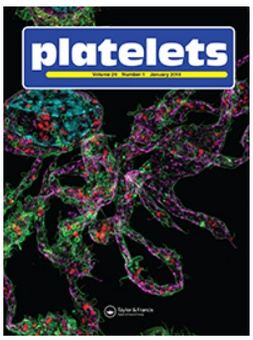

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SHORT COMMUNICATION

Physical inactivity and platelet function in humans and brown bears: A comparative study

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Abstract

Physical inactivity increases the risk of thromboembolism. However, good standardized human models on inactivity are in short supply and experimental models are few.

Our objective was to investigate how standardized bed rest affects platelet aggregation in humans and to investigate if aggregation is altered in a translational model system – the hibernating brown bear (*Ursus arctos*). We collected blood from (1) healthy male volunteers participating in a 21-day bed rest study in head-down tilt position (–6°) 24 h a day; (2) free-ranging brown bears captured during winter hibernation and again during active state in summer. We analyzed platelet function using multiple electrode platelet aggregometry. In total, 9 healthy male volunteers (age 31.0 ± 6.4 years) and 13 brown bears (7 females and 6 males, age 2.8 ± 0.6 years) were included. In hibernating bears adenosine diphosphate, arachidonic acid, thrombin receptor activating peptide, and collagen impedance aggregometry tests were all halved compared to summer active state. In human volunteers no statistically significant changes were found between baseline and the end of bed rest. In human male volunteers 3 weeks of bed rest did not affect platelet function. In hibernating brown bears platelet aggregation was halved compared to summer and we hypothesize that this is a protective measure to avoid formation of thrombi under periods of low blood flow.

Keywords

Thrombosis, Platelets, Platelet aggregation, Immobilization

History

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Introduction

Physical inactivity is a risk factor for venous and arterial thrombosis, and sedentary lifestyle increases the risk for cardiovascular disease [1,2]. In this respect the hibernating brown bear, *Ursus arctos*, could serve as a translational model. Indeed, while inactivity is a thromboembolic risk factor in humans, this is not the case for hibernating bears, which have almost no physical activity for 6 months each year [3–5]. In this study we analyzed how 21 days of bed rest, a long-term model of enforced physical inactivity, affects platelet aggregation in humans and whether platelet aggregation is differentially altered during hibernation compared to active state in brown bears.

Materials and methods

The European Space Agency and the German Aerospace Center conducted a head-down-tilt-bed-rest (−6°) study in 2011–2012. Ten healthy men were restrained to the metabolic ward of the German Aerospace Center (DLR) for a 7-day ambulatory control period followed by 21 days of bed rest in head-down tilt position (–6°) 24 h a day followed by a 7-day recovery period. One test subject disrupted the study for medical reasons. We analyzed samples from 9 male volunteers (age 31.0 ± 6.4 years and weight 77.2 ± 5.7 kg).

All volunteers were nonsmokers and free of any clinical/biomedical sicknesses. For inclusion test, subjects were obliged to have a negative thrombophilia screening panel test (antithrombin III, protein C and S, factor V Leiden, prothrombin mutation, and antiphospholipid antibody causing elevation in partial thromboplastin time) because of the long period of inactivity. Fasting (9 h) blood samples were collected in the morning. A detailed description of subject recruitment, screening, the participants, and metabolic ward conditions has been described by Buehlmeier et al [6]. This prospective, cross-over study was conducted under different dietary campaigns, but our study includes data from the conventional diet part of the study only. Blood samples for aggregometry were drawn at baseline and after 19 days of bed rest, and hematological tests were taken at baseline and after 10 days. This bed rest study was approved by the independent ethics committee of the Ärztekammer Nordrhein, Düsseldorf, Germany, and was performed according to the Declaration of Helsinki. All subjects participated after providing signed informed consent.

Blood was drawn from free-ranging brown bears (7 females and 6 males, age 2.8 ± 0.6 years) during winter hibernation in February (weight 48.1 ± 14.0 kg) and from the same bears when active in June (weight 50.9 ± 16.6 kg) 2010–2011 in Dalarna, Sweden, according to a protocol for capture and anesthesia [7]. Brown bears typically reach reproductive maturity before the age

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of 5 years and rarely live the age of 25 [8]. The study of bears was approved by the Swedish Ethical Committee on animal research.

Whole blood was analyzed with a platelet function analyzer (Multiplate impedance aggregometer, Dynabyte, Munich, Germany) [4]. Final agonist concentrations were adenosine diphosphate (ADP) 6.4 μM, arachidonic acid (ASPI) 0.5 mM, thrombin receptor activating peptide (TRAP) 32 μM, protease-activated receptor 4 (PAR-4; AYPGKF) 20 mM, or collagen (COL) 1 μg/mL. Aggregometry was performed within 1 h after blood sampling from the anaesthesized bears.

For statistical comparison, a paired t test was used and $p < 0.05$ was considered statistically significant.

Results

There was a small, but statistically significant increase in hemoglobin during bed rest compared to baseline due to the known hypovolemia resulting from the head-down tilt. Brown bears had statistically significantly higher levels of hemoglobin and lower levels of platelet count in winter compared to summer (Table I).

There were no differences in aggregation in humans between baseline and 19 days of bed rest. In bears, ADP, ASPI, TRAP, and collagen impedance aggregometry tests were all statistically significantly lowered by half during winter compared to summer (Table I). TRAP and PAR-4 are based on human peptide sequences and were less effective in bear than in human platelet activation as could be expected. There were no sex differences in aggregometry or hematology in the bear population. Rectal temperature of the bears was 33.4 ± 1.1°C in winter and 39.8 ± 0.8°C in summer which is approximately 2°C higher than normal body temperature in summer, caused by the bear running during the helicopter capture [9]. To test for the effect of temperature, aggregometry was performed in a subset of samples ($n = 6$) collected during winter, using identical blood samples examined at 33°C as well as 37°C. We could not document any significant differences (Table II).

Table II. Aggregation results in bears at different temperatures.

		33°C	37°C	p-Value
ADP test	AUC (±SD)	78.1 (26.0)	65.8 (23.6)	0.15[#]
ASPI test	AUC (±SD)	74.4 (26.9)	76.3 (18.2)	0.76[#]
TRAP test	AUC (±SD)	26.8 (9.0)	25.4 (12.9)	0.72[#]
COL test	AUC (±SD)	69.2 (24.5)	69.8 (12.6)	0.98[#]
PAR-4 test	AUC (±SD)	37.0 (13.2)	26.8 (10.9)	0.05[#]

Values are mean ± standard deviation.
ADP, adenosine diphosphate; ASPI, aspirin; AUC, area under curve; COL, collagen; PAR-4, protease-activated receptor 4; TRAP, thrombin receptor activating peptide.
[#]$n = 6$.

Discussion

Three weeks of bed rest did not affect platelet function in humans. In hibernating brown bears, platelet aggregation was halved compared to active state. We therefore speculate that brown bears, in order to compensate for lying still for 6 months with low circulatory state, have developed reduced platelet aggregation as a protection against thromboses.

In hibernating animals organ damage due to thrombosis has not been found [10], despite increased blood viscosity [11], low blood flow [12], low heart rate, [13] and physical inactivity. Bears had numerically lower platelet aggregation, even in summer, with all agonists except ADP compared with humans. This might be due to a species difference in amino acid sequence of the platelet receptor, enabling only partial activation of the agonists added. ADP and collagen provided the most consistent results between species in an earlier animal study [14]. As the metabolic rate during hibernation is only 25% of the summer activity rate [15], protein synthesis is reduced and central processes appear to be maintained by increased levels of very few key or broad-specificity proteins. Thus all coagulation factor levels were reduced to <80% except for the three key

Table I. Aggregation and hematology in humans versus bears.

	Humans ($n = 9$)				Bears				
	Bedrest	Baseline	Paired	Paired	n	Winter	Summer	Paired	Paired
	Mean ± SD	Mean ± SD	ratios	t test		Mean ± SD	Mean ± SD	W/S	t test
Red blood cells (10^9/L)	5 ± 0	5 ± 0	1.05	0.005	13	9.0 ± 0.5	6.6 ± 0.5	1.36	<0.001
Hemoglobin (Hgb) (g/L)	151 ± 8	144 ± 5	1.05	0.003	13	213 ± 12	165 ± 15	1.30	<0.001
Hematocrit (%)	44.3 ± 2.3	42.3 ± 1.5	1.05	0.004	13	54.8 ± 3.8	44.0 ± 3.3	1.25	<0.001
White blood cells (10^9/L)	7.4 ± 2.3	6.2 ± 2.2	1.23	0.111	13	5.9 ± 4.9	7.4 ± 2.3	0.83	0.322
Platelets (10^9/L)	245 ± 71	211 ± 37	1.16	0.084	13	174 ± 51	262 ± 61	0.69	<0.001
AUC (Ohm•min)									
ADP	57 ± 17	63 ± 26	0.01	0.494	12	33 ± 10	66 ± 23	0.55	0.001
ASPI	105 ± 16	101 ± 23	1.09	0.692	12	34 ± 9	68 ± 20	0.53	<0.001
TRAP	114 ± 30	140 ± 22	0.83	0.075	11	9.2 ± 6.9	18.5 ± 10.0	0.71	0.002
PAR-4	96 ± 14	96 ± 19	1.03	0.989	6	12.7 ± 7.1	22.5 ± 7.1	0.56	0.031
COL	84 ± 16	82 ± 17	1.04	0.729	12	30 ± 7	63 ± 22	0.53	<0.001
AUC/platelet count									
ADP	0.24 ± 0.05	0.30 ± 0.10	0.88	0.132	12	0.20 ± 0.07	0.26 ± 0.11	0.87	0.113
ASPI	0.46 ± 0.11	0.48 ± 0.10	0.98	0.609	12	0.21 ± 0.07	0.27 ± 0.11	0.83	0.084
TRAP	0.50 ± 0.18	0.69 ± 0.16	0.74	0.023	11	0.05 ± 0.03	0.07 ± 0.04	1.04	0.050
PAR-4	0.42 ± 0.11	0.47 ± 0.13	0.93	0.248	6	0.06 ± 0.04	0.08 ± 0.03	0.80	0.281
COL	0.36 ± 0.07	0.40 ± 0.10	0.93	0.225	12	0.19 ± 0.08	0.25 ± 0.11	0.83	0.127

ADP, adenosine diphosphate; ASPI, aspirin; AUC, area under curve; COL, collagen; PAR-4, protease-activated receptor 4; TRAP, thrombin receptor activating peptide.

components factors II, X, and fibrinogen, which were increased by 20% as shown by quantitative proteomics and verified by functional analyses [16]. This could be the most economical way to preserve a coagulation response during hibernation. Two different European space campaign bed rest studies on healthy volunteers showed no alterations in coagulation factors involved in secondary hemostasis at the end of bed rest compared to baseline [17–19].

A study by Broadley et al. documented that a short period of 45 minutes of supine rest reduced platelet aggregation, and the authors postulate that the reduction partly can be explained by a fall in plasma catecholamines [20]. The reverse, an increase in platelet aggregability and an increase in cathecolamine levels, has been observed after assumption of the upright posture [21]. Catecholamines can act on alpha-2 adrenoreceptors and promote platelet aggregation [22]. In longer bed rest studies psychological stress has been reported [23], but without changes in cathecolamine levels [24].

We observed a reduced platelet count from approximately 260 $\times$ 10^9/L to 170 $\times$ 10^9/L during hibernation. Studies on the influence of platelet count and platelet aggregometry have shown decreased aggregation when platelet count drops below 150 $\times$ 10^9/L [25] – beyond what we observed in this study (174 $\times$ 10^9/L $\pm$ 51). We therefore propose that the reduction in platelet aggregation in brown bears is not linked to platelet concentration reduction. In our previous aggregometry study (mid-April 2009 data) approximately 7–10 days after leaving the den, platelet aggregation was similar to hibernation in the present study although platelet count had a mean value of 207 $\times$ 10^9/L $\pm$ 24 [4]. The observed decrease in platelet count corresponds with studies of hibernating hamsters [26,27]. Upon arousal thrombocytopenia was reversed suggesting storage and release, possibly by margination of platelets during hibernation [26]. As hematocrit plays a role in the degree of margination [28], the reduced platelet count may in part be an effect of a higher hematocrit due to dehydration during hibernation.

In humans, hypothermia enhances agonist-induced platelet aggregation [29]. However, we found hibernating bears to have reduced platelet aggregation during moderate hypothermia and when we studied platelet function at different ex vivo temperatures there were no differences in aggregation.

In conclusion, in human male volunteers, 3 weeks of bed rest did not affect platelet function. In hibernating brown bears, platelet aggregation was halved compared to summer and we hypothesize that this is a protective measure to avoid formation of thrombi under periods of low blood flow.

Declaration of interest

The authors report no conflicts of interest.

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