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Plasmatic hemostasis at very high altitude – a thrombelastometric approach

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Original article

Abstract

Introduction: Changes in blood coagulation during exposure to high altitude are not well understood and studies of activation and consumption of specific coagulation factors in hypoxic humans have yielded conflicting results. In this study we used thrombelastometry (TEM) which allows a global evaluation of clot formation and lysis process to study blood coagulation profiles in volunteers exposed to prolonged hypobaric hypoxia at extreme altitudes.

Material and methods: We conducted a prospective observational study in 39 healthy volunteers during a research expedition up to an altitude of 7050 m. Plasma based thrombelastometric measurements and standard coagulation parameters were performed at different altitudes.

Results: TEM measurements showed an increase in clotting time (CT) and maximum clot firmness (MCF) at high altitudes, paralleled by an increase in international normalized ratio (INR) and activated partial thromboplastin time (aPTT). Fibrinogen concentration increased until 6022 m. D-Dimer and Thrombin-Antithrombin complex (TAT) increased with time exposed to severe hypoxia. For both measurements highest levels were found at 4844 m after acclimatization; in contrast, lower values were observed again at 7050m in the group of summiteers. Activated protein C resistance (APC-R) was slightly lowered at all altitudes.

Conclusion: Our results suggest that activation of the coagulation and fibrinolytic system occurs with increasing hypobaric hypoxia with concurrent use of coagulation factors indicating the occurrence of a consumption-coagulopathy phenotype.

Keywords

- hypobaric hypoxia
- thrombelastometry
- coagulation

Contribution

A – the preparation of the research project
B – the assembly of data for the research undertaken
C – the conducting of statistical analysis
D – interpretation of results
E – manuscript preparation
F – literature review

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Conflict of interest

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Introduction

An increasing number of people participate in mountaineering and trekking at high altitudes for recreational purposes. Climbers and trekkers who attempt ascents to high altitudes are usually healthy or suffer from minimal chronic conditions. Thromboembolic events such as deep vein thrombosis and pulmonary embolism have been reported in individuals without history for coagulation disorders or risk factors. It is generally assumed that exposure to hypobaric hypoxia is associated with a tendency to a more hypercoagulable state.[1-3]

Studies assessing coagulation profiles using conventional coagulation tests in the context of hypobaric hypoxia have reported conflicting results. While some demonstrated changes of coagulation parameters indicating hypercoagulability,[2-4] others reported a tendency towards a hypocoagulable state.[5-7] Potential explanations for a hypoxia induced hypocoagulability include defects or deficiencies of coagulation factors, or impaired interactions between coagulation components as observed in the context of disseminated intravascular coagulation (DIC).[7,8] In an earlier study, we demonstrated that both, hypercoagulation and hypocoagulation, occurred simultaneously in individuals during hypobaric hypoxia.[9]

However, conventional coagulation tests reflect in-vivo hemostasis poorly, since they only indicate deficits of coagulation factors greater than 50%. Thrombelastometry (TEM), offers an approach to investigate the interactions of coagulation factors with blood cells, their inhibitors and subsequent fibrinolysis and allows for global evaluation of the clot formation and lysis process. As a point-of-care method, it is used to detect coagulation defects in whole blood in real time while measuring viscoelastic changes during formation and lysis of a blood clot.[10-13]

TEM has been used to assess global viscoelastic properties of whole blood clot formation at high altitude.[7,14,15] Yet, the results of previous studies were inconsistent, possibly due to different and not standardized laboratory setups. Martin et al. demonstrated impairment of whole blood coagulation with increasing clotting time and clot formation time in the field.[7] On the other hand, Rocke et al. reported higher platelet activity with an increase of fibrinogen and a tendency to hypercoagulability caused by a change in platelet protein composition in high altitude while analyzing with TEM.[14]

To date, it has not been consistently established if coagulation disorders do occur frequently at high altitude and whether hypobaric hypoxia leads to a predominantly procoagulatory, hypocoagulatory or a disseminated intravascular coagulopathy-like phenotype. The goal of this study was therefore to measure routine coagulation parameters in combination with a laboratory-based plasma TEM in blood samples from a large cohort of healthy mountaineers exposed to altitudes of up to 7050 m.

Material and Methods

Expedition protocol and subjects

This study was performed in the context of the High-Altitude Medical Research Expedition Himlung Himal 2013 and was designed as a prospective, observational cohort study.

A total of 40 healthy volunteers (age 18-70 years) with sufficient mountaineering experience and level of fitness were included in the study. Exclusion criteria consisted of previous occurrence of severe mountain sickness at moderate altitude, preexisting chronic diseases (such as cardiovascular and pulmonary diseases or diabetes) or other reasons requiring regular medication. Mountaineers were instructed not to take any medication without consent of our independent expedition physician. Written informed consent was obtained from all subjects prior to study inclusion. The study was approved by the ethical committee of the Canton Bern, Switzerland (KEK 226/12).

Seven to eight weeks prior to the expedition all participants underwent a thorough medical assessment with baseline measurements (PR) at the University Hospital of Bern, Switzerland, at an altitude of 550 m above sea level. During the ascent to the 7126 m high Himlung Himal assessments were performed twice at base camp (4844 m, BC1 and BC2), at camp 2 (6022 m, C2), and at camp 3 (7050 m, C3). Post expedition measurements (550 m, PO) did take place five weeks after completion of the expedition at the University Hospital of Bern. Finger pulse oximetry was performed throughout the altitude stay in the morning and evening at rest in a sitting position (Onyx 9500 SportStat, Nonin Medical, Plymouth, USA). After establishing stable values during at least 3 minutes pulse-oximetric oxygen saturation (SpO_2) was recorded in a research diary by each subject.

Blood sampling and pre-test processing

Fasting blood samples were obtained from the subjects seated in an upright position after at least one overnight stay at the respective altitude always at the same time of day (between 7.15-9.15 am) to control for circadian changes in hemostatic markers. 10 ml blood

was drawn by a clean venipuncture using a safety butterfly 21G cannula (Sarstedt, Sevelen, Switzerland) and collected into sterile tubes containing a buffered sodium-citrate solution (Sarstedt, Sevelen, Switzerland) in a ratio of 1 : 9 sodium citrate (3.2%) / blood. The procedure of venipuncture and sample handling was carried out in a standardized fashion by trained personnel to ensure identical sample processing at all study sites. At each altitude, platelet-poor plasma was then obtained by centrifugation of the samples at 2000 G (EBA 20, Hettich AG, Bäch, Schweiz) during 10 min, plasma was aliquoted immediately and kept frozen at minus 40-60°C. The frozen specimens were transported to Switzerland and stored at minus 80°C until laboratory measurements. Continuous temperature logger devices were used to control those minus temperatures were maintained all the time.

Laboratory methods

The markers of activated coagulation were measured using commercially available assays according to the manufacturer's instructions and are described in Table 1. For TEM measurements, frozen plasma specimens were thawed using a water bath at a temperature of 37.2° for 7 minutes. Immediately afterwards, 300 µl of plasma were recalcified with 20 µl of star-TEM® reagent (containing 0.2 M $CaCl_2$). No further activator was added [so called non-activated test (NATEM)], and the analyses were run for 1 hour. The decision to evaluate the concentration of fibrinogen with an automated BCS XP was made *post hoc*.

All measurements were performed at the Center of Laboratory Medicine, Kantonsspital Aarau, Switzerland. An automated BCS XP (Siemens Healthcare Diagnostics, Marburg, Germany) was used for conventional coagulation tests. TEM was performed with a ROTEM delta® (Tem Innovations GmBH, Munich, Germany). Reagents were from Siemens Healthcare Diagnostics, Marburg, Germany and from Tem Innovations GmBH, Munich, Germany (for thrombelastrometric measurements).

Statistical analysis

The Shapiro-Wilk test was used for normality testing. In most cases the normality assumption was not rejected, therefore results are given as mean ± standard deviation. To take the longitudinal setting and the possibility of varying number of subjects per camp into account, Generalized Estimating Equations (GEE) modelling was applied to model the effects of altitude and pulse-oximetric oxygen saturation on the different coagulation parameters. The Sidak correction was used for pairwise comparisons between camps. Each relationship was in turn assessed for possible confounders such as hemoglobin, hematocrit, age and sex. Confounders were assessed one at a time, then only significant ones were entered in the GEE model using a parsimonious strategy regarding the number of independent variables in the model, given the sample size of the study. To analyze differences of the summiteers, a two-samples *t*-test was used. Two-sided *p*-values < 0.05 were considered statistically significant. To illustrate the relationship between the different parameters, scatterplot matrices were used. Data analysis was done using STATA 16.1 (STATA Corp. LLC, College Station, TX, USA).

Table 1. Description of the analyzed coagulation factors

Parameter	Description	Reagent
APC-R	**Activated protein C resistance:** Increased APC resistance (reflected by a lower APC-R ratio) occurs when the anticoagulant complex formed by activated protein C and its co-factor protein S, cannot sufficiently inactivate procoagulant Factors Va and VIIIa. It leads to an increased risk for venous thrombosis.	ProC Global
aPTT	**Activated partial thromboplastin time:** Test to measure the intrinsic system of coagulation. Increased aPTT indicates a delayed clotting.	Pathromtin SL
CT	**Clotting time:** Time period from initiation of test until the curve of the thrombelastometer reaches an amplitude of 2 mm. Increased CT indicates coagulation factors deficiencies.	star-TEM®
CFT	**Clot formation time:** Time period from 2 mm amplitude until the curve of the thrombelastometer reaches an amplitude of 20 mm. Increased CFT indicates reduced clot formation due to deficiencies in fibrinogen, factor XIII and thrombocyte interactions.	star-TEM®

Parameter	Description	Reagent
MCF	**Maximum clot firmness:** The highest value of the amplitude during measurement. Decreased MCF indicates reduced clot firmness due to deficiencies in fibrinogen and thrombocyte interactions	star-TEM®
D-Dimer	Fibrin degradation product. Increased D-Dimer indicate an activation of coagulation	INNOVANCE D-Dimer
FBG	**Fibrinogen:** Decreased FBG indicates a possible hypocoagulability	Multifibren U
PTR/INR	**Prothrombin time ratio / International Normalized Ratio:** Test to measure the extrinsic system of coagulation. Decreased PTR respectively increased INR indicates a delayed clotting.	Thromborel S
TAT	**Thrombin antithrombin III-Complex:** A proteinase-inhibitor complex that is formed by binding of antithrombin-III to thrombin. Decreased TAT indicates a progoagulatory state.	Enzygnost TAT micro

Reagents were from Siemens Healthcare Diagnostics, Marburg, Germany and from Tem Innovations GmBH, Munich, Germany (for thrombelastrometric measurements).

Results

Participants and course of the expedition

One participant had to be excluded from the expedition due to an incidental finding on brain MRI during baseline testing. A total of 39 subjects (26-70 years of age [45.5 ± 12.1]) were enrolled in the study, including 18 women, aged between 27 and 58 years (41.4 ± 9.4). Out of these 18 women 7 women were not using oral contraceptives, 7 women were on an older generation oral contraceptive, 1 subject used a progestin-only contraception, and 3 women entered menopause before expedition.

The expedition to Mount Himlung Himal started from Kathmandu (day 1). The mountaineers arrived at the basecamp (BC, 4844 m) on day 6 and the first medical testing was performed on day 7 (BC1). The examinations in Camp 2 (C2), 6022 m were done on day 13. Due to unfavorable weather conditions the ascent protocol had to be adapted and basecamp examinations 2 (BC2) were conducted on day 19 and 20 and in Camp 3 (7050 m) on day 23 and 24, for groups 1 and 2, respectively. Four to five weeks after the end of the expedition post-examinations were performed (PO) (Figure 1).

Blood and coagulation parameters

The number of participants, corresponding maximum reached altitudes, pulse-oximetric oxygen saturation, hemoglobin, hematocrit and coagulation parameters are reported in Table 2.

INR initially increased after exposition to high altitude, with a decrease after acclimatization; then showed an increase again with highest levels at 7050 m. After adjusting for sex, significant differences were found for BC1 vs. PR ($p < 0.001$), C2 vs. PR ($p < 0.001$), C3 vs. PR ($p = 0.012$), PO vs. PR ($p < 0.001$), BC2 vs. BC1 ($p < 0.001$), PO vs. BC1 ($p < 0.001$), BC2 vs. C2 ($p < 0.001$), PO vs. C2 ($p < 0.001$), C3 vs. BC2 ($p < 0.001$), PO vs. BC2 ($p = 0.004$), PO vs. C3 ($p < 0.001$).

aPTT initially increased significantly ($p < 0.0001$) at base camp and after a slight decrease, remained stable during the further course of the expedition.

TAT has a high preanalytical susceptibility. Unreliable conditions during PR measurements (very hot weather conditions, difficulties with blood sampling in female volunteers), might have led to increased TAT levels at PR. Together with the observation that apart from TAT, none of the other parameters in PR and PO were different, we decided to use post expedition values as baseline levels: We found an increase over the period of severe hypobaric hypoxia with a significant increase at 4844 m after acclimatization. Levels then decreased in the group of summiteers at 7050 m (Figure 2a).

The following significant changes were seen after adjusting for sex: BC2 vs. BC1 ($p = 0.047$), PO vs. BC2 ($p = 0.042$).

APC-R was lowered at all altitudes but increased again at post expedition values. The following significant differences were observed: C2 vs. PR ($p = 0.048$), BC2 vs. PR ($p < 0.001$), C3 vs. PR ($p = 0.006$), PO vs. PR ($p < 0.001$), BC2 vs. BC1 ($p < 0.001$), C3 vs. BC1 ($p = 0.007$), PO vs. BC1 ($p < 0.001$), BC2 vs. C2 ($p = 0.047$), PO vs. C2 ($p < 0.001$), PO vs. BC2 ($p < 0.001$), PO vs. C3 ($p < 0.001$).

D-Dimer increased with time exposed to severe hypoxia, showing highest levels at 4844 m after acclimatization; lower levels were measured at 7050 m in the

group of summiteers (Figure 2b). Significant differences were seen for the following camps: BC2 vs. PR ($p = 0.001$), BC2 vs. BC1 ($p < 0.001$), BC2 vs. C2 ($p = 0.024$), PO vs. BC2 ($p < 0.001$). When adjusted for hemoglobin, hematocrit and CRP, significant differences were seen for BC2 vs. PR (p = 0.028), BC2 vs. BC1 ($p = 0.002$), and PO vs. BC2 ($p = 0.007$).

Fibrinogen concentrations increased with altitude up to 6022 m, where they reached a peak value (Figure 2c). Adjusted for CRP, significant differences were found for C2 vs. PR ($p < 0.001$), BC2 vs. PR ($p < 0.001$), C2 vs. BC1 ($p < 0.001$), BC2 vs. BC1 ($p < 0.001$), BC2 vs. C2 ($p = 0.007$), C3 vs. C2 ($p = 0.001$), PO vs. C2 ($p < 0.001$), PO vs. BC2 ($p < 0.001$), PO vs. C3 ($p = 0.035$).

CT values were altitude dependent and showed highest levels at 7050 m. Significant differences were found for the following camps (indifferent if adjusted for hemoglobin or unadjusted): C3 vs. PR ($p < 0.001$), C3 vs. BC1 ($p < 0.001$), C3 vs. C2 ($p < 0.001$), C3 vs. BC2 ($p < 0.001$), PO vs. C3 ($p < 0.001$) (Figure 2d).

CFT showed no clear relationship with altitude. Only for the measurements at 4844 m and 7050 m we found a modest and non-significant decrease respectively increase (Figure 2e).

MCF showed significant camp- and time related changes with a maximal clot firmness after acclimatization at 4844m, lower levels were measured at 7050 m in the group of summiteers (Figure 2f). Significant changes between camps were found as follows: C2 vs. PR ($p < 0.001$), BC2 vs. PR ($p < 0.001$), C2 vs. BC1 ($p = 0.008$), BC2 vs. BC1 ($p = 0.003$), P0 vs. C2 ($p < 0.001$), P0 vs. BC2 ($p < 0.001$). Adjusting for hematocrit and hemoglobin revealed significant differences for C2 vs. PR ($p < 0.001$), BC2 vs. PR ($p = 0.004$), C2 vs. BC1 ($p = 0.001$), PO vs. C2 ($p < 0.001$), and PO vs. BC2 ($p = 0.001$).

To establish possible relationships between the different parameters, we created relation graphs for fibrinogen, MCF, CT, CFT and pulse-oximetric oxygen saturation (SpO_2), respectively (Figures 3 and 4). A significant negative correlation was found for fibrinogen and CFT ($p < 0.001$), and a significant positive correlation for fibrinogen and MCF ($p < 0.001$). No correlation was seen for fibrinogen and CT (Figure 3).

Regarding the relationship of CT, CFT, MCF and SpO_2 (Figure 4), there was a significant negative correlation for CT and SpO_2 ($p < 0.001$), as well as MCF and SpO_2 ($p < 0.001$). For CFT no correlation with SpO_2 was found ($p = 0.498$).

15 subjects (age 43.6 ± 13.8, 5 females) reached C3 and the summit. Table 3 depicts descriptive data on this sub-group at each camp. Compared to the subjects who did not reach the summit, significantly higher APC-R at PR (0.87 ± 0.09 vs. 0.76 ± 0.14) and BC1 (0.84 ± 0.06 vs. 0.76 ± 0.15), higher CT at PR (943.4 ± 154 sec vs. 818.8 ± 196 sec) and BC2 (1374.1 ± 841 sec vs. 927.0 ± 207 sec), as well as higher CFT at PO (713.8 ± 360 sec vs. 457.5±132 sec) were observed (TEM values in Tables 2 and 3 are only given as relative changes). No other significant differences were found when analyzing coagulation parameters between these two sub-groups.

Table 2. Time course of the expedition and results stratified by camp

Parameters	PR [550 m]	BC1 [4844 m]	C2 [6022 m]	BC2 [4844 m]	C3 [7050 m]	PO [550 m]	*p*–value	Reference range
Time (d)	–56/–63	7	13	19/20	23/24	59/66		
Subjects [n]	39	39	36	39	15	39		
SpO_2 [%]	97.6 ± 0.8	83.8 ± 4.6	72.7 ± 8.8	88.6 ± 2.3	68.8 ± 9.6	97.4 ± 0.7	< 0.0001	
Hb [g/dl]	14.79 ± 0.93	15.35 ± 0.90	16.03 ± 1.10	17.03 ± 0.89	17.5 ± 1.0	15.21 ± 0.91	< 0.0001	♀ 12.0–15.5 ♂ 3.5–17.2
Hct [%]	43.46 ± 2.8	45.11 ± 2.65	47.17 ± 3.30	50.19 ± 2.61	51.67 ± 3.11	44.74 ± 2.67	< 0.0001	♀ 35.5–45.0 ♂ 39.5–50.5
INR	1.11 ± 0.06	1.15 ± 0.07	1.14 ± 0.06	1.08 ± 0.06	1.2 ± 0.11	1.06 ± 0.08	< 0.0001	0.8–1.1
PTR [%]	85.34 ± 7.22	79.67 ± 7.12	79.74 ± 6.27	87.65 ± 7.59	75.38 ± 11.8	91.14 ± 7.00	< 0.0001	70–130
aPTT [sec]	34.5 ± 3.8	38.6 ± 5.4	36.9 ± 4.8	36.2 ± 4.80	36.13 ± 13.54	33.8 ± 6.40	0.0001	25–38
D–Dimer [mg/l]	0.41 ± 0.29	0.35 ± 0.19	0.55 ± 0.54	0.85 ± 1.32	0.49 ± 0.38	0.38 ± 0.32	< 0.001	< 0.5
APC–R	0.80 ± 0.13	0.79 ± 0.13	0.76 ± 0.13	0.75 ± 0.12	0.79 ± 0.08	0.85 ± 0.14	< 0.0001	> 0.7
FBG [g/l]	2.36 ± 0.40	2.47 ± 0.45	3.03 ± 0.54	2.78 ± 0.56	2.57 ± 0.49	2.28 ± 0.38	< 0.0001	1.8–3.5
TAT [µg/l]	6.4 ± 6.4	3.2 ± 1.6	4.2 ± 2.6	5.0 ± 5.5	4.0 ± 1.2	3.2 ± 2.5	0.0011	2.0–4.2

Parameters	PR [550 m]	BC1 [4844 m]	C2 [6022 m]	BC2 [4844 m]	C3 [7050 m]	PO [550 m]	*p*–value	Reference range
CT	1.00 (0.78–1.21)	1.25 (0.55–1.45)	1.35 (0.54–1.46)	1.28 0.46–1.54)	2.28 (0.36–1.64)	1.05 (0.79–1.21)	< 0.0001	
CFT	1.00 (0.32–1.68)	0.93 (0.35–1.65)	0.96 (0.45–1.55)	0.81 (0.41–1.59)	1.15 (0.23–1.77)	0.81 (0.51–1.49)	0.4859	
MCF	1.00 (0.78–1.21)	1.08 (0.76–1.24)	1.22 (0.83–1.17)	1.23 (0.73–1.27)	1.10 (0.82–1.19)	1.00 (0.81–1.19)	< 0.0001	

The time course is indicated as days after departure from Kathmandu. The number of subjects denotes the number of climbers who reached the respective camp. Standard coagulation parameters are given as mean ± standard deviation. Thrombelastometric values are given as relative changes to baseline values (PR) (in parentheses the corresponding range, again as relative changes). The *p*–values represent the overall difference across all altitudes. Abbreviations: PR, baseline measurements; BC1 and BC2, base camp 1 respectively 2 after 4 respectively 5 days of acclimatization; C2, camp 2; C3, camp 3; SpO_2, pulse oxygen saturation; Hb, hemoglobin; Hct, hematocrit; INR, international normalized ratio; PTR, prothrombin ratio time; aPTT, activated partial thromboplastin time; APC–R, activated protein C resistance; FBG, fibrinogen; TAT, thrombin antithrombin III–Complex; CT, clotting time; CFT, clot formation time; MCF, maximum clot firmness.

Table 3. Measurement results of the 15 mountaineers who reached CAMP 3 and the summit stratified by camp

Parameters	PR [550 m]	BC1 [4844 m]	C2 [6022 m]	BC2 [4844 m]	C3 [7050 m]	PO [550 m]	Reference range
SpO2 [%]	97.5 ± 1.0	82.8 ± 5.7	69.1 ± 9.9	88.4 ± 2.2	68.8 ± 9.6	97.6 ± 0.7	
Hb [g/dl]	15.18 ± 0.53	15.52 ± 0.93	16.23 ± 1.05	17.19 ± 0.64	17.5 ± 1.0	15.6 ± 0.55	♀ 12.0–15.5 ♂ 13.5–17.2
Hct [%]	44.67 ± 1.54	45.6 ± 2.72	47.67 ± 3.14	50.67 ± 1.80	51.67 ± 3.11	46.00 ± 1.77	♀ 35.5–45.0 ♂ 39.5–50.5
INR	1.13 ± 0.07	1.15 ± 0.06	1.17 ± 0.06	1.09 ± 0.07	1.2 ± 0.11	1.04 ± 0.06	0.8–1.1
PTR [%]	84.27 ± 8.83	78.67 ± 6.92	75.71 ± 6.01	84.57 ± 7.89	75.38 ± 11.8	91.87 ± 7.23	70–130
aPTT [sec]	35.73 ± 3.88	39.53 ± 5.30	39.07 ± 4.46	37.87 ± 4.91	36.13 ± 13.54	32.87 ± 2.99	25–38
D–Dimer [mg/l]	0.32 ± 0.15	0.34 ± 0.16	0.48 ± 0.28	0.54 ± 0.37	0.49 ± 0.38	0.40 ± 0.45	< 0.5
APC–R	0.87 ± 0.09	0.84 ± 0.06	0.81 ± 0.09	0.79 ± 0.06	0.79 ± 0.08	0.89 ± 0.07	> 0.7
FBG [g/l]	2.28 ± 0.29	2.59 ± 0.45	3.13 ± 0.68	2.65 ± 0.36	2.57 ± 0.49	2.20 ± 0.19	1.8–3.5
TAT [µg/l]	5.5 ± 4.7	2.9 ± 1.2	4.2 ± 2.9	4.5 ± 4.5	4.0 ± 1.2	3.9 ± 4.0	2.0–4.2
CT [sec]	1.00 (0.84–1.16)	1.28 (0.69–1.86)	1.31 (0.97–1.66)	1.28 (0.70–1.86)	1.20 (0.85–1.56)	0.96 (0.75–1.16)	
CFT [sec]	1.00 (0.46–1.54)	0.76 (0.47–1.04)	1.01 (0.54–1.47)	0.89 (0.36–1.42)	1.04 (0.24–1.84)	0.81 (0.51–1.49)	
MCF [mm]	1.00 (0.82–1.18)	1.16 (0.97–1.35)	1.21 (0.98–1.45)	1.24 (1.00–1.48)	1.22 (1.06–1.39)	0.96 (0.84–1.08)	

Standard coagulation parameters are reported as mean ± standard deviation. Thrombelastometric values are given as relative changes to baseline values (PR) (in parentheses the corresponding range, again as relative changes). Abbreviations: PR, baseline measurements; BC1 and BC2, base camp 1 respectively 2 after 4 respectively 5 days of acclimatization; C2, camp 2; C3, camp 3; SpO_2, pulse oxygen saturation; Hb, hemoglobin; Hct, hematocrit; INR, international normalized ratio; PTR, prothrombin ratio time; aPTT, activated partial thromboplastin time; APC–R, activated protein C resistance; FBG, fibrinogen; TAT, thrombin antithrombin III–Complex; CT, clotting time; CFT, clot formation time; MCF, maximum clot firmness.

Discussion

The present study provides evidence for the development of a consumption-coagulopathy phenotype of coagulation abnormality during progressive exposure to high altitudes of up to 7050 m caused by a hypoxia-induced pro inflammatory state. Specifically, we demonstrate an increase of CT and MCF. Additionally, we documented prolonged aPTT, higher INR and an increase of fibrinogen and D-Dimer levels during the course of the expedition and with increasing altitude. APC-R tends to decrease with a concomitant increase of TAT when assuming that the effective baseline values of TAT are those of post expedition. This indicates an increased inactivation of the procoagulant factors V and VIII and an increase of antithrombin-III, a negative acute phase protein. Both effects are consistent with the development of a pro-inflammatory state.[8]

Our study has the following limitations: Primarily, due to logistic reasons in the context of a high-altitude field study, the number of participants was restricted and not all climbers reached every altitude, leading to a loss of data and a selection bias. Despite the restricted size of our volunteer cohort, our study still represents one of the largest sample sizes in comparison to other published high-altitude research in the field. Secondly, due to the lack of stability of whole blood during storage and transport and in favour of a high measurement quality we decided to apply a highly standardized laboratory test setting using plasma instead of whole blood. TEM in plasma is feasible and has been compared to whole blood by Schoergenhofer et al.[16] The authors describe a prolongation of clotting time and a decrease of MCF in frozen plasma compared to whole blood specimen when the coagulation process is started by recalcification only. They also investigated the effect of the freezing process on the TEM-results and found that compared to whole blood only a slight prolongation of CT occurred. Although it has been shown that TEM is feasible in plasma, robust reference values have not been fully established. We therefore report measurement results relative to plasma baseline values.

Nevertheless, the lack of cellular interactions, a crucial part of hemostasis in vivo, is certainly a limitation of our study, so we may not have detected a potential prothrombotic effect induced by blood cells.[4] This may also be why the absolute values of MCF we measured, were generally lower than levels in whole blood, where platelets contribute to the firmness of a clot. Accordingly, we could not find any relevant changes for CFT: CFT represents the kinetics of clot formation, and provides information about the interactions of thrombin, factor XIII, the amount and function of platelets and fibrinogen. Therefore, the lack of CFT change could be due to a selection bias: First, the necessary 20 mm were not reached in all samples within the given hour of analysis; second, because of the lack of thrombocytes, a missing initial activation of clot formation could be suspected, leading to a loss of results. Moreover, it could be argued that because of this lack of activation, the study period of one hour was too short to initiate coagulation in all plasma samples and that therefore potential lysis may have been missed.

On the other hand, preanalytical sample handling and analysis for evaluation of blood cells such as leukocytes and especially platelets are complicated and require equipment that is not available in the setting of a high-altitude expedition. Standardized laboratory settings are indispensable in order to get meaningful results using TEM.[10, 12, 13] Unlike previous studies,[7, 14] and thanks to centrifugation and generation of platelet poor plasma, our measurements were performed in a state-of-the art laboratory setting after identical preanalytics ensuring that no systematic error would bias our results.

Although coagulation may start later if no activator is used as in INTEM or EXTEM – analysis, good discrimination of hypo- and hypercoagulation is possible when TEM is performed as a non-activated test (NATEM). The reason for this is that the activation of coagulation only occurs due to the contact of the plasma with the tube and pin.

Furthermore, a study postulated a possible endogenous heparin-like activity with increasing hypobaric hypoxia causing a delay of coagulation.[17] Unfortunately, we did not have sufficient material to further investigate this hypothesis. Finally, another confounder may have slightly affected our results, namely the use of an older generation oral contraceptive in 7 subjects before and during the expedition.

Previous studies have reported that exposure to high altitude and physical activity trigger an inflammatory response with an increase of pro-inflammatory cytokines and acute phase proteins such as IL-1, IL-6, CRP and factor VIII.[4, 18-22] This in turn leads to activation of coagulation via the tissue factor dependent extrinsic pathway, as well as inhibition of fibrinolysis.[23, 24] Collectively, these changes may induce endothelial dysfunction, resulting in activation of coagulation and causing a phenotype form of coagulation factor consumption.

In our study we now found evidence in favor of an activated coagulopathy: we report among others a prolongation of aPTT, INR and of clotting time (CT) with a negative correlation for SpO_2 and an increase of MCF with a positive correlation for SpO_2. This is in accordance to the Caudwell Everest Expedition,[7] that demonstrated a tendency towards hypocoagulability when analyzing

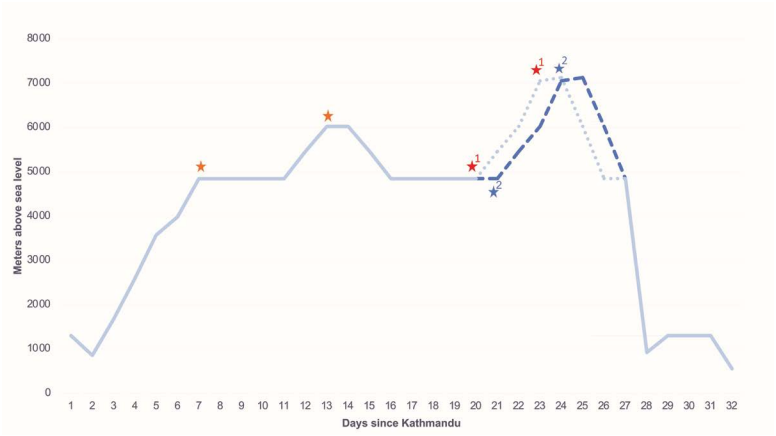

Figure 1. Figure 1. Altitude profile of the expedition: the sleeping height is marked for each day during the expedition

Note: * – stands for the day when measurements were conducted. Due to unfavorable weather conditions, group 2 had to extend the stay at base camp for one day, this is why the ascent protocol is slightly different in terms of timeline:
• • • – representing the ascent protocol of group 1;
▬ ▬ – representing ascent protocol of group 2.

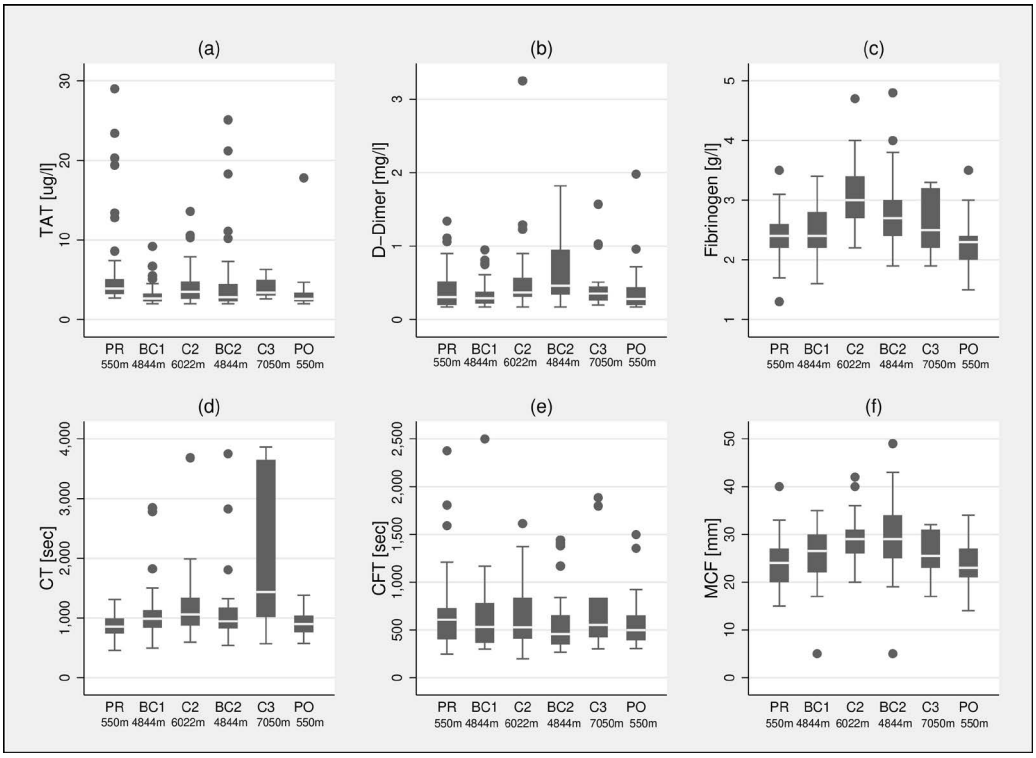

Figure 2. Changes in blood coagulation parameters during the expedition are given as camp-related changes for a) TAT b) D-Dimer c) fibrinogen d) CT e) CFT, and f) MCF, respectively

Note: Significance levels for camp-wise comparison are shown in the results section. The hinges correspond to the 25th and 75th percentile (thus are defined by IQR), and the whiskers depicts minimum and maximum values, unless there are outliers (defined as values beyond 1.5* IQR) from 75% (or 25%) quantile.

Abbreviations: TAT, thrombin-antithrombin; CT, clotting time; CFT, clot formation time; MCF, maximum clot firmness; IQR, interquartile range.

coagulation changes with a thrombelastograph during an ascent to Mount Everest base camp. We hypothesize that this effect is caused by the development of clotting factor deficiency with increasing altitude, triggered by initial activation through an inflammatory process, causing a delay in clot formation.

Fibrinogen – an acute phase protein – has been demonstrated to increase during physical activity at high altitude.[25, 26] In our study fibrinogen increased to a maximum at 6022 m. We hypothesize this initial increase to be part of the acute phase reaction, followed by a decrease, although going higher, due to the onset of a consumptive coagulopathy. Using TEM we showed a positive correlation between increasing fibrinogen and MCF, indicating stronger clot formation. Similar findings were described before by Rocke et al. who assessed coagulation in individuals at an altitude of 5200 m.[14] The results of the latter study indicate an increase in clot strength caused by higher fibrin interaction. Additionally, the authors report a change in thrombocyte proteome leading to a higher thrombocyte activity and consecutively an increase in fibrinogen synthesis.

Another possible mechanism for decreasing coagulation factors might be a compensation to balance for increased activity of thrombocytes and general hyperviscosity due to plasma volume reduction.[26, 27] Wang et al. found an overall decrease of coagulation factors, increase of prothrombin time and aPTT when comparing plasma and whole blood samples from lowland and highland populations.[26] Finally, higher hemoglobin and hematocrit levels in high altitude themselves lead to a relative dilution of coagulation factors, resulting in a prolongation of clotting time.[14]

Other confounding factors could contribute to a lower plasma concentration of coagulation factors. These include, fluid retention and edema formation, loss of protein into interstitial spaces due to endothelial permeability changes, as well as proteinuria, and catabolic metabolism.[28-30] However, since mean total body weight did not change significantly in our study group (data not shown), we do not assume that a catabolic state was a major confounding factor.

APC resistance depends on Protein C and S as well as von Willebrandt factor. After adjusting for CRP levels, we documented significant changes in APC resistance with lower values during hypobaric hypoxia. Off note, an increasing APC resistance is reflected by a lower APC-R test result. We hypothesize that these findings are caused by an upregulation of factor VIII, which has been shown to be particularly dependent on the intensity of physical activity,[22] and a concomitant decrease of Protein C and S at altitude.[9] We assume that this outbalancing effect is the reason for the only small changes in APC resistance. Furthermore, we conducted analyses on Thrombin-Antithrombin complex (TAT), a complex formed by the natural anticoagulant antithrombin-III neutralizing thrombin, indicating the rate of thrombin formation or degradation. However, TAT has a wide reference interval and a high preanalytical susceptibility. Regarding this, pre-expedition TAT measurements were complicated by the very hot weather conditions during the measurements, as well as difficulties with blood sampling in female volunteers (6 out of the 7 outliers at PR were women). Therefore, we used post expedition values as baseline levels. We believe that the TAT results are consistent with our hypothesis of consumptive coagulopathy in hypobaric hypoxia since we demonstrated an increase with higher altitude. Interestingly, for both D-Dimer and TAT measurements, although not significantly, lower levels were measured at 7050 m in the group of summiteers. These findings are in line with our previous study where we demonstrated that mountaineers reaching the summit had lower D-Dimer levels as well as increased aPTT levels.[9] In both studies one explanation could be a selection bias of the fittest. However, given the relatively small sample size, different volunteer cohorts and ascent profiles as well as technical differences in climbing conditions, results of these two studies should only be compared with caveats.

Finally, comparing changes in coagulation parameters of summiteers compared to the non-summiteers revealed some significant differences for APC-R, CT and CFT. However, we believe this post-hoc analysis is not sufficiently powered to make a definitive conclusion regarding coagulation parameters and changes at hypobaric hypoxia. We therefore assume that subjects reaching higher altitudes or the summit, were to a lesser extent affected by a phenotype form of consumptive coagulopathy, which is also in line with the descriptive data of both groups.

In summary, the data shown do not support a clinically overt consumptive coagulopathy, but we see these changes still in favor of a consumptive-coagulopathy-like phenotype: we noted a clear prolongation of clotting time, an initial increase of aPTT and INR, as well as an increase of D-Dimer. Contrary to a consumptive coagulopathy, fibrinogen did not decrease. As fibrinogen is an acute phase protein, it's possible that an acute phase reaction induced by hypobaric hypoxia masks fibrinogen consumption to some extent. What's more, we noted an initial increase up until an elevation of 6022 m, which could be interpreted by being part of the acute phase reaction, followed by a decrease, although going higher, due to the onset of a consumptive coagulopathy. Although, these assumptions remain speculative the findings could explain the co-existence of the higher risk of thrombotic events and bleeding complications in high altitude.

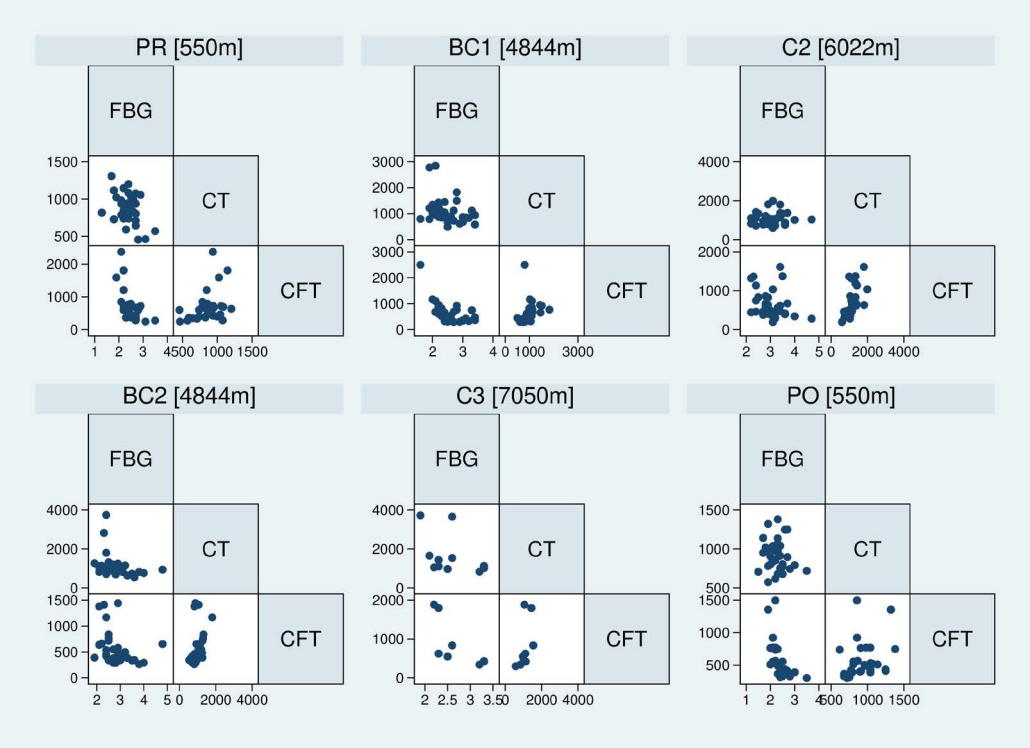

Figure 3. Scatterplot matrix depicts the relationship of FBG, MCF, CT and CFT for each campsite

Abbreviations: MCF, maximum clot firmness; CT, clotting time; CFT, clot formation time.

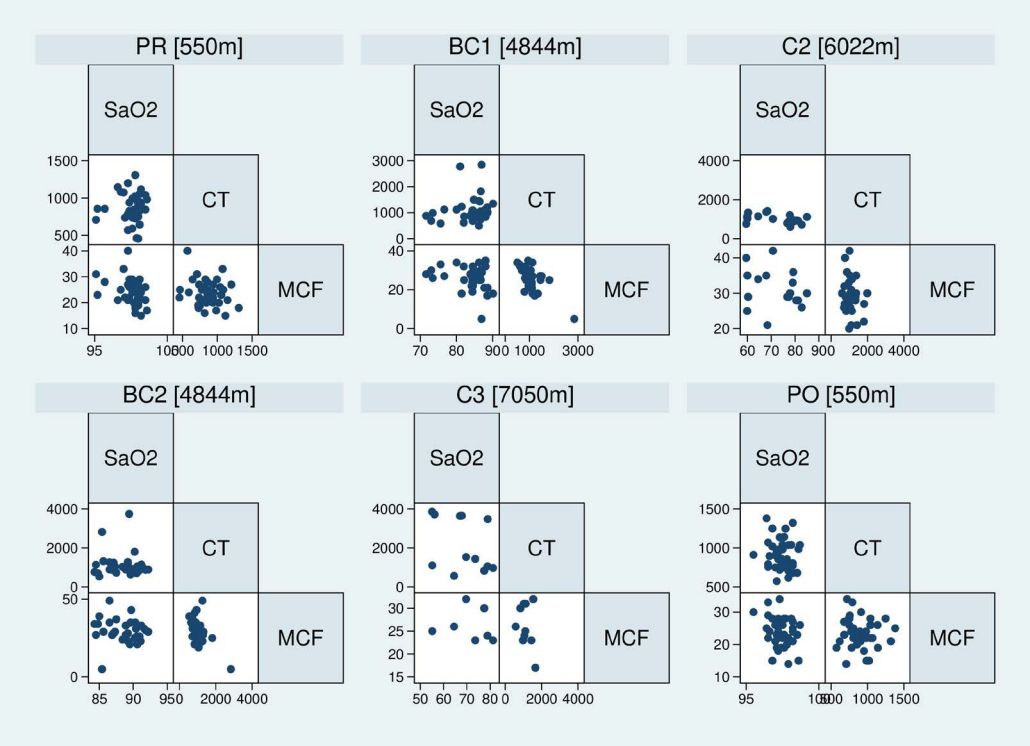

Figure 4. Scatterplot matrix depicts the relationship of SpO_2, MCF, CT and CFT for each campsite

Abbreviations: SpO_2, pulse-oximetric oxygen saturation; MCF, maximum clot firmness; CT, clotting time; CFT, clot formation time.

Conclusion

In conclusion, our findings indicate that activation of the coagulation and fibrinolytic system occurs with increasing altitude, with concurrent expenditure of coagulation factors indicating the development of disseminated intravascular coagulopathy-like phenotype. We interpret these changes as a result of the occurrence of a consumption- coagulopathy phenotype of coagulation abnormality caused by a hypoxia-induced pro inflammatory state.

Further studies should investigate the question, if endogenous heparinization at high altitude could play a role in delaying clotting time or hemostasis in general. Additionally further analyses including platelets using a thrombelastometric approach could be from great interest.

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