer, Stefan Zunzer, Stefan Spirk and Mario Frei for their continuous help, advice and an enthusiastic work environment. Thanks for the great three years I could be part of this institution!

My sincere thanks also go to Peter Hofmann for his continuous and reliable support in various issues as well as Wolfgang Domej for all his help regarding the hypoxia chamber and the Land STMK financed project.

Many thanks to Monika Siwetz, Nina Flieser, Bernhard Robinig, Peter Schönbacher, Rudolf Schmied and Beate Scheiber at the Institute of Cell Biology, Histology and Embryology for their assistance during the study, Peter Sedlmayr for his introduction to flow cytometry analysis as well as Gregor Weiss and Lukas Schinagl for their excellent stirring.

Thanks are also given to Robert Fuchs for his competent advice while publishing, Sieglinde Zelzer for her help regarding the oxidative stress level evaluation as well as Brigitte Poncza and Nathalie Allard for the determination of catecholamines.

I am very thankful to Elisabeth Stummer for broading my horizons regarding the philosophy of doing a PhD. Special thanks are given to Markus Auer and my friends for their maintaining motivation. Finally I thank my family for being my anchor in highs and lows.

http://oe1.orf.at/

In addition, I want to express my appreciation to Land STMK, Austrian Association for Alpine Medicine (ÖGAHM), Franz-Lanyar Stiftung and Doctoral School for Lifestyle-Related Diseases (Med Uni Graz) for their financial support.

Declaration

Hereby I declare that this thesis is my own original work and that I have fully acknowledged by name all of those individuals and organizations that have contributed to the research for this thesis. Due acknowledgement has been made in the text to all other material used. Throughout this thesis and in all related publications I followed the guidelines of "Good Scientific Practice".

Please note that parts of this thesis have already been published:

Kroepfl JM, Pekovits K, Stelzer I, et al. Exercise increases the frequency of circulating hematopoietic progenitor cells but reduces hematopoietic colony forming capacity. *Stem Cells Dev* 2012, 21(16):2915-25.

Date Signature

Graz, 21.12.2012