Eur J Appl Physiol
DOI 10.1007/s00421-015-3309-3

ORIGINAL ARTICLE

Increased endothelial microparticles and oxidative stress at extreme altitude

Jacqueline Pichler Hefti[1,2] · Alexander Leichtle[3] · Monika Stutz[4] · Urs Hefti[5] · Thomas Geiser[1] · Andreas R. Huber[6] · Tobias M. Merz[2]

Received: 9 July 2015 / Accepted: 8 December 2015

Abstract
Purpose Hypoxia and oxidative stress affect endothelial function. Endothelial microparticles (MP) are established measures of endothelial dysfunction and influence vascular reactivity. To evaluate the effects of hypoxia and antioxidant supplementation on endothelial MP profiles, a double-blind, placebo-controlled trial, during a high altitude expedition was performed.
Methods 29 participants were randomly assigned to a treatment group ($n = 14$), receiving vitamin E, C, A, and *N*-acetylcysteine daily, and a control group ($n = 15$), receiving placebo. Blood samples were obtained at 490 m (baseline), 3530, 4590, and 6210 m. A sensitive tandem mass spectrometry method was used to measure 8-iso-prostaglandin $F_{2\alpha}$ and hydroxyoctadecadienoic acids as markers of oxidative stress. Assessment of MP profiles including endothelial activation markers (CD62+MP and CD144+MP) and cell apoptosis markers (phosphatidylserine+MP and CD31+MP) was performed using a standardized flow cytometry-based protocol.
Results 15 subjects reached all altitudes and were included in the final analysis. Oxidative stress increased significantly at altitude. No statistically significant changes were observed comparing baseline to altitude measurements of phosphatidylserine expressing MP ($p = 0.1718$) and CD31+MP ($p = 0.1305$). Compared to baseline measurements, a significant increase in CD62+MP ($p = 0.0079$) and of CD144+MP was detected ($p = 0.0315$) at high altitudes. No significant difference in any MP level or oxidative stress markers were found between the treatment and the control group.
Conclusion Hypobaric hypoxia is associated with increased oxidative stress and induces a significant increase in CD62+ and CD144+MP, whereas phosphatidylserine+MP and CD31+MP remain unchanged. This indicates that endothelial activation rather than an apoptosis is the primary factor of hypoxia induced endothelial dysfunction.

Keywords Microparticles · Oxidative stress · Extreme altitude · Hypoxia · Endothelial dysfunction

Abbreviations

AMS	Acute mountain sickness
ANOVA	Analysis of variance
BH4	Tetrahydrobiopterin
CD31+MP	Cluster of differentiation 31 positive microparticle also known as platelet endothelial cell adhesion molecule positive microparticle
CD62+MP	Cluster of differentiation 62 positive microparticle also known as endothelial-selectin positive microparticle

Communicated by Fabio Fischetti.

✉ Jacqueline Pichler Hefti
Jacqueline.Pichler@Insel.ch

[1] Department of Pneumology, University Hospital and University of Bern, Bern, Switzerland

[2] Department of Intensive Care Medicine, University Hospital and University of Bern, Bern, Switzerland

[3] University Institute of Clinical Chemistry, University Hospital and University of Bern, Bern, Switzerland

[4] Department of Clinical Investigation, University Hospital and University of Bern, Bern, Switzerland

[5] Swiss Sportsclinic, Bern, Switzerland

[6] Center of Laboratory Medicine, Cantonal Hospital Aarau, Aarau, Switzerland

Published online: 28 January 2016

CD144+MP	Cluster of differentiation 144 positive microparticle also known as vascular endothelial-cadherin positive microparticle
HACE	High altitude cerebral edema
HAPE	High altitude pulmonary edema
HODE	Hydroxyoctadecadienoic acid
iPF2α-III	8-Iso-prostaglandin $F_{2\alpha}$
LLS	Lake Louise acute mountain sickness score
MP	Microparticles
NAC	N-acetylcysteine
NO	Nitric oxide
NOS	Nitric oxide synthase
PS	Phosphatidylserine
PS+MP	Phosphatidylserine positive microparticle
ROS	Reactive oxygen species
SpO_2	Pulse oxymetric arterial oxygen saturation
9-HODE	(±)9-Hydroxy-10E,12Z-octadecadienoic acid
13-HODE	13(*S*)-Hydroxy-9Z,11E-octadecadienoic acid

Introduction

In human subjects, exposure to hypoxia in the context of a stay at high altitudes is associated with increased levels of reactive oxygen species (ROS) (Pichler Hefti et al. 2013; Dosek et al. 2007; Chao et al. 1999; Schmidt et al. 2002) and altered activities of antioxidant systems (Chandel et al. 1998; Hoshikawa et al. 2001). Direct assessment of ROS is difficult due to their highly reactive and unstable nature. Lipid peroxidation markers such as isoprostanes are established markers of vascular oxidative stress, since they are produced by free radical induced peroxidation of the arachidonic acid backbone (Montuschi et al. 2004; Milne et al. 2007; Delanty et al. 1996; Montezano et al. 2015). Non-enzymatic ROS-induced formation of hydroxyoctadecadienoic acid (HODE) species, originating from the linoleic acid pathway, also represent relevant markers of oxidative stress (Yoshida and Niki 2006). ROS are known to mediate and modulate endothelial function in vascular diseases by reducing the nitric oxide (NO) availability in multiple ways. First, by scavenging NO by superoxides (Wingler et al. 2001); or indirectly by uncoupling of the enzyme nitric oxide synthase (NOS) (Wohlfart et al. 2008) and scavenging of tetrahydrobiopterin (BH4), which is the essential cofactor for NOS (Vasquez-Vivar et al. 1999; Ihlemann et al. 2003). Hypoxia can compromise the ability of activated endothelium to regulate the adequate synthesis of NO. Additionally, ROS can induce an increase of vasoconstrictive factors such as arachidonic acid metabolites, e.g., thromboxans (Delannoy et al. 2010; Fike et al. 2003) or hydroxyeicosatetraenoic acid compounds (Yu et al. 2015; Pichler Hefti et al. 2013).

Various pathophysiological conditions, e.g., pulmonary hypertension and shear stress, can lead to endothelial dysfunction (Budhiraja et al. 2004; Bull et al. 2003). Characteristics of endothelial dysfunction are the expression of adhesion molecules and tissue factor, secretion of cytokines and changes in vessel tone promoting mediators (e.g., prostacyclin derivates or NO). Clinically these changes may lead to an impairment of vessel tone regulation, increased procoagulatory activity, and altered vascular permeability (Deanfield et al. 2007). Relevant increases in ROS and subsequent changes in endothelial function, e.g. vascular leakage (Bailey et al. 2006, 2010) have been associated with the occurrence of different forms of altitude-related diseases. The formation of free radicals leading to increased permeability of the blood–brain barrier and subsequent vasogenic cerebral edema is a causative factor for the development of acute mountain sickness (AMS) and high altitude cerebral edema (HACE) (Bailey et al. 2009; Hooper et al. 2000). Similarly, increased capillary permeability due to endothelial dysfunction in combination with hypoxia induced increased pulmonary capillary pressure, is the main causative factor in the development of high altitude pulmonary edema (HAPE) (Maggiorini et al. 2001; Berger et al. 2005). Variations in individual ROS-levels and the resulting changes in pulmonary endothelial function have been associated with the variability in individual susceptibility for HAPE development in subjects with exaggerated hypoxic pulmonary capillary vasoconstriction (Burhop et al. 1988; Connolly and Aaronson 2010).

Microparticles (MP) are small membrane vesicles (sizes ranging 100–1000 nm) that are released from different cell types by exocytic blebbing of the plasma membrane. MP are constitutively shed from the surface of cells, but their formation can be up regulated in response to cellular activation or apoptosis (Lynch and Ludlam 2007) or as a result of increased oxidative stress (Hjuler Nielsen et al. 2015). MP contain bioactive phospholipids, cytoplasmic components, and various antigens originating from the parent cells and can influence vascular function—such as endothelium-dependent vasoregulation and endothelial permeability (Boulanger et al. 2001; Marcos-Ramiro et al. 2014). Most MP in human blood originate from platelets and endothelial cells. They represent markers of endothelial dysfunction and vascular injury (VanWijk et al. 2003; Montoro-Garcia et al. 2011). Increased levels are detectable in a variety of cardiovascular diseases, such as in diabetes (Tsimerman et al. 2011), pulmonary hypertension (Bakouboula et al. 2008), and in critically ill patients (Mastronardi et al. 2011). Elevated shear stress in vessel walls in the context of reduced NO-bioavailability (Vion et al. 2013) and increased oxidative stress affecting

endothelial antioxidant defense (Tushuizen et al. 2006; Mastronardi et al. 2011) induce shedding of MP into the blood stream. Different mechanisms leading to endothelial dysfunction are characterized by specific antigen expression on MP. Death and apoptosis of endothelial cells lead to the expression of platelet endothelial cell adhesion molecules also known as cluster of differentiation 31 (CD31+) and phosphatidylserine (PS) on the outer membrane of MP (PS+MP) (Zwaal and Schroit 1997; Werner et al. 2006). The loss of the membrane asymmetry and subsequent expression of PS on the outer membrane of platelet, MP is induced by the exposure to collagen (Thiagarajan and Tait 1991) and results in increased procoagulant activity and vascular dysfunction. In contrast, the activation of endothelial cells is associated with a membrane skeleton breakdown, which does not result in DNA degradation and consecutive cell death (VanWijk et al. 2003). Activated endothelium releases E-selectin (CD62+) positive MP (Jimenez et al. 2003). VE-cadherin (CD144+) is a calcium-dependent cell–cell adhesion glycoprotein and plays an important role in the cohesion and organization of the intercellular junctions and therefore regulates the tightness of the endothelium (Chichger et al. 2014). CD 144+ is involved in a downstream signaling pathway in the shear stress induced activation of integrins and cytoskeleton activation (Tzima et al. 2005). Endothelial dysfunction and increased vascular permeability are associated with modification or blocking of CD144+ on endothelial cells (Corada et al. 2001; Yuan 2002). CD144+ MP levels have been shown to correlate with increased pulmonary artery pressure (Amabile et al. 2008). Only a few studies have addressed the effects of sustained hypoxia on specific MP. Ayers et al. reported changes in various MP at a moderate altitude of 2590 m during short-term exposure (48 h). Subjects presented with mild hypoxemia (mean oxygen saturation 91 %) and decreased PS+MP were found, whereas MP of endothelial origin (CD62+MP and CD144+MP) did not change significantly (Ayers et al. 2014). Lichtenauer et al. found MP of apoptotic origin (CD31+/PS+MP) to be significantly increased during simulated hypoxia (5500 m) with a target oxygen saturation of 78 % (Lichtenauer et al. 2014). The protective effect of supplementation of antioxidants on endothelial integrity has been studied in different cardiovascular diseases (Wray et al. 2012; Chaumais et al. 2014; Traber and Stevens 2011; Kuiper et al. 2011). In the context of tissue hypoxia and increased oxidative stress, vitamin A and C have been postulated to offer enhanced protective effects (Palace et al. 1999; Hoyos et al. 2012; Schofield and Ratcliffe 2004). Vitamin C stabilizes BH4, an oxidation-sensitive co-factor of NO synthase, which regulates NO availability for vasomotor response. Both vitamin C and E protect against lipid peroxidation and therefore provide a protective effect on the integrity of the endothelial membrane (Pratico et al. 1998; Nunes et al. 1997).

The aim of this prospective, randomized, placebo-controlled double-blind trial was to examine the changes in MP profiles associated with vascular dysfunction in the context of severe and sustained hypoxia, and oxidative stress in healthy individuals in the context of a high-altitude field study. Second, the effect of an antioxidant treatment on endothelial function and MP was to be determined. We hypothesized that prolonged hypobaric hypoxia would lead to an increase in markers of endothelial dysfunction and vascular injury which can be attenuated by supplementation of antioxidants.

Methods

Subjects

Twenty-nine low-land residents participating in the Swiss high altitude expedition to Pik Lenin (7145 m) in Kirgizstan were included in the study. Subjects had to be healthy and not been taking any long-term medication during the study period. Subjects had to abstain from any high altitude exposure for a period of 6 weeks prior to the expedition.

Clinical parameters and procedures

Using a computer-based randomized allocation procedure, subjects were assigned in a double-blind fashion to receive either antioxidant dietary supplements or placebo over the period of 2 months prior to departure until completion of the climb. The antioxidant dietary supplements consisted of 800 I.E. vitamin E, 1000 mg vitamin C, 200,000 I.E. vitamin A, and 600 mg *N*-acetylcysteine (NAC) daily. Antioxidant dietary supplements and placebo of identical appearance were provided by the state pharmacy of the Canton of Aargau (Spitalapotheke, Kantonspital Aarau, Switzerland). Study group allocation was performed by the pharmacy; allocation was not revealed to the subjects or the investigators until completion of the data collection and the processing of the blood samples. Throughout the entire expedition food and fluids were provided in unlimited amounts to the study subjects. Baseline assessment was performed at 490 m. Altitude study sites were located at camp 1 (3550 m, expedition day 3), camp 2 (4590 m, day 6), and at camp 4 (6210 m, day 15). To assess signs and symptoms of AMS the Lake Louise AMS Score (LLS) was recorded at each study site (Roach et al. 1993). The participants had to rate their severity of symptoms of headache, gastrointestinal upset, fatigue/weakness, dizziness, and sleeping difficulties in a chart from 0 to 3. The LLS represents the sum of each of the five symptom classes. A LLS of minimum 3

and the presence of headache defines occurrence of AMS. Clinical examination of the study participants was performed by the research physicians at each altitude. Diagnosis of HACE was based on findings of encephalopathic symptoms and signs, including ataxic gait, severe lassitude, and progressive decline of mental function and consciousness. The diagnosis of HAPE was based on the typical clinical symptoms and lung auscultation. Pulse oximetry was performed in a sitting position with a finger pulse oximeter (Onyx 9500 SportStat, Nonin Medical, Plymouth, USA) after having stable values during at least 3 min. AMS scores and oxygen saturation (SpO_2) were recorded at each study site.

Blood samples and detection of microparticles

Venous blood samples were drawn at each study site with a 20 gauge needle (Butterfly Sarstedt, Seewelen, Switzerland) on 0.109 M citrate anticoagulant (vol. 9:1). Citrated plasma samples were centrifuged within 15 min after collection for 15 min at 2000×*g* (Rotana, Hettich AG, Bäch, Switzerland). The supernatants were carefully collected and the aliquots were stored at −30 °C and transported to laboratory facilities in Switzerland.

To generate a MP pellet, thawed citrated plasma samples were centrifuged for a second time at 16,000×*g* for 2 min at room temperature. 250 µl aliquots of the supernatant were pelleted by centrifugation at 16,000×*g* for 30 min at room temperature. 90 % of the supernatant was discarded and the pellets were washed with buffer (Tris–HCl 0.05 M/PBS 0.01 M, pH 7.2), and centrifuged again at 16,000×*g* for 30 min. Thereafter 90 % of the supernatant was discarded. The pellets were resuspended in 100 µl of buffer. To 50 µl of the required dilution of the antibody 5 µl of the MP suspension was added and incubated for 30 min. The number of PS+MP were determined by Annexin V labeling using Annexin binding buffer pH 7.4 (HEPES 10 mM, NaCl 150 mM, KCl 5 mM, MgCl2 1 mM, CaCl2 1.8 mM). For the detection of endothelial MP anti-CD62E-PE (Sigma, Saint Louis, USA), anti-CD31-FITC (Sigma-Aldrich, St. Louis, USA), and anti-CD144-FITC (BD Pharmingen, San Jose, USA) were used. Background fluorescence was compared with that of the isotype-control antibody. After addition of 900 µl PBS pH 7.2 samples were analyzed with a LSR II flow cytometer with FACSDiva software (BD Biosciences, San Jose, California, USA). On the basis of the events counted per 60 s MP concentration was calculated taking flow rate and dilution factor into account. Intra-assay and inter-assay variations were evaluated in ten independent experiments. Data sets were processed by FlowJo software 7.5 (Tree Star, Inc. Oregon, USA).

Markers of oxidative stress

Serum samples were used for the analysis of polyunsaturated fatty acid products. After extraction in aqueous acetonitrile containing deuterated internal standards the metabolites were analyzed by reverse phase high-performance liquid chromatography/electrospray ionization tandem mass spectrometry in negative Multiple Reaction Monitoring detection mode using an API 4000 QTrap mass spectrometer (WO/2008/145384). Non-enzymatic ROS-generated linoleic acid derivatives (±)9-hydroxy-10E,12Z-octadecadienoic acid (9-HODE), and 13(*S*)-hydroxy-9Z,11E-octadecadienoic acid (13-HODE), and a prostaglandin F2-like compound, 8-iso-prostaglandin $F_{2\alpha}$ (iPF2α-III) were used as markers of oxidative stress.

Statistics

The results are presented as mean and standard deviation or median and upper and lower limits of interquartile ranges (IQR) for parametric and non-parametric data, respectively. Normality testing was done using the D'Agostino & Pearson omnibus normality test. Factorial repeated measures analysis of variance (ANOVA) and post-hoc testing was used for data on MP-subtype data to test the differences between treatment groups and specific altitude-related changes in means. Standard statistical software packages were used for analysis of data (GraphPad Prism 5, GraphPad Software, USA and SigmaPlot version 12.0, Systat Software Inc., USA).

Ethics

The study was approved by the institutional review board (Ethics Committee of the Canton of Aargau) and adheres to the tenants of the Declaration of Helsinki. Written informed consent was obtained from all the subjects before the study inclusion. The trial has been registered on clinical trials.gov (NCT01571687).

Results

Participants and base line characteristics

Twenty-nine subjects (21 male, 8 female) participated in the high altitude expedition. After initial enrolment several subjects had to be excluded from the study. In the control group, one subject developed cardiogenic shock due to myocardial infarction, three subjects developed severe AMS, and one subject suffered from HACE, all these climbers had to abort the climb. In the treatment group,

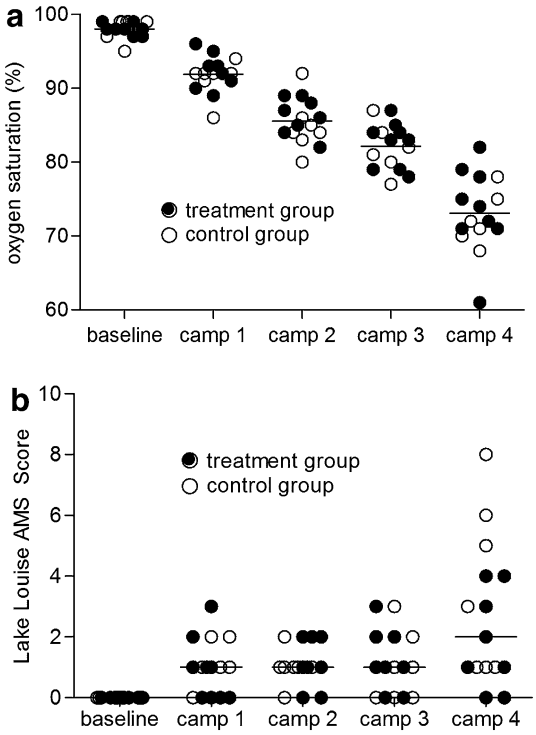

Fig. 1 **a**, **b** Pulse oxymetric arterial oxygen saturation (SpO_2) and acute mountain sickness scores at different altitudes stratified by treatment groups. A significant decrease in SpO_2 and an increase in Lake Louise AMS scores occurred with increasing altitude (both $p < 0.0001$). No significant difference was found between subjects supplied with dietary antioxidant supplements and controls for SpO_2 and Lake Louise AMS Scores. Severe AMS (≥5) only occurred in the highest camp at 6210 m in subjects of the control group

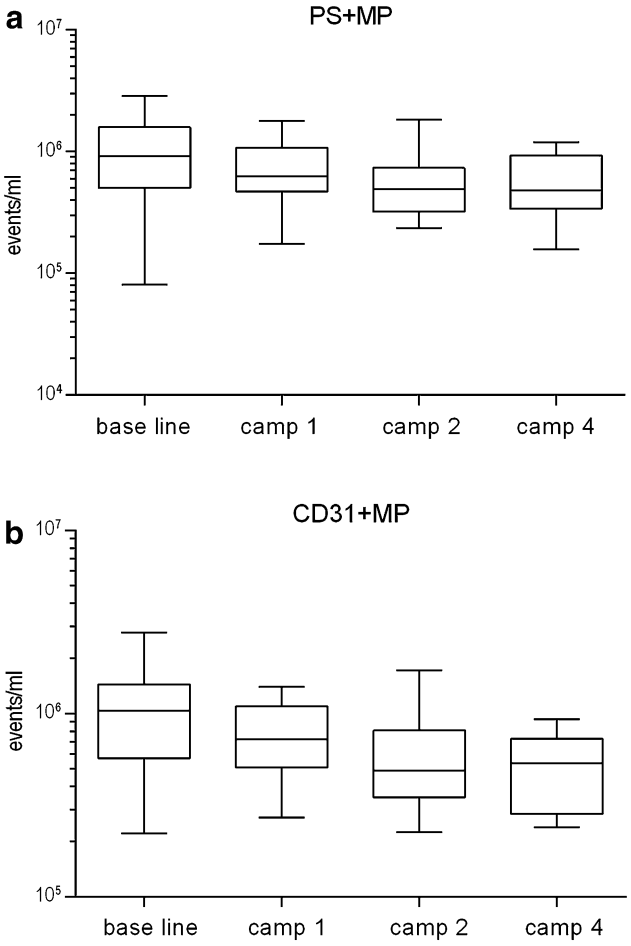

Fig. 2 **a**, **b** Markers of endothelial apoptosis of all subjects at different altitudes. Assessment of levels of PS+MP ($p = 0.1718$, **a**) and CD31+MP ($p = 0.1305$, **b**) revealed no statistically significant changes in altitude measurements. The whiskers correspond to the 5th and 95th percentile and the hinges reflect the 25th and 75th percentile, respectively

three subjects had to be excluded due to musculoskeletal and other non-altitude-related problems. The remaining 15 subjects (mean 48.8 years ± 6.7, 6 females, 9 treatment group, 6 control group), with a complete set of data and blood samples, were included in the final analysis.

Oxygen saturation and altitude illnesses

Mild AMS was seen in two-thirds of both groups on one examination day. Mean LLS increased from 0 ± 0 at base line to 1.0 ± 0.93 at camp 1, to 1.1 ± 0.70, and to 2.7 ± 2.35 at camp 2 and 4. Severe AMS (≥5) was seen only in the control group (3 subjects, respectively, 50 %). HACE was diagnosed in two women (both control group): in one subject after ascent to camp 4, and in one after reaching the summit. Both subjects were treated accordingly and descended to camp 1, leading to complete disappearance of the symptoms. None of the subjects developed clinical overt HAPE. SpO_2 significantly decreased from mean 98.0 % ± 1.16 to 91.9 % ± 2.42 at camp 1, to 85.6 % ± 3.07 at camp 2, and to 73.1 % ± 5.13 at camp 4 and did not differ between treatment groups. Oxygen saturation and AMS Scores of the study participants at different altitudes are shown in Fig. 1a, b.

Detection of microparticles

Assessment of PS+MP revealed median 919,650 (IQR 502,690–1.592 × 10^6) events/ml at 490 m. No statistically significant difference between treatment groups ($p = 0.751$) and no statistically significant changes in altitude measurements ($p = 0.1718$) were observed (Fig. 2a). Detection of CD31+MP yielded median 1.041 × 10^{-6} (IQR 5.725 10^5–1.451 × 10^6) events/ml at base line without any significant difference between treatment groups ($p = 0.576$) or changes at altitudes ($p = 0.1305$) (Fig. 2b). Median CD62+MP were at 17,620 (IQR 3390–127,846) events/ml during baseline testing and changed significantly at altitude measurements ($p = 0.0079$), whereas

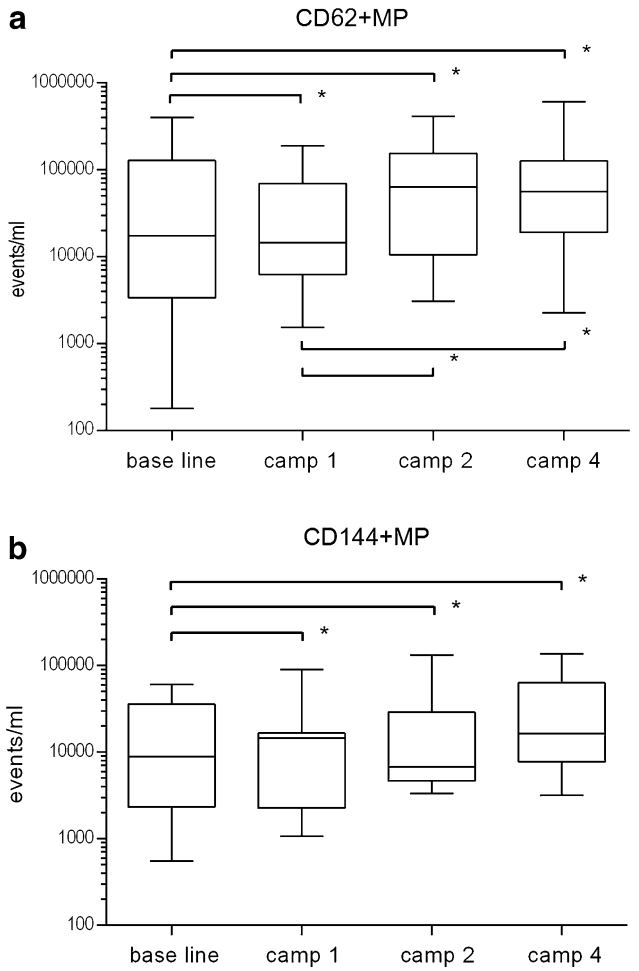

Fig. 3 **a**, **b** Markers of endothelial activation of all subjects at different altitudes. Levels of CD62+MP changed significantly at altitude measurements ($p = 0.0079$). Post-hoc analysis revealed a significant difference between baseline and all altitude measurements and between altitude measurements, except between camp 2 and 4 (**a**). CD144+MP changed significantly at altitude measurements ($p = 0.0315$). Post-hoc analysis showed a significant difference between baseline and all altitude measurements (**b**). *Asterisks* denotes statistically significant difference ($p < 0.05$) between altitudes in post-hoc testing. The whiskers correspond to the 5th and 95th percentile. The hinges are the 25th and 75th percentile, respectively

no significant differences between treatment groups were detected ($p = 0.102$). At camp 1, CD62+MP first decreased by −17.1 %, and increased thereafter by +258.7 % at camp 2, and by +219.4 % at camp 4 compared to the base line measurement (Fig. 3a). No significant difference between treatment groups was detected ($p = 0.102$). CD144+MP increased significantly from baseline median 8900 (IQR 2340–35,870) events/ml by +64.4 % at camp 1, and +85.5 % at camp 4, CD144+MP was 24.3 % lower at camp 2 compared to base line ($p = 0.0315$) (Fig. 3b). No significant effect of antioxidant supplementation on MP levels was detected ($p = 0.930$).

Markers of oxidative stress

Levels of 9-HODE and 13-HODE differed significantly in high altitude measurements compared to the low-land base line ($p = 0.0005$ and $p = 0.0003$, respectively), whereas no significant differences were found between different altitude measurements. Levels of iPF2α-III differed significantly between altitudes ($p = 0012$) and were higher at the altitudes of camp 1 and camp 4 when compared to baseline values and significantly higher at the altitude of camp 4 compared to all lower altitudes (Fig. 4). Markers of oxidative stress did not differ between treatment groups (all $p > 0.635$).

Discussion

The results of the study at hand can be summarized as follows: At altitude, we found a significant increase in CD144+MP and CD62+MP, both markers of endothelial activation. Levels of PS+MP and CD31+MP—representing markers of cell death—did not differ significantly when comparing measurements at different altitudes. Our results did not show a significant difference in markers of oxidative stress or MP levels between subjects supplied with antioxidant dietary supplements and subjects receiving a placebo preparation.

The main limitation of this trial is the relatively low number of subjects who reached all altitude study sites and were included in the final analysis. In high altitude field studies, a selection bias with a positive selection of the healthiest subjects and drop-out of subjects with high-altitude illness occurring at higher camps or insufficient physical fitness cannot be avoided. Conventional statistical imputation methods to account for missing data are based on the assumption that data are missing at random. These methods cannot be applied in the context of non-random subject drop-outs; therefore list wise deletion of subjects with missing values was applied. No statistically significant difference in AMS Scores, SpO_2 values, markers of oxidative stress, and MP levels was found between treatment groups in the subjects included in the final analysis. The lack of a significant effect of antioxidant supplementation on MP levels might be caused by a selection bias of healthy subjects at higher camps or by a lack of a protective effect, either due to insufficient mechanisms of action or inadequate supplement levels. The duration of antioxidant supplementation before the expedition was relatively long compared to other studies in the setting of high altitude sojourns (Bailey and Davies 2001; Baillie et al. 2009) and provided a high dose of vitamin A, C, and E when compared to other large studies in humans (Bjelakovic et al. 2012; Boaz et al. 2000; Stephens et al. 1996). Additionally, nutritional effects of hypobaric hypoxia—such as altitude-associated

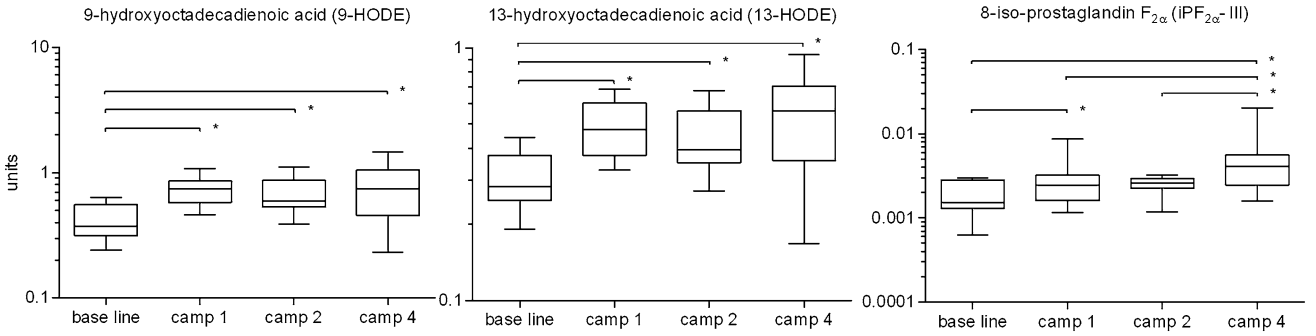

Fig. 4 Markers of oxidative stress of all subjects at different altitudes. Levels of 9-HODE and 13-HODE differed significantly in high altitude measurements compared to the low-land base line ($p = 0.0005$ and $p = 0.0003$, respectively), whereas no significant differences were found between different altitude measurements. Levels of iPF2α-III differed significantly between altitudes ($p = 0012$) and were higher at the altitudes of camp 1 and camp 4 when compared to baseline values and significantly higher at the altitude of camp 4 compared to all lower altitudes. Markers of oxidative stress did not differ between treatment groups ($p > 0.635$). The whiskers correspond to the 5th and 95th percentile. The hinges are the 25th and 75th percentile, respectively

anorexia (Westerterp-Plantenga et al. 1999)—might have influenced the effects of antioxidant supplementation. Standardized preanalytic procedure and measurement of MP does not exist, limiting the comparability and interpretation of absolute MP level values of different studies (Chandler et al. 2011; van der Pol et al. 2012). However, the preanalytic procedure was standardized throughout all study measurements and results are based on changes in MP levels at increasing altitudes and not on absolute values. The pathobiology of MP generation is not completely understood and there might be confounding factors which complicate the interpretation of the data. Lewis et al. examined the effects of hypobaric and normobaric hypoxia corresponding to 5050 m in 12 lowlanders on brachial endothelium-dependent flow-mediated dilatation and reported a persistent impairment in vascular dilation, which was mediated partially via oxidative stress and sympathoexcitation (Lewis et al. 2014). This effect was largely reversed following α1-adrenoreceptor blockade. Based on our data, we cannot exclude that sympathetic activation has an effect on endothelial cellular markers of activiation/apotosis. Toth et al. described gender and menstrual cycle dependent generation of platelet derived MP generation (Toth et al. 2007), but did not find an effect on endothelial derived MP. Our study includes six female subjects and focused on endothelial MP. However, based on published studies we cannot completely exclude the possibility that menstrual cycle induced hormonal effects influenced endothelial MP levels in our female subjects. Effects of altitude-associated hemoconcentration and resulting shear stress on endothelial MP are matter of debate and contradictory results have been published (Boulanger et al. 2007; Horigome et al. 2002). Based on previous work, we expect an increase of mean hematocrit values to 49 % at 6200 m compared to baseline values of 44 % (Pichler Hefti et al. 2010) which is mainly caused by an increase in red cell blood volume. Whether an increase in hematocrit influences endothelial MP concentration has not been established yet.

Data on MP in hypoxic humans are scarce and show an association between levels of MP-subtypes and hypoxemia. An increase in CD31+/Annexin+MP indicating apoptosis of endothelial cells during short-term exposure to normobaric hypoxia corresponding to an altitude of 5500 m has been found in one study (Lichtenauer et al. 2014). This result contrasts to our findings which shows unchanged CD31+MP and PS+MP, and increasing levels of CD62+MP suggesting that severe hypoxia in humans leads to an activation of the endothelial layer rather than to endothelial apoptosis. The shorter duration of hypoxic exposure in the former study, not allowing for sufficient altitude adaptation, as well as different molecular mechanisms induced by normobaric versus hypobaric hypoxia might explain these differing findings. In contrast to our results, Ayers et al. found a statistically significant decrease of PS+MP in prolonged hypobaric hypoxic conditions at a lower altitude of 2590 m (Ayers et al. 2014).

The increase in CD62+ and CD144+MP levels in our study subjects might be explained as follows: Increasing levels of CD62+ and CD144+MP have been shown to correlate with shear stress (Vion et al. 2013) and disease severity and outcome in pulmonary hypertension (Tual-Chalot et al. 2010; Akinnusi and El Solh 2009; Amabile et al. 2009). A hypoxia-induced increase in pulmonary capillary pressure is invariably found at higher altitude, potentially leading to increased CD62+MP and CD144+MP levels in this context by similar mechanisms. CD144 is known to be a key regulator of the endothelial permeability (Sidibe and Imhof 2014; Wallez and Huber 2008; Angelini et al. 2006). Not only internalization, but also shedding and cleavage of the external domain of CD144 correlate with the loss of endothelial leak tightness (Herren et al. 1998; Schulz et al. 2008). In hypobaric hypoxic

conditions, increased levels of CD144+MP might indicate an increased pulmonary vascular permeability in the context of HAPE. However, to answer the question if increased levels of CD62+MP and CD144+MP correlate with the extent of hypoxic pulmonary vasoconstriction and/or pulmonary or cerebral vascular leakage would require measurement of pulmonary vascular hemodynamics and lung fluid content, as well as assessment for brain edema, a task not easily performed at extreme altitudes.

Conclusions

In conclusion, our study shows a significant increase in levels of CD62+MP and CD144+MP and unchanged levels of PS+MP and CD31+MP during hypobaric hypoxia indicating that endothelial activation rather than an apoptosis is the primary factor of hypoxia induced endothelial dysfunction. The exocytosis of CD144+MP suggests an increase in vascular permeability, which would be in accordance with the understanding of the pathophysiology of HAPE and HACE. Further studies are needed to evaluate the potential associations of specific hypoxia induced MP profile changes and organ dysfunction and capillary leakage in the context of sojourns to extreme altitudes.

Acknowledgments We thank all the volunteers, mountain guides, and local staff for their invaluable support of this research project. Equipment was kindly provided by Hettich AG, Bäch, Switzerland. This study was supported by the Swiss Society of Mountain Medicine.

References

Akinnusi ME, El Solh AA (2009) Circulating endothelial microparticle levels and hemodynamic severity of pulmonary hypertension: is there a role for sleep apnea? Am J Respir Crit Care Med 179(4):328

Amabile N, Heiss C, Real WM, Minasi P, McGlothlin D, Rame EJ, Grossman W, De Marco T, Yeghiazarians Y (2008) Circulating endothelial microparticle levels predict hemodynamic severity of pulmonary hypertension. Am J Respir Crit Care Med 177(11):1268–1275. doi:10.1164/rccm.200710-1458OC

Amabile N, Heiss C, Chang V, Angeli FS, Damon L, Rame EJ, McGlothlin D, Grossman W, De Marco T, Yeghiazarians Y (2009) Increased CD62e(+) endothelial microparticle levels predict poor outcome in pulmonary hypertension patients. J Heart Lung Transplant 28(10):1081–1086. doi:10.1016/j.healun.2009.06.005

Angelini DJ, Hyun SW, Grigoryev DN, Garg P, Gong P, Singh IS, Passaniti A, Hasday JD, Goldblum SE (2006) TNF-alpha increases tyrosine phosphorylation of vascular endothelial cadherin and opens the paracellular pathway through fyn activation in human lung endothelia. Am J Physiol Lung Cell Mol Physiol 291(6):L1232–L1245. doi:10.1152/ajplung.00109.2006

Ayers L, Stoewhas AC, Ferry B, Latshang TD, Lo Cascio CM, Sadler R, Stadelmann K, Tesler N, Huber R, Achermann P, Bloch KE, Kohler M (2014) Circulating levels of cell-derived microparticles are reduced by mild hypobaric hypoxia: data from a randomised controlled trial. Eur J Appl Physiol 114(5):1067–1073. doi:10.1007/s00421-014-2837-6

Bailey DM, Davies B (2001) Acute mountain sickness; prophylactic benefits of antioxidant vitamin supplementation at high altitude. High Alt Med Biol 2(1):21–29. doi:10.1089/152702901750067882

Bailey DM, Roukens R, Knauth M, Kallenberg K, Christ S, Mohr A, Genius J, Storch-Hagenlocher B, Meisel F, McEneny J, Young IS, Steiner T, Hess K, Bartsch P (2006) Free radical-mediated damage to barrier function is not associated with altered brain morphology in high-altitude headache. J Cereb Blood Flow Metab 26(1):99–111. doi:10.1038/sj.jcbfm.9600169

Bailey DM, Evans KA, James PE, McEneny J, Young IS, Fall L, Gutowski M, Kewley E, McCord JM, Moller K, Ainslie PN (2009) Altered free radical metabolism in acute mountain sickness: implications for dynamic cerebral autoregulation and blood-brain barrier function. J Physiol 587(Pt 1):73–85. doi:10.1113/jphysiol.2008.159855

Bailey DM, Dehnert C, Luks AM, Menold E, Castell C, Schendler G, Faoro V, Gutowski M, Evans KA, Taudorf S, James PE, McEneny J, Young IS, Swenson ER, Mairbaurl H, Bartsch P, Berger MM (2010) High-altitude pulmonary hypertension is associated with a free radical-mediated reduction in pulmonary nitric oxide bioavailability. J Physiol 588(Pt 23):4837–4847. doi:10.1113/jphysiol.2010.194704

Baillie JK, Thompson AA, Irving JB, Bates MG, Sutherland AI, Macnee W, Maxwell SR, Webb DJ (2009) Oral antioxidant supplementation does not prevent acute mountain sickness: double blind, randomized placebo-controlled trial. QJM Mon J Assoc Phys 102(5):341–348. doi:10.1093/qjmed/hcp026

Bakouboula B, Morel O, Faure A, Zobairi F, Jesel L, Trinh A, Zupan M, Canuet M, Grunebaum L, Brunette A, Desprez D, Chabot F, Weitzenblum E, Freyssinet JM, Chaouat A, Toti F (2008) Procoagulant membrane microparticles correlate with the severity of pulmonary arterial hypertension. Am J Respir Crit Care Med 177(5):536–543. doi:10.1164/rccm.200706-840OC

Berger MM, Hesse C, Dehnert C, Siedler H, Kleinbongard P, Bardenheuer HJ, Kelm M, Bartsch P, Haefeli WE (2005) Hypoxia impairs systemic endothelial function in individuals prone to high-altitude pulmonary edema. Am J Respir Crit Care Med 172(6):763–767. doi:10.1164/rccm.200504-654OC

Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C (2012) Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases. Cochrane Database Syst Rev 3:CD007176. doi:10.1002/14651858.CD007176.pub2

Boaz M, Smetana S, Weinstein T, Matas Z, Gafter U, Iaina A, Knecht A, Weissgarten Y, Brunner D, Fainaru M, Green MS (2000) Secondary prevention with antioxidants of cardiovascular disease in endstage renal disease (SPACE): randomised placebo-controlled trial. Lancet 356(9237):1213–1218

Boulanger CM, Scoazec A, Ebrahimian T, Henry P, Mathieu E, Tedgui A, Mallat Z (2001) Circulating microparticles from patients with myocardial infarction cause endothelial dysfunction. Circulation 104(22):2649–2652

Boulanger CM, Amabile N, Guerin AP, Pannier B, Leroyer AS, Mallat CN, Tedgui A, London GM (2007) In vivo shear stress determines circulating levels of endothelial microparticles in end-stage renal disease. Hypertension 49(4):902–908. doi:10.1161/01.HYP.0000259667.22309.df

Budhiraja R, Tuder RM, Hassoun PM (2004) Endothelial dysfunction in pulmonary hypertension. Circulation 109(2):159–165. doi:10.1161/01.CIR.0000102381.57477.50

Bull TM, Golpon H, Hebbel RP, Solovey A, Cool CD, Tuder RM, Geraci MW, Voelkel NF (2003) Circulating endothelial cells

in pulmonary hypertension. Thromb Haemost 90(4):698–703. doi:10.1267/THRO03040698

Burhop KE, Selig WM, Malik AB (1988) Monohydroxyeicosatetraenoic acids (5-HETE and 15-HETE) induce pulmonary vasoconstriction and edema. Circ Res 62(4):687–698

Chandel NS, Maltepe E, Goldwasser E, Mathieu CE, Simon MC, Schumacker PT (1998) Mitochondrial reactive oxygen species trigger hypoxia-induced transcription. Proc Natl Acad Sci USA 95(20):11715–11720

Chandler WL, Yeung W, Tait JF (2011) A new microparticle size calibration standard for use in measuring smaller microparticles using a new flow cytometer. J Thromb Haemost 9(6):1216–1224. doi:10.1111/j.1538-7836.2011.04283.x

Chao WH, Askew EW, Roberts DE, Wood SM, Perkins JB (1999) Oxidative stress in humans during work at moderate altitude. J Nutr 129(11):2009–2012

Chaumais MC, Ranchoux B, Montani D, Dorfmuller P, Tu L, Lecerf F, Raymond N, Guignabert C, Price L, Simonneau G, Cohen-Kaminsky S, Humbert M, Perros F (2014) N-acetylcysteine improves established monocrotaline-induced pulmonary hypertension in rats. Respir Res 15:65. doi:10.1186/1465-9921-15-65

Chichger H, Duong H, Braza J, Harrington EO (2014) p18, a novel adaptor protein, regulates pulmonary endothelial barrier function via enhanced endocytic recycling of VE-cadherin. FASEB J Off Publ Fed Am Soc Exp Biol. doi:10.1096/fj.14-257212

Connolly MJ, Aaronson PI (2010) Cell redox state and hypoxic pulmonary vasoconstriction: recent evidence and possible mechanisms. Respir Physiol Neurobiol 174(3):165–174. doi:10.1016/j.resp.2010.08.016

Corada M, Liao F, Lindgren M, Lampugnani MG, Breviario F, Frank R, Muller WA, Hicklin DJ, Bohlen P, Dejana E (2001) Monoclonal antibodies directed to different regions of vascular endothelial cadherin extracellular domain affect adhesion and clustering of the protein and modulate endothelial permeability. Blood 97(6):1679–1684

Deanfield JE, Halcox JP, Rabelink TJ (2007) Endothelial function and dysfunction: testing and clinical relevance. Circulation 115(10):1285–1295. doi:10.1161/circulationaha.106.652859

Delannoy E, Courtois A, Freund-Michel V, Leblais V, Marthan R, Muller B (2010) Hypoxia-induced hyperreactivity of pulmonary arteries: role of cyclooxygenase-2, isoprostanes, and thromboxane receptors. Cardiovasc Res 85(3):582–592. doi:10.1093/cvr/cvp292

Delanty N, Reilly M, Pratico D, FitzGerald DJ, Lawson JA, FitzGerald GA (1996) 8-Epi PGF2 alpha: specific analysis of an isoeicosanoid as an index of oxidant stress in vivo. Br J Clin Pharmacol 42(1):15–19

Dosek A, Ohno H, Acs Z, Taylor AW, Radak Z (2007) High altitude and oxidative stress. Respir Physiol Neurobiol 158(2–3):128–131. doi:10.1016/j.resp.2007.03.013

Fike CD, Kaplowitz MR, Pfister SL (2003) Arachidonic acid metabolites and an early stage of pulmonary hypertension in chronically hypoxic newborn pigs. Am J Physiol Lung Cell Mol Physiol 284(2):L316–L323. doi:10.1152/ajplung.00228.2002

Herren B, Levkau B, Raines EW, Ross R (1998) Cleavage of beta-catenin and plakoglobin and shedding of VE-cadherin during endothelial apoptosis: evidence for a role for caspases and metalloproteinases. Mol Biol Cell 9(6):1589–1601

Hjuler Nielsen M, Irvine H, Vedel S, Raungaard B, Beck-Nielsen H, Handberg A (2015) Elevated atherosclerosis-related gene expression, monocyte activation and microparticle-release are related to increased lipoprotein-associated oxidative stress in familial hypercholesterolemia. PLoS One 10(4):e0121516. doi:10.1371/journal.pone.0121516

Hooper DC, Scott GS, Zborek A, Mikheeva T, Kean RB, Koprowski H, Spitsin SV (2000) Uric acid, a peroxynitrite scavenger, inhibits CNS inflammation, blood-CNS barrier permeability changes, and tissue damage in a mouse model of multiple sclerosis. FASEB J Off Publ Fed Am Soc Exp Biol 14(5):691–698

Horigome H, Hiramatsu Y, Shigeta O, Nagasawa T, Matsui A (2002) Overproduction of platelet microparticles in cyanotic congenital heart disease with polycythemia. J Am Coll Cardiol 39(6):1072–1077

Hoshikawa Y, Ono S, Suzuki S, Tanita T, Chida M, Song C, Noda M, Tabata T, Voelkel NF, Fujimura S (2001) Generation of oxidative stress contributes to the development of pulmonary hypertension induced by hypoxia. J Appl Physiol (1985) 90(4):1299–1306

Hoyos B, Acin-Perez R, Fischman DA, Manfredi G, Hammerling U (2012) Hiding in plain sight: uncovering a new function of vitamin A in redox signaling. Biochim Biophys Acta 1821 1:241–247. doi:10.1016/j.bbalip.2011.06.014

Ihlemann N, Rask-Madsen C, Perner A, Dominguez H, Hermann T, Kober L, Torp-Pedersen C (2003) Tetrahydrobiopterin restores endothelial dysfunction induced by an oral glucose challenge in healthy subjects. Am J Physiol Heart Circ Physiol 285(2):H875–H882. doi:10.1152/ajpheart.00008.2003

Jimenez JJ, Jy W, Mauro LM, Soderland C, Horstman LL, Ahn YS (2003) Endothelial cells release phenotypically and quantitatively distinct microparticles in activation and apoptosis. Thromb Res 109(4):175–180

Kuiper HC, Bruno RS, Traber MG, Stevens JF (2011) Vitamin C supplementation lowers urinary levels of 4-hydroperoxy-2-nonenal metabolites in humans. Free Radic Biol Med 50(7):848–853. doi:10.1016/j.freeradbiomed.2011.01.004

Lewis NC, Bailey DM, Dumanoir GR, Messinger L, Lucas SJ, Cotter JD, Donnelly J, McEneny J, Young IS, Stembridge M, Burgess KR, Basnet AS, Ainslie PN (2014) Conduit artery structure and function in lowlanders and native highlanders: relationships with oxidative stress and role of sympathoexcitation. J Physiol 592(Pt 5):1009–1024. doi:10.1113/jphysiol.2013.268615

Lichtenauer M, Goebel B, Fritzenwanger M, Forster M, Betge S, Lauten A, Figulla HR, Jung C (2014) Simulated temporary hypoxia triggers the release of CD31+/Annexin + endothelial microparticles: a prospective pilot study in humans. Clin Hemorheol Microcirc. doi:10.3233/CH-141908

Lynch SF, Ludlam CA (2007) Plasma microparticles and vascular disorders. Br J Haematol 137(1):36–48. doi:10.1111/j.1365-2141.2007.06514.x

Maggiorini M, Melot C, Pierre S, Pfeiffer F, Greve I, Sartori C, Lepori M, Hauser M, Scherrer U, Naeije R (2001) High-altitude pulmonary edema is initially caused by an increase in capillary pressure. Circulation 103(16):2078–2083

Marcos-Ramiro B, Oliva Nacarino P, Serrano-Pertierra E, Blanco-Gelaz MA, Weksler BB, Romero IA, Couraud PO, Tunon A, Lopez-Larrea C, Millan J, Cernuda-Morollon E (2014) Microparticles in multiple sclerosis and clinically isolated syndrome: effect on endothelial barrier function. BMC Neurosci 15:110. doi:10.1186/1471-2202-15-110

Mastronardi ML, Mostefai HA, Meziani F, Martinez MC, Asfar P, Andriantsitohaina R (2011) Circulating microparticles from septic shock patients exert differential tissue expression of enzymes related to inflammation and oxidative stress. Crit Care Med 39(7):1739–1748. doi:10.1097/CCM.0b013e3182190b4b

Milne GL, Yin H, Brooks JD, Sanchez S, Jackson Roberts L 2nd, Morrow JD (2007) Quantification of F2-isoprostanes in biological fluids and tissues as a measure of oxidant stress. Methods Enzymol 433:113–126. doi:10.1016/S0076-6879(07)33006-1

Montezano AC, Dulak-Lis M, Tsiropoulou S, Harvey A, Briones AM, Touyz RM (2015) Oxidative stress and human hypertension: vascular mechanisms, biomarkers, and novel therapies. Can J Cardiol 31(5):631–641. doi:10.1016/j.cjca.2015.02.008

Montoro-Garcia S, Shantsila E, Marin F, Blann A, Lip GY (2011) Circulating microparticles: new insights into the biochemical

basis of microparticle release and activity. Basic Res Cardiol. doi:10.1007/s00395-011-0198-4
Montuschi P, Barnes PJ, Roberts LJ 2nd (2004) Isoprostanes: markers and mediators of oxidative stress. FASEB J Off Publ Fed Am Soc Exp Biol 18(15):1791–1800. doi:10.1096/fj.04-2330rev
Nunes GL, Robinson K, Kalynych A, King SB 3rd, Sgoutas DS, Berk BC (1997) Vitamins C and E inhibit O_2-production in the pig coronary artery. Circulation 96(10):3593–3601
Palace VP, Hill MF, Khaper N, Singal PK (1999) Metabolism of vitamin A in the heart increases after a myocardial infarction. Free Radic Biol Med 26(11–12):1501–1507
Pichler Hefti J, Risch L, Hefti U, Scharrer I, Risch G, Merz TM, Turk A, Bosch MM, Barthelmess D, Schoch O, Maggiorini M, Huber AR (2010) Changes of coagulation parameters during high altitude expedition. Swiss Med Wkly 140(7–8):111–117
Pichler Hefti J, Sonntag D, Hefti U, Risch L, Schoch OD, Turk AJ, Hess T, Bloch KE, Maggiorini M, Merz TM, Weinberger KM, Huber AR (2013) Oxidative stress in hypobaric hypoxia and influence on vessel-tone modifying mediators. High Alt Med Biol 14(3):273–279. doi:10.1089/ham.2012.1110
Pratico D, Tangirala RK, Rader DJ, Rokach J, FitzGerald GA (1998) Vitamin E suppresses isoprostane generation in vivo and reduces atherosclerosis in ApoE-deficient mice. Nat Med 4(10):1189–1192. doi:10.1038/2685
Roach RC, Bartsch P, Hackett PH, Oelz O (1993) Hypoxia and molecular medicine. In: The Lake Louise acute mountain sickness scoring system. Suton JR, Coates G, Houston Ch, Burlington
Schmidt MC, Askew EW, Roberts DE, Prior RL, Ensign WY Jr, Hesslink RE Jr (2002) Oxidative stress in humans training in a cold, moderate altitude environment and their response to a phytochemical antioxidant supplement. Wilderness Environ Med 13(2):94–105
Schofield CJ, Ratcliffe PJ (2004) Oxygen sensing by HIF hydroxylases. Nat Rev Mol Cell Biol 5(5):343–354. doi:10.1038/nrm1366
Schulz B, Pruessmeyer J, Maretzky T, Ludwig A, Blobel CP, Saftig P, Reiss K (2008) ADAM10 regulates endothelial permeability and T-Cell transmigration by proteolysis of vascular endothelial cadherin. Circ Res 102(10):1192–1201. doi:10.1161/CIRCRESAHA.107.169805
Sidibe A, Imhof BA (2014) VE-cadherin phosphorylation decides: vascular permeability or diapedesis. Nat Immunol 15(3):215–217. doi:10.1038/ni.2825
Stephens NG, Parsons A, Schofield PM, Kelly F, Cheeseman K, Mitchinson MJ (1996) Randomised controlled trial of vitamin E in patients with coronary disease: cambridge Heart Antioxidant Study (CHAOS). Lancet 347(9004):781–786
Thiagarajan P, Tait JF (1991) Collagen-induced exposure of anionic phospholipid in platelets and platelet-derived microparticles. J Biol Chem 266(36):24302–24307
Toth B, Nikolajek K, Rank A, Nieuwland R, Lohse P, Pihusch V, Friese K, Thaler CJ (2007) Gender-specific and menstrual cycle dependent differences in circulating microparticles. Platelets 18(7):515–521. doi:10.1080/09537100701525843
Traber MG, Stevens JF (2011) Vitamins C and E: beneficial effects from a mechanistic perspective. Free Radic Biol Med 51(5):1000–1013. doi:10.1016/j.freeradbiomed.2011.05.017
Tsimerman G, Roguin A, Bachar A, Melamed E, Brenner B, Aharon A (2011) Involvement of microparticles in diabetic vascular complications. Thromb Haemost. doi:10.1160/TH10-11-0712
Tual-Chalot S, Guibert C, Muller B, Savineau JP, Andriantsitohaina R, Martinez MC (2010) Circulating microparticles from pulmonary hypertensive rats induce endothelial dysfunction. Am J Respir Crit Care Med 182(2):261–268. doi:10.1164/rccm.200909-1347OC
Tushuizen ME, Nieuwland R, Scheffer PG, Sturk A, Heine RJ, Diamant M (2006) Two consecutive high-fat meals affect endothelial-dependent vasodilation, oxidative stress and cellular microparticles in healthy men. J Thromb Haemost 4(5):1003–1010. doi:10.1111/j.1538-7836.2006.01914.x
Tzima E, Irani-Tehrani M, Kiosses WB, Dejana E, Schultz DA, Engelhardt B, Cao G, DeLisser H, Schwartz MA (2005) A mechanosensory complex that mediates the endothelial cell response to fluid shear stress. Nature 437(7057):426–431. doi:10.1038/nature03952
van der Pol E, van Gemert MJ, Sturk A, Nieuwland R, van Leeuwen TG (2012) Single vs swarm detection of microparticles and exosomes by flow cytometry. J Thromb Haemost JTH 10(5):919–930. doi:10.1111/j.1538-7836.2012.04683.x
VanWijk MJ, VanBavel E, Sturk A, Nieuwland R (2003) Microparticles in cardiovascular diseases. Cardiovasc Res 59(2):277–287
Vasquez-Vivar J, Hogg N, Martasek P, Karoui H, Pritchard KA Jr, Kalyanaraman B (1999) Tetrahydrobiopterin-dependent inhibition of superoxide generation from neuronal nitric oxide synthase. J Biol Chem 274(38):26736–26742
Vion AC, Ramkhelawon B, Loyer X, Chironi G, Devue C, Loirand G, Tedgui A, Lehoux S, Boulanger CM (2013) Shear stress regulates endothelial microparticle release. Circ Res 112(10):1323–1333. doi:10.1161/CIRCRESAHA.112.300818
Wallez Y, Huber P (2008) Endothelial adherens and tight junctions in vascular homeostasis, inflammation and angiogenesis. Biochim Biophys Acta 1778(3):794–809. doi:10.1016/j.bbamem.2007.09.003
Werner N, Wassmann S, Ahlers P, Kosiol S, Nickenig G (2006) Circulating CD31+/annexin V+ apoptotic microparticles correlate with coronary endothelial function in patients with coronary artery disease. Arterioscler Thromb Vasc Biol 26(1):112–116. doi:10.1161/01.ATV.0000191634.13057.15
Westerterp-Plantenga MS, Westerterp KR, Rubbens M, Verwegen CR, Richelet JP, Gardette B (1999) Appetite at "high altitude" [Operation Everest III (Comex-'97)]: a simulated ascent of Mount Everest. J Appl Physiol (1985) 87(1):391–399
Wingler K, Wunsch S, Kreutz R, Rothermund L, Paul M, Schmidt HH (2001) Upregulation of the vascular NAD(P)H-oxidase isoforms Nox1 and Nox4 by the renin-angiotensin system in vitro and in vivo. Free Radic Biol Med 31(11):1456–1464
Wohlfart P, Xu H, Endlich A, Habermeier A, Closs EI, Hubschle T, Mang C, Strobel H, Suzuki T, Kleinert H, Forstermann U, Ruetten H, Li H (2008) Antiatherosclerotic effects of small-molecular-weight compounds enhancing endothelial nitric-oxide synthase (eNOS) expression and preventing eNOS uncoupling. J Pharmacol Exp Ther 325(2):370–379. doi:10.1124/jpet.107.128009
Wray DW, Nishiyama SK, Harris RA, Zhao J, McDaniel J, Fjeldstad AS, Witman MA, Ives SJ, Barrett-O'Keefe Z, Richardson RS (2012) Acute reversal of endothelial dysfunction in the elderly after antioxidant consumption. Hypertension 59(4):818–824. doi:10.1161/HYPERTENSIONAHA.111.189456
Yoshida Y, Niki E (2006) Bio-markers of lipid peroxidation in vivo: hydroxyoctadecadienoic acid and hydroxycholesterol. BioFactors 27(1–4):195–202
Yu X, Li T, Liu X, Yu H, Hao Z, Chen Y, Zhang C, Liu Y, Li Q, Mao M, Zhu D (2015) Modulation of pulmonary vascular remodeling in hypoxia: role of 15-LOX-2/15-HETE-MAPKs pathway. Cell Physiol Biochem Int J Exp Cell Physiol Biochem Pharmacol 35(6):2079–2097. doi:10.1159/000374015
Yuan SY (2002) Protein kinase signaling in the modulation of microvascular permeability. Vascul Pharmacol 39(4–5):213–223
Zwaal RF, Schroit AJ (1997) Pathophysiologic implications of membrane phospholipid asymmetry in blood cells. Blood 89(4):1121–1132