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Microvascular Perfusion Measured by Orthogonal Polarization Spectral Imaging is Well Maintained during Exposure to High Altitude in Trained Mountaineers*

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Abstract
The objective of this study was to examine microvascular perfusion during hypobaric hypoxia and physical exercise. We used orthogonal polarization spectral imaging for the non-invasive visualization and assessment of the sublingual mucosal microcirculation in twelve healthy altitude acclimatized mountaineers. Red blood cell velocity (RCV), microvascular diameter (Dia), functional capillary density (FCD) and the number of rolling leukocytes were studied at baseline and after (I) a climb to an altitude of 3196 m, (II) a passive ascent to the same altitude by helicopter and (III) an exercise program at an altitude below 2100 m in the European Alps. Exposure to high altitude and exercise resulted in an increased heart rate (Trial I: 64 [54–66] vs. 95 [84–100]; median [interquartile range]; $P<0.05$) and decreased oxygen saturation (Trial I: 98 [98–99] vs. 90 [88–92]; $P<0.05$). However, RCV, Dia and FCD did not change significantly. Furthermore, no enhanced rolling of leukocytes in postcapillary venules could be observed (Trial I: 6.2 [4.4–6.8] vs. 7.8 [4.3–6.7]). In the pooled data of all three trials of this study we could show a significant positive correlation between oxygen saturation and red blood cell velocity ($r = 0.25$; $P = 0.02$). These results indicate that orthogonal polarization spectral imaging can be a useful tool for the microcirculatory assessment of man under hypoxic conditions. We could show that in trained, acclimatized subjects microvascular perfusion is well maintained during hypobaric hypoxia at an altitude of 3196 m and no evidence for an increased postcapillary leukocyte adhesion was seen.

Key words: Microcirculation, venules, capillaries, altitude, hypobaric hypoxia, white blood cells, exercise

Introduction

Hypoxia can have profound influences on vascular tone and modulates microvascular oxygen delivery to the tissue. The balance between the local dilatory effect of hypoxia and the neural control of vascular tone depends on species, vascular region and the degree of hypoxia [1]. During exercise the sympathetic activity is further enhanced [2], however, hypoxia also prevents sympathetic vasoconstriction [3,4]. Animal studies using intravital microscopy for the assessment of microvascular perfusion during hypoxia have shown a vasodilatory response of arterioles and venules in the hamster cheek pouch [5], whereas a sympathetic vasoconstriction of the gastrointestinal circulation [6] and unchanged diameter of mesenteric venules have been reported in rats [7]. Also the venules of the rat's cremaster muscle do not show significant changes in diameter during hypoxia [8]. However, reduced microcirculatory red blood cell velocity seem to be a common feature of systemic hypoxia in those three animal models [5, 7, 8].

Hypoxia also promotes a rapid increase in leukocytes rolling and adherence to the venular endothelium followed by transmigration and a concomitant increase in microvascular permeability [9, 10]. Wood et al. demonstrated that systemic hypoxia, induced by breathing 10% oxygen, resulted in an enhanced adhesion of leukocytes to mesenteric venules of non-acclimatized rats, whereas animals that were adapted to hypoxia did not show these changes [11]. Furthermore, exercise training also reduced the leukocyte endothelial cell interaction during hypoxia [8].

Whilst numerous animal studies have investigated changes of microcirculatory hemodynamics and leukocyte-endothelial cell interaction, only recently the direct visualization of the human microvasculature became available. Orthogonal polarization spectral (OPS) imaging enables non-invasive assessment of the microvascular perfusion without the use of dye [12]. OPS imaging has been used to identify microcirculatory impairment in sepsis [13, 14], cardiogenic shock [15] and anemic preterm infants [16].

We tested the hypothesis that exposure to high altitude activates inflammatory reactions and changes microvascular perfusion. Furthermore, we wanted to answer the question if exercise alters this response to hypobaric hypoxia. We recently showed in the same set of volunteers that during moderate hypobaric hypoxia (altitude of 3196 m) expression of CD18 adhesion molecules on circulating leukocytes and production of

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reactive oxygen species was increased. Activation of this potentially cytotoxic effect was strongly inhibited by physical exercise [17]. In the same group of acclimatized volunteers, increased total calf blood flow was associated with a reduced microvascular equilibrium pressure assessed by venous congestion plethysmography, which could be explained by a reduction in normal post-capillary leukocyte margination [18].

In the present part of the study, we wanted to investigate whether these observed changes in leukocyte activation [17] and microcirculatory balance [18] translate into alterations of sublingual microvascular perfusion and leukocyte-endothelial cell interaction visualized with OPS imaging. The pattern of the response of human sublingual microcirculation to moderate hypobaric hypoxia (altitude of 3196 m) after (I) an active and (II) a passive ascend and (III) the effect of exercise at low altitude were studied.

Methods

Subjects

The data presented in this paper are part of a complex study and the protocol has been described in detail previously [17,18]. Briefly, twelve young, healthy male members of the South Tyrolean Mountain Rescue (age: 24 to 38 years; body mass index: 18.6 to 26.7 kg m^{-2}) gave written informed consent and took part in the procedures, which were approved by the local ethical committee. All volunteers were residents of the region where the study was performed, but did not permanently live at altitudes higher than 1200 m. However, as a result of their training regimes, all were acclimatized to altitudes up to 3000 m.

Study Protocols

In the first trial (*Active Ascent*), subjects ascended in 3.5–5.5 h from 1416 m (barometric pressure 634–637 mmHg corresponding to a partial oxygen pressure of 124 mmHg) to the Becherhaus refuge at 3196 m (barometric pressure 514–517 mmHg corresponding to a partial oxygen pressure of 108 mmHg) where they stayed over-night. The same subjects were airlifted from the base camp to the Becherhaus six weeks later (flight time 8 min), where they rested without undergoing any exercise program (*Passive Ascent*). The third leg of the study was conducted 4 months later when subjects performed hiking exercise below an altitude of 2100 m of similar duration (4–4.5 h) and effort as the climb to the Becherhaus (*Exercise at lower altitude*).

The microcirculatory assessments were done one day prior to the exposure to hypoxia or exercise (T_0), in the first 45 minutes upon arrival at the Becherhaus or after the exercise program (T_1) and 15 to 22 hours later, after sleeping either at an altitude of 3196 m (*Active* and *Passive Ascent*) or the base camp altitude (1416 m) in the *Exercise at lower altitude trial* (T_2). Basic non-invasive hemodynamic monitoring of heart rate, blood pressure, oxygen saturation was performed (SC6002, Siemens Medical Solution, Munich, Germany) during OPS recordings.

Orthogonal Polarization Spectral (OPS) Imaging

Orthogonal polarization spectral imaging (Cytometrics, Philadelphia, PA, U.S.A.) was developed as a non-invasive technique for the investigation of the human microvasculature [12]. Briefly, linearly polarized light is projected into the tissue. Only the scattered fraction of the remitted light that has been depolarized by 90° passes a second (orthogonal) polarizator and is recorded by the CCD camera. Thus, in essence a virtual light source is created at a depth of approximately 1 mm within the tissue. The wavelength used (548 nm) is absorbed by hemoglobin, yielding an image of the erythrocytes and, therefore, the perfused vessels in negative contrast. The imaging has a resolution of approximately 1 μm (see figure for a typical OPS image). The method has been validated for quantitative measurements of microcirculatory parameters in an animal model in direct comparison with intravital microscopy [19].

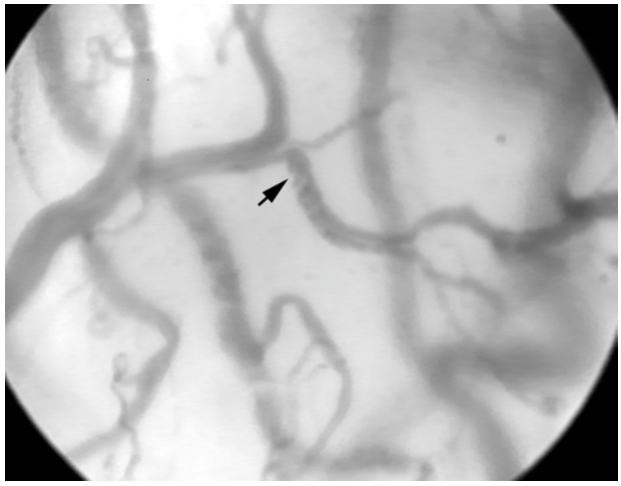

Fig. 1. Typical OPS image of the sublingual microcirculation. Capillaries and postcapillary venules are contrasted by the light absorbing erythrocytes. Activated leukocytes (arrow) appear as bright dots that move along the edge of the vessel lumen with a slower velocity than perfusing erythrocytes.

Images were obtained from the sublingual mucosa, the most frequently used site in adults [13, 14, 20, 21], where the probe was put in direct contact with the tissue. Once blood vessels were discernible, the OPS imaging probe was manually focused and, by stabilizing it on the volunteers' teeth, it was possible to reduce movement artifacts. Since the probe had to be in direct contact with the mucosa the exerted pressure could impair the sublingual tissue blood flow. Thus, the contact pressure was minimized to a point where the venules and capillaries remained in focus and showed maximum flow judged by the online image depicted on a 9-inch monitor (PVM-97, Sony, Japan). During measurements subjects rested supine on a bed in a quiet, temperature-controlled room (20 ± 5°C) covered with blankets to guarantee maximum comfort.

During a 20 to 30 minute period, at least 20 OPS imaging sequences of 10 seconds duration with minimal movement artifacts were recorded from each subject on video tapes. The tapes were subsequently analyzed off-line by an investigator blinded to the study protocol in each case.

Calculation of Vessel Diameter and Red Blood Cell Velocity and Functional Capillary Density

Vessel diameter (Dia) and red blood cell velocity (RCV) were measured with video analysis software (CapiScope, KK Technology, Devon, UK) in all focused vessels per observation area. Video sequences with little movement artifacts were used for the analysis of RCV with a spatial correlation technique. Dia was calculated automatically in selected vessels by perpendicular grey level profiles where the steepest slope in the grey level values is taken as being the edge of the vessel.

Functional capillary density (FCD) was defined as the length of small vessels (diameter $\leq$ 20 μm) in one observational field that showed flow of red blood cells during the 10 second observation period, divided by the area of the observation field. It is expressed as cm per cm^2.

Numbers of Rolling Leukocytes

Since the monochromatic light of the OPS device is mainly absorbed by the erythrocytes, leukocytes and plasma appear as gaps in the visualized erythrocyte column. Activated rolling leukocytes reveal a slow pattern of movement along the vessel wall. We made a quantitative assessment of the numbers of rolling leukocytes by subdividing the screen of a high resolution 20 inch monitor (PVM-20M2MDE, Sony, Japan) into nine rectangles ("fields" of 13 cm by 10 cm) corresponding to an area of approximately 0.26 mm by 0.20 mm in the image. Rolling leukocytes were defined as leukocytes that moved at a velocity significantly less than that of erythrocytes and plasma gaps in a given vessel. We counted the number of post capillary venules in each square and the number of rolling leukocytes which could be identified in each post capillary venule, expressed in rolling leukocytes per 10 seconds per vessel.

Statistical Analysis

Not all data were distributed normally (Kolmogorov-Smirnov test). Also, in view of the small numbers of volunteers, data is given as median and interquartile rage (IQR). SigmaStat (SPSS Inc., Chicago, IL, USA) was used for data analysis. Vessels were grouped according to their diameter for the analysis using 20 μm as a cut off and the distribution of microvessels with different diameter was calculated. RCV was measured in all vessels and results were grouped based on vessel diameter. Data was compared using Friedman analysis of variance by ranks. *Post hoc* analysis was performed by using Dunn's method. Linear relationships between macro- and microcirculatory parameters were tested using Spearman's rank correlation. Significance was assumed when $P < 0.05$.

Results

Macrohemodynamic data of the volunteers during the OPS recording period are given in Table 1. None of the volunteers showed symptoms of acute mountain sickness, i.e. none had headaches and a Lake Louise Scores > 3 points or either high altitude cerebral or pulmonary edema. Due to uncontrollable movement of the subjects tongue, OPS recordings of 10 or 11 volunteers could be analyzed. Dia and RCV were measured in a total of 3004 microvessels. The median number of analyzed vessels per time point was 31 (range 27–33).

Active Ascent

After the climb to the mountain refuge (T_1) oxygen saturation decreased significantly compared to baseline values (T_0) and was still reduced after 15 to 22 hours at high altitude (T_2; see Table 1). Inversely, heart rate increased at T_1 and was still elevated at T_2 (Table 1). Whilst median systolic and even more pronounced median diastolic blood pressure decreased after the strenuous ascent, these changes did not reach significance (Table 1).

RCV as well as FCD and the number of rolling leukocytes were unchanged within this trial (Table 2).

Passive Ascent

Air-lifting the volunteers to the high altitude refuge caused a significant, however, slightly less pronounced hypoxemia (T_1). Similar to the Active Ascent oxygen saturation was still reduced after 15 to 22 hours at high altitude (T_2). Heart rate was significantly increased during hypobaric hypoxia (T_1 and T_2), whereas blood pressure tended to increase (see Table 1). Microvascular perfusion (RCV and FCD) and the number of visualized activated leukocytes were unchanged (see Table 2).

Exercise at Lower Altitude

After completion of the exercise program at an altitude below 2100 m (T_1) SpO_2 was slightly, however significantly reduced, but normalized until the next morning (T_2). The significant reduction of systolic and diastolic blood pressure at T_1 was more pronounced than after the comparable exhausting climb to the Becherhaus, whereas the increase in heart rate was less pronounced (see Table 1). Microvascular OPS parameters RCV and FCD were unchanged (Tables 2). We could not visualize a change in the numbers of activated leukocytes (Table 2).

Correlations

For correlation analysis data from the three trials was pooled and linear regression analysis for OPS imaging parameters, oxygen saturation as a surrogate for the severity of hypoxia and macrohemodynamic parameters were performed. We found a weak, however significant positive correlation between the RCV and SpO_2 ($r = 0.25$; $P = 0.02$; Fig. 2), whereas microvascu-

Table 1. Hemodynamic data and oxygen saturation. Data is given as median and IQR. Friedman test with post hoc Dunn's method; P<0.05: * vs. T_0, # vs. T_2.

	Active Ascent (n = 11)	Passive Ascent (n = 11)	Exercise at Lower Altitude (n = 10)
	Systolic Blood Pressure, mmHg		
T_0	121 [118–124]	115 [111–120]	118 [114–121]
T_1	116 [108–120] #	126[115–132]	112 [110–114] *
T_2	1245 [115–126]	130 [118–139]	114 [111–119]
	Diastolic Blood Pressure, mmHg		
T_0	72 [70–76]	64 [58–71]	69 [65–73]
T_1	67 [60–76] *	70 [61–73]	64 [59–68] *
T_2	69 [63–75]	75 [65–78]	67 [58–72]
	Heart Rate, min^{-1}		
T_0	64 [54–66]	62 [57–68]	65 [53–72]
T_1	95 [84–100] *	73 [64–80] *	86 [83–90] *,#
T_2	75 [72–82]	74 [66–84] *	63 [56–66]
	Oxygen Saturation, %		
T_0	98 [98–99]	98 [97–99]	97 [96–98]
T_1	90 [88–92] *	93 [91–93] *	96 [96–97] *
T^2	93 [92–95]	94 [93–96]	98 [97–99]

Table 2. Microvascular parameters assessed by orthogonal polarization spectral imaging and concentration of circulating white blood cells. Data is given as median and IQR. Friedman test with post hoc Dunn's method; P<0.05: * vs. T_0, # vs. T_2.

	Active Ascent (n = 11)	Passive Ascent (n = 11)	Exercise at Lower Altitude (n = 10)
	Red Blood Cell Velocity, Small Vessels (0–20 μm), μm/s		
T_0	426 [379–461]	404 [353–471]	529 [477–631]
T_1	381 [377–474]	415 [341–475]	552 [486–597]
T_2	406 [357–466]	423 [389–537]	529 [511–603]
	Red Blood Cell Velocity, Large Vessels (>20 μm), μm/s		
T_0	495 [471–540]	465 [418–603]	659 [635–692]
T_1	471 [353–490]	446 [426–644]	568 [510–637]
T_2	410 [393–479]	464 [423–592]	564 [547–623]
	Functional Capillary Density, cm/cm^2		
T_0	27 [25–32]	29 [28–30]	27 [25–28]
T_1	28 [25–30]	30 [27–32]	29 [25–30]
T_2	29 [23–34]	30 [28–31]	27 [26–28]
	Numbers of Rolling Leukocytes, n/vessel/10s		
T_0	6.2 [4.4–6.8]	7.3 [3.2–9.0]	7.8 [6.6–9.6]
T_1	7.8 [4.3–9.0]	7.5 [5.5–10.1]	8.8 [4.8–9.2]
T_2	6.7 [4.4–6.7]	5.8 [4.8–10.2]	8.0 [5.0–8.6]
	Circulating White Blood Cells, 10^6/l		
T_0	5,800 [5,225–6,450]	7,000 [5,425–7,475]	5,885 [4,915–7,670]
T_1	11,750 [9,900–13,100] #	6,300 [5,900–7,075]	12,530 [10,988–16,148] *,#
T_2	5,500 [4,600–6,650]	5,900 [4,850–7,025]	5,890 [5,050–6,583]

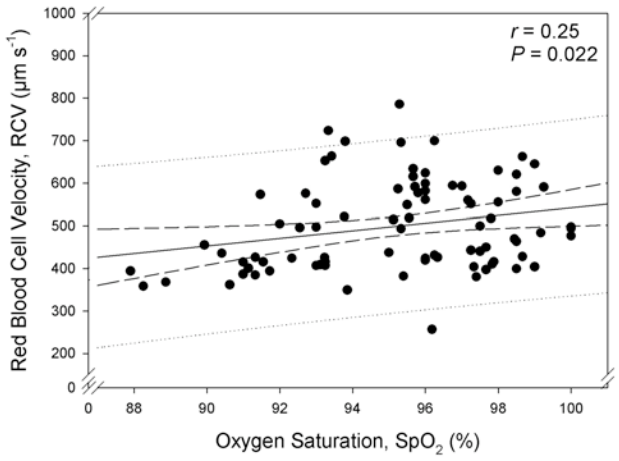

Fig. 2. Correlation between red blood cell velocity (RCV) and oxygen saturation (SpO_2) of the pooled data from all three trials is shown.

lar diameter (r = 0.016; P>0.2) and FCD was not influenced by hypoxia (r = -0.072; P>0.2).

Discussion

To our knowledge, this is the first field study using intravital microscopy for the assessment of microvascular perfusion in healthy volunteers during exposure to hypobaric hypoxia. The purpose of this study was to investigate whether microvascular perfusion is altered at high altitude and after exercise under normoxic and mild hypoxic conditions. The primary finding of the presented current investigation is that mild hypoxia, exercise and the combination of both stimuli do not appear to alter microvascular perfusion of sublingual tissue in healthy, altitude acclimatized volunteers.

Microvascular Perfusion

Various animal studies investigated the microvascular response to hypoxia [5, 7, 8, 22]. However, owing to the absence of appropriate techniques enabling the direct visualization of the human microcirculation, little is known about microvascular perfusion during hypoxia in man.

Microvascular reaction to tissue hypoxia has been described as a locally mediated vasodilatation and a capillary recruitment [23, 24]. The response to systemic hypoxia is complicated by a sympathetically mediated vasoconstriction initiated by chemoreceptor stimulation and hypoxia of the central nervous system itself [25]. In humans, the net effect of acute systemic hypoxia on a limb is thought to be vasodilatation [2, 26]. In animal studies using intravital microscopy for direct visualization of the microvasculature, however, reports on alterations of microvascular perfusion during acute hypoxia differ between species and the microvascular region under study [5, 7, 8]. Whereas the circulation of the hamster cheek pouch exhibits vasodilatation throughout the microvascular bed [5], changes in the gastrointestinal microcirculation of rats are characterized by arteriolar vasoconstriction but unchanged venular diameters [7]. Whilst a reduction of red blood cell velocity was shown in both species, this may have been attributable the effect of anesthesia in these hypoxia studies [5, 7, 8]. Anesthesia is supposed to have an influence on the hypoxia induced centrally mediated peripheral vasoconstrictor tone leading to a pronounced decrease in perfusion pressure and, thus, red blood cell velocity during systemic hypoxia [25]. This reduction in microvascular blood flow was not found to be influenced either by acclimatization [7] or by exercise training [8].

In contrast to these animal studies we have not found alterations in microvascular perfusion during hypobaric hypoxia. OPS imaging enables visualization of sublingual microcirculation in conscious volunteers. No significant change in blood pressure was observed during the period of hypoxia, nor was red blood cell velocity significantly altered. These observations supported the hypothesis that microvascular perfusion is well maintained during moderate hypoxia in acclimatized subjects. This assumption is further strengthened by our observation of an unchanged functional capillary density throughout the study protocol. Only capillaries, venules and small veins can be visualized in this region due to the microvascular architecture of the sublingual mucous membrane. Thus no direct observations regarding arteriolar perfusion were made.

We also did not observe any significant changes in red blood cell velocity or functional capillary density in the volunteers after exercise under either hypoxic or normoxic conditions.

The effects of systemic hypoxia on macrohemodynamics and limb perfusion have been intensively studied in man [1, 2, 26]. Local vasodilator and sympathetic vasoconstriction influence vascular tone. The balance of both reactions is dependent on the vascular region under study and the degree of hypoxia. Moreover, a local vasodilatation may be masked by centrally mediated sympathetic nerve activity. While some authors have demonstrated increased forearm blood flow during hypoxia [26], a similar degree of hypoxia (arterial oxygen saturation of 80%) did not change leg perfusion in another investigation [2]. We previously showed that total calf blood flow was not changed during mild hypobaric hypoxia that give rise to a mean oxygen saturation of 90%. However, the expected increase in limb blood flow after exercise was more pronounced under hypoxic conditions [18].

Leukocyte Endothelial Cell Interaction

In animal studies, even short periods of acute hypoxia (10 minutes of 7.5 to 10% inspired oxygen concentration) have been shown to cause a significant increase in the number of adherent leukocytes in mesenteric [11] and cremaster [8] venules. This activation of leukocytes is followed by a migration into the extravascular space and a concomitant increase in microvascular permeability [7, 10, 11]. Reactive oxygen species and nitric oxide seem to play a major role in the magnitude of leukocyte adherence and transmigration [10]. By contrast to these studies, we found no change in the number of rolling leukocytes in the sublingual venules, as assessed by the OPS imaging technique, af-

ter exposure to high altitude. There are several reasons that might account for this discrepancy:

Firstly, the hypoxic stimulus may have not been strong enough to activate the immunological cascades resulting in increased leukocyte activation. An inspired oxygen concentration of 10% (as used in most of the animal studies) gives rise to an oxygen partial pressure that is equivalent to normal ambient air at a pressure of approximately 350 Torr or an altitude of approximately 5000 to 5500 m. The studies at the Becherhaus were conducted at an altitude of 3196 m only (average 515 Torr during the study periods, equivalent to a 15% oxygen concentration at sea level). Nevertheless, we have previously shown that the rapid passive ascent to the Becherhaus by helicopter caused an increased expression of CD18 and an elevated superoxide anion production in the circulating polymorphonuclear neutrophils [17]. Thus we concluded that the level of hypoxia had been sufficient to activate the leukocytes. However, it may not have been strong enough to cause an increase in the adhesion and transmigration of leukocytes. This notion is further supported by the fact that we observed no increase in microvascular permeability in the plethysmographic studies on these subjects [18].

Second, despite not living at altitudes higher than 1200 m, the volunteers frequently had to ascend to altitudes greater than 2500 to 3000 m as part of their training. Thus, they may be considered as acclimatized to hypobaric hypoxia equivalent to the altitude of the Becherhaus. Acclimatization is known to significantly reduce or even abolish the leukocyte activation in response to acute exposure to hypoxia [7], thus acclimatization may have had an influence on the magnitude of leukocyte activation or modify vascular endothelium in these volunteers.

Third, exercise training prior to the exposure to hypoxia has been shown to attenuate hypoxia-induced inflammation [8]. Different mechanisms, such as increased nitric oxide availability [27,28], increased levels of endogenous antioxidants [29] and/or heat shock proteins [30] may account for this anti-inflammatory effect. In addition, oxygen delivery to the tissues may be improved by vascular hyperplasia in the trained musculature [31]. Indeed we found indirect evidence for an increased microvascular surface area in the calves of our subjects [18], who all underwent regular endurance training.

Fourth, sublingual microcirculation may not be the preferable vascular bed to assess changes after exercise. However, the sublingual microcirculation shows a good correlation with sublingual and gastric tonometry, surrogates for alterations of the splanchnic perfusion in septic patients [32] and hemorrhagic shock [33]. Thus OPS imaging of the sublingual microcirculation is considered valuable tool for the assessment of systemic changes in microcirculatory perfusion. This notion is further supported by significant correlation between SpO_2 and RCV in the pooled data of our study. Moreover using OPS imaging we have previously described the detection of rolling and sticking leukocytes in patients undergoing cardiopulmonary bypass [21]. Nevertheless, a possibly increased number of activated white cell may have been trapped in organs like the lung, liver, and spleen and may not be visible in the sublingual microcirculation.

Limitations of this Study

The altitude exposure of 3196 m in our study was relative moderate and the degree of hypoxemia was mild with the volunteers' oxygen saturation hardly decreased below 90 %. However, as a result of improvements in touristy infrastructure, the number of people who access high altitudes of 3000 to 3500 m is steadily rising. Furthermore, acute mountain sickness can occur at altitudes as low as 1500 m in highly susceptible subjects [34] and is evident in most subjects that are prone to this condition at altitudes greater than 2500 m [35]. Thus we believe that studies at this level of altitude are needed, since they address the majority of tourist exposed during summer and winter sport activities.

Volunteers in our study were well trained and acclimatized to hypobaric hypoxia at the study altitude. Thus maintenance of microvascular perfusion may not be guaranteed in non-acclimatized subjects exposed to the same degree of hypobaric hypoxia. Further studies with non-acclimatized volunteers or subjects susceptible for acute mountain sickness or high altitude edemas at higher altitudes or exposure to more severe hypoxia are needed to evaluate the role of microvascular perfusion in mountain sickness.

The sublingual microcirculation may not reflect other microcirculatory beds in the specific conditions of hypobaric hypoxia. Especially cerebral and pulmonary microvascular perfusion which is of special interest in acute mountain sickness and high altitude cerebral or pulmonary may not show similar alterations. Furthermore with OPS imaging, only superficial microvessels in mucous membranes can be assessed non-invasively in adults. Thus no conclusion on the pathogenesis of these disorders can be drawn from our study.

The sublingual microcirculation is the most commonly investigated area in man. Only capillaries and postcapillary venuels can be visualized at this site but arterioles usually appear out of focus. Whilst especially postcapillary venuels are the site where leukocyte-endothelial interaction occurs, only arterioles actively change diameter but can not be investigated.

Studies with OPS imaging of sublingual microvessels have another significant limitation - in man it is not possible to study the same vessel over time, therefore no direct comparisons can be made. Similar to other groups we aimed to reduce the resulting error by studying a large numbers of different vessels in order to obtain reliable data. We assessed a total of 3004 capillaries and postcapillary venules in this study and measured the diameter and velocity in an average of 31 vessels at each time point in each subject.

In conclusion, we found no evidence for an impaired microvascular perfusion of the sublingual mucous membrane in trained, acclimatized mountaineers during acute hypoxia and physical exercise under hypoxic or normoxic conditions. Despite the fact that the hypoxic stimulus at an altitude of 3196 m was

strong enough to increase the expression of leukocyte adhesion molecules and reactive oxygen species production [17] this activation was not followed by an increase rate of rolling leukocytes in these subjects in sublingual tissues. Similarly, whilst an increase of total calf blood flow after normoxic and – even more pronounced – after hypoxic exercise had been observed [18], it did not translate into a change in sublingual microvascular blood flow.

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