Combined effect of acute altitude exposure and vigorous exercise on platelet activation

Running title: High altitude exercise-induced platelet activation

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Summary:

Background: Exposure to high altitudes and exercise alters body's physiology and may cause acute cardiovascular events. Platelet activation is one of the key players in these events. Therefore, we investigated the effect of vigorous exercise at higher altitude (2650 m) on platelet aggregation and serum markers of platelet activation.

Methods: 14 healthy subjects performed a step incremental ergometer test until exhaustion at the Environmental Research Station (UFS, 2650m) at Zugspitze. Platelet aggregation and serum levels of endothelin-1, soluble p-selectin, platelet factor4 and Chromogranin A were measured.

Results: Platelet activation was significantly enhanced after exercise at high altitude compared to measures immediately prior exercise. We detected significantly enhanced serum levels of endothelin-1 and soluble p-selectin whereas chromogranin A and platelet factor 4 remained unchanged.

Conclusion: This effect might be due to increased endothelin-1 levels causing pulmonary vasoconstriction, rheological changes and direct platelet activation. This might be of clinical relevance, especially in patients with pre-existing diseases.

Keywords: Platelet activation, high altitude, exercise, recreational athletes, pulmonary vasoconstriction

Introduction

Alpine tourism has increased to approximately 120 million visits per year recently. A large amount of these visitors and recreational athletes participate in sports at higher altitudes (e.g. skiing, Nordic skiing, mountaineering or modern adventure sports such as mountain-biking). Among those are patients with known or unknown underlying cardiovascular diseases including Coronary Artery Diseases (CAD). Exposure to higher altitude changes body's physiology especially of the cardiovascular system and the blood system profoundly. Response to hypobaric hypoxic environmental conditions increases heart rate and pulmonary and systemic blood pressures and changes autonomic nervous system [1]. Adaptation to chronic hypoxemia increases red blood cell count and haemoglobin [2]. Few studies have been published so far evaluating the effect of acute or chronic altitude exposure on the development of cardiovascular diseases and events. Results show conflicting findings most likely due to distinct confounders [3]. Hypoxemia combined with increased myocardial oxygen demand due to elevated heart rates at altitude might create a situation where myocardial oxygen supply cannot be sufficient. This might lead to exaggerated symptoms of CAD and increase possibility for cardiac events [4]. Adequate acclimatization, optimal medical therapy and a graded exercise test at sea level have been proposed to ensure safety for patients at risk for cardiac events at high altitude [5].

The exact mechanisms leading to potentially enhanced cardiovascular events at acute altitude exposure remain unclear. Platelet activation and aggregation is one of the key players in the pathogenesis of cardiovascular events such as myocardial infarction or stroke. Recently, we were able to show increased platelet activation at high altitude exposure [6]. Platelet aggregation in general plays a crucial role in primary haemostasis. Different influences have been identified to cause platelet activation - also in the absence of required haemostasis: In this context, platelet activation can be initiated a) by metabolic changes or inflammation, b) by rheological changes or increased vascular resistance [6], c) in the context of physical stress [7, 8] d) by environmental stress such as altitude exposure [6]. Therefore, in the present pilot study, we aimed to investigate the combined effect of vigorous exercise and acute altitude exposure on platelet activation in a healthy cohort as this might have a substantial impact on cardiovascular events in patients with underlying vascular and coronary diseases at acute altitude exposure.

Material and Methods

Study Population and Ethics: We included fourteen healthy volunteers (4 women, 10 men) with a mean age of 35.6 years (range: 24-56 yr) and without any known (in particular cardiac or pulmonary) disease. Written informed consent in accordance with the Declaration of Helsinki was obtained from all volunteers before enrolment. The study protocol was approved by the local ethics committee (*Ethikkommission der Medizinischen Fakultät der LMU München*).

Exercise testing: We performed a step protocol with a cycle ergometer starting at 40 W (women) or 60 W (men), respectively. Step changes took place every three minutes with workload increasing by 20 W. Mean maximal power output was 216 W. Exercise tests were conducted at the Environmental Research Station Schneefernerhaus (UFS) at the Zugspitze (2650 m, 715 mbar) within one hour after arrival to avoid adaptation. Blood was taken from an antecubital vein prior exercise and immediately after termination of exercise testing.

Measurement of platelet activation: Aggregation was assessed by an impedance aggregometer (Multiplate, Roche, Basel, Switzerland). The detailed testing principle and procedure is described elsewhere [8]. Adenosine diphosphate (ADPtest) and arachindonic acid (ASPItest) served as stimulants of platelet aggregation. During the measurement, increases of impedance were recorded. The results are given as the area under the curve (AUC) of the ensuing plot with the arbitrary unit “aggregation”. Different serum markers have been identified to mirror platelet activation: Large quantities of platelet factor 4 (PF4) are released at sites of platelet activation [9] and soluble p-selectin has been demonstrated to be a reliable marker of platelet aggregation [10]. Therefore, serum levels of soluble p-selectin and platelet factor 4 were measured with a standard ELISA kit according to the manufacturer’s instruction (Biocat Germany).

Measurement of stress parameters: With respect to a potential underlying pathophysiological role, we measured Chromogranin A (CGA) and Endothelin-1 (ET-1) as markers for catecholamine secretion and pulmonary vasoconstriction . CGA is known to be an essential part of secretory vesicle in endocrine cells, neurons and neuroendocrine cells. Increased levels of CGA have been associated with physical stress [8, 11] serving as a marker for sympato-adrenergic activation in our current survey. Endothelin-1 (ET-1) is produced by endothelial cells, smooth muscle cells, monocytes and macrophages [12]. ET-1 acts as a vasoconstrictor on pulmonary vessels in response of acute hypoxia [13]. Increased ET-1 levels have been shown after vigorous exercise [8] as well as at acute altitude exposure [6]. Serum levels of CGA and ET-1 were measured with a standard ELISA kit according to the manufacturer’s instruction (CGA: Antikoerper online, Germany; P-Selectin: Biocat, Germany).

Statistical analysis:

Data was evaluated for normal distribution by the Anderson–Darling test. As no normal distribution was found results are presented as mean and interquartile range (IQR), Wilcoxon test was performed to test for statistical significance, Values of $P < 0.05$ were considered statistically significant.

Results:

After vigorous exercise at high altitude until fatigue we detected a significant increase of both ADP-induced (97.5 [IQR: 85.6-109.5] vs. 78 [IQR: 63.2-90.2], p<0.01, figure 1A) and ASPI-induced platelet aggregability (122.5 [109.3-131.8] vs. 110.5 [IQR: 97.5-123.5]; p=0.02, figure 1B) in Multiplate testing compared to levels at high altitude before exercise. This increase was accompanied by significantly elevated levels of soluble p-selectin (47.1 [IQR: 34.1-59.7] vs. 40.1 [IQR: 23-55.1] pg/mL; p=0.02, figure 1C). Levels of PF4 (11386 [IQR: 9845-12786] vs. 10043 [IQR: 8901-11623] ng/mL; p=0.2, figure 1D) showed a numerical increase but no statistical difference. Whereas CGA was not altered relevantly (105 [IQR: 61.3-156.8] vs. 108 [IQR: 67.8-193] ng/mL; p=0.57, figure 1E), Endothelin-1 serum levels increased significantly from 0.95 [IQR: 0.77-1.42] to 1.22 [IQR: 1.06-2.14] pg/mL (p=0.02, figure 1F) after exercise at high altitude.

Discussion

To the best of our knowledge, this is the first study, which elucidates the effect of a combination of acute altitude exposure and vigorous exercise on platelet aggregation and serum markers of platelet activation. Evaluation of platelet activation at higher altitudes seems to be highly relevant, as the effect of a hypobaric hypoxic environment on cardiovascular events still needs to be determined. Increasing numbers of visitors participate in mountain sports or exercise at higher altitudes with unknown risks for those with known or unknown coronary and vascular diseases. In the present study with 14 healthy subjects (mean age 35.6 years), we detected a pronounced activation of platelets in Multiplate testing with ADP and ASPI that was accompanied by a significant increases of serum levels of ET-1 and soluble p-selectin.

In a previous study we were able to detect the effect of acute altitude exposure without exercise on platelet activation. We demonstrated, that acute altitude exposure may activate platelets in healthy young individuals without sympato-adrenergic activation but with a 44% increase of ET-1 [6]. This platelet activation might be of clinical relevance in patients with pre-existing cardiovascular diseases. For example Isik et al. could describe a higher rate of reinfarction after ST-elevating myocardial infarction in patients living at an altitude of 1960m alt. compared to patients living at sea level [14].

In our present study, we were able to demonstrate a further increased platelet activation after exercise at high altitudes compared to levels at high altitude before exercise.

Exercise is known to influence platelet aggregation: Exercise with low maximum workload in a cohort with pre-existing coronary heart disease increases ADP-induced Multiplate tests associated with an increase of CGA as measure of sympato-adrenergic activation and cardiac burden [7]. Additionally, it has been shown, that extreme cardiac burden such as marathon running causes platelet activation accompanied with both, an increase of CGA and also of ET-1 [8, 15]. In our study we found an association of increased levels of ET-1 serum levels with increased platelet activation. These finding may be explained by platelet-activating properties of ET-1 [16]. Besides, also rheological changes as a consequence of hypoxic vasoconstriction could cause platelet activation. ET-1 is an important mediator of hypoxic pulmonary vasoconstriction (HPV), which serves to optimize ventilation–perfusion matching in focal hypoxia and may improve pulmonary gas exchange [13]. During global hypoxia as given at altitude exposure, HPV induces general pulmonary vasoconstriction, which may raise pulmonary total vascular capacity [13], lead to rheological changes and activate platelets.

As CGA remained unchanged, it seems to be of secondary importance at altitude compared to exercise at sea level.

Conclusion:

Our current pilot study on young healthy subjects could demonstrate a pronounced platelet activating effect of exercise at acute altitude exposure (2650 m). In contrast to previous studies at normal altitude, sympato-adrenergic activation seems to be of only secondary importance. Instead, our survey gives hint for a role of ET-1 either as direct platelet activating agent or as a marker of rheological changes causing platelet activation. These findings might implicate clinical relevance in patients travelling to and exercising at higher altitudes with underlying cardiovascular disease as increased platelet activation could be a trigger for acute vascular events. Since this current work represents only a pilot study with a small number of study subjects, these findings need to be confirmed in larger cohorts with additional confirmation of the hypothesis of increased pulmonary pressure via non-invasive examinations. In addition, further investigations in diseased cohorts and studies addressing an acclimatization effect to attenuate platelet aggregability are needed.

Author contributions:

K. Lackermair: study design, conduction of experiments, draft of manuscript

D. Schüttler: conduction of experiments, draft of manuscript

A. Kellnar: conduction of experiments, revision of manuscript for relevant intellectual content

CG. Schuhmann: conduction of experiments, revision of manuscript for relevant intellectual content

LT. Weckbach: conduction of experiments, revision of manuscript for relevant intellectual content

S. Brunner: study design, conduction of experiments, revision of manuscript for relevant intellectual content

Source of founding:

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All authors declare no conflicts of interest.

Figures:

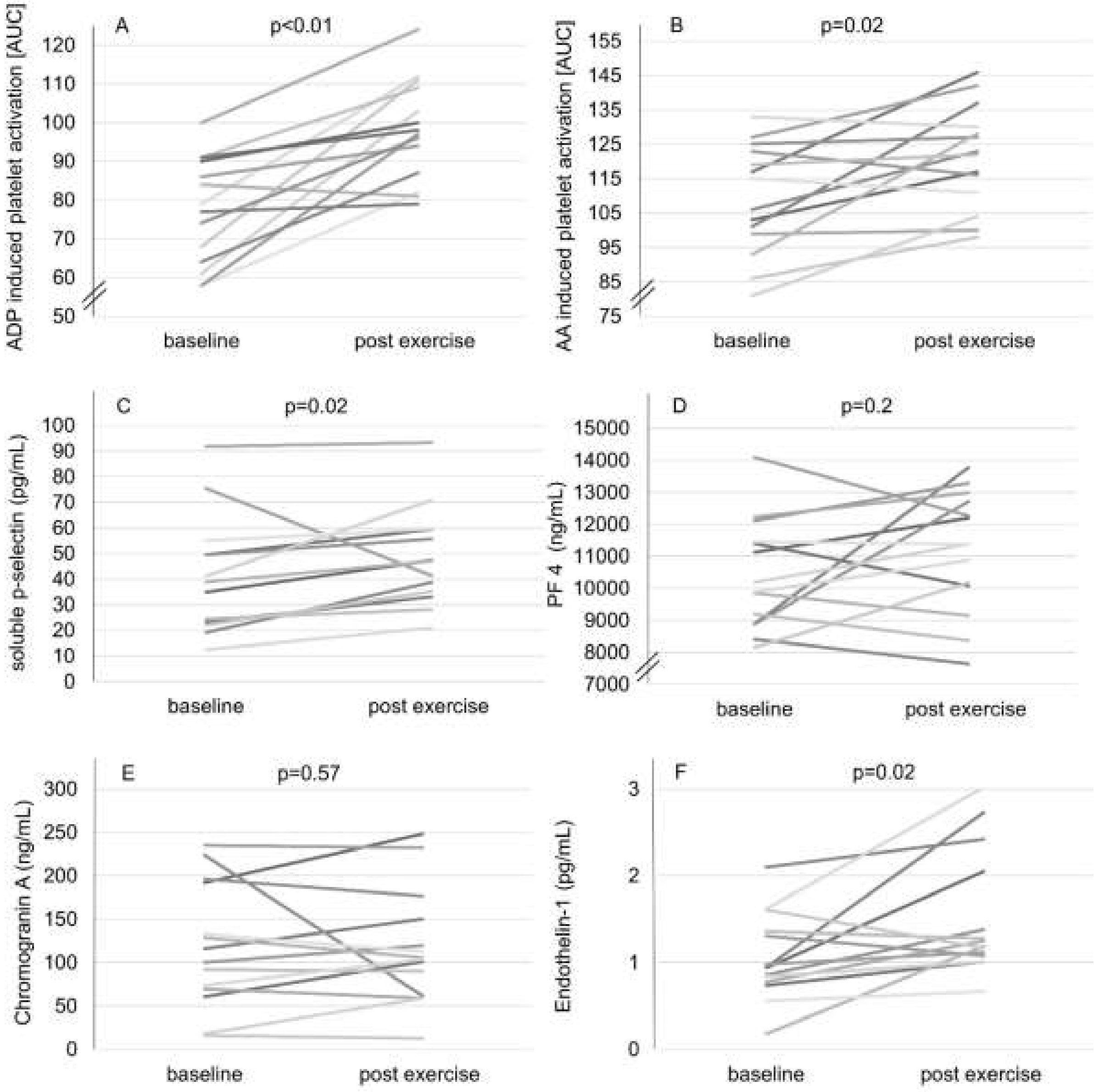

Figure 1: Impedance aggregometric measurement of platelet activation stimulated with adenosine diphosphate (ADP, panel A) and arachidonic acid (AA, panel B) is depicted. Results are given as Area Under Aggregation Curve (AUC). Results show levels of soluble p-selectin

(panel C),PF4 (panel D) , Chromogranin A (panel E) and Endothelin-1 (panel F) before and after exercise testing.-.

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