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Changes of cytokines under the influence of

high altitude hypobaric hypoxia and physical activity

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1. Summary

The human immune response on either hypobaric hypoxia alone or combined with exercise at very high altitude has not been described in detail before. Studies implicate that hypoxia as well as exercise trigger both pro- and anti-inflammatory processes. This study, explores the relationship between biomarkers under hypobaric hypoxia in rest and under exercise conditions. This work is one part of a research leading to two different doctoral theses. One with focus on proinflammatory cytokines and chemokines, the other with focus on growth factors under the mentioned conditions. The twenty-five healthy volunteers participated in a series of resting and exercise trials both under normoxia and hypobaric hypoxia using a non-randomized, non-blinded design. Each trial included peripheral blood sampling at rest or before and 180 minutes after cessation of an incremental step test with 12 minutes of duration both at sea level and at 5050m (median barometric pressure was 760mmHg and 563mmHg, respectively). Pro-inflammatory [IL-1beta, IL-2, IL-6, IL-8, IL-12p40, IL-1ra, sIL-2ra, IFNgamma, TNF-alpha, MCP-1, MIP-1alpha, MIP-1beta, and IP-10] and anti-inflammatory (IL-4 and IL-10) biomarkers were assessed. Hypobaric hypoxia alone caused a significant increase in IL-8, IL-1ra, sIL-2ra, TNF-alpha, MCP-1, and MIP-1beta and a significant decrease in IFNgamma, IL-12p40, and MIP-1alpha. The same markers were measured before and after exercise both at sea level and at high altitude. IL-12p40 showed a significant increase at high altitude. Other biomarkers were unchanged. Hypobaric hypoxia is a potent trigger of changes in cytokine concentrations. Conversely, exercise seems to have minor effects on cytokine regulation. A trainings effect seems to be responsible for the unchanged cytokine plasma levels under the high altitude exercise test.

2. List of abbreviations

IL-1beta	Interleukin 1 beta
IL-2	Interleukin 2
IL-6	Interleukin 6
IL-8	Interleukin 8
IL-12p14	subunit beta of interleukin 12
IL-2ra	interleukin-2 receptor alpha
sIL-2ra	soluble IL-2 receptor alpha
IL-1ra	interleukin- 1 receptor antagonist
IFNgamma	interferon gamma
TNF alpha	tumor necrosis factor alpha
MCP-1	monocyte chemoattractant protein-1
MIP-1alpha	macrophage inflammatory protein alpha
MIP-1beta	macrophage inflammatory protein beta
IP-10	Interferon gamma-induced protein 10
IL-4	interleukin 4
IL-10	interleukin 10
LPS	Lipopolysaccharide
NGF	nerve growth factor
ICAMS	intercellular adhesion molecule
Ig M/G/A	immunoglobulin M/G/A
GM-CSF	granulocyte macrophage colony-stimulating factor

2. List of abbreviations

APC	antigen presenting cell
PAMP	pathogen-associated molecular pattern
VCAM	vascular cell adhesion molecules
BAL	bronchoalveolar lavage
AMS	acute mountain sickness
ARDS	acute respiratory distress syndrome
HAPE	high altitude pulmonary edema
MHC I/II	major histocompatibility complex I/II
Fc region	fragment crystallizable region
CD 80/86/40	cluster of differentiation 80/86/40
Rpm	revolution per minute
MFI	median fluorescent intensify
COPD	chronic obstructive pulmonary disease
ROS	reactive oxygen species
TLR	toll like receptor
HIF	hypoxia inducible factor
LPS	lipopolysaccharide
MNC	mononuclear cell
PMA	phorbol-12-myristate-13 –acetate
nf κB	nuclear factor kappa B
LLS	Lake Louis Score

3. Introduction

"I have not at all the feeling of having really got acclimatized

which would certainly be the first necessary step toward improvement."

From The Magic Mountain by Thomas Mann (1939)

3.1. Humans and environmental extremes in the mountains

Mountains exist on all continents and cover 27% of the earth´s surface. Human beings have to go to high altitude for different reasons. People settled down in high altitude since prehistoric time e.g. in the Andes [1].

Mountains and high altitude areas e.g. the Himalayans, the Andes, the Alps and the Atlas of Africa demand special adaptation of people. High altitude residents developed a physique that enables them to resist severe conditions since generations. They have to confront to extreme cold or extreme temperature variations, Hypoxia, oxygen lack and solar radiation, wind speed increase and humidity decrease [2].

In addition, high altitude residents who are adapted genetically, large numbers of people travel to high altitude for recreation, mountaineering, trekking, and skiing. Professionals like military personnel in Asia and in the latest time in Afghanistan and Kashmir as well as athletes or mountaineers in remote mountain areas are confronted with altitude problems, developing acute mountain sickness (AMS) and are in the focus of high altitude research. Especially the influence of hypobaric hypoxia and exercise in the immune system and the participation in the pathophysiology of AMS is the main part of research [1, 2].

The motivation of high altitude research is first to keep people in high altitude healthy and second to find out more about the complicated human physiology and pathology under hypobaric hypoxia. The high altitude model may be used as a paradigm especially in changes of the immune competence in cellular hypoxia as base for research in critical care medicine [3].

3.2. The immune system

The human immune system is an intricately regulated and interconnected complex network of circulating and fixed cells, lymphoid organs, cytokines and humoral factor that protect the human body from disturbing [4, 5]. The human defense consists of the innate and adaptive immune system that differ in speed and specificity in the immune reaction [5]. After a pathogen managed to pass through the mechanical, microbiological and physical barriers, which are part of the non-specific defense of the skin and natural surface barriers, it is confronted first by the cellular part of the innate system [4-6].

The innate immune system is a more basic system of cells and proteins that secures an immediate host defense [4, 5]. The adaptive immune response is a highly specialized cellular network acting via immunoglobulins in response organs (lymph nodes, spleen, and mucosa associated tissues). The two parts of the immune response are inseparable compounded and interact with each other [4, 5].

3.3. Cytokines

Cytokines are small, non-enzymatic, non-structural proteins. Currently, about 130 members of the cytokine family are known [7]. Originally, they were called lymphokines and monokines in order to indicate their cellular origin [8].

They represent a multi-diverse family of polypeptide regulators of a low molecular weight which are produced throughout the body [9]. Through environmental challenges such as hypoxia, hypothermia, and pathogenic microorganisms, monocytes and macrophages are stimulated to release cytokines in order to mediate physiological adaptations to changes in homeostasis [10].

Cytokines are a heterogeneous group which involves interferons, interleukins, the chemokine family, growth factors, the tumor necrosis factor, and apokines [9].

They act in a hormone-like manner in nano- to picomolecular concentrations [8]. They are intercellular regulators and mobilize cells of the innate and adaptive immune system. Cytokines influence cell growth, cell death, angiogenesis, cellular development and repair processes in the aim of restoration of homeostasis [11]. They are secreted from different cell and act on other cells

fulfilling the definition of a hormone. However, unlike hormones which are produced in specialized primary tissues, cytokines are secreted ubiquitously. In general, every cell can both produce and respond to a cytokine, with the exception of erythrocytes [9].

3.3.1. Cytokine receptor and signaling pathways

Cytokines act via a complicated network and are regulated by changing conditions imposed by other simultaneously acting cytokines. The net effect may be synergetic or contrasting. As mediators of inter-and intracellular communication, they are secreted by cells to stimulate other cells in a paracrine manner or to stimulate and alter their own function in an autocrine manner. While most cytokines are released into the extracellular interstitial fluid after biosynthesis, small amounts of cytokines are associated with the cellular membrane. Subsequently, extracellular binding of a cytokine to cell surface receptors initiates an intracellular signaling cascade eliciting nuclear events including regulation of proliferation, differentiation, and function of the cell [8].

Table 1 Cytokine receptors

Cytokine family	Members	Receptor
Hematopoietins	IL-2,IL-3, IL-4 , IL-6, , IL-10, IFN alpha	Cytokine receptor class 1
TNF	TNF-alpha, TNF-beta	NGF/ TNF receptor
Chemokines	IL-8, MIP-1alpha, MIP-1beta, MCP-1	Rhodopsinsuperfamily
Beta-trefoil	IL-1alpha, IL-1beta, IL-1ra	Split tyrosine kinase/ IL-1 receptor

3.3.2. Pro-inflammatory cytokines

Cytokines are grouped due to their biological functions in different classes [12]. If they promote proinflammatory reactions, they are called "proinflammatory cytokines", to demonstrate their role during infection and inflammation [12]. Proinflammatory cytokines are essential to start, accelerate and coordinate the immune response to protect the homeostasis against pathogens. They influence the cytotoxic effect and the humoral-, cellular- and allergic response [13].

3.3.3. Acute phase cytokines

IL-1 is a paradigmatic cytokine illustrating the pleiotropic effect of cytokines. There exists a complicated network of interactions between family members and their receptors [14].

The IL-1 family includes three different peptides including IL-1beta, IL-1alpha and IL-1ra. IL-1ra is the antagonist of the IL-1 mediated effect.[14] Both IL-1alpha and IL-1beta, as their nomenclature shows, share similar proinflammatory activities. IL-1 is produced by activated macrophages from different sources (alveolar macrophages, Kupffer cells, adherent spleen and peritoneal macrophages), by peripheral neutrophils and T-and B-cells [14]. Together with IL-6 and TNF-alpha they are called "acute phase cytokines" [15, 16]. IL-1 stimulates the central nervous system as an endogenous pyrogen, interferes with metabolism via increased sympathetic tone and activates the production of acute phase proteins [14]. Triggers for the IL-1 stimulation are bacterial agents such as LPS(lipopolysaccharide) of the bacterial wall or nonbacterial stimulators such as hypoxia, thermal changes, radiation, other cytokines (TNF-alpha, IL-2, IL-3, IL-12) and multiple chemical attractants[14].

TNF-alpha is released by monocytes, macrophages, neutrophils, lymphocytes, natural killer cells, endothelial cells, astrocytes, and microglial cells. The main stimulators for TNF-alpha are LPS and endotoxin. Other stimulators include viral, fungal, and bacterial antigens, C5a anaphylaxin, immune complexes, and the synergistic IL-1 release of TNF-alpha. TNF-alpha can also trigger its own release. [17].

3. Introduction

TNF-alpha exerts a pleiotropic effect on a large number of target cells. The result of persisting TNF-alpha synthesis is cachexia. Via stimulation of metabolic enzymes, adipocytes and skeletal myocytes within the body undergo catabolism. Processes involved include glycogenolysis, lipolysis and increased protein turn over resulting in anemia and anorexia. This condition is present in cancer patients, acquired immune deficiency syndrome, parasite infections, and heart failure.[17]

In septic shock, TNF-alpha and co-stimulatory factors such as IL-1 trigger the production of platelet activating factor, eicosanoids, and leukotriene which act as secondary effectors and lead to symptoms of shock. TNF-alpha acts synergetic with IL-1, affecting endothelial activation via leukocyte stimulation and binding to ICAMS (intercellular adhesion molecule) [18].This results in vascular leakage due to structural reorganization triggered by prostacyclin and endothelial derived factor. The structural change plays a role both in vasodilation and fluid sequestration as seen in shock. By acting as a growth factor, TNF-alpha triggers healing from inflammatory processes and stimulates fibroblasts for mesenchymal cell proliferation. In a cytotoxic effect, it mediates cell apoptosis and necrotic cell lysis. Referring to its name, some tumor cells are sensitive to this effect. Additionally, TNF-alpha affects the activity of natural cytotoxic cells. As a stimulator of immune response, activation of phagocytosis, leukocyte recruitment, and chemotaxis play a role in the innate immune response. TNF-alpha augments the production of superoxide anion and degranulation. Additionally, the production of IL-1 and IL-6 are stimulated. Finally, T-cells are activated which improves the immune response [12, 19-21].

The main sources of IL-6 are endothelial cells, fibroblasts, and monocytes/macrophages, especially during inflammation. Stimulators for IL-6 secretion by fibroblast and endothelial cells are IL-1 and TNF-alpha (co-stimulator). LPS is an additional trigger in monocytes. Recently, an IL-6 release from the contracting muscle during exercise was found [22]. It is known as the most potent stimulator of the acute phase reaction, acting via hepatocytes. Additionally, it influences B-cells and T-cells. In contrast, it also has anti-inflammatory effects [23-25].

As a major regulator of the acute phase reaction, the syntheses of acute phase proteins are activated via hepatocyte induction in synergy of IL-6 and IL-1. IL-6, compared to IL-1 and TNF-alpha, is the only stimulator of all acute phase proteins [24]. Thus, it plays a key role in the acute phase response. It initiates the leukocyte migration. IL-6 does not influence neutrophil infiltration but it stops the

accumulation [25]. The second role of IL-6 is to accelerate induction of apoptosis in neutrophils in order to end the first part of immune response. Hence,IL-6 is acting as a mediator between the innate and adaptive immune response [25].

IL-6 together with IL-4 and IL-5 activates B-cells to differentiate and leading to a sufficient secretion of immunoglobulins (IgM, IgG, IgA). While only activated B-cells are stimulated via IL-6, it has no influence on resting B-cells. In synergy with IL-3, IL-4, and GM-CSF (granulocyte macrophage colony-stimulating factor), IL-6 stimulates multipotential hematopoietic stem cells [26].

T-cells in the thymus are co-stimulated by IL-6 and IL-1 for proliferation. Additionally, IL-6 synergistically with IL-1 is necessary in the primary antigen dependent T- cell proliferation. Finally, T-cytotoxic cells are activated by IL-6 [24].

3.3.4. Interferon family

Characteristic of interferons is the ability to interfere with viral expansion [13]. The family is divided into two groups, type 1 (Interferon alpha and beta) and type 2 (interferon gamma) [6]. Group one has major antiviral activities; it is produced by fibroblasts and monocytes. A trigger mechanism leads to intracellular protection from infected viral RNA and up-regulation of MHC I, to enhance the destruction by cytotoxic T-cells from viral infected host cells [6].

Type 2 include interferon gamma. It was first mentioned as a secretion product of Th1 lymphocytes, CD4 helper cells, CD8 cytotoxic cells and neutrophil killer cells. It is the main cytokine of the TH1 response [27].

Stimulation of MHC I was the first effect known for IFNgamma. It enables the CD8 cytotoxic cell to recognize the intracellular pathogen [27]. In addition, it triggers the release of IL-12 production from infected APC (antigen presenting cell). At the same time CD4 cell differentiation in the TH1 response is accelerated. Through all these actions IFNgamma links the innate to the adaptive immune response [27].

IFNgamma mediated activation of macrophages causes an elevation in its biological activity [27]. The aforementioned link between the innate and adaptive immune system is important for the adequate antiviral response. Additionally, specific antiviral proteins are stimulated by IFNgamma. IFNgamma

acts as a chemokine, attracting leukocytes to the site of inflammation. Together with IL-1 and TNF-alpha, chemokine activation is elevated by IFNgamma [27].

IL-2 and its receptor belong to the group of interferons. It plays a crucial role in T-cell activation and it is known as a T-cell growth factor and a stimulator in the cellular immune response [28].

In the pleiotropic sense, it is also responsible for down regulation of the T- cell response.

Together with IFNgamma and IL-15, the Th1 response is supported by IL-2. The migration of the undifferentiated or partly differentiated T-cells to secondary lymphoid organs is especially triggered by IL-2. With IL-2's influence, CD8 T-cells are accumulated and their differentiation is also completed in the lymph nodes [28]. Additionally, IL-2 is essential for priming T-cells and for sufficient development of T-memory cells [29]. In the case of a chronic infection, IL-2 promotes activation of induced cell death (apoptosis) to end the immune response and down regulate the T- cell response. Otherwise, a few activated T-cell are kept alive and will activate the host's defense with a specialized T-cell unit and to recruit new undifferentiated T-cells [29].

Beside the mentioned IL-2 receptor that is expressed on the surface of activated T-cells, a soluble receptor form is known. The soluble receptor consists of 2 subunits, the alpha chain and the beta chain, both are able to bind IL-2, and together they build a high affinity unit. The alpha chain is only expressed after a T cell or B- cell stimulation and its elevation gives a pattern of immune activity [30].

IL-12p40 is a subunit utilized by IL-12 and IL-23. They both have a heterodimeric structure and share the larger IL-12p40 units.IL-12 and IL-12p40 are often interchangeably used [13].

IL-12 is secreted by dendritic cells, Langerhans cells and mononuclear phagocytic cells as well as B-cells and mast cells. In synergy with IL-18 it is an activator of the Th1 cell response. This happens via differentiation of T-cytotoxic cells and stimulation of IFNgamma as the paradigmatic cytokine of the TH1 response.[13] During the innate immune response, APC in contact with LPS and pathogen associates molecules (PAMP) start to secret p40 and p35. This initiates an IL-12 secretion in APC´s that results in the secretion of IFNgamma and stimulation of T- cell differentiation [31].

With the first antigen contact at the port of infection, IL-12 is only triggered by PAMPs and is the first molecule secreted independently of cytokine stimulation. Therefore the immune response starts before presentation of the APC in secondary immune organs and via the interferone release, the adaptive response and specific T-cell answer is triggered.[31]

3.3.5. Chemokines

Chemokines belong to a small group of the cytokine family and are subdivided into different groups according to their cystein tertiary structure [7]. Their main task is the recruitment of leukocytes to the site of infection, a process called chemotaxis. Via stimulation of the leukocytes, chemokines augment the local immune response and potency. The migration of neutrophils is transendothelial along the gradient of the chemokine concentration.[6, 15, 32]. Parallel to cell recruitment, adhesion molecules in the vascular endothelium are up-regulated [32]. In order to concentrate the immune cells to the center of local immune response. Today, it is known that the task of chemokines is more complex and pleiotropic. They lead lymphocytes during hematopoiesis to sample antigens in secondary lymphoid tissue [7].

Simplistically, the function of the chemokine family is subdivided in pro-inflammatory and homeostatic actions. However; chemokines may have both functions (i.e. MCP-1) [33].

The currently known important chemokines are the monocyte chemoattractant protein-1 (MCP-1, CCL2), macrophage inflammatory protein-1alpha/ CCL3 (MIP-1alpha), macrophage inflammatory protein-1beta/ CCL4 (MIP-1beta) and interleukin 8 (IL-8, CXCL8) [13].

MIP-1alpha and MIP-1beta are released from mostly monocytes and macrophages by proinflammatory cytokines (IL-1beta, TNF-alpha and IFNgamma) and bacterial triggers like LPS [34]. Inhibition of MIP-1 secretion is transmitted via anti-inflammatory cytokines including IL-4, IL-10 and T-cells. MIP-1alpha augments the potency of the Th1 response by induction of the IFNgamma release in activated T-cells. MIP-1beta influences the adhesion of T- cells at the site of infection via vascular cell adhesion molecules (VCAMs) [34].

MCP-1 is produced primarily by monocytes; however, many other cells release MCP-1, too. In contrast to the other chemokines, the main purpose of MCP-1 is attracting monocytes to the site of inflammation and is one of the crucial parts of the innate immune response [35].It also activates and influences the chemotaxis of T-cells, monocytes, and natural killer cells [30]. MCP-1 is responsible for lymphocyte recruitment to lymph nodes, where T- cells undergo further differentiation [6]. The first contact between T-cells and antigens takes place in secondary lymphoid organs such as lymph nodes.

Stimulated by MCP-1, native T-cells (TH 0-cells) differentiate into Th2-cells. [6]MCP-1 and histamine release from eosinophils has been established [36].

IL-8 is produced by stimulated monocytes but not by tissue macrophages and T-cells. Mononuclear phagocytes release IL-8 upon stimulation by other pro-inflammatory cytokines including TNF-alpha, IL-1, IL-3, IL-7, GM-CSF, immune complexes and bacteria [37].

Mononuclear phagocytes are the main source for IL-8 secretion [13]. It is the most potent chemoattractant for neutrophils [13]. In addition, IL-8 release lysosomal enzymes from neutrophils in the context of the acute phase reaction and inhibits the histamine release from human basophils [37].

Interferon inducible factor 10 (IP-10, CXCL10) plays a crucial role in the development of the neural system.IP-10 influences embryogenesis and the development of the nervous system by recruiting of neural cells and inducing apoptosis in glial cells [38]. IP-10 mediated increase in cell growth is also seen in bronchial cells, astrocytes, glioma cells and some cancer cell lines [38]. The anti-proliferative and antiangiogenetic effect mostly influences tumor cells and inhibits cancer growth. It acts as a counterpart to the angioproliferative IL-8 to maintain balance between the two effects. Endothelial cells are the cells most affected [38].

3.3.6. Anti-inflammatory cytokines

To regulate an adequate immune response, it is important to balance pro-and anti-inflammatory cytokines. After confronting the pathogen, a limitation of inflammatory response is necessary to prevent the body from its own destruction. An uncontrolled immune response can result in toxic shock with massive cellular damage [61]. In contrast to the majority of pro-inflammatory cytokines, IL-10 and IL-4 mainly exert anti-inflammatory effects. The lack of both cytokines results in total immunolysis during the proinflammatory response and it is responsible for multiple autoimmune diseases such as inflammatory bowel diseases and rheumatoid arthritis [61].

The crucial task of IL-10 is the inhibition of the acute inflammation response. This is accomplished by inhibiting the interacting proinflammatory cytokines. IL-10 directly inhibits IL-1 and TNF-alpha, the main activators of the acute phase response, as well as IL-1beta, IL-1alpha and the chemokine family

[62]. The inhibition works via two points of action. First is the inhibition of proinflammatory cytokines and the second is activation of the production in natural antagonist, like IL-1ra [61, 62].

IL-4 is a T-cell activator and influences B- cell growth .The main source of IL-4 is T- cells, especially activated CD4 T- helper cells. IL-4 influences Th2 cells in its pathway to maturation from primordial Th0 cell by up-regulation at the surface of Th2 cells, where IL-4 stimulates the cell maturation in the autocrine way. Other IL-4 sources are mast cells, eosinophils and basophiles, which release IL-4 via crosslinking of the Fc- receptor at the mast cell surface [63-65].

The mast cells, which fulfill a less specialized differentiation, contain IL-4 as a granule associated peptide in a preform and release IL-4 in the context of an allergic reaction. With the release of IL-4 during the first antigen contact, the adaptive immune response is started in the antigen dependent priming of native CD4 cells and differentiation to Th2 cells and B cell proliferation is started [13, 65].

The Th2 response is important for stimulation of the humoral immune response and activation of B-cells. It negatively controls the Th1 response that defines the anti-inflammatory task of IL-4 [65].

IL-4 influences B-cell lymphopoiesis by activation, differentiation and proliferation of B-cells, as well as augmentation of the resting B-cell numbers. By stimulating MHC II, B7/CD 72 (CD80/CD86), CD 40 and IgM the antigen presenting capacity of B-cells to T- cells is increased [64]. CD40 activated native B- lymphoid cells secret IgE in response to an IL-4 stimulation, which is a consequence of an isotype switch through deletional recombination in the B- cells DNA [66].

3.4. Hypoxia in high altitude

Hypoxia is defined as the reduced partial pressure of oxygen in the inspiratory air that alters and influences the homeostasis of the body system. The relationship of altitude to partial O2 pressure is inverse, and a chain of physiological processes is initiated [39]. With higher altitude, the partial pressure of oxygen is reduced; however, the percentage of oxygen remains 20.93% in the atmosphere at every altitude [40, 41].

A reduced inspired oxygen level results in a pathway of adaption processes, first in organ systems and later in cellular mechanism. The path of the oxygen from the air through the lungs to the blood stream and later to the mitochondria of the cell is called the oxygen cascade. When there is a

reduction of inspired oxygen, the peripheral chemosensor in the carotid body detects the lack of oxygen and stimulates an increase in the respiratory rate, a process called ventilatory acclimatization [42-44].

At the cellular level, hypoxemia triggers the secretion of erythropoietin that augments the number of red blood cells to increase the number of oxygen carriers in the blood stream.

On the molecular level, HIF-1 (hypoxia inducible factor-1) is released to trigger angiogenesis via cytokines and growth factors, to support continuing oxidative metabolism [42-44].

Acclimatization to hypoxia is mediated predominantly through HIF-1 and its subunits. HIF-1protects against tissue hypoxia at the molecular level and the HIF-1 pathway has been identified to regulate several hundred genes by direct or indirect modulation of gene expression. HIF-1 induced gene products modify various cell functions including metabolic functions and angiogenesis [42, 43]. All response mechanisms' goal, whether it is hematopoietic, vascular or cardiorespiratory, are to maintain an adequate tissue oxygenation [45].

3.5. Hypoxia and cytokines

Acute exposure to high altitude changes immunological parameters [39, 40]. Lymphocytes and phagocytes are known to show adaptation to hypobaric hypoxia via cytokine release [46].

Hypobaric hypoxia orchestrate the activity of immune cells. The T-cell mediated immune response is impaired more by hypoxia than natural killer cells that exhibit temporary changes [46]. In general, the phagocytic cells (monocytes, macrophages, neutrophils and mast cells) are stimulated by hypoxia, and are the first line of defense. There is a survival advantage to have immunological defenses stimulated by hypoxia from inflammation e.g. burns, trauma and pancreatitis [45]. Hypoxia stimulates phagocytosis, chemotaxis and increases neutrophils [46-48]. Hypoxia sensitized monocytes and T-cells respond with an elevation in the release of the proinflammatory cytokines like TNF-alpha, IL -1alpha, and IL-1beta [49].

The involvement of hypoxia triggered cytokine response as the cause for high altitude illness is a long held presumption. To date, there is no real evidence for cytokine involvement [49].In vitro hypoxia studies show that endotoxin stimulated mononuclear cells cause increased levels of IL-1alpha, IL-1

beta and TNF-alpha, and it is speculated that this is the origin of acute respiratory distress syndrome (ARDS) and high altitude pulmonary edema [16].TNF-alpha and the other proinflammatory cytokines have been found in increased concentrations in BAL (bronchoalveolar lavage fluid) in patients with high altitude edema and ARDS [50, 51].

IL-6 plays a key role in the acute phase response. The role of pluripotential IL-6 during hypoxia in the context of EPO (erythropoietin) stimulation and not in its inflammatory task [52]. A correlation of elevated acute phase proteins or other pro-inflammatory cytokines (IL-1, IL-1beta and TNF-alpha) and the co-stimulatory effect of IL-6 on EPO production in hepatocytes is known [52]. The signaling mechanisms remain unknown, however [53].Hypoxia is identified as a co-stimulator of the pro-inflammatory cytokine production. The elevation in systemic proinflammatory cytokine levels is from local inflammation such as inflammation in the lung or the brain. The increase in inflammatory cells and cytokine levels are for the immune system to better react to the insult [54].

Local inflammatory reactions having systemic effects have been studied. In 1999, chemokines released from cerebral ischemia was first described by Bona et al [55]. In 2012, Cowell et al. [56] showed that MIP-1alpha was increased in cerebral hypoxic ischemic lesions five days after the hypoxic event in neonatal rat brains. This animal model showed a correlation of hypoxic time and increase in MIP-1 alpha. It is assumed that cerebral microglia and activated monocytes are secreting MIP-1 alpha [56].

Present research tries to link a coherence of systemic inflammation from alveolar macrophages is triggered by alveolar hypoxia. Not expectantly, systemic inflammation is not influenced by hypoxia in the tissue, but is influenced by the release of MCP-1 from macrophages playing a central role in systemic inflammation. MCP-1 is released into the blood stream by macrophages and travels to the target tissue to trigger mast cell degranulation. This is the first step of immune response due to hypoxia [36,57,58].

High altitude pulmonary edema (HAPE) and ARDS are associated with elevated levels of IL-8. The exact mechanism remains unclear, but it is speculated that IL-8 attracts leukocytes to the ischemic areas [59]. This theory is supported by the work of Karakurum et al. [60] who could prove that under hypoxic conditions endothelial cells produce IL-8 which triggers an increase of migration of neutrophils to the site of ischemia. Signal transmission is via nfĸB-pathway. Further factors increased are IP-10, IFNgamma, TNF-alpha, and IL-2.

3.6. Physical Exercise and the immune system at high altitude

Exercise and hypoxia are two different, independent stressors for the immune system. It is suggested that exercise under hypoxic conditions provokes a more pronounced immunological stress than exercise under sea level conditions. In addition to high altitude, exercise has a severe and transitory impact in the immune system and longs for a cooperation of the immunological, metabolic and sympatho-adrenal response to maintain the homeostasis [39].

Acute and chronic exercise has a transitory effect on the circulating immune cells.

Neutrophils are increased immediately after exercise; a second delayed increase follows after a few hours. The first increase is catecholamine triggered and the second wave is assumed to be a cortisol dependent bone marrow release. Exercise stimulated hypoxia increases phagocytosis, chemotaxis and the reactive oxygen species (ROS) effect and is known as the hypoxic effect [61]. Circulating monocytes are increased about 2 hours after exercise and catecholamines are likely responsible by causing the demargination of immature monocytes. With a down-regulation of TLR (toll like receptor) there is a concomitant dependent decrease of IL-1, IL-6 and TNF-alpha [67, 68].

A post exercise lymphocytosis is well known and the increase is dependent on the intensity and duration of the exercise. The increase in T-and B- cells are mostly catecholamine dependent and normal levels return within 24 hours. The consensus of multiple studies have demonstrated an insignificant elevation in T-and B-cells [67-70].

Cytokine change due to exercise is different than the typical inflammatory cytokine release. The acute phase cytokines such as TNF-alpha and IL-1beta are normally not elevated after exercise. A different cascade in cytokine release compared to inflammatory cytokine pathways is assumed. IL-6 is one of the first cytokines to be released with exercise. Its source is the contracting muscle cells and they have a role in lipid metabolism. TNF-alpha, normally released after endotoxin stimulation during exercise, is generally decreased. This observation support the theory that the immune system is activated after exercise, but its role is more metabolic than immunologic.

Anti-inflammatory cytokines, such as IL-10 and IL-1ra, are generally elevated after exercise. In total, an anti-inflammatory milieu is produced and is influenced by catecholamines, cortisol, and growth factors that influence cytokine metabolism [69, 70].

3.7. Hypothesis

The current work tries to illuminate the effect of hypobaric hypoxia on cytokine plasma levels measured by Luminex xMAP Multiplexing Technology under different circumstances.

In detail, the following questions should be answered:

1) Is there a change in cytokine levels in hypobaric hypoxia?

2) Is there a change in cytokine plasma levels after the additional trigger exercise under normoxia and under hypobaric hypoxic conditions in high altitude?

3) Is there an effect on cytokine release after a quick ascent from 5050m to 6035m?

4. Materials and methods

All samples and entailing data sets have been acquired during both data collection sessions in Bangor [United Kingdom (UK)] in June 2008 and an international medical expedition (Hidden Valley Expedition 2008) in the Western Himalayan mountains (Dhaulagiri Circuit, Nepal) in September 2008. Both was organized by Medical Expeditions.

The following methods are identical with the doctoral thesis of Sven Dietrich with focus on the change of growth factor under the mentioned conditions.

Data presented in this study originates from a pool shared by the following cross-over study presenting complementary findings with respect to cytokine interaction during a high altitude (5050 m) sojourn.

4.1. Materials

4.1.1. Equipment

Sterican® 20-Gauge needle (B. Braun Melsungen AG, Melsungen, Deutschland), EDTA Vacutainer™ (BD, Franklin Lakes, New Jersey, USA), Heparin Vacutainer™ (BD, Franklin Lakes, New Jersey, USA), Eppendorf tubes of 1.5 ml (Eppendorf AG, Hamburg, Germany), pipette (Genex B) (Genex Laboratories, Torquay, UK), centrifuge (Eppendorf MiniSpin) (Eppendorf AG, Hamburg, Germany), pulseoximeter (Nonin Onyx 9500) (Nonin Medical Inc., Plymouth, Minnesota, USA), barometric pressure wrist watch (Suunto X3HR) (Suunto Oy, Vantaa, Finland), ratings of perceived exertion (Borg, 1982) and mood (Feeling scale -5/+5: Hardy and Rejeski, 1989), SigmaStat and SigmaPlot (Systat Software, Inc., San José, California, USA), heart rate monitor (Polar S120) (Polar, Kempele, Finland), Eppendorf Thermomixer comfort (Eppendorf AG, Hamburg, Germany), Luminex 100 (Luminex Corporation, Austin, Texas, USA), hemoglobin-photometer (HemoCue Plasma/Low Hb Photometer, Sweden/UK), centrifuge (HaematoSpin 1400, Hawksley, UK), hematocrit reader (Tube reader, Hawksley, UK), new Neubauer chamber.

4. Materials and methods

4.1.2. Chemicals

20-plex Premixed Human Cytokine Milliplex Map Panel Immunoassay (Cat. # SPRPMX21) including EGF, FGF-2, G-CSF, GM-CSF, IFNgamma, IL-1ra, IL-2, sIL-2ra, IL-4, IL-6, IL-8, IL-10, IL-12p40, IFNgamma-induced protein (IP)-10, monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein (MIP)-1alpha, MIP-1beta, TNF-alpha, Human Cytokine/Chemokine Standard, Human Cytokine Quality Controls 1 and 2, Serum Matrix, Assay Buffer, Wash Buffer, Human Cytokine Detection Antibodies, Streptavidin-Phycoerythrin (Merck Millipore, Billerica, Massachusetts, USA), and Sheath Fluid (Luminex)

4.2. Methods

4.2.1. Study population

The study formed one of a series completed on the "Hidden Valley Expedition 2008" to Nepal. The study was approved by both the Coventry Ethics Committee and the Nepal Health Research Council, and all participants provided written informed consent.

All 25 subjects of the study were healthy moderately active expedition members. For further demographic data see table 1.

Table 1 Demographic data

The table shows demographic data of expedition members who participated in the present study. Values are expressed as mean (standard deviation), except for gender distribution (absolute numbers).

M male, *F* female, *BMI* body mass index [body weight in kilograms per square meters (kg x m2)].

	Whole Group	Group 1 Resting	Group 2 Exercise	Group 3 Damphus Peak
Sex, M : F	16 : 10	15 : 10	5 : 3	4
Age, years	33.1 (± 11.74)	32.8 (± 11.33)	32.8 (± 8.44)	26.0 (± 3.91)
BMI, kg x m2	23.33 (± 12.90)	22.48 (± 13.57)	27.12 (± 14.43)	14.25 (± 11.78)

4.2.2. Study design

The study shows a non-randomized, non-blinded design with two parallel groups and an additional third group. The differing total numbers of participants in the 3 groups are due to the fact that individual subjects either took part in all groups or two or only one and due to the fact that some subjects only took part at sea level (69 m) but not at high altitude (5050 m). That is why for instance in group 2 as many as 10 subjects took part at sea level (69 m), but only 8 subjects at high altitude (5050 m). For further detail see table 2.

Table 2 Number of participants in the 3 groups studied

The table shows the distribution of participants and the strength of the different groups both at sea level (69 m) and high altitude (5050 m). Values are expressed as absolute numbers of participants.

	Whole Group	Group 1 Resting	Group 2 Exercise	Group 3 Damphus Peak
Total	26	25	10	4
Sea level (69 m)	25	25	10	-
High altitude (5050 m)	18	18	8	4

Group 1 members represent the reference group at rest. This group was comprised of 25 participants at sea level (69 m) and 18 at high altitude (5050 m).

Group 2 members underwent an incremental exercise test and totaled to 10 at sea level (69 m) and 8 at high altitude (5050 m).

In addition, a third group of 4 participants underwent a one-day climb from Hidden Valley Base Camp (5050 m) to Damphus Peak (6035 m) and descending back to Hidden Valley Base Camp.

The difference in altitude from ascent to Damphus Peak (6035 m) to descent to Base Camp (5050 m) totaled to 1970 meters.

In accordance with ethical approval from Coventry University Ethics Committee, participants gave their written informed consent to participate in the study in order to verify that they were fully informed of the study's requirement and had been notified of and understood the possible risks. In addition, participants completed a health screen questionnaire prior to conducting each of the two trials.

It has to be taken into account that subjects may or may not have participated in other double-blinded studies of which one applied carbohydrate supplementation and the other inhaled Iloprost.

4.2.3. Inclusion and exclusion criteria

Inclusion criteria were normal hemoglobin and hematocrit values (quantified prior to commencing the expedition). Subjects were also asked to refrain from consuming food for at least 2-3 hours prior to exercise. All subjects were non-smokers over the age of 18 years.

Exclusion criteria were a history of cardiovascular, respiratory, nervous, renal, liver, skeletal/muscular and metabolic disease. Additionally, none of the participants suffered from immunosuppression, was on any regular medication, or had suffered recently an allergic reaction. Other medical conditions were well controlled and individuals were free of symptoms at the time of the expedition's departure.

4.2.4. Data collection protocol

The study consisted of two trials of data collection. The first trial was conducted at Bangor University, UK in July/August 2008 (69 m, barometric oxygen pressure (pO_2) 760 mmHg) and the second trial took place at Hidden Valley Base Camp, Nepal in October 2008 (5050 m, pO_2 550 mmHg).

4. Materials and methods

4.2.5. Expedition itinerary

The main route of ascent corresponded to the Dhaulagiri Circuit in the Western part of the Nepalese Himalayas. On the way to Hidden Valley Base Camp at 5050 m expedition members were exposed to various climatic conditions such as hot weather zones, humid subtropical climate, monsoon rain, and finally a permafrost zone above 3000 m. Expedition members were divided into four trekking groups, each of which departed on a different day.

Table 3 and figure 1 show the complete expedition itinerary.

Route				Altitude (m)			Barometric Pressure (mmHg)			Comments	
Date (dd.mm.yy)	Day	From	To	Start	End	Difference	Start	End	Difference		
02.10.08	1.	Beni	Babichar	826	970	144	914	900	14		
03.10.09	2.	Babichar	Phedi	970	1007	37	904	885	19		
04.10.08	3.	Phedi	Muna	1007	1701	694	880	826	54		
05.10.08	4.	Muna	Jugapani	1701	1433	268	826	831	5		
06.10.08	5.	Jugapani	Bogara	1433	2110	677	855	793	62		
07.10.08	6.	Bogara	Bogara	2110	2110	0	792	793	1	Rest	
08.10.08	7.	Bogara	Dobang	2110	2475	365	792	756	36		
09.10.08	8.	Dobang	Dobang	2475	2475	0	759	758	1	Rest	
10.10.08	9.	Dobang	Salagari	2475	3000	525	759	703	56		
11.10.08	10.	Salagari	Italian Base Camp	3000	3606	606	705	683	22		
12.10.08	11.	Italian Base Camp	Italian Base Camp	3606	3606	0	665	664	1	Rest	
13.10.08	12.	Italian Base Camp	Japanese Base Camp	3606	4220	614	661	613	48		
14.10.08	13.	Japanese Base Camp	Dhaulagiri BC	4220	4691	471	616	580	36		
15.10.08	14.	Dhaulagiri BC	Hidden Valley	4691	5050	359	579	580	1		
16.10.08	15.	Hidden Valley	Hidden Valley	5050	5050	0	580	512	68	Research	
17.10.08	16.	Hidden Valley	Hidden Valley	5050	5050	0	554	512	42	Research	
18.10.08	17.	Hidden Valley	Hidden Valley	5050	5050	0	NA	550	NA	Research	
19.10.08	18.	Hidden Valley	Hidden Valley	5050	5050	0	553	555	2	Research	
20.10.08	19.	Hidden Valley	Hidden Valley	5050	5050	0	553	NA	NA	Research	Damphus Peak (6035m)
21.10.08	20.	Hidden Valley	Hidden Valley	5050	5050	0	NA	NA	NA		Damphus Peak (6035m)
22.10.08	21.	Hidden Valley	Yak Kharka	5050	3885	1165	NA	643	NA		
23.10.08	22.	Yak Kharka	Marpha	3885	2670	1215	NA	NA	NA		
24.10.08	23.	Marpha	Jomson	2670	2720	50	NA	NA	NA		

Table 3 Expedition itinerary

The expedition itinerary is outlined by showing starting and end points of each expedition day with corresponding altitudes.

BC Base Camp, *m* meters

4. Materials and methods
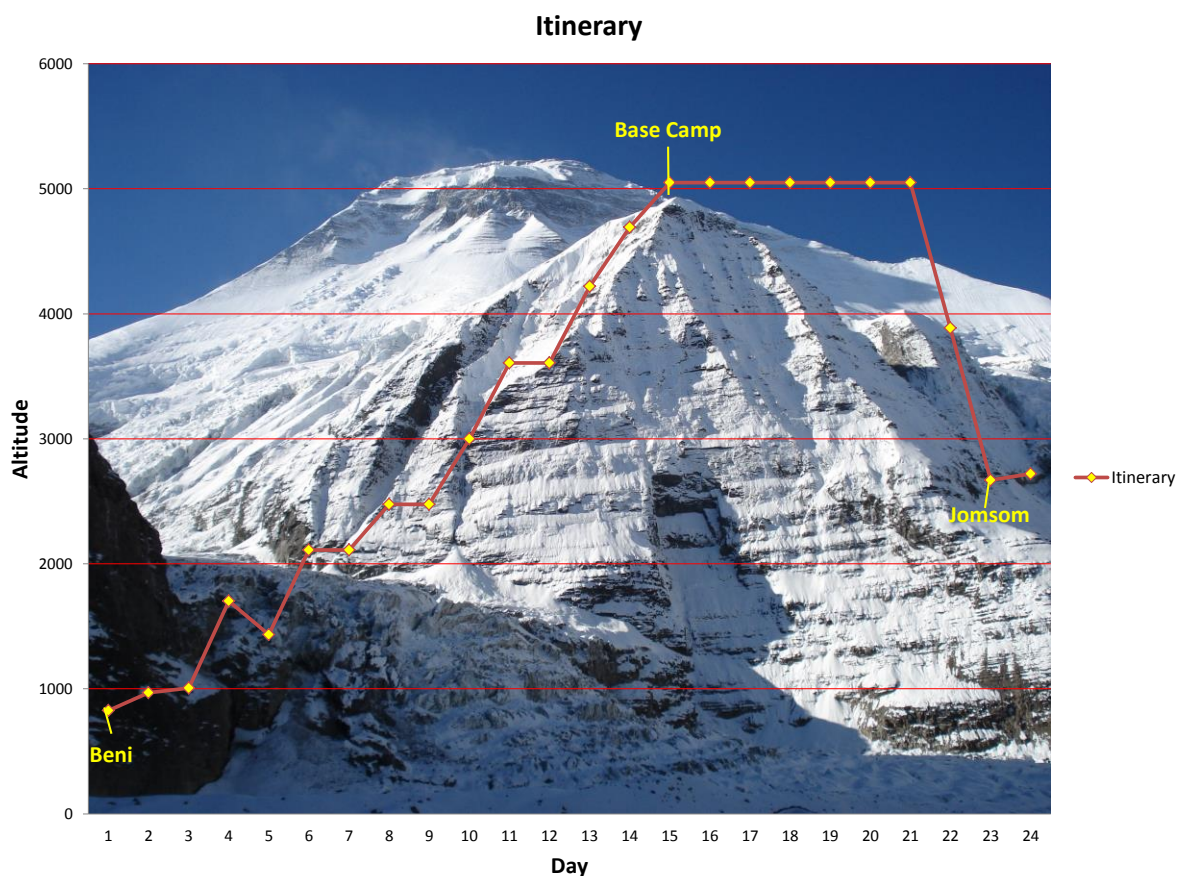
Figure 1 Expedition itinerary

The expedition in the Nepalese Himalayas started at Beni (826 m), went via the Dhaulagiri Circuit for 14 days in order to arrive at Hidden Valley Base Camp (5050 m). After 6 days of research the expedition went on and ended in Jomsom (2720 m).

4.2.6. Collection of blood samples

4.2.6.1. Set-up

Baseline resting blood samples were taken from a population of 25 participants (10 women, 15 men) in Bangor (UK, 69 m) and of 18 participants (7 women, 11 men) in Hidden Valley Base Camp (Nepal, 5050 m) who were all overnight fasted (minimum 8 hours fast). Participants remained in a seated position for 15 minutes prior to venipuncture from an antecubital vein by means of a 20-Gauge needle. A total amount of 6 ml blood was distributed into 1 EDTA and 1 Heparin Vacutainer™ of 3 ml each.

Subjects who underwent an incremental step test were comprised of a population of 10 participants in Bangor (69 m) and 8 participants in Hidden Valley Base Camp (5050 m). Participants were fasted at least for 2-3 hours prior to the first and remained fasted until the last phlebotomy.

After the first phlebotomy an incremental step test was undertaken. Each participant stepped on and off a 25 centimeter metal step over a period of twelve minutes in total subdivided into three intervals of four minutes each. An acoustic beeping signal indicated the step-frequency which was for the first interval 13 steps per minute, for the second 26 steps per minute, and with initiation of the third interval the beeping signal was turned off and the subject was asked to apply a step-frequency as high as possible. One entire stepping cycle consisted of putting one foot on the step with the first beep, followed by the second foot with the second beep, so that the participant was standing with both feet on the step. With beep three and four, the feet were brought down in the same fashion one by one. Every minute the leading foot was changed in order to prevent exhaustion of one particular lower limb. Immediately prior to and during exercise heart rate (Polar, S120), ratings of perceived exertion and mood (Feeling scale -5/+5) were recorded at minute intervals. Four more blood samples were taken directly after, 15 min, 90 min, and 180 min after cessation of exercise. A total of 30 ml of blood was collected per participant.

A supplementary third group was formed by four subjects (all male) who undertook a one-day climb from Hidden Valley Base Camp (5050 m) to Damphus Peak (6035 m). After an overnight fast, blood samples were taken from participants at 03:30 a.m. (local time) prior to ascent and directly after return from the Damphus Peak summit. During their climb participants did not remain fasted. Meters covered on ascent and descent totaled to 1970. Duration of ascent and descent averaged to 12 hours (from 03:30 a.m. until 3:30 p.m.).

4.2.6.2. Processing of Blood Samples

Immediately after venipuncture the utilized EDTA Vacutainer^{TM} was aliquoted into 2 Eppendorf tubes of 1.5 ml each by means of a pipette (Genex B). Same procedure was applied to the utilized Heparin Vacutainer^{TM}. Within 30 minutes after venipuncture Eppendorf tubes were spun in a centrifuge (Eppendorf MiniSpin) for 10 minutes at sea level (69 m) and for 5 minutes at high altitude (5050 m)

at a relative centrifugal force of 12,000. Afterwards, plasma from the 2 EDTA and 2 Heparin aliquots was distributed to 6 and 2 Eppendorf tubes of 0.5ml each, respectively.

4.2.6.3. Storage and Transport of Blood Samples

After processing, blood samples were sealed in plastic bags and kept at -26° Celsius (C) in a freezer. At Hidden Valley Base Camp (Nepal, 5050 m), the portable freezer was insulated with a styrofoam case and cooled via a gas cartridge on the day of descent. Transportation of the freezer by foot to the nearest town with electrical supply for cooling took 8 hours. During transportation a remote sensor in the freezer allowed for real time temperature readings and re-cooling via a gas cartridge provided stable temperatures of at least -10° C or below. Subsequently, samples were transported by plane from Nepal to the UK, where samples from the first trial were added and finally sent via air mail to the laboratory in Homburg (Germany).

4.2.7. Immunoassay

In order to analyze specimen Luminex xMAP Multiplexing Technology was employed. Following cytokines were measured simultaneously: IL-1beta, IL-2, IL-6, IL-8, IL-12p40, IL-1ra, sIL-2ra, IFNgamma, TNF-alpha, MCP-1, MIP-1alpha, MIP-1beta, IP-10 and anti-inflammatory (IL-4 and IL-10). Additionally, the growth factors from Sven Dietrichs study were analyzed.

4.2.7.1. Luminex xMAP Multiplex Technology

Luminex allows simultaneous analysis of exact quantities of a large number of small-volume samples. It is a highly specific, sensitive, and reliable assay. Luminex is based on flow cytometry and employs as the key principle the fluorescent color coding of microspheres (polystyrene particles) allowing their identification and precise correlation with their respective population through optical analysis. Through combination of red and infrared fluorescent dyes 100 (10^2) different fluorescent color tones are created. One unique bead class is made up by one microsphere dyed with one of the 100 different fluorescent color tones and its specific capture antibody. Specific reaction partners (analytes) bind to

the microsphere´s capture antibody. The resulting complex (bead + capture antibody + analyte) is recognized by a detection antibody and subsequently labeled with so-called reporter molecules. The median fluorescence intensity of the reporter molecule is then used to quantify the amount of analyte bound to the bead. In this manner, a single sample of a volume as small as 25 µl can be incubated with up to 100 different bead classes at the same time on a 96-well-microtiter plate [71, 72].

After the binding reaction is completed, microspheres are suctioned from the microtiter plate. While two lasers are directed at the particle stream, mirrors direct fluorescence discriminator signals emitted from microspheres and detection signals emitted from reporter molecules to detectors. Figure 2 shows the set-up of the bead (microsphere) technology. Photodiodes classify the microspheres and detect side scatter signals of the particles, which allows determination of the particle size and exclusion of fluorescent contaminants or microsphere aggregates (e.g., doublets) from the results, respectively. A special laser excites the fluorescent dye of the reporter molecules and the intensity of the emitted light allows for quantification of the analyte bound to a particular microsphere. Finally, optical signals are translated into digital signals. With the Luminex analysis system, up to 20,000 events per second can be amplified, processed and recorded.
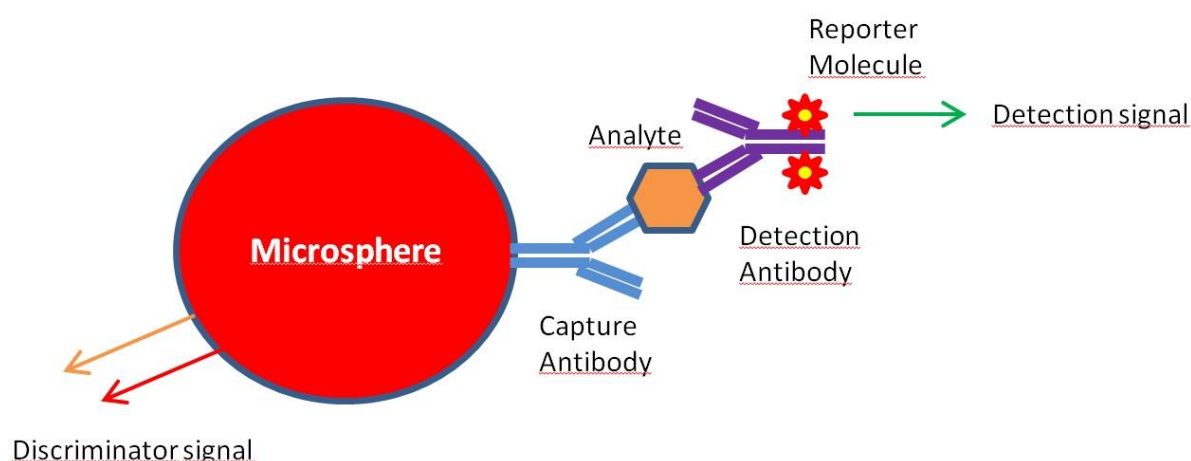
Figure 2 The bead (microsphere) technology of Luminex

Luminex employs as the key principle the fluorescent color coding of microspheres (polystyrene particles) allowing their identification and precise correlation with their respective population through optical analysis. One unique bead class is

made up by one microsphere dyed with one of the 100 different fluorescent color tones and its specific capture antibody. Specific reaction partners (analytes) bind to the microsphere´s capture antibody. The resulting complex (bead + capture antibody + analyte) is recognized by a detection antibody and subsequently labeled with a so-called reporter molecule. While two lasers are directed at the particle stream, mirrors direct fluorescence discriminator signals emitted from microspheres and detection signals emitted from reporter molecules to detectors. Photodiodes classify the microspheres and detect side scatter signals of the particles. The median fluorescence intensity of the reporter molecule is then used to quantify the amount of analyte bound to the bead. Optical signals are finally translated into digital signals.

4.2.7.2. Assay

Before analysis, samples were prepared as recommended by the manufacturer's instructions (see figure 3). Premixed antibody-immobilized beads were sonicated for 30 seconds and vortexed for 1 minute. Quality controls 1 and 2 were generated by adding 250 µl deionized water, mixing and vortexing the vials (rest before use: 5-10 min) and pipetting them into test tubes. At room temperature 30 ml of wash buffer were diluted with 270 ml deionized water. 1.0 ml of deionized water was mixed into the bottle containing lyophilized Serum Matrix (rest before use: 10 min). Adding 250 µl deionized water resulted in human cytokine standard (10,000 pg/ml). Working standards were generated by serial dilutions of the standard dilution (10,000 pg/ml). Five test tubes were filled with 200µl of Assay Buffer (0 pg/ml). 50 µl of the 10,000 pg/ml standard was added to tube 1 (= 2000 pg/ml). 50 µl of tube 1 was added to tube 2 (= 400 pg/ml). 50 µl of tube 2 was added to tube 3 (= 80 pg/ml). 50 µl of tube 3 was added to tube 4 (= 16 pg/ml). 50 µl of tube 4 was added to tube 5 (= 3.2 pg/ml).

4. Materials and methods

Standard Concentration (pg/mL)	Volume of Deionized Water to Add	Volume of Standard to Add
10,000	250 µL	0

Standard Concentration (pg/mL)	Volume of Assay Buffer to Add	Volume of Standard to Add
2000	200 µL	50 µL of 10,000 pg/mL
400	200 µL	50 µL of 2000 pg/mL
80	200 µL	50 µL of 400 pg/mL
16	200 µL	50 µL of 80 pg/mL
3.2	200 µL	50 µL of 16 pg/mL
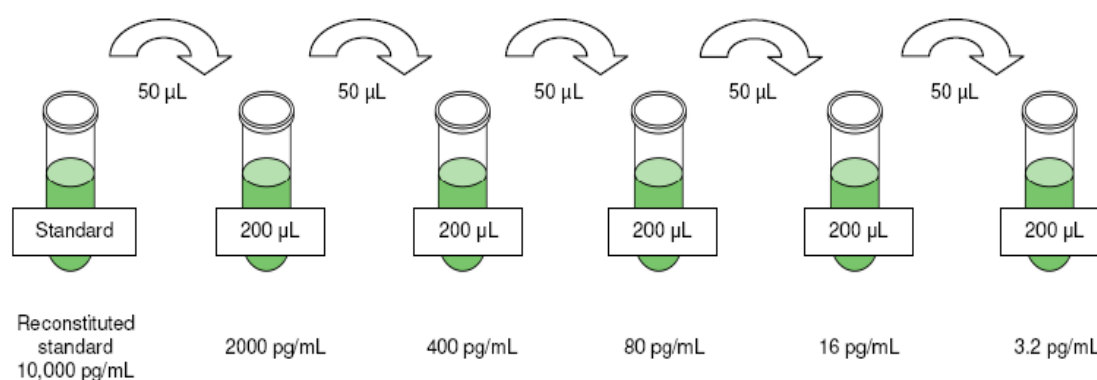
Figure 3 Assay preparation

Before analysis, samples were prepared as recommended by the manufacturer's instructions. 1.0 ml of deionized water was mixed into the bottle containing lyophilized Serum Matrix and 250 µl deionized water was added. The resulting human cytokine standard (10,000 pg/ml). Working standards were generated by serial dilutions of the standard dilution (10,000 pg/ml). Five test tubes were filled with 200 ìl of Assay Buffer (0 pg/ml). 50 µl of the 10,000 pg/ml standard was added to tube 1 (= 2000 pg/ml). 50 µl of tube 1 was added to tube 2 (= 400 pg/ml). 50 µl of tube 2 was added to tube 3 (= 80 pg/ml). 50 µl of tube 3 was added to tube 4 (= 16 pg/ml). 50 µl of tube 4 was added to tube 5 (= 3.2 pg/ml).

pg pictogram, *ml* milliliter, *µl* microliter

After adding 200 µl of Assay Buffer into each of the 96 wells the microtiter plate was sealed and mixed on a plate shaker (Eppendorf Thermomixer comfort) at 500 revolutions per minute (rpm) for 10 minutes at room temperature (20-25° C). Assay Buffer was then removed and 25 µl of each Standard and Quality Control solution were pipetted into the respective wells. Assay Buffer was used for the 0 pg/ml standard (background). Each sample well was filled with 25 µl of Assay Buffer and 25

µl of Serum Matrix solution was added to each of the Background, Standards, and Quality Control wells. Eventually, 25 µl of each sample was pipetted into the appropriate wells. The bottle containing the premixed beads was vortexed and 25 µl of the bead mix were added to each well.

After incubation (60 min at 20-25° C) on the plate shaker the fluid phase was removed from the microtiter plate and each well was washed 2 times with 200 of Wash Buffer.

Before a second incubation process (30 min at 20-25° C) began, 25 µl of detection antibodies were added to each well. After pipetting 25 µl of Streptavidin R-Phycoerythrin into each well, a third incubation period (30 min at 20-25° C) on the plate shaker began. All fluid was removed from the microtiter plate and each well was washed 2 times with 200 µl of Wash Buffer. Each well was then filled with 150 µl of Sheath Fluid, the plate was put on a plate shaker for 5 minutes, and ultimately inserted into the Luminex 100™ IS. The median fluorescent intensity (MFI) data was saved and cytokine concentrations in samples were calculated.

4.2.7.3. Software

Data was analyzed using Luminex IS 2.3 and both MFI and calculated concentration values were reported for each analyte.

4.2.8. Statistical method

Statistical analysis was performed using SigmaStat 3.1 (Systat Software, Point Richmond, CA, USA). Differences between sea level (69 m) and high altitude (5050 m) values as well as between pre-exercise and post-exercise values were analyzed using the Wilcoxon test. Values of $p < 0.05$ were considered statistically significant. Results are presented as median (Min; Max). We considered 3.2 pg/ml, the lowest dilution on the standard curve, to be the lower limit of detection.

4.2.9. Additional data

While leucocyte counts were obtained by means of microscopic visualization in a new Neubauer chamber, data about differential counts was provided kindly by the laboratory of Heartlands hospital (Birmingham, UK).

Hemoglobin content of blood samples was measured by means of a hemoglobin-photometer (HemoCue Plasma/Low Hb Photometer). By means of both a centrifuge (HaematoSpin 1400) and a hematocrit reader (Tube reader), hematocrit measurements were obtained.

Data sets from the participants´ data collection booklets were provided by the University of Bangor (Wales, UK). Data include cold symptoms, Bristol stool scale, resting heart rate, arterial oxygen saturation, barometric pressure, and the Lake Louise Score (LLS).

Cold symptoms in the last 24 hours were reported by the participant in person in the evenings throughout the entire sojourn. For each of the following items participants gave subjective score points with 0 = none, 1 = mild, 2 = moderate, and 3 = severe: sneezing, runny nose, blocked nose, sore throat, cough, chilliness, and malaise.

Gastrointestinal voiding was self-reported on a daily basis by employing the Bristol Stool Scale (number of motions per day and consistency of motions on a 1–7-point Likert scale).

Resting heart rate and arterial oxygen saturation was recorded both in the mornings while fasted and seated for 10 minutes and in the evenings while seated for 10 minutes by means of a pulse oximeter (Nonin Onyx 9500). Barometric pressure was read from a wrist watch (Suunto X3HR).

The LLS (Lake Louis Score)was filled in by the participant in person in the mornings throughout the entire sojourn. For each of the following five symptoms participants gave subjective score points in increasing severity from 0 to 3: headache, gut symptoms, fatigue/weakness, dizzy/lightheaded, difficulty sleeping (see table 4).

4. Materials and methods

Score	Headache	Gastrointestinal symptoms	Fatigue/ weakness	Dizziness/ lightheadedness	Difficulty Sleeping
0	None	None	None	None	Slept as well as usual
1	Mild	poor appetite or nausea	Mild	Mild	Did not sleep as well as usual
2	moderate	moderate nausea or vomiting	Moderate	Moderate	Woke many times, poor night's sleep
3	Severe	severe nausea and vomiting, incapacitating	Severe	Severe	Could not sleep at all

Table 4 Lake Louise Score

The Lake Louise Score uses five symptoms, each scoring from 0 to 3 with increasing severity in order to detect AMS.

The diagnosis of AMS is based on a rise in altitude within the last four days and the application of the scoring system according to the LLS. In order to diagnose AMS, headache is obligatory and at least one other symptom of the LLS has to be present (gastrointestinal symptoms, fatigue/weakness, dizziness/lightheadedness, difficulty sleeping). Finally, a total score of 3 or more from the questionnaire confirms the diagnosis of AMS. 3 to 5 points mean mild AMS and scores of 6 and above signify severe AMS

5. Results

Results for cytokines and additional parameters like blood cell counts and saturation were obtained after statistical calculation.

According to the hypothesis, the results are subdivided into three parts.

At first, data from resting subjects was analyzed and sea level values were related to high altitude values. Additionally, data from subjects who undertook a standardized incremental exercise test was utilized to check values before exercise against values 180 minutes after cessation of exercise, both was confirmed at sea level and high altitude. At last, data obtained from a one-day climb totaling to an altitude difference of 1970 meters was evaluated to compare values before ascent with values after descent.

As the major finding, proinflammatory cytokines (sIL-2ra, IFNgamma, TNF-alpha), especially chemokines (IL-8, MCP-1, MIP-1alpha, MIP-1beta) showed in general a significant changes predominantly when comparing sea level values with high altitude values.

Compared to sea level values, high altitude plasma levels were elevated in the following cytokines: IL-8, IL-1ra, sIL-2ra, TNF-alpha, MIP-1alpha and MCP-1. A decrease in high altitude plasma levels was found in IFNgamma, IL-12p40 and MIP-1alpha.

There was no major impact on cytokine levels through exercise both at sea level and at high altitude. The additional short term altitude difference of 970 m had no impact in cytokine plasma levels.

5.1. Group 1 (Resting at Sea Level versus High Altitude)

Following table shows all data from participants that were at rest both at sea level and at high altitude.

Variable (units) n=25	Sea Level	High Altitude	p value	Rise ↑/ Fall ↓
IL-1beta	6.345 ± 7.466	10.84 ± 18.884	NS	↑
IL-2	6.566 ± 12.54	6.6 ± 16.401	NS	↑
IL-4	3.178 ± 0.108	4.958 ± 6.493	NS	↑
IL-6	13.001 ± 34.257	13.492 ± 38.112	NS	↑
IL-8	5.786 ± 2.528	10.186 ± 4.693	<0.001	↑
IL-10	27.086 ± 108.326	28.332 ± 110.762	NS	↑
IL-12p40	48.711± 81.042	33.382 ± 64.249	0.011	↓
IL-1ra	19.139 ± 43.677	36.991 ± 80.163	0.002	↑
sIL-2ra	40.974 ± 94.385	118.477 ± 192.003	<0.001	↑
IFNgamma	19.561 ± 17.214	11.691 ± 9.087	0.001	↓
TNF-alpha	8.328 ± 4.154	11.929± 6.613	0.002	↑
MCP-1	255.493± 64.942	353.895± 179.509	0.001	↑
MIP-1alpha	26.974± 27.287	19.864± 17.028	0.015	↓
MIP-1beta	33.025 ± 20.668	65.951± 58.777	<0.001	↑
IP-10	571.147± 257.908	665.549± 291.548	NS	↑
Leucocytes/µl	3475 ± 1627,41	4414.47 ± 2292.58	NS	↑

Lymphocytes (%)	49 ± 13.34	40.29 ± 13.46	**0.048**	↓
Monocytes (%)	2.81 ± 2,01	4.06 ± 2.51	**NS**	↑
Neutrophils (%)	45.43 ± 11,93	55.35 ± 12.87	**0.022**	↑
Eosinophils (%)	3.53 ± 3,56	1.58 ± 0.9	**NS**	↓
Basophils (%)	3 ± NA	NA	**NA**	**NA**
O2 Saturation (%)	95.04 ± 4.80	79.82 ± 8.6	**<0.001**	↓
pO2 (mmHg)	760 ± 0	554.25 ± 0.44	**<0.001**	↓
Hgb (g/L)	141.08 ± 16.11	157.13 ± 17.94	**<0.001**	↑
Hct (%)	39.83 ± 5.20	44.2 ± 4.6	**NS**	↑
BMI	23.28 ± 2.75	23.69 ± 3.56	**NS**	↑

Mean ± standard deviation rounded values of 25 subjects are shown.

IL-1beta interleukin (IL) 1 beta, *IL-12p40* subunit beta of interleukin IL-12, *sIL-2 ra* soluble IL-2 receptor alpha, *IFNgamma* Interferone gamma, *TNF-alpha* tumor necrosis factor alpha, *MCP-1* monocyte chemoattractant protein-1, *MIP-1 alpha* macrophage inflammatory protein 1 alpha, *IP-10* interferone inducible factor 10, *O2* oxygen, *pO2* barometric oxygen pressure, *Hgb* hemoglobin, *Hct* hematocrit, *BMI* body mass index, *NS* not significant, *NA* not available

Table 5 Cytokines, differential counts and additional data of resting subjects at sea level versus high altitude.

In subjects that were at rest, significant changes occurred when comparing sea level data with values at altitude (5050m) in the following proinflammatory cytokines: IFNgamma, IL-12p40, TNF-alpha, sIL-2ra. There were no marked changes in the other proinflammatory cytokines including IL-2, IL-4, IL-6, and IL-1beta.

Significant change within the anti-inflammatory group occurs in IL-1ra, while IL-4 and IL-10 were not significantly elevated.

5. Results

Significant changes in chemokines at high altitude were seen in IL-8, MIP-1beta, MIP-1alpha, and MCP-1. Analysis of differential counts showed significant changes in neutrophils and lymphocytes. Insignificantly altered were leucocytes, monocytes, and eosinophils. Additionally, O_2 saturation and partial oxygen pressure along with hemoglobin counts did show significant changes. However, neither BMI, nor hematocrit changed significantly.

Variable (units) n=25	Sea Level	High Altitude	p value	Rise ↑ / Fall ↓
IL-8	5.786 ± 2.528	10.186 ± 4.693	<0.001	↑
IL-12p40	48.711 ± 81.042	33.382 ± 64.249	0.011	↓
IL-1ra	19.139 ± 43.677	36.991 ± 80.163	0.002	↑
sIL-2ra	40.974 ± 94.385	118.477 ± 192.003	<0.001	↑
IFNgamma	19.561 ± 17.214	11.691 ± 9.087	0.001	↓
TNF-alpha	8.328 ± 4.154	11.929± 6.613	0.002	↑
MCP-1	255.493± 64.942	353.895± 179.509	0.001	↑
MIP-1alpha	26.974± 27.287	19.864± 17.028	0.015	↓
MIP-1beta	33.025 ± 20.668	65.951± 58.777	<0.001	↑
Lymphocytes (%)	49 ± 13.34	40.29 ± 13.46	0.048	↓
Neutrophils (%)	45.43 ± 11,93	55.35 ± 12.87	0.022	↑
O2 Saturation (%)	95.04 ± 4.80	79.82 ± 8.6	<0.001	↓
pO2 (mmHg)	760 ± 0	554.25 ± 0.44	<0.001	↓
Hgb(g/L)	141.08 ± 16.11	157.13 ± 17.94	<0.001	↑

Mean ± standard deviation rounded values of 25 subjects are shown.

5. Results

IL-8 interleukin (IL) 8, *IL-12p40* subunit beta of interleukin IL-12, *sIL-2 receptor alpha* soluble IL-2 receptor alpha, *IFNgamma* Interferone gamma, *TNF-alpha* tumor necrosis factor alpha, *MCP-1* monocyte chemoattractant protein-1, *MIP-1 alpha* macrophage inflammatory protein 1 alpha, *MIP-1 beta* macrophage inflammatory protein beta, *O2* oxygen, *pO2* barometric oxygen pressure, *Hgb* hemoglobin

Table 6 Significant changes of cytokines, differential counts and additional data of resting subjects at sea level versus high altitude

In resting subjects, a rise of most of the cytokines was observed at high altitude when compared to values at sea level. The proinflammatory cytokines, TNF-alpha (p <0.002) and sIL2-ra (p<0.002), and the chemokines MCP-1 (p =0.001), MIP-1beta (p <0.001) and IL-8 (p<0.001) showed a significant rise at high altitude.

IFNgamma (p<0.001) and the chemokines MIP-1alpha (p<0.0015) decreased significantly.

The anti-inflammatory cytokine IL-1ra was the only with significant elevation in plasma level at altitude (p<0.002).

Analysis of differential blood counts showed significant changes shown by a decrease in lymphocytes (p=0.048) and an increase in neutrophil counts (p=0.022) at high altitude. Finally, significant results of additional data include a decrease in partial oxygen pressure (p<0.001) and oxygen saturation (p<0.001), while hemoglobin levels were significantly elevated (p<0.001) at high altitude.

Additional parameters such as BMI did not change significantly nor hematocrit. However, O2 saturation, partial oxygen pressure decreased significantly, when hemoglobin was elevated in high altitude.

5.2. Group 2 (Exercise)

Following table shows all data of subjects who underwent an incremental exercise step test both at sea level and at high altitude.

Variable (units)n=8	Sea Level				High Altitude		
	Pre	**180min post**	**p**	**↑/↓**	**Pre**	**180min post**	**p**
IL-1beta	6.279 ± 5.408	6.373 ± 5.888	**NS**	↑	16.74 ± 26.707	6,248 ± 5,65	**NS**
IL-2	4.604 ± 3.372	3.589 ± 1.08	**NS**	↓	3.622 ± 0.782	3.152 ± 0.134	**NS**
IL-4	3.132 ± 0.191	3.2 ± 0	**NS**	↑	3.2 ± 0	3.544 ± 0.972	**NS**
IL-6	9.794 ± 8.832	10.169 ± 8.704	**NS**	↑	8.489 ± 6.046	7.57 ± 7.02	**NS**
IL-8	6.46 ± 3.681	6.25 ± 2.962	**NS**	↓	6.945 ± 2.425	6.562 ± 2.302	**NS**
IL-10	7.114 ± 7.34	7.054 ± 7.582	**NS**	↓	7.694 ± 9.172	7.137 ± 7.538	**NS**
IL-12p40	58.06 ± 81.747	54.844 ± 79.743	**NS**	↓	30.969 ± 47.65	42.055 ± 58.566	0.031
IL-1ra	32.918 ± 47.106	34.211 ± 50.737	**NS**	↑	39.868 ± 59.089	39.829 ± 61.341	**NS**
sIL-ra	22.025 ± 39.217	34.07 ± 67.94	**NS**	↑	47.377 ± 77.395	55.39 ± 81.64	**NS**

IFNgamma	22.05 ± 21.331	20.77 ± 18.28	**NS**	↓	17.031 ± 17.093	9.766 ± 5.944	**NS**
TNF-alpha	7.652 ± 5.142	6.967 ± 4.299	**NS**	↓	7.925 ± 3.537	6.735 ± 2.345	**NS**
MCP-1	253.903 ± 57.399	214.316 ± 62.444	**NS**	↓	349.311 ± 159.12	257.021 ± 80.782	**NS**
MIP-1alpha	33.885 ± 26.316	34.165 ± 20.164	**NS**	↑	27.28 ± 19.885	18.471 ± 15.737	**NS**
MIP-1beta	29.067 ± 10.411	27.9 ± 11.194	**NS**	↓	32.903 ± 14.081	32.368 ± 13.858	**NS**
IP-10	428.356 ± 156.268	412.285 ± 156.359	**NS**	↓	547.191 ± 394.251	548.722 ± 352.491	**NS**
Leucocytes/µl	4281.25 ± 1613.766	6818.75 ± 1933.342	0.016	↑	4256.25 ± 752.348	8543,75 ± 3485,422	0,008
Lymphocytes (%)	45.143 ± 16.807	38 ± 12.617	NS	↓	44.5 ± 9.739	39,75 ± 10,348	NS
Monocytes (%)	3.125 ± 1.553	5.333 ± 4.131	NS	↑	3 ± 2.673	2,333 ± 1,033	NS
Neutrophils (%)	49 ± 14.306	56.5 ± 15.476	NS	↑	51 ± 8.485	58,125 ± 10,602	NS
Eosinophils (%)	2.857 ± 4.18	2 ± 1.549	NS	↓	2.4 ± 1.14	1 ± 0	NS
Basophils (%)	NA	NA	NA	NA	NA	NA	NA
O2 Saturation (%)	96 ± 1.069		NA	NA	85.313 ± 6.665		NA
pO2 (mmHg)	760 ± 0		NA	NA	553.75 ± 1.035		NA
Hgb (g/l)	140.25 ± 19.219	142.875 ± 18.78	NS	↑	167.333 ± 16.042	173 ± 15,427	NS
Hct (%)	41.25 ± 5.308	40.75 ± 2.403	NS	↓	48.5 ± 4.243	NA	NA
BMI	23.63 ± 0.984		NA	NA	26.232 ± 4.995		NA

AMS	0 ± 0	NA	NA	0 ± 0	NA

Mean ± standard deviation rounded values of 8 subjects are shown.

IL-1beta, interleukin (IL) 1 beta,*IL-2 , interleukin 2*,*IL-1ra, interleukin 1 receptor antagonist,* **IL-12p40**subunit beta of interleukin IL-12, *sIL-2 ra* soluble IL-2 receptor alpha, *IFNgamma* Interferone gamma, *TNF-alpha* tumor necrosis factor alpha,*MCP-1*monocyte chemoattractant protein-1, *MIP-1 alpha* macrophage inflammatory protein 1 alpha, *IP-10*interferone inducible factor 10, *O2*oxygen, *pO2* barometric oxygen pressure, *Hgb*hemoglobin, *Hct*hematocrit, *BMI* body mass index, *NS* not significant, *NA* not available, ↑ increase, ↓ decrease

Table 7 Cytokines, differential counts and additional data of exercising subjects at sea level and high altitude

Two factors measured in exercising subjects presented values of statistical significance. While we could show a significant increase after 180 minutes after exercise at high altitude for IL-12p40, it was not different in a significant magnitude at sea level. Secondly, the leucocyte count showed a significant increase 180 minutes after exercise both at sea level and high altitude.

5.2.1 Sea level at rest versus 180 minutes post exercise

There was no significant change in any of the cytokine at sea level after 180min.post exercise.

Only the leucocytes showed a significant elevation (p = 0.016).

5.2.2. High altitude pre versus 180 minutes post

At high altitude no significant changes occurred in all cytokines in plasma levels when compared before and 180 minutes after cessation of exercise except IL-12p40 (p=0.0031). However, leucocytes showed significant increase (p= 0.008).

5.3. Group 3 (Damphus Peak)

No significant changes, neither in cytokines, nor in differential counts nor in additional data, could be shown after intense exertion while climbing to Damphus Peak at 6035m. The difference in altitude from ascent to descent totaled to 1970 meters.

Variable (units)n=4	Pre	Post	p value	Rise ↑/ Fall ↓
IL-1 beta	4.055 ± 1.037	4.270 ± 1.196	NS	↑
IL-2	3.200 ± 0.000	3.105 ± 0.190	NS	↓
IL-4	3.200 ± 0.000	3.200 ± 0.000	NS	→
IL-6	4.210 ± 2.033	6.277 ± 2.636	NS	↑
IL-8	8.465 ± 3.969	8.170 ± 0.739	NS	↓
IL-10	6.758 ± 4.600	5.298 ± 2.471	NS	↓
IL-12p40	4.982 ± 3.565	4.063 ± 1.725	NS	↓
IL-1ra	57.403 ± 88.926	59.750 ± 102.388	NS	↑
sIL-2ra	166.938 ± 205.895	154.355 ± 10.176	NS	↓
IFNgamma	14.833 ± 10.157	14.435 ± 11.178	NS	↓
TNF-alpha	8.183 ± 2.374	7.218 ± 2.575	NS	↓
MCP-1	586.735 ± 376.423	409.728 ± 191.185	NS	↓
MIP-1alpha	17.038 ± 6.099	18.795 ± 11.087	NS	↑
MIP-1beta	47.830 ± 20.418	31.470 ± 12.255	NS	↓
IP-10	583.018 ± 110.445	450.520 ± 119.833	NS	↓

Leucocytes/µl	5025 ± 1330.1	9987.5 ± 3416.23	**NS**	↑
Lymphocytes (%)	49.25 ± 8.921	24 ± NA	**NA**	NA
Monocytes (%)	7 ± 1.633	3 ± NA	**NA**	NA
Neutrophils (%)	39.5 ± 6.028	72 ± NA	**NA**	NA
Eosinophils (%)	4.25 ± 1.708	1 ± NA	**NA**	NA
Basophils (%)	NA	NA	**NA**	NA
O2 Saturation (%)	NA	NA	**NA**	NA
pO2 (mmHg)	553 ± 0	553 ± 0	**NS**	→
Hgb(g/L)	170.5 ± 11.121	175 ± NA	**NA**	NA
Hct (%)	39.83 ± 5.20	44.2 ± 4.6	**NS**	↑
Leucocytes/µl	5025 ± 1330.1	9987.5 ± 3416.23	**NS**	↑

Mean ± standard deviation rounded values of 4 subjects are shown.

IL-1 beta interleukin (IL) 1 beta, *IL-12p40* subunit beta of interleukin IL-12, *sIL-2ra* soluble IL-2 receptor alpha, *IFNgamma* Interferone gamma, *TNF-alpha* tumor necrosis factor alpha, *MCP-1* monocyte chemoattractant protein-1, *MIP-1 alpha* macrophage inflammatory protein 1 alpha, *IP-10* interferon inducible factor 10, *O2* oxygen, *pO2* barometric oxygen pressure, *Hgb* hemoglobin, *Hct* hematocrit, *BMI* body mass index, *NS* not significant, *NA* not available

Table 8 Nonsignificant changes of cytokines, differential counts, and additional data after a one-day climb

6. Discussion

The goal of this research is to investigate if hypobaric hypoxia influences pro- and anti-inflammatory cytokine and chemokine plasma levels and if exercise has any additional effect.

The exposure to high altitude is a well-known environmental stress with physiological consequences, with the major stressor being hypobaric hypoxia. Nevertheless cytokines are pleiotropic and their production, stimulation and release is influenced by multiple external and internal stimuli like temperature, changes in pathogen exposure, stress hormones, exercise , and circadian rhythm. These facts are the crux of in vivo investigations that researchers attempt to master for an interference free study environment.

Prior investigations tried to measure the change in behavior and activity of immune cells under different environmental conditions. Measuring cytokine levels is one way to show changes in immunocompetent cells in a hypoxic condition [46, 73].

In acute mountain sickness triggered by hypobaric hypoxia as a causative agent, inflammation has long been suspected as a second trigger for its pathogenesis. Nevertheless, most investigations failed to demonstrate a correlation [54, 74].

The change in cytokine production is a way of measuring the cells adaptation to the hypoxic stimuli. Studying the effects of hypoxic conditions will contribute to our emerging understanding of the physiological changes observed in hypobaria and hypoxia, as well as in malignancy and in inflammation. Exercise as an additional stressor failed to demonstrate any observed plasma level changes between sea level and high altitude [46].

What makes this research remarkable is the data comes from in vivo hypobaric hypoxic condition in high altitude, whereas most studies to date used normobaric hypoxic chambers. It is one of the widest biomarker studies under hypobaria and exercise as additional stimulator [75].

6.1. Proinflammatory cytokines under hypoxic conditions

To date research postulates an augmentation in the production of proinflammatory cytokines under hypobaric hypoxia as it is common in pathophysiological conditions like malignancy, strokes, wound healing and sepsis [76].

A long standing hypothesis is the increase in proinflammatory cytokines and chemokines lead to manifestations of AMS triggered by changes in the blood brain barrier homeostasis that results in the cerebral edema seen in HAPE. Nevertheless there is no proof of mentioned biomarker involvement in the pathogenesis [49].

The major finding of this study is an elevation of TNF-alpha, IL-12p40, sIL-2ra, IL-1ra and the chemokines under hypobaric hypoxia, while IFNgamma decreased significantly in this condition.

While Julian et al.[49] failed to show changes in TNF-alpha under hypobaric hypoxia under similar conditions, this study confirmed significant increases in TNF-alpha after exposure to high altitude. Compared to the biomarker study of Julian et al.[49] the probands were not treated with immunomodulation medication as acetazolamide and dexamethasone, which are known to reduce plasma levels of TNF-alpha.

The results are in agreement with both human (from bronchoalveolar lavage) and animal studies [77, 78].TNF-alpha augmentation was also reported in rat brains after intermittent hypoxia [79]. It has also been found to be elevated in hypoxia-mediated clinical conditions such as patients with obstructive sleep apnea and cardiovascular remodeling after a hypoxic trigger resulting in right ventricular hypertrophy in lung fibrosis [80, 81]. In the work of Smith et al.[82], TNF-alpha levels change when measured in acute and chronic hypoxia in mice. TNF-alpha is augmented in hypoxic conditions and triggers a complicated pathophysiological process, inducing a hypoxia mediated pulmonary vasoconstriction and initiating an IL-6 release in animal models after chronic intermittent hypoxia (10 % pO2 , Duration of 21 days)[82].

This study failed to show an increase in IL-6; however recent work presents the novel finding. There are significant changes in TNF-alpha under hypobaric hypoxia.

Several studies showed a TNF-alpha increase during hypoxia in alveolar cells and a similar correlation in ARDS and HAPE is assumed [50,51, 83].The data is controversial as Svenson et al.[84] couldn`t find any change in proinflammatory cytokines especially, TNF-alpha in the BAL of probands after a rapid ascent to 4559 m or in HAPE patients. However, they failed to measure plasma cytokine levels. Hempel et al. [83] found under LPS stimulation of human alveolar macrophages under hypoxia (02< 0.05%) an augmentation of TNF-alpha release, compared to room air.

In Summary, the elevation of TNF-alpha during hypoxia could be seen as an experimental model for other hypoxic pathological conditions such as rapid tumor growth, in wounds, and the development of ARDS and AMS [85].

Since it is already known in pulmonary artery hypertension, hypoxia caused by lung fibrosis that leads to right ventricular hypertrophy is triggered by a TNF-alpha mediated process. Further investigations could lead to therapy with a TNF-alpha antagonist after the role of TNF-alpha in hypoxia induced pathophysiological processes is better defined[16].

In conclusion, TNF-alpha is augmented while the corresponding cytokines, IL-1 and IL-6 remain unaffected. Unfortunately we could not correlate an involvement of TNF-alpha in high altitude associated cachexia and anorexia as it is typically seen in tumor patients. The BMI decreased insignificantly, but this could be due to an elevation in muscle mass and a decrease in body fat after the ascent to Hidden Valley Base camp[18].

IL-6, one of the best investigated proinflammatory cytokine did not show a change under hypobaric hypoxia. This finding is in agreement with Pavlicek et al.[86]and Svenson et al. [87].

Currently, the data is controversial. Hartmann, Schoene, Klausen and Mazzeo et al. found elevated plasma levels after different altitude exposure time (> 2 days) [42, 52, 54, 88].

Elevated levels in IL-6 under hypoxia are correlated to a nonspecific inflammation which might be an expression of organ prone hypoxia triggered inflammation. Alveolar endothelial cells, cardiomyocytes and leukocytes are known to release IL-6 in response to hypoxia [54]. A correlation of hypoxic sensitive organs and cytokine release in response to the development of AMS is assumed [52, 54]. In animal and in vitro cell studies, the effect of hypoxemia as an individual trigger of IL-6 release has been shown

[89].IL-6 is known to stimulate EPO production in hepatocytes during hypoxia [53]. IL-6 seems to fulfill local proinflammatory, adaptive and a pathophysiological task during hypoxia. The probands of this study underwent a 15 day trip in altitude to get adapted to the final altitude of 5050m. During this time, probands were already exposed to two different stimuli, altitude and physical exercise by walking. Hartmann et al. [54] tried to differentiate between active and passive ascent to high altitude in different altitudes. No change of cytokines plasma level was found in the higher altitude, which supports the assumption of an acclimatization effect [54]. The collected data (high altitude baseline sample) was already influenced by active ascent. To date unfortunately, no studies of a long-term sojourn in high altitude and IL-6 levels are known.

For future investigations, blood samples should be taken during the ascent to the maximum research altitude to verify any cytokine change.

Nevertheless, this study and the comparable work of Julian et al. [49]challenge the question of IL-6's participation as a key proinflammatory cytokine in hypobaric hypoxia. Both did not show an elevation of IL-6 in hypoxic conditions, however Julian et al. reported elevated levels after a nine hour exposure at an altitude of 4875m in AMS resistant probands. The reason is still unknown and needs further investigations.

In analogy to Fritzenwanger et al., Klokker et al. and Julian et al.[45, 49, 90] found no change in IL-1beta plasma levels in hypobaric hypoxia, simulated hypoxia in a decompression chamber and under real altitude conditions in vivo.

Conflicting data was presented by Ghezzi et al. [16]. An increase in IL-1beta and IL-1alpha under hypoxia was found. But instead of hypobaric hypoxia, Ghezzi et al.[16] exposed MNC in cell culture to anoxia (FiO2 0%), which presumably exerted a stronger hypoxic effect. Dziurla et al.[91] created a hypoxic cell culture in vitro of stimulated CD4 cells and found increased levels of IL-1beta, IL-10 and IL-8.

Becker et al.[10] investigated IL-1beta release under various conditions including hypobaria, normobaric hypoxia, and hypobaric hypoxia. An altitude of 6069 m was simulated in a hypobaric chamber. Only after stimulation with LPS changes were found. Only under hypobaria and hypobaric hypoxia, IL-1beta levels decreased significantly; normobaric hypoxia had no influence in IL-1beta plasma levels. This finding leads to the conclusion that hypobaria is a crucial stimulus, while hypoxia alone is insufficient to cause changes

in IL-1beta levels [10]. The same results were observed by Julian et al.[49], when IL-1beta levels fell below detection limits.

Unlike this study, all other investigators, except Wang et al.[92] , used artificial hypoxic conditions, which is not comparable with hypobaric hypoxia in altitude conditions. Cell cultures stimulated with PMA (phorbol-12-myristate-13-acetate) were used by the other investigators; however, this study used whole blood and couldn´t differentiate the effects by specific cell type. Compared to the short impaired hypoxia models, this study had a longer acclimatization to altitude. As already mentioned, the interference prone cytokines might cause different results under changed environmental conditions and a field study is not comparable to the in vitro conditions [92].

Another theory supports the data presented here and explains a physiological reason for a missing proinflammatory cytokine response. There is a known inhibitory effect of proinflammatory cytokines, mainly IL-1beta, in hypoxic triggered erythropoetin production from hepatocytes[93]. Faquin et al.[53] and Ferucci et al.[94] have shown that IL-1beta is a dose dependent and a powerful inhibitor of hypoxia triggered EPO production in hepatocytes. One assumption and possible explanation for the missing IL-1 beta elevation under hypoxia might be due to a physiological adaption to hypoxia from an increase of EPO.

IFNgamma levels decreased from sea level to high altitude level. The data presented here is in agreement with the results of Facco et al.[46] that also showed a decrease of IFNgamma plasma levels in acute and chronic hypoxia (day 1, day 21 and after returning to sea level) in young female subjects. They also differentiated white blood cells and showed a decrease of IFNgamma release from T cells in hypoxia and a decrease of CD4 and CD8 T- lymphocytes. Fritzenwanger et al.[45] has also shown that exposure to high altitude results in a decrease in IFNgamma plasma levels.

However, intermittent hypoxia over a period of 10 days, but in an isobaric atmosphere in a mixed chamber (SaO2 80% added) did not show a change in plasma levels of IFNgamma[80].

The data of this study supports the hypothesis of animal studies and the work of Facco et al. [46] Hypoxia downregulates the expression of IFNgamma from T-cells [75]. The findings of this study supports this hypothesis as a decrease in lymphocytes at high altitude (51% of white blood cells at sea level and only 39 % in high altitude) was observed, although the decrease is not statistically significant.

In summary, IFNgamma plasma level is decreased by hypoxia, and this is believed to cause an alteration in cellular and immunological parameters [46].

Data from this study demonstrate a significant elevation of sIL-2ra after exposure to hypobaric hypoxia; however IL-2 plasma levels remain unchanged. After a review of the literature, this study is the first investigation to examine sIL-2ra in vivo under hypobaric hypoxia conditions. IL-2 plays a crucial role in T-cell activation and it is known as a T-cell growth factor and a stimulator in the cellular immune response [28]. Elevated levels are correlated with the activity of T-cell mediated disease like sarcoidosis. For that reason, sIL-2ra is known as marker for T-cell activation and the sIL-2ra serum levels are currently believed to be elevated in T- helper cell induced alveolitis [95]. An assumption leads to a possible correlation of alveolar hypoxic conditions and a possible consequence for the immune response [95].

To date no research reports the effect of hypoxia on sIL-2ra and further investigation is needed to explain these findings [30].

6.2. Chemokines under hypoxic conditions

Chemokines in general fulfill proinflammatory and angiogenetic tasks. One assumption of the pathogenesis of AMS is an increased brain volume with diminished buffer capacity that leads to symptoms of brain swelling. Proinflammatory mediators are known to weaken the blood- brain barrier and are also known to be released during a local hypoxic condition, such as a stroke [49, 56, 96].

MCP-1 and MIP-1alpha are known to weaken the tight-junctions in the blood-brain barrier during hypoxic induced inflammation and induce a leukocyte attraction in the brain parenchyma. MIP-1beta was recently detected as an AMS related chemokine [49].

An elevation in plasma levels was expected as a systemic response to hypoxia probably initiated by hypoxia sensitive organs as the lung and the brain [49].

In agreement with Patuccini et al. [97] a significant elevation of IL-8 plasma levels in high altitude condition was found.

After a review of the current literature, there are only a few known in vivo studies. IL- 8 is a chemotactic factor and in hypoxic conditions, endothelial cells are able to release IL-8 to trigger the migration of neutrophils to the locus of ischemia [60]. It also has angiogenetic properties in tumor growth and metastasis [98].

IL-8 is known to be involved in the pathophysiologic of ARDS as it is responsible for neutrophil migration. Elevated IL- 8 levels have been reported in the BAL of ARDS patients and correlate with the severity [99].

Since HAPE and ARDS are both correlated with elevated levels of IL-8, it is assumed that the ischemic focus attracts leukocytes with the help of IL-8. The exact mechanism remains unclear [59].This theory is supported by the work of Karakurum et al.[60] who have demonstrated a hypoxic triggered release of IL-8 by endothelial cell will cause the migration of neutrophils to the site of ischemia.

Patitucci et al.[97] measured IL-8 plasma levels at sea level, Mt. Everest base camp (6000m) and Mt. Everest summit (8848m). Plasma levels were elevated 2.4 times in base camp, with normalization of the plasma levels in higher altitudes. (5000m , 6000m, 7000m , 8000m) . This finding is in agreement with the results measured in this study. Additional evidence was found when a group of Sherpa's demonstrated higher levels of IL-8 than the westerners [97]. The current belief is that participation of IL-8 in the acclimatization to hypobaric hypoxia is accomplished by the influence in the nfκB pathway for gene transcription [97].

HIF 1 alpha indirectly implicates the nfκB pathway and the IL- 8 levels are elevated due to it angiogenetic properties [97]. In natives living at high altitudes, the increased levels of angiogenetic IL-8 is seen as a measurable factor for adaption to high altitude [97].

MIP-1alpha/1-beta and MCP-1 are predominantly proinflammatory chemoattractants released by resting macrophages and monocytes [15,32,34,35]. The role of chemokines under hypoxia in cerebral ischemia was first detected by Bona et al.[55] and Cowell et al.[56] and demonstrated elevated MIP-1alpha concentrations in cerebral hypoxic ischemic lesions after hypoxic events in neonatal rat brains [55]. Cerebral microglia and activated monocytes are the source of MIP-1alpha release [55]. In the recent findings, MIP-1beta and MCP-1 plasma levels were increased under hypoxic conditions, especially when

MIP-1alpha was significantly decreased. The results correlate with the findings of Julian et al. [49]. Elevated MIP-1beta plasma levels were concurrent to the development of AMS in AMS sensitive probands [49]. Chemokines influence the development of AMS by attraction of macrophages to hypoxic brain areas [96]. MIP-1beta also has an influences in the permeability of the brain blood barrier and enables leukocyte migration to the brain parenchyma [49].MIP-1alpha plays a role in hypoxic brain injury and assists in parenchyma repair after stroke by recruiting monocytes [56].

Together with the finding of Julian et al. [49] and from an animal study [56] , the elevation of MIP-1beta during hypoxic conditions could underline the hypothesis of the development of HAPE.

During hypoxic conditions, MCP-1 was found to be significantly elevated in this study.

This is a new finding because there are no known reports of the quantitative measurement of chemokines under in vivo conditions in hypobaric hypoxia. Current research is trying to explain the function of MCP-1 during different hypoxic condition in tumor growth and in other ischemic scenarios, e.g. brain lesions [96]. It is well known that the major role of MCP-1 is recruiting leucocytes to the hypoxic areas of the tumor, and can become the main component of the tumor mass. MCP-1 also plays a crucial role in tumor biology in tumor growth, angiogenesis, and metastasis [96] Chao et al. [57] has reported a coherence of systemic inflammation triggered by alveolar hypoxia through release of MCP-1 from alveolar macrophages. MCP-1 is responsible for the contribution of systemic inflammation, triggered by local hypoxic conditions in alveolar macrophages [36]. Other mast cells (peritoneal and blood mast cells) do not release MCP-1 during hypoxia. That supports the special key position of alveolar macrophages as the first cell to detect and respond to hypoxic conditions [56].

The key issue in this finding is the release of proinflammatory mediators. In animal studies, TNF-alpha, MIP-1beta and MCP-1 where released by alveolar cells [96].These proinflammatory mediators caused the activation of systemic inflammation. Many pathophysiologic conditions start with alveolar hypoxia that leads to systemic inflammation (e.g. pneumonia, COPD, and high altitude illness). The result of Chao et al. [57]which identified MCP-1 as mediator of systemic inflammation triggered by low alveolar pO2 concentration, and the data presented from this study support the idea of an hypoxia triggered systemic inflammation [58].

Further research is necessary to identify pathways of hypoxia induced inflammation and the physiological benefit of a proinflammatory situation, especially in the strategy of acclimatization [49, 57, 58].

6.3. Anti-inflammatory cytokines under hypoxic conditions

In accordance with Julian et al.[49] and Facco et al.[46] IL-10, IL 1beta and IL-4 were not infected by hypobaric hypoxia, however, a significant increase in IL-1ra was seen at high altitude when compared at sea level.

IL-1ra is known as the highly selective antagonist of the IL-1 triggered proinflammatory cytokine response. It is released by different immune cells like neutrophils which were found significantly elevated after exposure to hypobaric hypoxia in this study.

According the innovative finding from Julian et al. [49] a correlation with the increase of IL-1ra levels and a particular resistance in the development of acute mountain sickness was shown.

The pathophysiology of AMS assumes an increase in proinflammatory cytokines that interferes with the blood-brain-barrier by impairing tight junctions which leads to the development of brain edema [49, 100, 101]. In animal studies, IL-1ra is known for its neuro- protective properties in cerebral ischemia [102]. IL-1ra antagonizes the hypoxic triggered IL-1 driven neuroinflammation and accelerates healing after acute hypoxia [103].IL-1ra administered to stroke victims may have a beneficial impact on stroke recovery [103].

In theory, cytokines with the protective role as an anti-inflammatory should be elevated to prevent AMS. Julian et al. [49] revealed the anti-inflammatory effect of IL-1ra in the prevention of AMS. Nevertheless, it is difficult to explain a complicated pathophysiological process like the multifactorial and oligosymptomatic AMS with the change in one single plasma cytokine level.[49] An increase in IL-1ra after the first altitude exposure was found, with no change after the second ascent. The effect of high altitude acclimatization is the assumed explanation for expected increase in IL-1ra that did not occur in additional to altitude boost [54].

In summary, IL-1ra elevation during hypoxia is an interesting finding and further investigation is needed to understand the clinical relevance.

6.4. Cytokines and exercise

Exercise under hypoxic conditions at high altitude is more stressful compared to exercise at sea level. In theory this additional stressor is expected to have a greater impact on immune function and cytokine levels. Hypoxic exercise should have higher influence in cytokine release than hypoxia without exercising [39, 40, 104].

This study investigated if there are changes in pro- and anti-inflammatory cytokine plasma levels after physical exercise when compared to resting values and whether changes occur at high altitude when compared to sea level. Furthermore, a small group of 4 male probands were exposed to an additional ascent to 6035 m.

Neither the 12 minute exercise protocol at sea level and at high altitude, nor a 12 hour climb from Hidden Valley Base Camp (5050m) to Damphus Peak (6035m) and back produced significant changes in cytokine levels. However, an elevation in IL-12p40 plasma levels post- exercise was measured at high altitude (5050m) conditions.

The recent reviews of Suzuki et al.[105] and Walsh et al. [67, 68]demonstrated that in the majority of the investigations reported in the literature, exercise alone didn´t change IL-1beta, TNF-alpha, and IFNgamma plasma levels.

However, IL-6 and IL-1ra plasma levels are, in general, increased after exercise. The chemokines IL-8, MCP-1 as well as MIP-1alpha present conflicting data. The literature reports vary on the plasma levels of IL-10 and, to the author's knowledge, changes to IL-4 levels hasn't been reported [106].

In the recent work, the plasma cytokine levels of IL-6 remain uneffected by exercise in normoxia and high altitude. In comparison with the study of Hagobian et al. [107], the study design is similar. The differences in Hagobian et al.'s study design is a testing level at a lower altitude (4300 m), with only one night to acclimatize (at 1860m) before the first testing day in 4300m . Additionally, another exercise

mode (ergometric cycling) was chosen. Carbohydrate supplements and antioxidants were given to the probands, with no significant influence in the measured IL- 6 and TNF-alpha levels. Hagobian et al [107]reported a significant elevation of IL-6 after exercise 2, 4 and 20 h post exercise (42 times higher than at sea level)at the first day in high altitude. In the recent study, the blood sample was taken 180min. post exercise after 3 weeks continuous walking to the altitude of 5050m and it is possible that the elevation of cytokines seen in Hagobian et al.'s study was missed due to the time of sampling. In Hagobians study, the measurement of IL-6 plasma level were made at day 1 (altitude 4300m) and day 13 at the same altitude. At day 1, IL-6 levels were elevated 42-fold, but the high plasma levels were not persisting during the high attitude sojourn. There might be the explanation for our findings. A long sejourn under high altitude conditions decrease the plasma levels in IL-6.

In future investigations, post exercise data needs to be measured more frequently to get a better understanding of cytokine distribution [107].

It has been reported that hypoxia and exercise induced elevation in plasma cytokine are removed from the systemic circulation via a renal elimination pathway to the urine [108].

The time of detection of the cytokine elimination product in the urine is variable and depends on the time of elevation in the serum [109]. Often cytokines are not detectable in the plasma after a trigger, but an elevation in the urine can be found.

IL-6 which peaks during exercise, (what we couldn´t measure because of the mode of exercise) and directly after exercise and it is measureable in the urine about 1 hour after exercise. Different elimination times are assumed, but not known, for each of the cytokines. An assumption leads to the conclusion that the sampling 180 min. post exercise could be to late for a detection of elevated plasma levels in IL-6.

That supports the finding of the unchanged cytokine situation, independent of hypoxic environment. In the further investigation, sampling time needs to be early after the trigger, because of the short half life of cytokines [105, 109-111].

In correlation to Hagobian et al. [107], TNF-alpha did not show a change under any condition. The antioxidant supplementation had no influence in cytokine plasma levels in the mentioned study. This is controversial data. Nieman et al. [112] found lower levels in the anti-inflammatory cytokines IL-10 and

IL-1ra and lower cortisol levels and they have shown the expected increase in IL-6 is reduced by ingesting carbohydrates [113]. Carbohydrates influence the immune system through an increased blood glucose level that cause enhanced fat oxygenation and elevated stress hormone release [113]. The hormone-pituitary axis exerts its effect through adrenal gland activation causing the release of cortisol, glucose and epinephrine which interferes with the release of proinflammatory cytokines [114].

The probands in this study also participated in other studies during the research expedition. One study was a double blind experiment using carbohydrate supplementation versus placebo. The high caloric carbohydrate supplement could have influenced cytokine release and interfered with collected results.

The current literature has demonstrated cytokine elevation only after extreme exercise (e.g.marathon) at sea level [115, 116] According to the latest review of Suzuki et al. the majority of studies have not found a change in TNF-alpha after exercise [67, 68, 112]. As we had no change after the step test in sea level and at high altitude, in correlation to others, the theory is supported that the mentioned exercise isn´t sufficient to trigger a TNF-alpha release.[69, 70, 115, 117].

An early proinflammatory cytokine, IL-1beta, wasn´t found to be elevated after exercise in this investigation and is in agreement to the latest reports in the literature [19, 20, 108].

It has been shown that there is no correlation in IL-1beta secretion with changes in mode and intensity of exercise. Sprenger at al.[108]found no change in IL-1beta after a strenuous 20 Km marathon race. However, there is conflicting data showing an increase of IL-1beta in the muscle after a downhill running race. That supports the theory of local cytokine release from the contracting muscle [118]. During hypoxia, Wang et al.[92] performed an exhausting exercise test using a bicycle ergometer and demonstrated an increase of IL-1 beta; however, plasma levels of IL-1beta decreased with exercise under severe hypoxic conditions.

Since IL-6 and IL-10 were increased post-exercise and post-hypoxia, a buffer effect of anti-inflammatory cytokines was proposed [70]. Strangely, IL-1beta, one of the early proinflammatory cytokines was only increased after eccentric exercise [92].No effect in plasma levels of IL-6 and IL-10 was seen after submaximal exercise [20]. It is assumed that the release of IL-1beta from the working muscle is stopped by the anti-inflammatory effect of IL-6 and IL-10. This study could not show a change in IL-6, IL-10 or IL-

1 beta, but this observation correlates with the current thinking that the inflammatory cytokines IL-1beta and TNF-alpha are not stimulated in the classical cascade after exercise and under hypoxia [70].

Contrary to the findings presented in this study, Wang et al. [92]and Smith at al. [119]found a decrease in IL-1beta after exercise.

This study's design may explain the observed difference. Perhaps the rapid excretion of IL-1beta into the urine is could the reason why no change in IL-1beta in the serum after exercise was detected. 180min. after excersice [104, 108].

Another reason might be that the local production of IL-1beta from the exercising muscle could not be detected in the blood [118].

IL-6's role to increase the immune response has been assumed for a longtime and it is known to be the first cytokine to increase during exercise and up to a100-fold increase in plasma levels can be detected after exercise [104, 112, 115]. IL-6 was almost augmented in all exercise studies in literature [120].However, there is no evidence in the literature to support the notion that IL-6 is produced as a modulator of the immune system in the classical acute phase response [120]. The exercise cascade begins with a peak in IL-6 elevation during exercise and then a rapid decrease; this is followed by increase of IL-1ra, IL-8, MIP-1alpha and MIP-1beta [22]. It differs from the classical immune cascade because TNF-alpha is mostly unchanged in any post-exercise condition and IL-6 and TNF-alpha are initiators of the classical proinflammatory cascade. The elevated plasma levels of IL-6 and TNF-alpha are likely due to metabolic demands [22, 67, 68, 104].

Immune cells are not the only source of IL-6. Contracting muscle has also been shown to release IL-6 [67, 68, 120].In addition to IL-6's immune function, it is postulated to have a metabolic role to modulate glucose uptake and fat oxygenation in the working muscle [22, 115].

IL-6 is also known to inhibit the production of TNF-alpha and IL-1 in combination with exercise that results in an anti-inflammatory effect on the exercise [115].

The reason why no changes of IL-6 levels were measured in this investigations could be due to the time of sampling. It has been reported that according to the mode of exercise, either eccentric (lengthening contraction) or concentric (shortening contraction), levels of IL-6 can vary [20]. In concentric training,

the levels of IL-6 decrease within a few hours post exercise, but with, eccentric exercise plasma levels IL-6 remain elevated for a few days [20]. A correlation of work intensity and IL-6 release by muscle contraction is assumed [20, 115] [121].

In this work the levels of anti-inflammatory cytokines did not show a change after exercise in normoxia and hypobaric hypoxia.

No change in IL-1ra plasma level was found after exercise. These results correlate with those of Hirose at al.[20] There is, however, conflicting data reported in the literature. Ostrowski et al.[104] and Schobensberger et al. [122]have demonstrated an elevation in IL-1ra after a marathon race .Similar to this study design, an ultramarathon in the Alps was performed (67 km in distance at a maximum height of 2700m). Compared to the mode of exercise use in this study, both used a more strenuous exercise. The reported running time of the participants was between 2 hours 40 minutes to 8 hours (average was 3 hours 27 minutes). In the study presented here, the participants had an ascent of 15 days allowing for acclimatization and a positive training effect that could be responsible for the observed unchanged plasma level.

It is assumed that extensive exercise releases more cytokine from more micro-trauma muscle damage. During exercise, blood flow to the internal organs is reduced. Effective renal plasma flow is reduced and is related to the intensity of exercise and renal blood flow may decrease to 25% of the resting flow. The redistribution in blood flow influences the cytokine clearance and augments cytokine level in prolonged exercise [20, 105].

In summary, there was no change in plasma cytokine levels in pro- and anti-inflammatory cytokines after a standardized maximal step test over a 12 minutes except for an increase of IL-12p40 post-exercise at high altitude.

Reasons for the unchanged cytokines levels are multifactorial. The mode of exercise is important-eccentric or concentric training, the amount of contracting muscle mass and the duration of training influence the cytokine release [20, 115, 121]. Another confounding factor is the timing of blood sampling. The urine clearance is different for every cytokine and depends on the renal blood flow which is reduced during maximum exercise to the benefit of a better muscle perfusion [20, 105].

The 15-day long ascent to the altitude of 5050 m by continuous walking on an uphill gradient served as submaximal training and allowed for a metabolic acclimatization to hypoxia and hypoxic exercise and is assumed to have influence the data results. There is most probably a trainings effect in continuous walking over 3 weeks and a slow acclimatization to hypoxia, that explains the unchanged plasma cytokine situation. Further investigation is needed to demonstrate the metabolic role of proinflammatory cytokines during exercise. Additional studies are needed to measure metabolic parameters as blood glucose, cortisol and stress hormone levels.

6.5. Summary and further directions

This investigation is one of the most comprehensive biomarker studies for cytokines and chemokines under in vivo conditions.

It included pro- and anti-inflammatory cytokines and chemokines in both normobaric and hypobaric hypoxia and includes markers (that to the best of this author's knowledge) that have not been reported under these conditions like sIL-2ra and IL-12p40. The study design had data collection at sea level and in high altitude, and an additional second higher altitude exposure with a standardized exercise test as additional stressor and trigger for cytokine and chemokine release. Compared to preceding investigations, this expedition offered a sufficient and significant amount of probands that enhances the validity of data.

This study has shown that hypoxia triggers an increase in TNF-alpha and most of the chemokines, with the only exception being the decrease in MIP-1alpha.

TNF-alpha is known as the key cytokine of the classical proinflammatory cytokine cascade and was elevated without a change in IL-6 or IL-1beta levels. It challenges the question if TNF-alpha is elevated because of a pathophysiological process like the development of AMS (25 % of the probands suffered temporarily from AMS) since it is known that other hypoxic triggered processes, such as OSAS, COPD, right ventricular hypertrophy and remodeling [16, 50], or from the interruption in homeostasis due to hypoxia as an immune system response [80].

Another remarkable result is the increased plasma level in IL-1ra with its protective anti-inflammatory properties. This finding supports the hypothesis of a protective effect of IL-1ra in the development of AMS [49]. Additionally, IL-8 was elevated in hypoxia. In correlation with the recent literature, this finding supports the hypothesis of IL-8 involvement in the acclimatization to high altitude and the increase elevation observed in natives. Further research is necessary to identify the nuclear mechanism that influences EPO release triggered by IL- 8.

The remaining chemokines are well known to be elevated in hypoxic conditions such as after a stroke. This interesting finding needs further investigation to explain the role of chemokines in the development of AMS and especially HAPE, by their interference in the blood brain barrier [103].

A new finding is the increase of sIL-2ra which can serve as a marker for T cell activity during hypoxia.

All cytokines did not show any change after exercise in sea level, high altitude and in an additional ascent over 6000 m. A possible explanation is, that the mode of exercise is not a sufficient trigger for cytokine release. The unchanged cytokine situation in sea level conditions underlines this assumption.

Another explanation is that the probands were already acclimatized from the 15 day ascent to 5050 m and there was a trainings effect by continuous walking.[97]

Further investigations are needed to explain the physiological meaning of changes to cytokine levels during acclimatization, adaption to hypoxic conditions, protection of the homeostasis and for future clinical applications of this knowledge for the pathophysiological process of other hypoxic conditions as stroke, pneumonia, COPD , lung fibrosis and sepsis.

7. References

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8. Publications and acknowledgments

9. Curriculum vitae

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01/2012-2015   Resident physician, internal medicine gastroenterology/pulmonology

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09/2010- 01/2012   Resident physician, internal medicine pulmonology

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05–08/2008   Tutor Internal Medicine, Saarland University (Germany)

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University and education

05/2010   2. State examination

2009   Universidad Católica de Santa Maria, Arequipa (Peru) (internship)

2009   University of Loma Linda, Loma Linda (USA)(internship)

10/2006 - 07/2007 ERASMUS exchange student program Université libre de Bruxelles

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2003 – 2010   Medical studies at Saarland University

06/2003   School leaving examination,

Warndtgymnasium Geislautern, Saarland (Germany)

1990 – 1994   Elementary School Großrosseln (Germany)

Doctoral thesis

From 07/2008   Changes of cytokines under the influence of high altitude hypobaric hypoxia and physical activity

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Special Skills

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