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Pro- and anti-coagulatory effects of bed rest

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Abstract

Background: 6 degrees head-down bed rest (HDBR) is routinely used in laboratories worldwide to simulate spaceflight deconditioning. Coagulatory changes have been associated with bed rest immobilization in addition to cephalad fluid shifts, muscle atrophy, osteoporotic changes. The exact mechanisms of bed rest induced coagulatory changes are, however, not clearly elucidated. For example, while pro-coagulatory changes in bed rested and immobilized patients are commonly known and treated with anti-coagulants to prevent hyper-clotting, it is still unclear which exact endothelial and hemostatic effects occur during bed rest over time. We examined whether medium-term bed rest of 21 days leads to anti-/pro-coagulatory effects.

Methods: The blood samples were collected under simulated microgravity conditions (HDBR) from 12 male subjects over a period of 21 days at the Institute for Space Medicine and Physiology (MEDES), Toulouse, France. None of the participants suffered from any pathological condition or were under treatment. Blood collection was done in the morning between 7 a.m. and 8 a.m., 5 days before bed rest (baseline data collection) and during bed rest on the 2^{nd} (HDT+2), 7^{th} (HDT+7), 14^{th} (HDT+14), 21^{st} (HDT+21) and just before standing up (R 0) on the first day of recovery. Post-bed rest samples were taken on the second day of recovery (R+2). Data from thrombelastometry (coagulation time (CT), clot formation time, α-angle and maximum clot firmness (MCF)) and other coagulation parameters determined in platelet poor plasma (endogenous thrombin potential (ETP), thrombin peak (TP), time to peak (ttPeak), slope, lag time, start tail, factor II, factor VII, factor VIII activity, thrombin-antithrombin complex (TAT), prothrombin fragment 1+2 (F1+2) and tissue-type plasminogen activator (t-PA)) were collected.

Results: CT was significantly prolonged throughout the whole time course. The α-angle was significantly reduced. ETP was decreased from HDT+7 onwards. TP and slope were significantly elevated on HDT+21 together with a decrease in start tail and ttPeak values. Lag time was prolonged on HDT+2, but showed no changes on other sampling points. Prothrombin showed no change during the bed rest, but a decrease on R+2. F1+2 showed no change except for a reduction on HDT+7 and HDT+21. Factor VIII was slightly elevated on R 0. TAT, t-PA, factor VII and MCF showed no significant change. No parameter showed pathological signs.

Discussion: According to the results of this study there is no pro-coagulant effect in healthy subjects undergoing 21 days of strict 6 degree head-down bed rest. These results are in contrast to the broad belief that bed rest and immobilization induces pro-coagulatory effects. As our subjects were healthy volunteers it is perhaps in those who have an imbalance in the anti- and pro-coagulatory system that tip towards increased clotting during immobilization. The question arises whether all patients unexceptionally need treatment to prevent thromboembolic events during immobilization.

Zusammenfassung

Hintergrund: Um Schwerelosigkeit zu simulieren und die damit verbundene Dekonditionierung zu erforschen, wird weltweit das Modell der sechs Grad Kopftieflage angewendet. Bettruhe und Immobilisation haben Volumenverschiebungen, Muskelatrophie und Knochenabbau zur Folge und werden mit Veränderungen im Gerinnungssystem in Zusammenhang gebracht. Die zugrundeliegenden Mechanismen der durch Bettruhe induzierten Gerinnungsveränderungen sind nicht hinreichend erforscht. Prokoagulatorische Veränderungen bei immobilisierten und bettlägerigen Patienten sind dokumentiert und werden mit Thromboseprophylaxe behandelt. In dieser Studie wurden die pro- und antikoagulatorischen Veränderungen bei 21 tägiger Bettruhe in sechs Grad Kopftieflage untersucht.

Methoden: Es wurden Blutproben von zwölf gesunden männlichen Probanden über 21 Tage simulierter Schwerelosigkeit am Institut de Médecine et de Physiologie Spatiales in Toulouse, Frankreich, genommen. Keiner der Probanden befand sich zum Zeitpunkt der Studie aufgrund einer Erkrankung in medizinischer Behandlung. Die Blutabnahmen erfolgten zwischen 7.00 Uhr und 8.00 Uhr, fünf Tage vor Beginn der Bettruhe (BDC-5), am zweiten (HDT+2), siebten (HDT+7), 14. (HDT+14), 21. (HDT+21) Tag der Bettruhe, am ersten Tag der Erholungsphase unmittelbar vor dem Aufstehen und am zweiten Tag der Erholungsphase (R+2). In thrombozytenarmem Plasma wurden Parameter der Thrombelastometrie (Coagulation Time (CT), Clot Formation Time, α-angle und Maximum Clot Firmness (MCF)), Parameter des Thrombingenerierungstests (Endogenes Thrombin Potential (ETP), Thrombin Peak (TP), Time to Peak (ttPeak), Slope, Lag Time und Start Tail) und des Weiteren Faktor II, Faktor VII, Faktor VIII, Thrombin-Antithrombin-Komplex (TAT), Prothrombin Fragment 1+2 (F1+2) und Tissue-type Plasminogen Activator (t-PA) bestimmt.

Resultate: Die CT war während der Bettruhe signifikant verlängert. Der α-angle war signifikant vermindert. ETP zeigte geringere Werte ab HDT+7. An HDT+21 waren die Werte von TP und Slope erhöht, während die Messwerten von Start Tail und ttPeak erniedrigt waren. Die Lag Time war zum Zeitpunkt HDT+2 verlängert, zeigte ansonsten jedoch keine Unterschiede. Prothrombin war unverändert während der Bettruhe, war aber in der Erholungsphase (R+2) leicht erniedrigt. F1+2 zeigten niedrigere Werte an den Tagen HDT+7 und HDT+21. Faktor VIII war an R 0 leicht erhöht. TAT, t-PA, Faktor VII und MCF zeigten keine signifikanten Veränderungen. Kein Parameter ließ auf Pathologien schließen.

Diskussion: Die Ergebnisse dieser Studie zeigen, dass kein prokoagulatorischer Effekt während der 21 tägigen Bettruhe in sechs Grad Kopftieflage bei gesunden männlichen Probanden auftritt. Die Ergebnisse stehen im Gegensatz zur weit verbreiteten Meinung, dass Bettruhe und Immobilisation prokoagulatorische Auswirkungen haben. Da an dieser Studie gesunde Probanden teilnahmen, reagieren vielleicht nur Patienten, die bereits ein Ungleichgewicht in ihrem Gerinnungssystem haben, auf Bettruhe und Immobilisation mit erhöhter Gerinnungsneigung. Es drängt sich die Frage auf, ob alle Patienten ausnahmslos mit Thromboseprophylaxe behandelt werden müssen, sobald sie immobilisiert werden.

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Abbreviations

µg	Microgram
ALAT/SGPT	Alanine aminotransferase
AP	Alkaline phosphatase
aPTT	Activated partial thromboplastin time
ASAT/SGOT	Asparatate aminotransferase
ATIII	Antithrombin
BCT®	Behring Coagulation Timer
BDC	Baseline Data Collection
CAT	Calibrated automated thrombogram
CAT	Calibrated Automated Thrombography
CBC	Cell blood count
CFT	Clot formation time
CNES	Centre national d'études spatiales
CO	Cardiac output
CRP	C-reactive protein
CT	Coagulation time
DBP	Diastolic blood pressure
ELISA	Enzmye-linked immuno sorbent assay
ESA	European Space Agency
ETP	Endogenous thrombin potential
F1+2	Prothrombin-fragment 1 and 2
FII	Prothrombin
FIIa	Thrombin
FXa	Factor Xa
g	Gravity of Earth
GGT	Gamma glutamyl transferase
GOD	Glucose oxidase
HDBR	6 degrees head-down bed rest
HDT	Head-down tilt
HR	Heart rate
HRP	Horseradish

LBNP	Lower body negative pressure
LI	Lysis index
MAP	Mean arterial pressure
MCF	Maximum clot firmness
MEDES	Institute for Space Medicine and Physiology
min	Minute
ml	Milliliter
mm	Millimeter
ng	Nanogram
nM	Nanomolar
NO	Nitric oxide
PAI-1	Plasminogen-activator-inhibitor-1
PAI-2	Plasminogen-activator-inhibitor-2
PGI2	Prostacyclin
pmol	Picomole
PPP	Platelet poor plasma
PT	Prothrombin time
R	Recovery
ROTEM®	Rotational thrombelastometry
s	Second
SBP	Systolic blood pressure
SD	Standard deviation
STD	Standard deviation
SV	Stroke volume
TAFI	Thrombin-activatable fibrinolysis inhibitor
TAT	Thrombin-antithrombin-complex
TEG®	Thrombelastography
TEM	Thrombelastometry
TF	Tissue factor
TFPI	Tissue factor pathway inhibitor
TP	Thrombin peak
TP	Thrombin peak
t-PA	Tissue plasminogen activator
TPR	Total peripheral resistance

TTP	Time to peak
u-PA	Urokinase-type plasminogen activator
US	Ultrasound
VTE	Venous thromboembolism
vWF	Von Willebrand factor
α	α-angle

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1 Introduction

Immobilization over longer periods can have a huge impact on a patient's state of health. Patients of all ages suffering from trauma, acute or chronic illnesses but also disabled and geriatric patients are often confined to bed for long periods. Although bed rest is initially beneficial for the recovery from an illness, it can induce a cascade to dependency especially in the elderly, which is unrelated to the initial cause for confinement (1). Effects of immobilization due to bed rest concern amongst others the muscular and skeletal system. Young men are reported to lose 1% of muscle strength each day in bed (2). Due to reduced plasma volume, reduction in baroreceptor sensitivity caused by old age and resulting orthostatic intolerance, the elderly are at great danger of falls (1). Physical restraint leads to bone loss. All factors combined, bed rest increases the risk for fractures followed by even longer periods of immobility.

It is broadly believed that immobility also induces a procoagulatory state. Virchow's triad defines the risk for developing thromboembolic events. Blood vessel injury, abnormal blood flow and imbalance in the anti- and procoagulatory system increase the risk for venous thromboembolism (3). Stasis due to decreased muscle pump when in supine posture is considered to contribute to the procoagulatory state (4) as well as hemoconcentration induced by bed rest (5).

Masoud et al. showed that orthostatic stress and shear stress lead to a hypercoagulable state in comparison to supine posture (6). The mechanisms involved are possibly hemoconcentration due to plasma shifts and endothelial activation due to higher shear stress (6).

In a later study Masoud et al. investigated solely the influence of hemoconcentration on the procoagulatory effect of prolonged standing. They increased the plasma volume by intravenous infusion of 1.5 l of isotonic fluid (7). Again plasma shifts occurred during standing, but the hematocrit values stayed similar to those in supine position (7). The hemodilution apparently had no effect on the procoagulable state provoked by standing. This led to the assumption that the endothelial influence due to increased shear stress is more striking.

In a related study conducted by Cvirn et al. effects of graded orthostatic stress under passive standing conditions on the coagulation system were examined, including analysis of the recovery phase (8). It could be shown, that the hypercoagulable values measured in whole blood during standing even persisted

after returning to supine position. As shear stress is terminated by supine position, it was concluded that hormones may have influences on hemostasis, which are not yet understood (8).

Plasma shifts and intravascular pressure changes, which occur during alteration of posture under gravitational conditions on Earth, influence the coagulation system. It can be expected, that - under microgravity conditions - these effects alter from those on Earth. At least, by eliminating the factor gravity, it can be investigated, whether hydrostatic forces do have an impact on coagulability and to what extent. Supine position and microgravity conditions eliminate the factor shear stress and the endothelial response because of decreased hydrostatic pressure in capacitance vessels. It can be assumed that both conditions have similar effects on hemostasis.

In general, hospitalized patients receive antithrombotic treatment as a prophylaxis as soon as they are immobilized. Attempts to classify risk groups and adapt treatment accordingly have been made, but have not been widely accepted (9).

It is still unclear which exact endothelial and hemostatic effects occur during bed rest over time. This study was conducted to monitor the alterations of pro- and anti-coagulatory parameters during bed rest.

1.1 Gravitation and spaceflight

Gravitation is a force which describes the attraction between two physical bodies. Newton's law of universal gravity defines the gravitational force as:

$$F = f \frac{m_1 m_2}{r^2}$$

F: Gravitational force [N]

m_1: Mass of physical body 1 [kg]

m_2: Mass of physical body 2 [kg]

r: Distance the center of masses of both physical bodies [m]

f: Gravitational constant = 6,673 10 $\cdot 10^{-11} \frac{\mathrm{Nm}^2}{\mathrm{kg}^2}$

Gravitational force is dependent on the mass of the planet and - in case of human spaceflight – the mass of an astronaut. It is influenced by the distance between the bodies, and decreases the further apart the two objects are.
Because the mass of a human being compared to the mass of a planet such as Earth can be neglected, the two effective influences on the load of an astronaut are the distance to and the mass of the surrounding planets.
Each planet is in the center of its own gravitational field. The field strength determines the load that acts on a body within the field.

$$\vec{g} = \frac{\vec{F}}{m}$$

$\vec{g}$: Gravitational field $[\frac{\mathrm{N}}{\mathrm{kg}}]$

$\vec{F}$: Gravitation force [N]

m: mass of a body in within the field [kg]

The gravitational field equals the acceleration due to gravity, which an astronaut is exposed to within his current environment. On Earth the acceleration due to gravity is defined as 9,80665 $\frac{\mathrm{m}}{\mathrm{s}^2}$. G-force is the multiple of gravitational acceleration and is understood as a measurement of load a human being has to bear during acceleration. 1 g - referred to as “normal gravity” - is defined as 9,80665 $\frac{\mathrm{m}}{\mathrm{s}^2}$ (10).
Any load above 1 g is called hypergravity, any load beneath microgravity (10). To be exact microgravity is reached at $1 \cdot 10^{-6}$ g. While hypergravity in the context of spaceflight is a rather short experience during the launching of a rocket or when returning to Earth, microgravity is a stimuli an astronaut is exposed to most of the time when on a mission in space.
Therefore studying the effects of microgravity on the human physiology has become a great field of interest to research. Not only does it help to protect astronauts in foreign environment enabling long duration missions, but also it provides answers by eliminating the factor gravity and its influence on biological systems (10).

To experience microgravity one either has to leave the gravitational field of Earth, which at the moment is impossible to conduct for humans, as it demands a travel of 6.37 million kilometers away from earth (10), or travel in free fall. Gravity is the only force acting on an object in a vacuum, when in free fall. Free fall applications on Earth such as the Zero Gravity Research Facility at NASA Research Center in Brookpark, Ohio, USA, are limited to very short periods of time (seconds). Creating an environment of microgravity for long periods requires a permanent free fall. This can be accomplished by orbiting Earth. Once an object has left Earth and entered an atmosphere with low friction, two forces act upon it (10). The centrifugal force - created by the velocity of the object - drags the object further into space and Earth's gravity field pulls the object back to the surface. If the object's velocity creates the necessary centrifugal force to equal gravitational force, the object orbits at a constant distance from Earth on a trajectory (see Figure 1). The speed is dependent on the distance from Earth and the remaining g-force. The object is now in a permanent free fall. Any objects within an orbiting space ship experience free fall and therefore are exposed to microgravity.

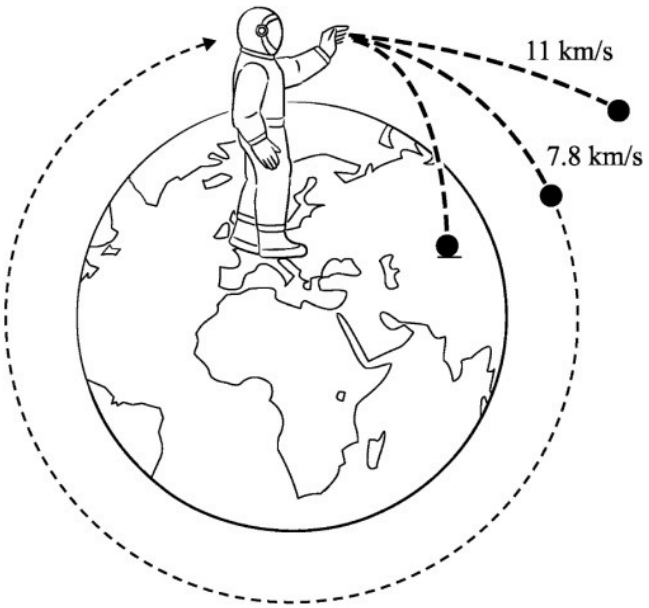

Figure 1: Spaceships or habitable space station orbiting Earth, obtained from *Fundamentals of Space Medicine* by Clément (10)

1.1.1 Gravitational effects on human cardiovascular physiology

The human body is adjusted to gravity on Earth. Changing posture of the human body towards the vector of Earth's gravitational field has an immediate impact on

the behavior of bodily fluids. One directly noticeable reaction is changing posture from lying supine to standing upright and triggering an orthostatic reaction. About 400-600 ml of blood (10% of total volume) is pulled towards the legs from the thorax (11), which leads to a decrease in stroke volume, preload and blood pressure.

In order to maintain constant blood flow to the brain and sustain adequate tissue perfusion pressure, the heart and the blood vessels have to adapt to the demands of the gravitational forces and keep the mean arterial pressure constant, when changing posture from supine to upright.

Mean arterial pressure (MAP) is the product of cardiac output (CO) and total peripheral resistance (TPR). Cardiac output is the product of heart rate (HR) and stroke volume (SV).

$$MAP = CO \times TPR$$

$$CO = HR \times SV$$

Mean arterial pressure is monitored by baroreceptors in the carotid sinus and aortic arch and stretch receptors in the right atrium. Cerebral ischemia leads to a central nervous reaction with an increase in blood pressure (e.g. Cushing-Reflex) (12). These mechanisms prepare for an immediate response within seconds to either sudden blood pressure drops or peaks. Baroreceptors trigger sympathetic and parasympathetic responses of the heart and modify TPR and capacitance vessels (12). Stretch receptors sense blood volume and pressure in the atrium. Increased volume raises HR (Bainbridge-effect) and reduces blood volume by increasing renal water excretion. The central nervous system reacts to low oxygen levels and decrease in blood pressure by increasing sympathetic neuronal activity which leads to vasoconstriction.

Long-term adaptation is conducted by reducing or increasing blood volume involving kidney function.

MAP is dependent on the three quantities: Heart rate, total peripheral resistance and stroke volume.

The heart rate underlies sympathetic and parasympathetic influences. A sympathetic response is positive chronotropic and increases the heart rate. A parasympathetic response reduces the heart rate (negative chronotropic) (13).
The total peripheral resistance is the force the heart has to overcome with every contraction. It is equal to afterload. Its force is influenced by contraction or dilation of small arteries and arterioles.
The stroke volume is the blood volume ejected from the left ventricle into the aorta during systole with every contraction. It is influenced by the preload, the contractility of the heart muscle and the afterload. Hence, the stroke volume can be increased by sympathetic activity and the Frank-Starling law of the heart (14). The heart muscle charges its contraction according to the degree by which the myofilaments are stretched by venous blood (preload) during the filling phase (diastole). The higher the end-diastolic volume, the greater the force of the ventricle contraction will be during the systole with increased stroke volume. This mechanism copes with increased volumetric load as well as it does with increased pressure load (afterload). Increased hydrostatic pressure in the aorta during systole (afterload) causes a higher end-diastolic volume, a higher muscle fiber stretch and subsequently a more forceful systole which pushes an adequate volume against higher pressure (14). This mechanism ensures a balance between venous return and cardiac output (14). The other mechanism to increase stroke volume is stimulated by the sympathetic division of the autonomic nervous system. Positive inotropic agents such as epinephrine and norepinephrine increase contractility independently of the Frank-Starling mechanism, but much faster. At the same time these hormones increase the heart rate (positive chronotropic response). The sympathetic response adapts cardiac output to physical work, whereas the Frank-Starling mechanism responds to alteration in venous return (14).
The hydrostatic pressures within the blood vessels throughout the body have a large variation when in upright standing position. Mean arterial pressures range from 50 mmHg at the head to 180 mmHg at the feet. The heart is just above the point of hydrostatic indifference (see Figure 2) with 100mmHg.

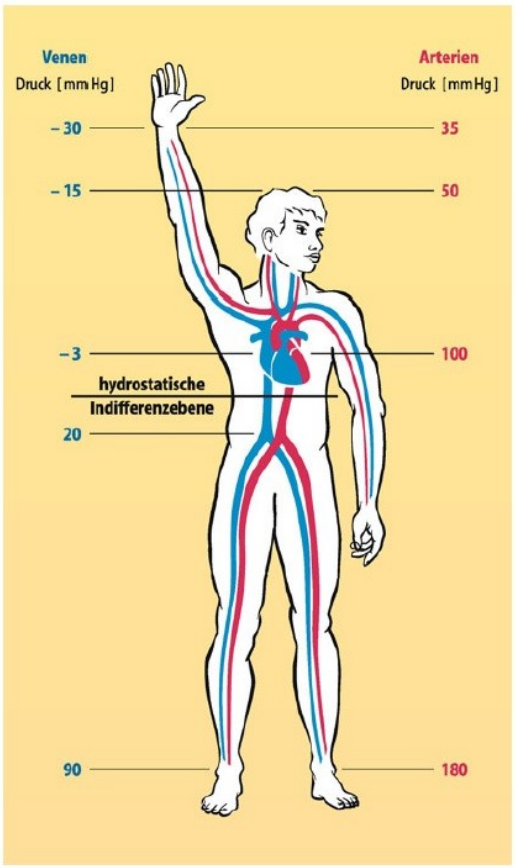

Figure 2: Mean arterial and venous pressure in standing upright position; obtained from *Physiologie des Menschen* by Schmidt (13)

Venous pressures are relatively lower underneath the indifference point compared to arterial pressures and negative above. The indifference point is often referred to as a plane or axis. This model can only serve as a simplification. The location of the indifference point is dependent on the type of postural change, the specific surrounding tissue (arterial, venous), the blood volume and the blood vessel compliance. If a body rotates around one axis, then indifference is a line which conforms to the relevant body axis. If the posture change is more complex and the rotation is across at least two axes, then we speak of a point of indifference (15).
Standing upright, under gravitational conditions on Earth, would cause venous pooling in leg veins, if the only force acting on venous blood is the negative pressure due to inspiration and the suction of the heart's right atrium and ventricle. As sustaining a sufficient preload and venous return for the heart's right ventricle is only possible by pumping the blood volume actively upwards against gravity, a more powerful force is needed. The calf muscles together with venous valves act jointly and effectively on keeping up venous blood stream towards the heart

against gravity to maintain an adequate preload and prevent venous pooling in the legs (see Figure 3).

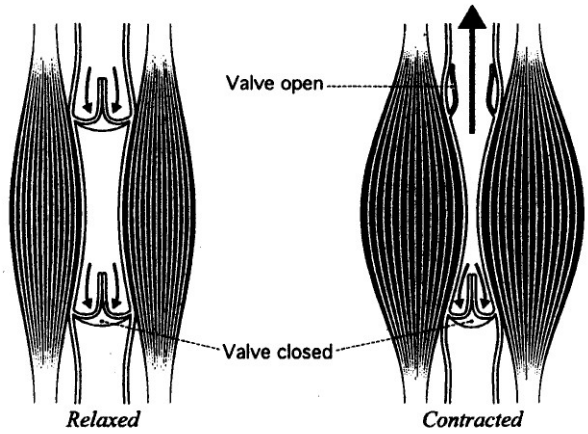

Figure 3: Principle of the calf muscle pump, obtained from *Fundamentals of Space Medicine* by Clément (10)

In supine position the differences between cephalic and lower body venous pressures are much smaller (see Figure 4). The same accounts for arterial pressures.

As already mentioned, standing up from supine position leads to adjustments of the cardiovascular system to the new posture within seconds. The posture change causes a redistribution of blood volume from the thorax to the venous capacitance vessels in the legs, which leads in fact to a decreased preload. The stroke volume initially drops (Frank-Starling mechanism), which reduces the mean arterial pressure and consequently decreased baroreceptor stimulation increases sympathetic activity. Thus, there is an acceleration of heart rate, an increase in the total peripheral resistance by mainly vasoconstriction in the musculature, skin and mesenteric blood vessels. Mean arterial pressure picks up again (16).

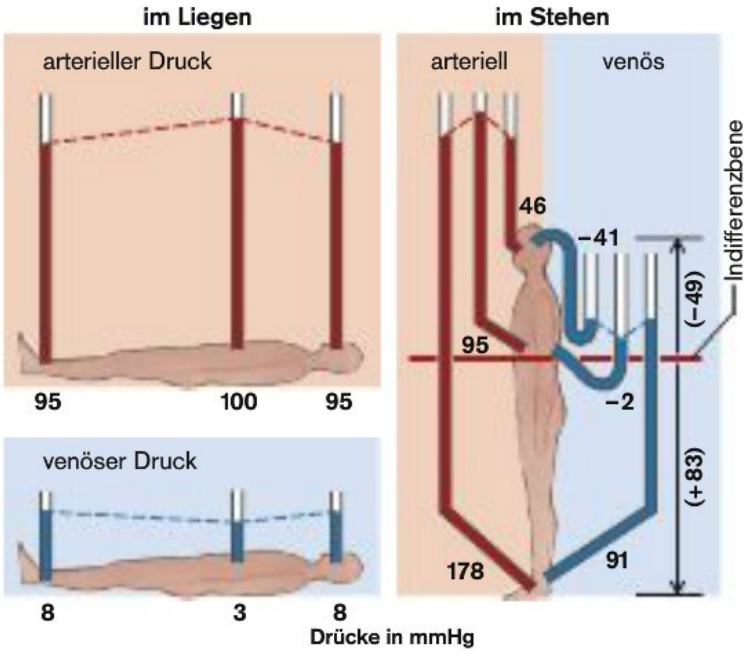

Figure 4: Hydrostatic pressures according to posture (standing/supine), obtained from *Physiologie* by Klinke (16)

Pressure changes within blood vessels cause alternating transmural forces on the blood vessel wall and activates endothelial cells. Also long standing periods increase hydrostatic pressure in arteries and veins in the legs. Both increase shear stress and shift about 12% of plasma volume from the intravascular compartment towards the interstitial compartment (6).

1.1.2 Microgravity effects on the cardiovascular system

Space flight challenges the human cardiovascular system and its responses. Firstly the system has to cope with hypergravity during launching. Once the ship is in free fall, bodily fluids are exposed to microgravity and the human physiology is initially challenged to adapt to the circumstances. When returning to Earth astronauts suffer from cardiovascular deconditioning and have to withstand the relative increase of g-force back on Earth and its effects on the hydrostatic pressure.

During take-off and re-entry space travelers are exposed to a maximum of 3 g (10). Astronauts have to bear hypergravity during launching for about eight minutes and during re-entry for about 3 minutes. By placing astronauts with legs

tucked vertically to the resulting g-forces, plasma shifts along the longitudinal axis can be avoided, and drops in stroke volume and fainting are prevented (10).

By the time the ship reaches orbit, microgravity markedly affects blood flow. The first three days of weightlessness seem to be the most challenging. Neck veins look dilated; the face looks puffy (10). Some astronauts complain about headaches. They claim to be less thirsty and the circumference of the thigh is reduced. All those effects or symptoms originate from the fluid shift towards the head. The forces which drag blood towards the ankles are eliminated in space. The heart is not yet adapted to that situation and pumps each stroke volume with the same force like on Earth. As a consequence body fluids accumulate around the head and the thorax (see Figure 5).

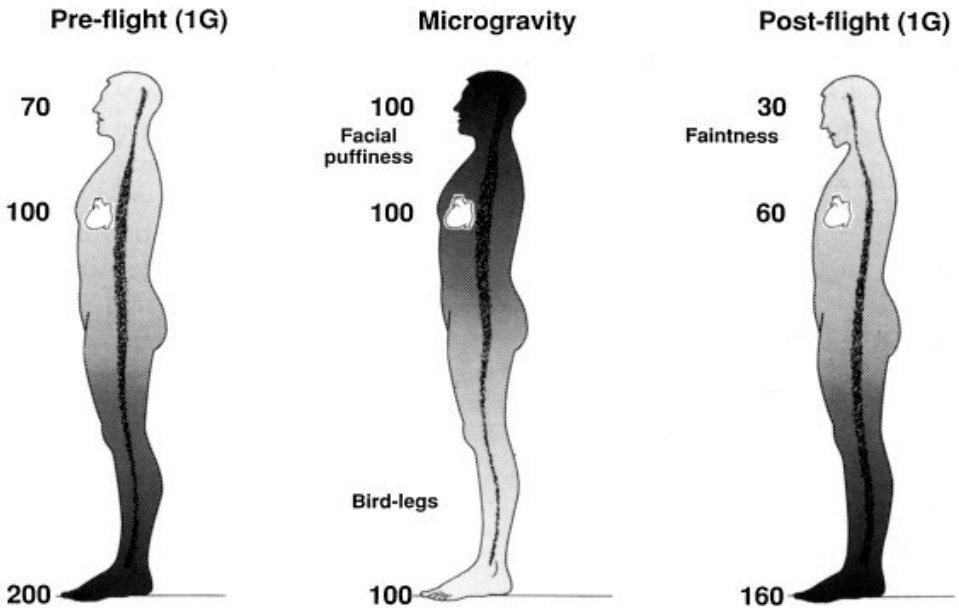

Figure 5: Fluid shifts before, during and after space flight. Blood pressure data are estimated values; obtained from "Cardiovascular adaptations, fluid shifts, and countermeasures related to space flight" (17)

Hargens et al. suggest also an increased filtration of blood into the surrounding tissue. They assume that the reduced tissue weight gives way to the still increased blood pressure within capillaries, which increases interstitial fluid volume (17) and leads amongst other things to an easily recognizable puffy face.

After some days human regulatory mechanisms counteract the altered forces (10). The blood volume is decreased, plasma volume by 22% (10). Water excretion is increased by the kidneys (10). Although the total body water is not altered, one to two liters of fluid is pulled out of the legs and moved towards thorax and head (10).

The above mentioned increased capillary filtration rate and subsequently increased interstitial fluids are pushed into the cells (10). Reduction of fluids leads to a drop in central venous pressure (10). The sympathetic nervous system is on a higher activity level. Norepinephrine is elevated together with the cardiac output, which is a sign of decreased arterial resistance (10). The baroreceptors' firing activity seems disturbed and the heart rate is elevated with a decreased variability (10). During the adjustments to microgravity the heart volume markedly increases due to higher preload (10). Together with the fluid shifts and the loss of blood volume the heart volume shrinks to a smaller volume (cardiac atrophy) than on Earth before launching (10). Cases of dysrhythmia during space flight and after return have also been reported (10).

All these adjustments cause cardiovascular deconditioning when astronauts return to Earth. Many astronauts suffer from orthostatic intolerance due to the adaptation to low gravity. Gravity pulls blood to the feet, the preload decreases and the heart rate in return is markedly increased, which helps to raise the cardiac output (10). The blood vessels increase the total peripheral resistance to maintain an adequate mean arterial pressure in order to prevent hypotension (10). In some astronauts these mechanisms fail or do not fully compensate the new forces (10). The symptoms range from lightheadedness, dizziness, headache and fatigue to presyncope or syncope (10).

1.1.3 Ground-based simulated microgravity and effects on the cardiovascular system

Investigating and understanding effects of microgravity on the human physiology is mandatory since Yuri Gagarin entered space in 1961. Health in space has to be guaranteed to successfully carry out missions on space stations, the Moon or even Mars. Knowledge of adaptation processes due to weightlessness prevents injury of astronauts, maintains health and ensures a space mission's success. Monitoring physiological adjustments in space is hardly feasible. Reproducibility and subject numbers cannot meet scientific demands well enough. Ground-based simulation models of hyper-, hypo- and microgravity have been tested and some challenge the human cardiovascular system as much as space environment.

At first water immersion was used to alter g-load on humans. It shares the effect of fluid shift with space habitants, but to the center of the body (18). Breathing under water is not compatible with weightless conditions. Hydrostatic pressure on the thorax influences the active and passive movements of breathing (18). Temperature affects the diameter of venous vessels. Therefore water has to be kept thermoneutral, to avoid volatility of venous volume (18). Lying in water for long periods affects the skin. Diffusion of fluids crossing the skin barrier in both ways cannot be monitored (18). Long-term periods demand wrapping (dry immersion beds) the subjects in water protected sheets (e.g. plastic) (18). Water immersion is not easily performable and not applicable for monitoring long-term effects.

Lower body negative pressure (LBNP) is a method which increases venous pooling in the lower limbs independently of any gravitational force. The subject's lower body beneath the iliac crest is fitted into a chamber. The negative pressure inside the chamber can be modified. This model enables to study orthostatic tolerance without the influence of calf muscles (18). LBNP can be applied in supine position or upright posture or any degree in between. In connection with a tilt table this model provided useful data for both clinical and space medicine.

Lower body positive pressure simulates conditions for central fluid shifts and their responses (18).

G-suits are designed to counteract influences of g-forces on the fluid shifts. It consists of inflatable compartments in the area of the legs and the abdomen. Adapting pressure to the exposed g-forces helps to keep the blood flow to the brain constant. As this model only alters the total peripheral resistance, its use for studying microgravity responses is limited (18).

The above mentioned models shed light on the immediate effects of gravitational alteration. Thus, for studying adaptational processes over weeks a better suited approach is needed. When cosmonauts returning from the first long-term missions complained about difficulties sleeping, the position of the bed was adapted to their well-being. It turned out that tilting the bed towards the head increased sleep quality during the re-adaptation to gravity after return (19). Further research led Russia to the conclusion, that 6 degrees head-down bed rest (HDBR) serves as the best model for simulating microgravity on Earth (20).

None of the ground based analogues to simulating space flight conditions can simulate forces during launching, re-entry or psychological effects and social stress of isolation connected to leaving Earth all together in one model (19). But HDBR is the best model on Earth to study effects of and responses to adjustments to microgravity.

When comparing space flight to HDBR it shows that alterations of the cardiovascular system are similar concerning upward fluid shift, plasma volume and development of orthostatic intolerance (19). The difference lies in the amount of intravascular volume loss, which is increased during space flight compared to HDBR (19). The reason for this could be the acting of Gx forces on Earth, which cannot be eliminated in bed rest studies. There is no diuresis on space missions compared to HDBR (19).

As in space flight, heart volume and stroke volume are significantly reduced in HDBR and total peripheral resistance is increased (19).

During bed rest the red cell mass is reduced together with reduced erythropoietin concentration (19). Blood viscosity and hematocrit are increased (19). While long-term bed rested patients are expected to develop venous thromboembolism, there has not yet been any case reported in HDBR studies (19).

Reduced thickness of anterior tibial tunica media and intima are reported (21).

1.1.4 Countermeasures in space

In the course of studying the deconditioning of space flight, considerations also went in the direction of preventing those effects by introducing countermeasures applicable to space. Regular fluid intake of recommended amounts to counterbalance blood volume loss is standard. Nutritional countermeasures, like high protein nutrition, are still being tested to counteract muscle loss (22). Resistive exercise, like treadmill and cycle ergometers maintain strength (10). Gravitational effects on the cardiovascular system can be carried out by applying artificial gravity using LBNP or short-arm centrifuges (19).

1.2 Hemostatic system

1.2.1 Overview

The hemostatic system controls two vital conditions: Bleeding and clotting. Anti-coagulatory mechanisms maintain a constant blood flow. The viscosity is kept at an optimum. Pro-coagulatory reactions counteract bleeding. In case of injury blood loss has to be minimized and continuous blood flow has to be restored. Keeping balance of these complex reactions depends on the interdependency of cellular (endothelium, thrombocytes) and plasmatic properties.

Anti-coagulatory mechanisms involve endothelial reaction, preventing thrombocytes from aggregating and inhibition of coagulatory enzymes and blood coagulation activators (23).

Pro-coagulatory effects involve contraction of arterial blood vessels to induce local stasis, presentation of receptors on thrombocytes and injured tissue including secretion of pro-coagulatory enzymes, regional limitation of the clotting process and delayed fibrinolysis (23).

1.2.2 Endothelium

The endothelium is the inner layer of the arterial and venous blood vessel and part of the tunica interna (intima). It serves as a barrier between the lumen and the vessel wall with selective permeability and provides a non-adhesive surface.

It has an anti-thrombogenic feature by producing and secreting blood coagulation reducing agents (24). Nitric oxide (NO) and prostacyclin (PGI_2) inhibit platelet aggregation (23). Heparin sulfate and thrombomodulin (integral membrane protein) act anti-coagulatory (23). Endothelial cells induce fibrinolysis by releasing tissue plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA) or inhibit fibrinolysis by releasing plasminogen-activator-inhibitor (PAI-1) (24).

Inducing blood coagulation by injury leads to vasoconstriction by contraction of smooth muscle fibers in the tunica media. Endothelial cell damage uncovers subendothelial collagen, which thrombocytes bind to by von Willebrand factor (vWF). Tissue factor, which is presented by macrophages, fibrocytes and smooth muscles fiber in the tunica media, kicks off the plasmatic (extrinsic) coagulation cascade (23,25).

1.2.3 Thrombocytes

Thrombocytes (platelets) evolve from megakaryocytes stimulated by thrombopoietin (26). The life cycle is seven to ten days long (26). Thrombocytes increase in number due to massive blood loss or inflammatory processes (26). They lack a cell nucleus, but have limited protein biosynthesis through remains of megakaryocyte mRNA and mitochondria (26). Normal blood concentration is 150000-35000/µl (26). One third of platelets are pooling in the spleen (26).

Inactive thrombocytes remain in a disc shape until they are activated and develop pseudopods and a more spherical morphology (26).

Platelets carry pro- and anti-coagulatory agents in their granules, like tissue factor pathway inhibitor (TFPI), protein C, protein S, α_2-Makroglobulin, factor XIII, factor V, factor XI and calcium (26).

Binding of thrombocytes to vWF and thrombin is done using the GPIb/V/IX-Complex (26). Binding to the wound demands the glycoprotein Ia/IIa (26). Fibrinogen and thrombocytes are linked to each other by the GPIIb/IIIa (26).

Platelet adhesion and aggregation is triggered by collagen, thrombin, thromboxane A_2, ADP, GPIIb/IIIa bound fibrinogen, GPIIb/IIIa bound vWF, and epinephrine (26). Aggregation is counteracted by PGI_2 (26).

Adhesion: Endothelial damage attracts thrombocytes to bind to subendothelial vWF and collagen. Collagen binding to GPIa/IIa activates the thrombocytes, apart from thrombin (26). Activation causes shape change, induces aggregation and thromboxan-A2 production and changes phospholipid configuration in thrombocytes membrane (26).

Aggregation: After adhesion platelets establish a stable binding to each other and the subendothelium via the GPIIb/IIIa receptor and fibrinogen.

Thrombocytes induce a membrane change and switch phospholipids to the surface (flip-flop mechanism), to which coagulation factors can bind to (27). Fibrin (factor Ia) cross-links thrombocytes (26).

1.2.4 Thrombin generation

Thrombin (factor IIa) catalyzes fibrinogen (factor I) to fibrin (fibrin Ia) through a fast proteolytic process (28). Thrombin formation is locally restricted (28). A multienyzme complex is linked to phospholipids in the membrane of platelets by

calcium and catalyzes the proteolysis of prothrombin to thrombin (28). The reactions that are catalyzed by the complex reinforce themselves after a few cycles and have been described as an “amplifier system” (29). The process is referred to as the coagulation cascade.

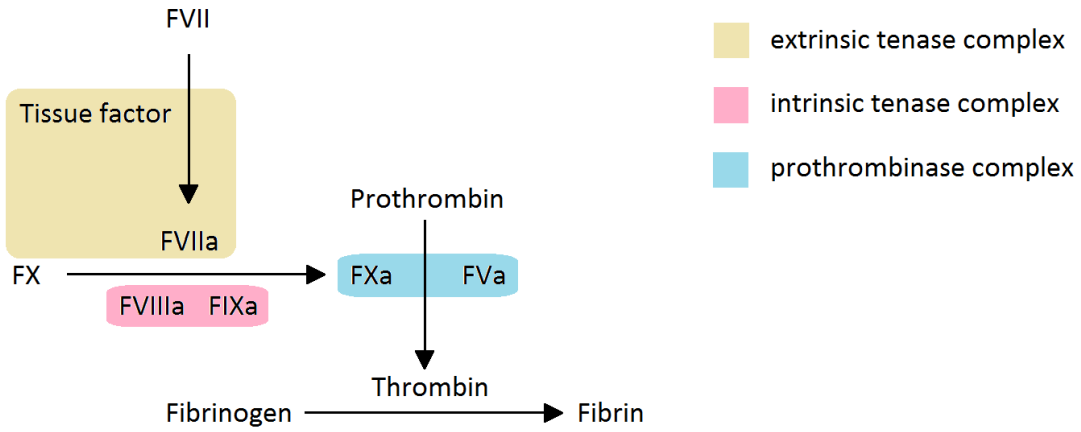

Figure 6: Coagulation cascade, multienzyme complex

The three main enzyme complexes that form thrombin are extrinsic tenase complex, intrinsic tenase complex and prothrombinase complex (see Figure 6) (28). Each complex is linked to the thrombocytes’ membrane and needs calcium and phospholipids for activation. Factor II (prothrombin), factor VII, factor IX and factor X are vitamin K-dependent (28).

The broad belief of a cascade with an extrinsic and intrinsic branch, that cycles through isolated zymogenic reactions, has been replaced by introducing the idea of a cell membrane associated amplification and propagation of thrombin (30). An initial marginal thrombin formation is amplified and reinforces further synthesis of thrombin on the surface of platelets (30).

Amplification: The initial thrombin formation is triggered off by blood reacting with tissue factor. Factor VII is activated and activates factor X (extrinsic tenase complex; see Figure 6) (28). Factor Xa and factor Va (prothrombinase complex; see Figure 6) catalyze prothrombin to thrombin (28). This phase is stopped by tissue factor pathway inhibitor (TFPI) shortly after the initiation (28). The hereby processed amount of thrombin is too weak to form fibrin (28). Thus, traces of thrombin are enough to burst thrombin generation on surrounding thrombocytes (28).

Propagation: Thrombin activated platelets generate increasing amounts of thrombin in sudden bursts (28). Thrombin activates factor V, factor VIII and factor XI (28) (see Figure 7).

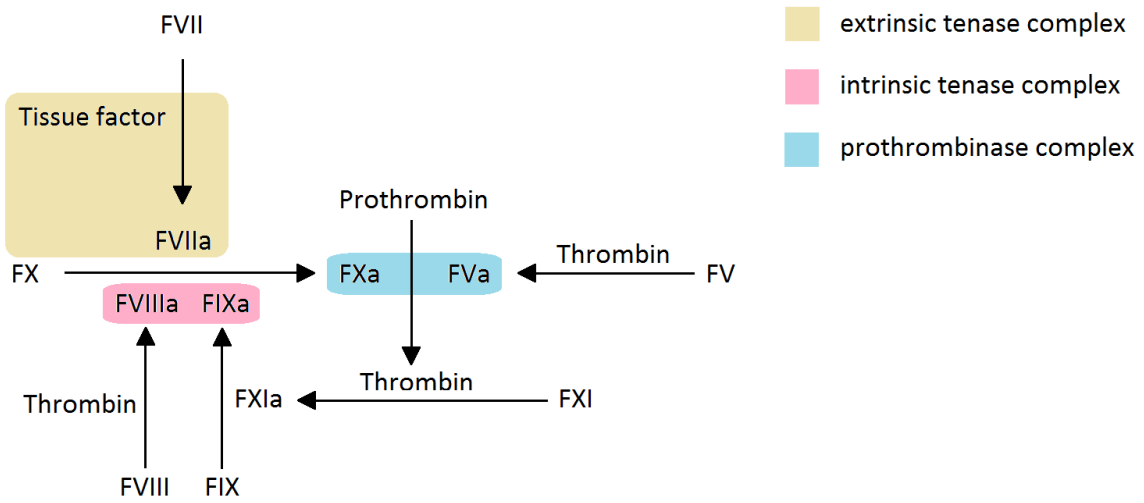

Figure 7: Thrombin activation of intrinsic tenase complex

Factor XIa activates factor IX. Factor IX is also activated by the extrinsic tenase complex (factor VIIa + tissue factor) (28). This is where the traditional extrinsic and intrinsic pathways meet (Josso pathway) (31). After a few cycles the process is accelerated and active thrombin is released into the blood stream (28).
Factor XII is part of the contact system. It is auto-activated by negative cell surface charge or by thrombin, kallikrein and plasmin (28). It is inhibited by C1-inhibitor (28). It activates factor XI and factor VII, and splits plasminogen (28). Factor XII both circulates free in the blood and is associated to the platelet membrane (28). Factor XII and factor XI both accelerate the thrombin generation (28).

1.2.5 Fibringeneration

Fibrinogen is synthesized in the liver and circulates freely in the blood stream and can be bound to platelet membrane and endothelial cells by the GPIIb/IIIa receptor (32). It is also found in the extracellular matrix and extravascular space, serves its purpose for wound healing and plays a role in inflammatory processes as acute-phase protein (32). Fibrinogen influences the blood viscosity (33). Thrombin splits fibrinogen into fibrin, fibrinopeptide A and fibrinopeptide B (see Figure 7). Fibrinogen is water soluble, while fibrin on its own is not (32). Fibrin offers binding

sites to fibrinogen to reach water solubility (32). Chains of fibrin connect to each other composing a net. Factor XIII is activated by thrombin. Factor XIIIa cross-links fibrin fiber to stabilize the mesh and prevent early lysis of fibrin (32). This is most likely done by factor XIIIa linking plasminogen activator inhibitor-1 (PAI-1) and plasminogen activator inhibitor-2 (PAI-2) to the fibrin mesh (34).

1.2.6 Inhibitors of hemostasis

To counteract an uncontrolled bleeding or clotting process, the hemostatic system uses inhibitors. The most important inhibitors belong to the group of serine proteases (serpins) (35). Serpins build an irreversible complex with their substrate in a 1:1 ratio, irreversibly inhibiting the substrate (35). Vitronectin binds serpine inhibitor complexes and either discards them in the vessel wall or passes them to the liver for decomposing (36).

Antithrombin (ATIII) ranks first among physiological inhibitors (35). It mainly inhibits free thrombin by forming the thrombin-antithrombin-complex (TAT) and factor Xa, but also factor IXa, factor XIa, factor XIIa, kallikrein and plasmin (35).

Protein C and protein S inhibit targeted factors (factor VIIIa and factor Va) by limited proteolysis (35). The activation of protein C to form protein Ca is done in two steps (35). Thrombomodulin, an endothelial receptor, binds thrombin to build a thrombin-thrombomodulin-complex (35). This complex activates protein C (35). Protein S enhances protein Ca (35). This mechanism turns thrombin and its pro-coagulatory potential into an anti-coagulatory agent (35).

1.2.7 Fibrinolysis

Fibrinolysis is the process of dissolving an already established clot. It limits the size of blood clots and keeps blood vessels free of blockages. Coagulation and fibrinolysis are in balance under physiological conditions. Plasmin degrades fibrin threads and fibrinogen. Tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA) activate plasminogen to form plasmin. Endothelial cells secrete t-PA and its activity is enhanced in the presence of fibrin. U-PA is found in the urinary tract (25).

Apart from dissolving blood clots, plasmin affects the coagulation by inhibiting prothrombin, factor VIII, factor IX, factor XI and factor XII.

To control overshooting of fibrinolysis effects, they are counteracted by fibrinolysis inhibitors. Plasminogen-activator-inhibitor-1 (PAI-1) is a serpin and claims the leading part of fibrinolysis inhibitors. PAI-1 is produced in endothelial cells and released into the blood. It inactivates t-PA and u-PA immediately. Thrombin-activatable fibrinolysis inhibitor (TAFI) is activated by the thrombin-thrombomodulin complex and stabilizes fibrin by preventing plasminogen and t-PA from binding to fibrin. Plasmininhibitor is bound to fibrin by factor XIIIa. Plasmininhibitor inactivates plasmin very fast. Plasminogen-activator-inhibitor-2 (PAI-2) inhibits t-PA and u-PA and is also connected to fibrin by factor XIIIa.

2 Aims and Objectives

It is generally believed, that long-term immobilization increases the risk for thromboembolic events. But it is still unclear which exact endothelial and hemostatic changes occur during this situation.

Bed rest and simulated weightlessness have effects on the cardiovascular system. Fluid shifts are counterbalanced by adaptations to the blood pressure (18). Equally adaptations regarding the hemostatic system are more than likely. It is expected that the hemostatic system adapts to the immobilization.

This study aims at understanding whether the hemostatic system counteracts a hypo- or hypercoagulable state to maintain equilibrium of hemostasis corresponding to the particular posture of lying in bed.

3 Methods

3.1 MEDES Clinique

The study was carried out at the Institute for Space Medicine and Physiology, MEDES Clinique d'Investigation, Toulouse, France. The Space clinic was founded in 1989 by European engineers and doctors from CNES (Centre national d'études spatiales) in order to develop a European expertise in space medicine and improve healthcare on earth by applying space research to ground healthcare challenges.

The MEDES Clinique is involved in Space missions, clinical research, health applications and telemedicine (37).

The clinic has great experience in carrying out scientific experiments especially bed rest campaigns. The bed rest campaigns at MEDES are usually planned for the duration of 5 days, 21 days or 60 days of complete and strict bed rest.

3.2 Subjects

3.2.1 Number of Subjects

The sample size was 12 to allow for dropouts and still gain significant results.

3.2.2 Recruitment and Selection of subjects

Candidates were recruited using public media, MEDES and ESA homepage announcements. Selection was done in two phases:

- Preliminary selection (Based on the candidates application form):
  Subject's way of life, educational level, professional experience, personal and family medical history
- Selection session (at MEDES Space Clinic):
  Comprehensive clinical and paramedical examination

Inclusion criteria

- Healthy male volunteer (see 5.2.2.1 for description of medical tests and laboratory analysis performed at the selection visit)
- Age 20 to 45 years

- No overweight nor excessive thinness with BMI (weight Kg/ height m^2) between 20 and 26
- Height between 158 cm and 190 cm
- No personal nor family past record of chronic or acute disease or psychological disturbances which could affect the physiological data and/or create a risk for the subject during the experiment
- Fitness level assessment:
    - if age < 35 years: 35 ml/min./kg < VO_2max < 60ml/min./kg
    - if age > 35 years: 30 ml/min./kg < VO_2max < 60ml/min./kg
- Active and free from any orthopedic, musculoskeletal and cardiovascular disorders
- Non smokers
- No alcohol, no drug dependence and no medical treatment
- Covered by a Social Security system
- Have signed the information consent
- Free of any engagement during the three hospitalization planned periods

Non-inclusion criteria

- Past record of orthostatic intolerance
- Cardiac rhythm disorders
- Chronic back pains
- History of hiatus hernia or gastro-esophageal reflux
- History of thyroid dysfunction, renal stones, diabetes, migraines
- Past records of thrombophlebitis, family history of thrombosis or positive response in thrombosis screening procedure
- Abnormal result for lower limbs echo-doppler
- History of or active claustrophobia
- History of genetic muscle and bone diseases of any kind
- Bone mineral density: T-score ≤ -1.5
- Osteosynthesis material, presence of metallic implants
- History of knee problems or joint surgery/broken leg
- Poor tolerance to blood sampling
- Having given blood (more than 8ml/kg) in a period of 8 weeks or less before the start of the experiment

- Special food diet, vegetarian or vegan
- History of intolerance to lactose or food allergy (milk proteins...)
- Positive reaction to any of the following tests: HVA IgM (hepatitis A), HBs antigen (hepatitis B), anti-HVC antibodies (hepatitis C), anti-HIV_{1+2} antibodies
- Echocardiography: inappropriate thoracic acoustic window
- Subject already participating or in the exclusion period of a clinical research
- Refusal to give permission to contact his general practitioner
- Incarcerated persons
- Non-compliant subjects or subjects who will most likely be non-compliant during the study, maybe due to language problems or poor mental health
- Subject who has received more than 4500 Euros within 12 months for being a research subject
- Subject under guardianship or trusteeship

Drop-out criteria:

- Recent illness or disease
- Evidence that the continuation of the study would not be justified
- At any time upon request by the participant

3.2.2.1 Selection description

3.2.2.1.1 Medical examination

The medical check-up included:

A medical and surgical history including habits life, alcohol, caffeine, tobacco consumption, previous medication, a complete clinical examination, a measurement of systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) in both supine position (after the subject has rested comfortably for at least 10 minutes) and standing position after 3 and 10 minutes (Stand Test). These tests will check the absence of orthostatic hypotension, a familiarization test with the Tilt table, a 12 leads electrocardiogram, a measurement of maximal oxygen consumption (VO_2 max test), an interview with a psychologist, a DEXA measurement of the bone density, an alcohol breath test and a biological screening:

Biochemistry

Fasting glucose, sodium, potassium, chloride, calcium, HCO_3^-, total protein, albumin, total cholesterol, HDL cholesterol, LDL cholesterol, triglyzerides, urea, creatinine, total bilirubin, asparatate aminotransferase (ASAT / SGOT), alanine aminotransferase (ALAT / SGPT), alkaline phosphatase, gamma glutamyl transferase (GGT), uric acid, C-reactive protein (CRP), TSH.

Hematology

Cell blood count (CBC), platelets, reticulocytes, fibronogen, PTT, PT, phlebitis markers (anti thrombin III, S-protein, C-protein), molecular screening (research for the factor V Leiden mutation and the mutation 20210 of the prothrombin gene). A positive result for phlebitis markers or the presence of one of these mutations will be considered as a non inclusion criteria and this information will be given to the subject during a consultation with a hematologist.

Vitamins and mineral status

Fat-soluble vitamin: retinol, retinyl-palmitate, beta-carotene, apha-carotene, serum phylloquinone, α-tocopherol, gamma-tocopherol.

Water-soluble vitamin status: erythrocyte glutatione reductase, vitamin B6, red cell folate, vitamin C, vitamin B12, 25 OHD (Vit D).

Serum iron, ferritin, transferin saturation, transferin receptor, selenium, copper and zinc.

Serology

Search for the hepatitis markers (A, B, C) and serological HIV (the volunteers are informed of the realization of these researches before the test).

Urine drug screen

Nicotine, barbiturates, benzodiazepines, opiates and cannabis.

Urinalysis

Urinalysis is made on strips: hematuria, leucocyturia, glucose, ketonuria, proteinuria, bilirubine, urobilinogen, nitrites, pH, and density.

Echo-doppler measurements of the lower limbs were performed in order to eliminate the venous deficiencies, chest radiography front and side, cardiac US scan tests were performed in order to make sure of the quality of the collected images (appropriate acoustic window).

3.2.2.1.2 Psychological screening

Each candidate was psychologically screened beforehand to detect persons with psychopathological tendencies and also to find candidates with the best suitable psychological profile to participate in the experiment. The tests were conducted in written form. Each subject was asked to write a biography and a personal history. Additionally a standardized personality test was done, all under the supervision of a skilled psychologist. The screening ended with a face to face interview.

3.3 Ethical Considerations

The local ethics committee at MEDES, Toulouse, France gave their ethical approval prior to the conduct of this study. Informed consent was obtained.

3.4 Study Design

This prospective study investigated the effects of strict medium term bed rest (following the ESA framework for bed rest studies) on the coagulation system (22). 12 healthy male subjects underwent 21 days of continuous 6 degrees head-down bed rest, which serves as a model for simulated cardiovascular weightlessness/microgravity and induces space flight deconditioning (19). Subjects are lying down with their heads 6 degree below horizontal, shown in Figure 8.

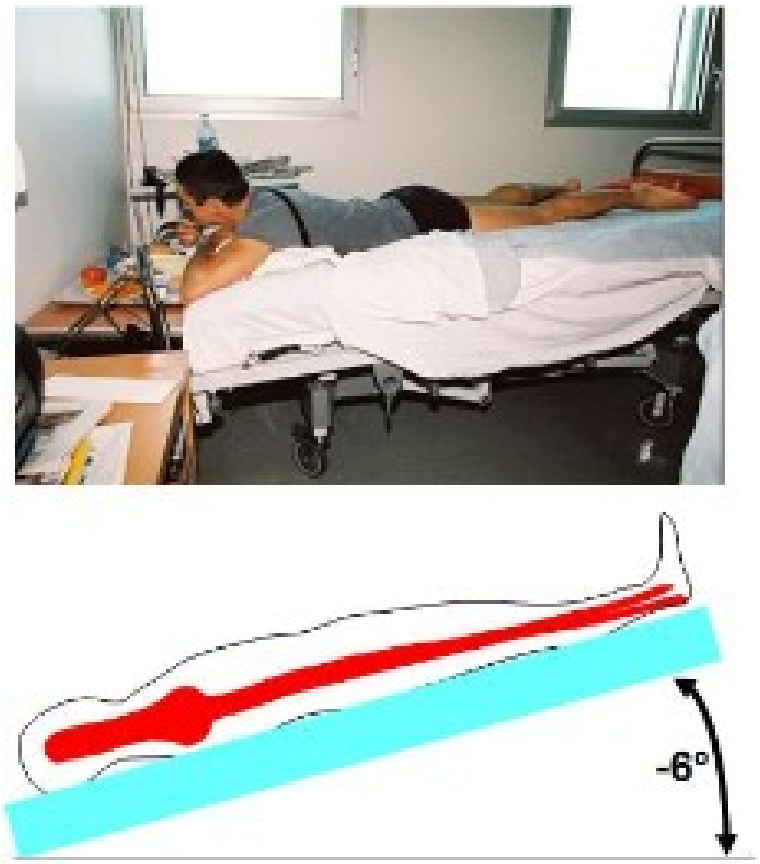

Figure 8: 6 degrees head-down tilt posture, obtained from MEDES – Institute for Space Medicine and Physiology (38)

The effects on the cardiovascular system are similar to those of long time immobilization.

The subjects arrived at the MEDES clinic 7 days before the bed rest started for collection of baseline data and left on the 7th day post bed rest.

Blood was collected in the morning hours between 7 a.m. and 8 a.m. 5 days before the bed rest started, on the 2nd, 7th, 14th, 21st day during 6 degrees head-down tilt bed rest, 1st day after the bed rest and the 2nd day of recovery. The blood collection on the 1st day after the bed rest (R 0) was in fact still in head down position, just before the subjects were tilted supine and were mobile again.

Table 1: Blood sampling time points

Pre – bed rest	Bed rest				Post – bed rest	
Day -5	*Day 2*	*Day 7*	*Day 14*	*Day 21*	*Day 0*	*Day 2*
			Code:			
BDC-5	HDT+2	HDT+7	HDT+14	HDT+21	R 0	R+2

Citrated blood samples were centrifuged and the platelet poor plasma was analyzed. CT, CFT, MCF and α-angle were measured by thrombelastometry and concentrations of TAT and F1+2 using ELISA. Quantitative one-stage photometric assays were carried out on factor II, factor VII and factor VIII using Behring Coagulation Timer (BCT®). ETP, Lag Time, Velocity Index (Slope), Thrombin Peak and Time to Peak were performed using a thrombin generation assay on the Calibrated Automated Thrombography (CAT).

3.5 Measurements and Calculations

3.5.1 Blood Collection and preparation of plasma

The blood sampling took place five days before the bed rest started (BDC-5), on the 2nd (HDT+2), the 7th (HDT+7), the 14th (HDT+14), the 21st (HDT+21) of bed rest and finally the day the subjects stood up from bed (R 0) and the 2nd day of recovery (R+2), see Figure 9. Blood collection was carried out in the mornings between 7 a.m. and 8 a.m., venous 17G-1.4x40mm Teflon catheter in the antecubital vein and Greiner VACUETTE® coagulation tubes 4,5mL were used. The VACUETTE® contained 500 µl of 3,8% sodium citrate and was filled with blood. The whole blood tube was centrifuged with 2000 g for 20 minutes at room temperature. 500µl of plasma were taken from the supernatant and transferred into up-to five labeled Eppendorf tubes depending on the plasma volume and immediately frozen and stored at -80° C.

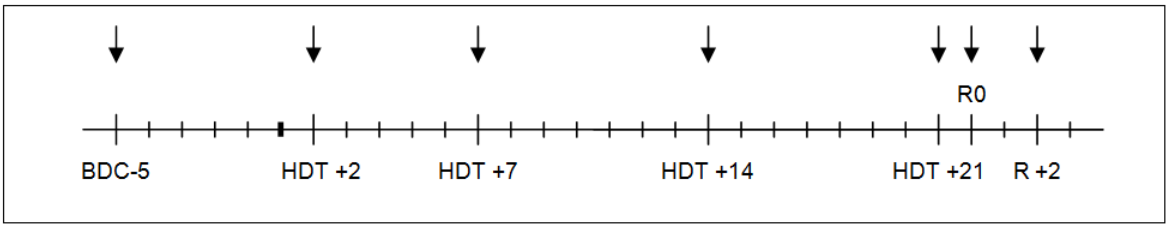

Figure 9: Days of blood sampling (arrows)

Due to the logistics of this study the blood samples had to be centrifuged, frozen and stored in Toulouse for the duration of the campaign and were subsequently shipped frozen to Graz for analysis. For this reason it was impossible to use whole blood. The time frame within to analyze whole blood would have been excessively overrun due to the logistical challenge. However, platelet poor plasma was a sufficient source for the investigation. So the results concentrated on the secondary hemostasis.

3.5.2 Thrombelastometry

3.5.2.1 ROTEM® 05 – device description

Rotational thrombelastometry (ROTEM®) is a method used for analyzing hemostasis in vitro and graphically displaying the development of the viscoelasticity in whole blood and plasma over time when inducing pro- and anti-coagulatory activity (39). Effects of coagulation factors, pro-coagulatory effects, fibrinolysis and each of their inhibitors are recorded over time. The ROTEM® 05 (Matel Medizintechnik, Graz, Austria) was used for the measurements and is based on the thrombelastography (TEG®) which was invented by Professor Helmut Hartert in 1948 (40). Since then it has been further developed and has reached its importance as a clinical application (e.g. point-of-care-testing) (39,41). Compared to the conventional thrombelastography (TEG®) the rotational thrombelastometry, which the ROTEM® 05 is based on, is not sensitive to vibrations and/or kicks against the chassis (42).

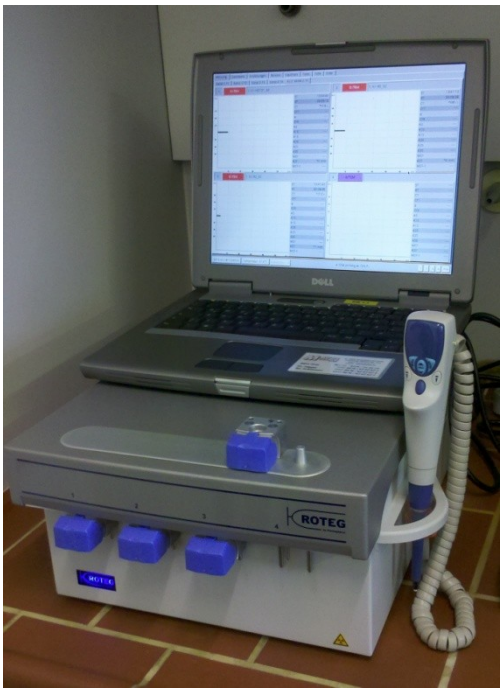

Figure 10: ROTEM® 05

The ROTEM® 05 consists of the measurement device including an attached laptop, see Figure 10. The device is controlled via a software interface which communicates between the device and the laptop. The data is saved on the laptop and the results can be retrieved later. The ROTEM® 05 has a heated pre-warming (37° C) position, on which the blood samples are prepared for the measurement. An electronic pipette is attached to the device to minimize alteration due to manual pipetting. The device also offers to connect a printer to directly print results.
The ROTEM® 05 has four measuring channels which can be used simultaneously and help increase the workflow (39).

Usually whole-blood (300µl), which is anti-coagulated with citrate prior to usage, is filled into a small cuvette, which is placed into a heated (37° C) metal cuvette holder. The coagulation process is triggered off by adding reagents like tissue factor to the blood. A thin sensor pin is dipped into the cuvette and oscillates back and forth (4,75°) around its axis. When the clotting begins, the viscosity increases due to long intertwining fibrin threads aggregating with platelets around the pin. This affects the oscillation. Increased clot firmness leads to increased restriction of the rotating movement of the pin (see Figure 11) (39).

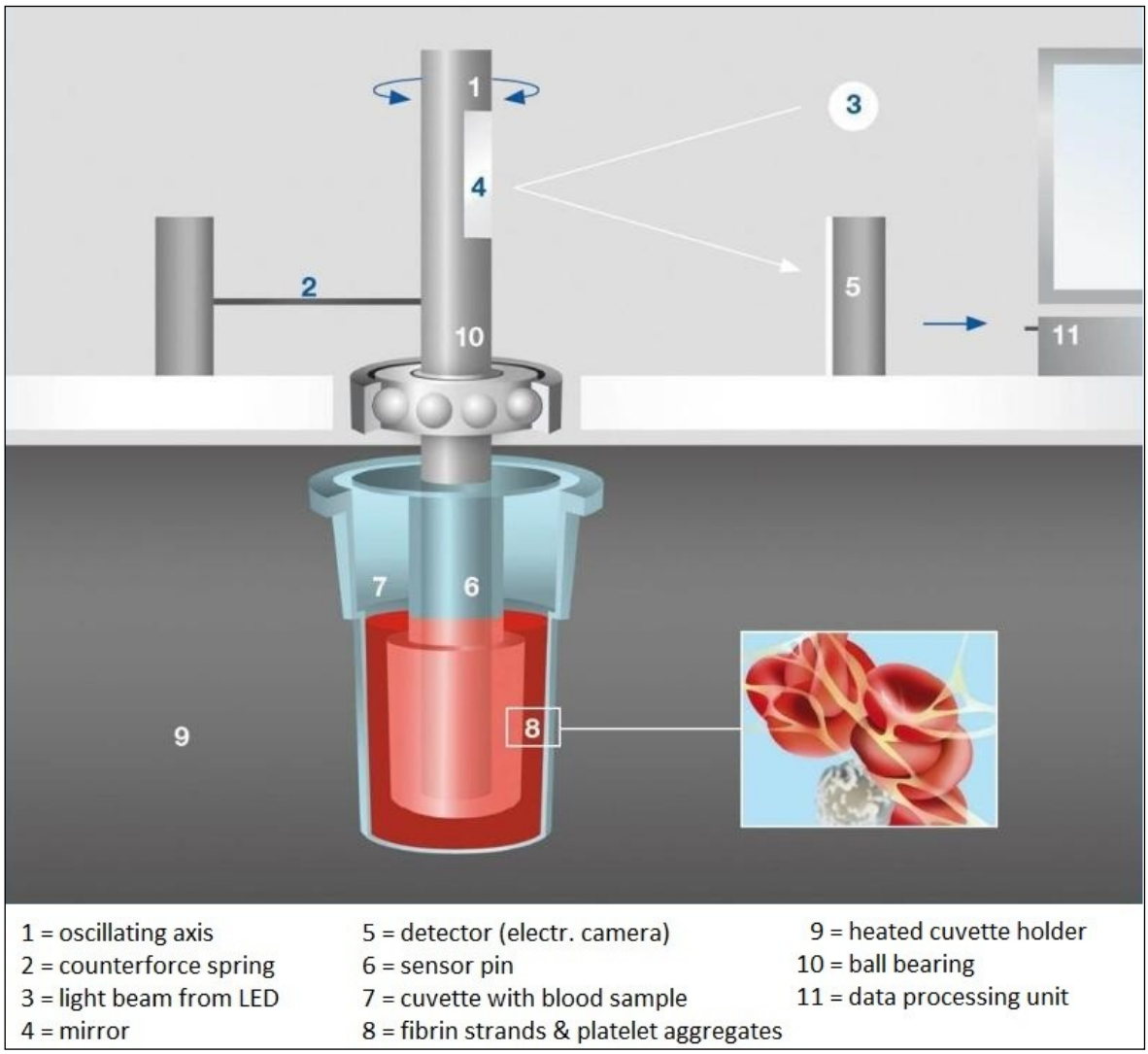

Figure 11: Measuring Principle, obtained from TEM International GmbH (43)

These changes are recorded by an optical detector, evaluated by a software and are graphically displayed.

3.5.2.2 Thrombelastometry – parameters and assays

If the pin turns without restriction the amplitude is 0mm. When the clot builds up, the amplitude increasingly rises until it reaches its maximum which reflects the maximum clot firmness (MCF) in millimeter. The coagulation time (CT) is the duration between adding coagulation activating agents (e.g. tissue factor) and the initial forming of the clot, i.e. >2mm amplitude. The time it takes for the amplitude to reach 20mm, is called the clot formation time (CFT). The α-angle is defined as the angle between the tangent to the curve and the zero baseline just at the end of the clot formation time. It describes the kinetics of the build-up of the clot and

cross-linking (8). If the analysis period exceeds 30 minutes and more, the fibrinolytic parameters Lysis Index 30 (LI 30), Lysis Index 60 (LI 60) and Maximum Lysis (in percentage of MCF) can be recorded (see Figure 12) (39,44). The time period between starting the test and reaching MCF is considered the coagulation period, which is then followed by the fibrinolysis phase (45).

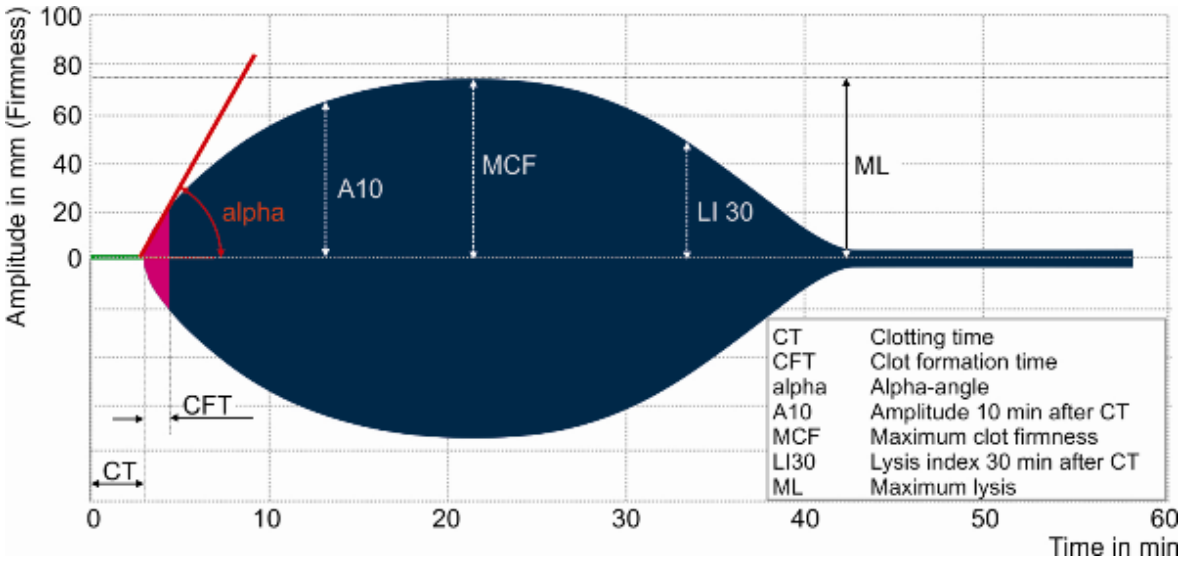

Figure 12: Thrombelastometry analysis, obtained from TEM International GmbH (46)

The thrombelastometry was initially designed to use native whole blood. In a clinical context however blood is often influenced by heparin, hemodilution or oral anticoagulants. Also the usage of citrated blood, which is a common laboratory coagulation assay, helped to improve the importance of the test by standardizing the method (39,42). The ROTEM® 05 therefore provides 5 different assays (EXTEM, INTEM, HEPTEM, FIBTEM, APTEM) to appropriately adapt the measuring process to the coagulation influencing agents and gain results and a concluding diagnosis fast (39).

Table 2: ROTEM-assay and clinical field of application (39,47)

EXTEM	Extrinsic activation through tissue-factor, not influenced by aprotinin or heparin
INTEM	Intrinsic activation, sensitive to heparin
HEPTEM	Heparinase added agent antagonizes heparin effect. Heparin effect can be evaluated when comparing HEPTEM to INTEM
FIBTEM	Inactivation of thrombocytes; plasmatic influence on the clot firmness
APTEM	Aprotinin inhibits early lysis; superior test for hyperfibrinolysis compared to D-dimer testing

CT can be prolonged due to heparin effect, hemodilution or reduced coagulation factors and can be considered the equivalent to activated partial thromboplastin time (aPTT) or prothrombin time (PT) (39). PT and aPTT are global tests. PT describes the time period from the activation of factor VII to the building of fibrin (48). APTT captures the activity of all enzymatic processes involved in the coagulation cascade except for factor VII and measures the time until fibrin formation (48). A reduction of α-angle (or an elevated CFT) states lack of fibrinogen. MCF is a marker which reflects the platelet function. If the firmness of a clot is weak and the MCF is low, it refers to a reduced platelet function (45). The relation between these parameters and the anti- or pro-coagulatory effects are summarized in Table 3.

Table 3: ROTEM parameters, effect on coagulation

	Pro-Coagulatory effect	**Anti-Coagulatory effect**
CT	↓	↑
CFT	↓	↑
MCF	↑	↓
α-angle	↑	↓

↑: prolonged/elevated
↓: reduced/diminished

The reference values are dependent on the assay, which is used for analysis; see Table 4.

Table 4: ROTEM reference values, obtained from Possibilities and limitations of thrombelastometry/-graphy (39)

	CT (s)	CFT (s)	MCF (mm)	α-angle
INTEM	100-240	30-110	50-72	70-83
EXTEM	38-70	34-160	50-72	63-83
FIBTEM	-	-	8-24	-
HEPTEM	equivalent to INTEM/EXTEM			
APTEM				

3.5.2.3 Thrombelastometry – measurement of MCF, CT and α-angle

As mentioned above the blood sampling and centrifugation was carried out at the MEDES Clinique in Toulouse, France and the thrombelastometry measurements were done at the laboratory of the Physiological Chemistry Department of the Medical University Graz, Austria. Therefore platelet poor plasma was the substrate for the measurements. EXTEM assay was used to kick off the coagulation. The 'activation solution' had a volume of 40µl and contained 3.5µg/ml collagen, 0.35pmol/l TF (Innovin®, Dade Behring Marburg GmbH), 3mmol/l CaCl2. The sample volume was 340µl in total, 300µl of PPP and 40µl of 'activation solution'. The pipetting was done manually. Every sample analysis was run for 60 minutes.

3.5.3 ELISA – Enzyme-linked Immuno Sorbent Assay

3.5.3.1 ELISA-principle

The Enzyme-linked immuno sorbent assay (ELISA) is an immunological biochemical test. It is an immunoassay which was first described by Peter Perlman and Eva Engvall in 1971 (49). The principle was derived from the radioimmunoassay, which had many disadvantages due to the difficult handling of the radioactivity involved (50). Those were solved with this technique which highlights the targeted molecule and in this way reveals its concentration by an enzyme-catalysed color changing reaction (51).

Immunoassays utilize the antibody-antigen reaction principle. In most cases an antibody or immunoglobulin is used which detects a target molecule (analyte) with its specific binding site and binds that analyte (52). It is used on biological fluids, such as urine, blood or serum (52). Immunoassays are very specific and highly sensitive towards measuring concentrations (52).

The ELISA is mostly used to analyse the pure existence or quantity of viruses, proteins, hormones or toxins in bodily fluids (52). In the ELISA either the antigen or the antibody is marked with an enzyme like horseradish (HRP), alkaline phosphatase (AP) or glucose oxidase (GOD). After the incubation antibodies are bound to the antigen. In a second step, a chromogenic substrate like for example para-Nitrophenylphosphate for AP is added (52). AP catalyses the reaction towards para-nitrophenol and induces a color change from colorless to yellow (52). The intensity is measured using a spectrophotometer at a certain wavelength and the concentration can be calculated using Beer–Lambert law.

For the analysis of the F1+2 and TAT concentration only direct sandwich type ELISA were used (53). This method is a non-competitive ELISA test using two antibodies. The first antibody (coating-antibody) is fixed to a plate in a known concentration. The antigen (target molecule) is added and incubated. After the incubation the plate is washed. Only antigen which is bound to the coating antibodies remains on the plate. Now the second antibody (detection-antibody) which is carrying the enzyme is poured over the plate. The detection antibody also binds the antigen, but at a different epitope than the coating-antibody. After a second wash, the chromogenic substrate is added and the color change is measured, as shown in Figure 13 (52).

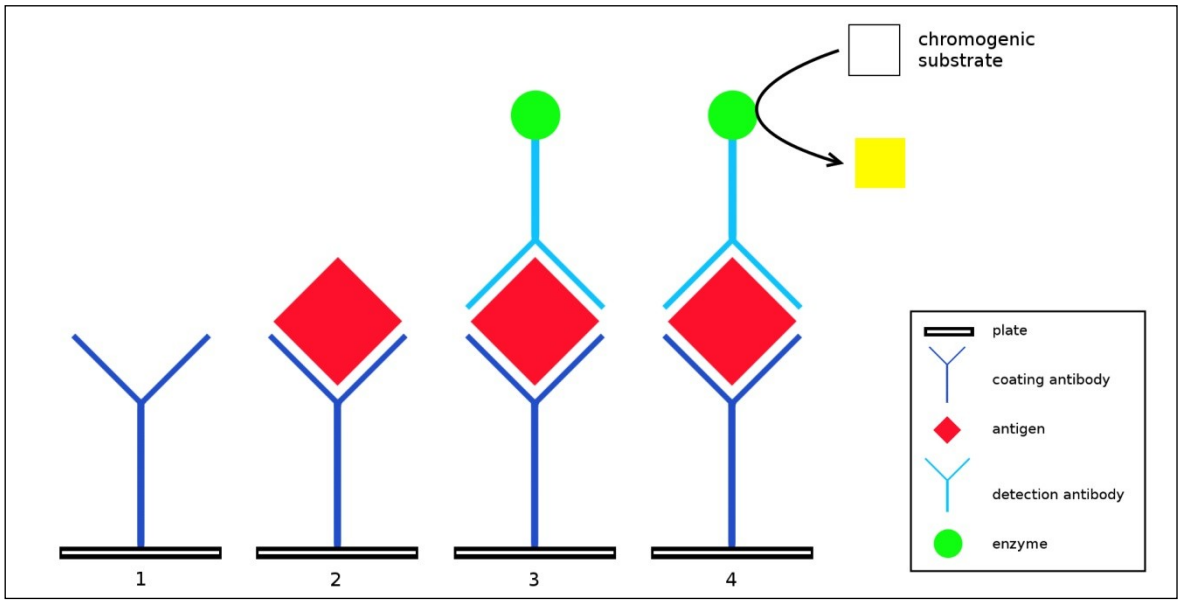

Figure 13: Non-competitive direct sandwich ELISA

3.5.3.2 Measurement of F1+2 concentration

Prothrombin (coagulation factor II, FII) is the vitamin K dependent zymogen of thrombin (coagulation factor IIa, FIIa). Factor Xa (FXa) splits prothrombin into α-thrombin and prothrombin-fragment 1 and 2 (F1+2), see Figure 14. Each prothrombin reacts to one α-thrombin and one F1+2. Therefore the concentration of F1+2 equals the concentration of thrombin.(54)

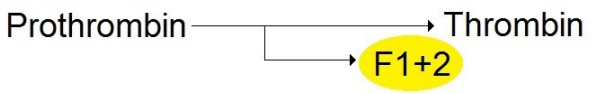

Figure 14: Thrombin activation

Prothrombin-fragment 1 carries the calcium- and phospholipid-binding fraction of prothrombin, whereas prothrombin-fragment 2 interacts with coagulation factor V. This test has been mainly developed to reveal hypercoagulability. Abnormal low concentrations of F1+2 have not been reported and are not clinically relevant.
The measurement was carried out using the ELISA-kit Enzygnost® F1+2 micro, Dade Behring, Marburg, Germany.
During the first incubation the fixed coating-antibodies bind F1+2. After the wash the detection-antibodies connect to the prothrombin-fragment 1 of F1+2. The detection antibodies carry peroxidase. The chromogen is added and the color intensity is measured at 492nm. The intensity is proportional to the F1+2 concentration (53).
The reference range of this test is 69-229 pmol/l (53).

3.5.3.3 Measurement of TAT

Antithrombin (ATIII) is the most important physiological inhibitor of the hemostasis. It inhibits Thrombin, FXa and most of other coagulation factors. The inhibition is slow and irreversible. Antithrombin reacts with thrombin and forms the thrombin-antithrombin-complex (TAT) in a 1:1 ratio, see Figure 15 (55). The TAT concentration reflects initial thrombin concentration. The TAT ELISA test is directed to diagnose hypercoagulability, and is only common for scientific purposes and not for clinical use. Abnormal low concentrations of TAT are without clinical relevance (56).

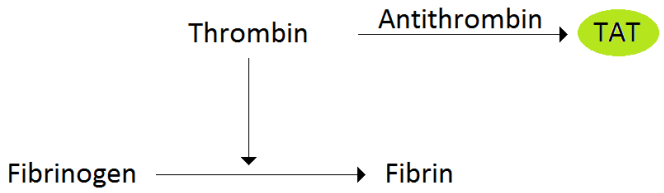

Figure 15: Thrombin-Antithrombin reaction

The measurement was carried out using the ELISA-kit Enzygnost® TAT micro, Dade Behring, Marburg, Germany.

The coating antibody binds the thrombin domain of TAT. After the wash the detection antibody, which carries peroxidase, reacts with the antithrombin III fraction of TAT. The extinction is measured at 492nm. The intensity is proportional to the TAT-concentration (53).

The reference range of this test is 2,0-4,2 µg/l (53).

3.5.3.4 Measurement of tissue plasminogen activator (t-PA)

Tissue plasminogen activator (t-PA) is a serine protease and the most important activator of plasminogen and inducts fibrinolysis. It is built in endothelial cells and stimulated by venous stasis. The plasma concentration in vivo underlies a circadian rhythm, with the lowest concentration during the night and the morning hours.

T-PA determination was done using IMUBIND tPA ELISA kit from American Diagnostica (Pfungstadt, Germany).

The reference range of t-PA in plasma is 1,4-8,4 µg/l (57).

3.5.4 One-stage PT and APTT method procedure for single factor determination

The one-stage method is based on measuring the clotting time of a sample. The more coagulation factors there are in a sample the faster it clots. If clotting factors are less or not present at all, the time it takes for a clot to form increases. The clotting is dependent on the conversion of fibrinogen to fibrin by thrombin. The development of fibrin is less in plasma with deficient coagulation factors.

Dependent on which factor is the target, either the prothrombin time (PT) (for Factor II, V, VII, X) or the activated partial prothrombin time (APTT) (for factor VIII, IX, XI) is examined.

In order to get the concentration of a single coagulation factor in a certain plasma sample, the PT or APTT of factor deficient plasma is measured and compared to the sample. Factor deficient plasma lack just one single coagulation factor. Therefore the PT or APTT will be increased. Once the PT or APTT of the deficient plasma is measured in a dilution series, the procedure is repeated with the sample plasma added to the deficient plasma. With rising sample plasma concentration, PT or APTT continuously decreases if the factor concentration is normal. Results are plotted on logarithmic-linear graph paper, as the relationship between APTT/PT and the serial dilutions are exponential. The lines should appear parallel. The sample plasma PT or APTT is then compared with a reference plasma dilution series.(54,58-60)

Factor II, factor VII and factor VIII were measured using the Behring Coagulation Timer (BCT®) (60).

3.5.5 Thrombin generation

Thrombin generation tests monitor the activity of thrombin over time. It results in a graphical curve from which further information on the process of the coagulation cascade can be derived (see Figure 16). In contrast to classical coagulation tests, which only measure time until the first appearance of thrombin, thrombin generation tests provide information on almost any factors influencing the cascade. In a clinical context this test provides a great advantage by not only detecting hypo- as well as hypercoagulability but also monitoring the influence of coagulation modifiers. Nevertheless the interpretation of the results requires skill and practice due to numerous influences on test conditions and measurands.

The test gathers more and more importance in drug testing regarding security and effectiveness (61).

3.5.5.1 Thrombin generation – method

To monitor the thrombin generation the coagulation system has to be activated carefully by a small amount of clotting activator like tissue-factor. The slow

activation ensures a more accurate result and mimics the in vivo coagulation as physiologically as possible. Today thrombin activation can be carried out continuously as fluorogenic assays were introduced and the accuracy was improved by the so called calibrated automated thrombogram (CAT). Technically this method does not measure the concentration of thrombin, but its ability to catalyze a reaction. Nevertheless it reflects the thrombin concentration. Therefore it respects direct thrombin inhibitors as well as it gives information on each reactant. In vivo thrombin catalyzes the reaction from fibrinogen to active fibrin monomers and is subsequently followed by clot formation. In this test the conversion rate of a fluorogenic substrate is measured using a photometer. Each plasma sample needs to be controlled by its own calibrator measurement because there is no direct correlation between the intensity of the fluorogenic signal and the thrombin concentration. Also the influence of the color of the plasma can have disturbing impacts on the fluorogenic intensity (61-64).

3.5.5.2 Thrombin generation – variables

As shown in Figure 16 after adding tissue factor at time 0 min, thrombin concentration exponentially increases after a certain lag time, followed by a rather linear growth until it reaches its maximum activity at the thrombin peak. The concentration of thrombin then continuously falls until there is no free thrombin available.

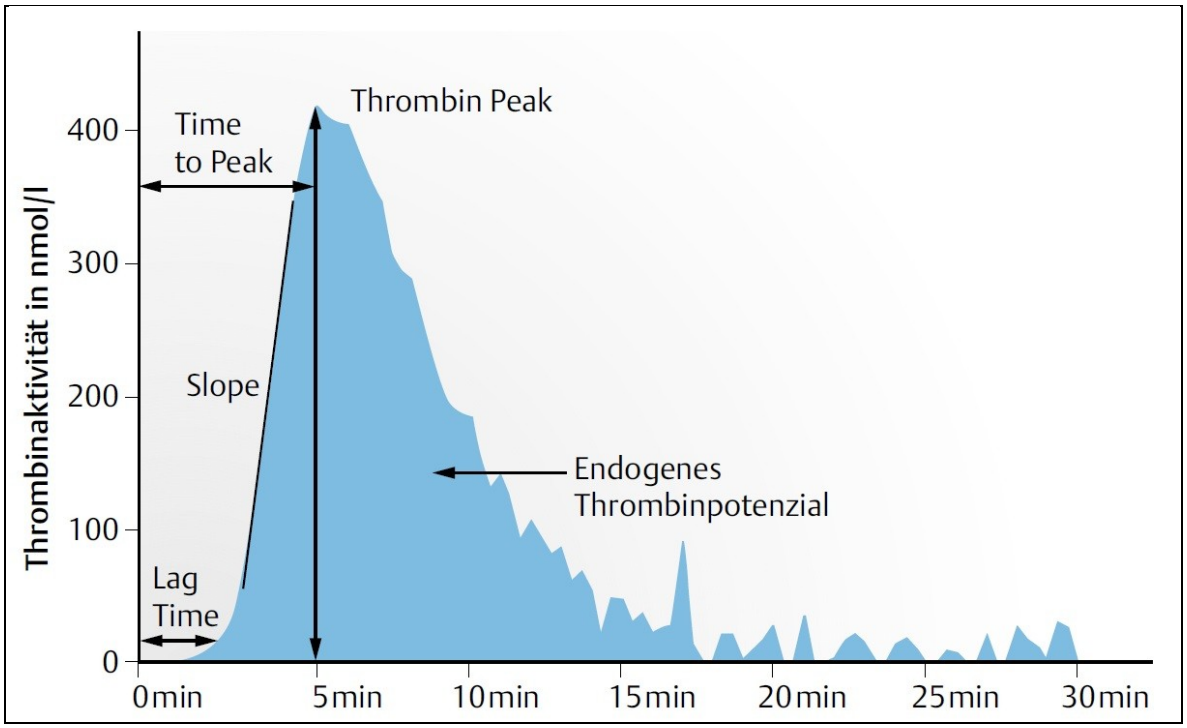

Figure 16: Thrombin generation curve, obtained from *Das Gerinnungskompendium: Schnellorientierung, Befundinterpretation, klinische Konsequenzen* by Barthels (61)

The variables of this curve are:

Lag Time: Time between adding the start reagent and the building of thrombin (minutes or seconds)

Time to Peak (TTP): Time between adding the start reagent and the reaching of the maximum activity of thrombin (minutes or seconds)

Thrombin Peak (TP): Maximum thrombin activity (nmol/l)

Endogenous thrombin potential (ETP): Area under the curve which can be referred to as the total amount of thrombin

Slope (Velocity Index): Build-up speed of the curve (nM/min); maximum or mean slope

Start Tail: Time between peak and half of peak (min) (descending) (65)

3.5.5.3 Measurement of ETP, Lag Time, ttPeak and Peak

Calibrated automated thrombography (CAT) (Thrombinoscope BV, Maastricht, the Netherlands) was used for monitoring thrombin generation curves. The thrombin generation of each sample was analyzed to get values for the parameters ETP, lag time, TTP, Slope, TP and start tail.

Five pmol/l of Tissue Factor (TF) was added to the samples to induce the coagulation (8).

Table 5: Thrombingeneration; reference values, obtained from *Das Gerinnungskompendium: Schnellorientierung, Befundinterpretation, klinische Konsequenzen* by Barthels (61)

Thrombingeneration Reference values	
ETP (nM/min)	1693-2629
Lag Time (min)	1,1 – 5,1
Time to Peak (min)	5,2 – 9,8
Thrombin Peak (nM)	225 – 545

3.6 Statistics

Calculations were performed by SPSS 18.0 (SPSS Inc., Chicago, Illinois, USA) and Microsoft Excel 2007 (Microsoft Inc.). Data are presented as mean ± SD. Differences between the sampling points were analyzed by means of t-test for independent samples in case of normally distributed variables, otherwise the Mann-Whitney U-test was applied (66). A P-value less than 0.05 was considered as statistically significant. $P < 0,05$ (*), $p \leq 0,01$ (**), $p \leq 0,001$ (***), $p \leq 0,0001$ (****).

4 Results

All 12 subjects successfully completed the 21 days of strict 6 degrees head down bed rest.

4.1 Anthropometrics and age of the subjects

The 12 subjects were all male, 34,25 years old on average with a standard deviation of ±8,34 years. The average height was 1,76 m ±0,06 m and the average weight was 69,8 kg ±8,0 kg. The average Body Mass Index was 22,4 ±1,7. The mean average BMI was 22,2.

Table 6: Height, weight and age of the subjects

Subject	Age	Height (m)	Weight (kg)	BMI
A	44	1,75	76,3	24,9
B	40	1,69	61,1	21,4
C	42	1,77	78,5	25,1
D	36	1,90	80,7	22,4
E	41	1,72	59,0	19,9
F	41	1,69	62,8	22,0
G	40	1,77	71,2	22,7
H	20	1,77	65,0	20,7
I	24	1,75	64,2	21,0
J	25	1,74	71,8	23,7
K	29	1,84	81,8	24,2
L	29	1,75	65,1	21,3

4.2 Raw data results of each subject

The results of the measurements of all parameters of subject A to subject L are shown in Table 29 to Table 40 in the appendix.

4.3 Thrombelastometry parameters

The following parameters were measured using thrombelastometry: Coagulation time (CT), Clot formation time (CFT), Maximum clot firmness (MCF) and α-angle (α). Only CT and α-angle showed statistically significant changes.

4.3.1 Coagulation time

Coagulation times (CT) have been measured using thrombelastometry and EXTEM assay. CT gives information on the extrinsic system and reveals loss of clotting factors. Increased values reflect pro-coagulatory state. Table 7 shows results for each sampling point. Mean, standard deviation and p-value are listed in Table 8 and graphically displayed in Figure 17.

The coagulation time increasingly rises and reaches its maximum of 826,833 ± 396,733 sec (P = 0,0017) on the 14th day (HDT+14) of bed rest. On the 21st day (HDT+21) it falls back and continually drops down to 587,083 ± 0,0113 sec (P = 0,0113) until the end of the bed rest, but does not reach baseline. On the 2nd day of recovery (R+2) the CT was almost twice as high as baseline.

Table 7: Coagulation time [s] results

Subject	BDC-5	HDT+2	HDT+7	HDT+14	HDT+21	R 0	R+2
A	202	227	206	487	484	211	572
B	258	374	374	656	752	322	453
C	251	551	457	1864	462	401	306
D	323	380	917	939	557	605	721
E	350	788	803	588	530	429	583
F		772	711	487	766	699	448
G	482	729	1122	859	786	665	900
H	279	491	650	506	622	556	604
I	216	539		593	505	336	1331
J	518	723		1073	742	730	794
K	668	1171	884	1139	1038	1546	812
L	390	736	1055	731	815	545	787

Table 8: Coagulation time [s]; mean, standard deviation (STD), p-value

Sampling point	Mean	STD	P-value
BDC-5	357,909	145,458	
HDT+2	623,417	250,683	0,0002
HDT+7	717,900	298,969	0,0020
HDT+14	826,833	396,733	0,0017
HDT+21	671,583	173,404	0,0000
R 0	587,083	343,242	0,0113
R+2	692,583	267,298	0,0016

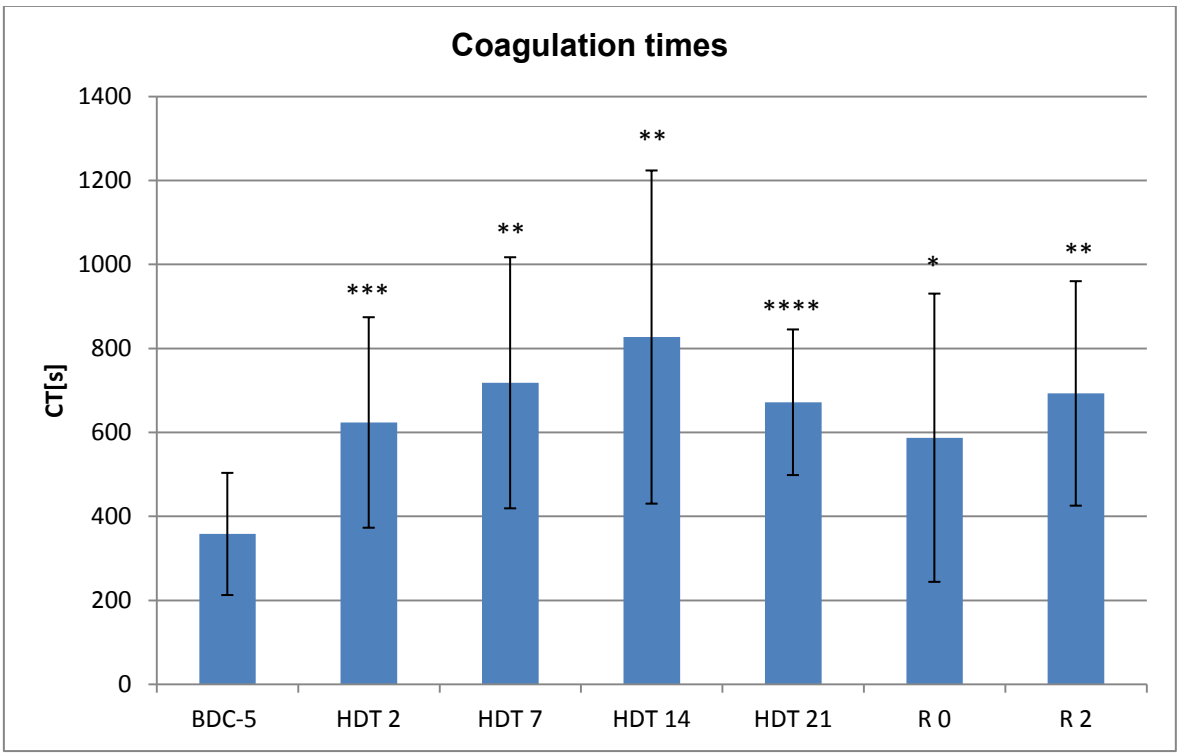

Figure 17: Coagulation times [s]

4.3.2 α-angle

The α-angle value gives information on the availability of fibrinogen in the plasma. Table 9 shows α-angle results at each sampling point for each subject. Mean, standard deviation and p-value are listed in Table 10 and graphically displayed in Figure 18. Every sampling point showed decreased values for α-angle during bed rest and even on the second day of recovery.

Table 9: α-angle [°] results

Subject	BDC-5	HDT+2	HDT+7	HDT+14	HDT+21	R 0	R+2
A	59	51	43	33	21	44	16
B	41	25	22	27	14	35	34
C	18	6		5	16		
D	34	17	12		27	10	18
E	27	16		12	22	39	20
F		11	5	10	9	12	7
G	16	12	18	12	15	17	17
H		12					
I	38	12		18	16	30	13
J	17	18		10		17	18
K	15	8	20	9		10	10
L	31	13		15	12	27	14

Table 10: α-angle [°]; mean, standard deviation (STD), p-value

Sampling point	Mean	STD	P-value
BDC-5	29,600	14,081	
HDT+2	16,750	11,871	0,0010
HDT+7	20,000	12,853	0,1494
HDT+14	15,100	8,698	0,0004
HDT+21	16,889	5,533	0,0155
R 0	24,100	12,583	0,1454
R+2	16,700	7,258	0,0238

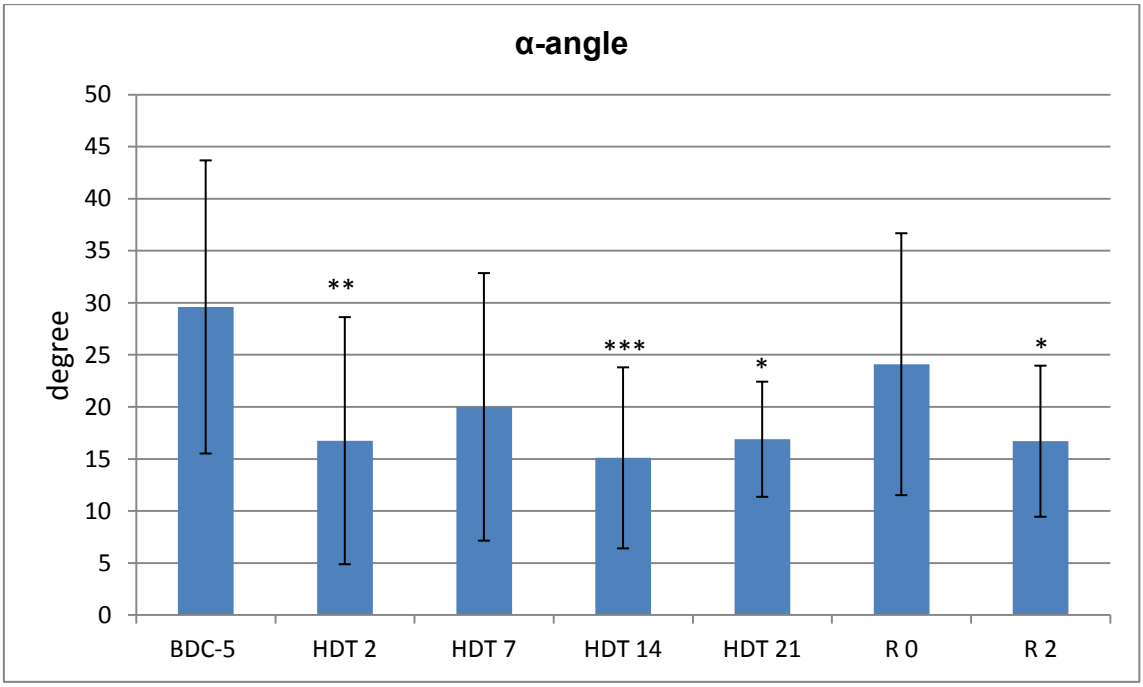

Figure 18: α-angle [°]

4.4 Thrombin generation parameters

Lagtime, endogenous thrombin potential, thrombin peak, time to peak and start tail were analyzed by thrombin generation test. Each test showed values in the results, which were statistically significant and conclusive.

4.4.1 Endogenous thrombin potential (ETP)

The ETP reflects the total amount of thrombin. The ETP values continually dropped from initially 1353,265 ± 136,059 nM×min on BDC-5 to 1252,163 ± 106,103 nM×min (*P*=0,0035) on HDT+21. During recovery ETP dropped from 1205,136 ± 106,103 nM×min (*P*=0,0006) on R 0 to 1201,522 ± 128,112 nM×min (*P*=0,0014) on the second day of recovery (R+2).

Table 11 shows the ETP results of each subject at each sampling point. Mean, standard deviation and p-value are listed in Table 12 and graphically displayed in Figure 19.

Table 11: ETP [nM×min] results

Subject	BDC-5	HDT+2	HDT+7	HDT+14	HDT+21	R 0	R+2
A	1491,50	1524,10	1421,99	1397,08	1403,26	1309,12	1305,73
B	1318,82	1147,80	1188,82	1170,12	1188,71	1016,95	1101,59
C	1398,26	1426,95	1285,87	1275,06	1175,60	1224,22	1153,37
D	1493,97	1384,76	1233,43	1290,36	1335,92	1245,43	1212,31
E	1504,11	1093,87	1306,42	1208,80	1284,11	1239,26	1230,02
F	1176,79	1295,50	1307,24	1248,36	1299,79	1228,75	1313,02
G	1438,45	1490,67	1507,76	1419,46	1336,31	1459,75	1401,48
H	1098,36	1022,58	1040,27	1104,54	1108,89	1022,41	1040,06
I	1320,46	1301,21		1163,18	1199,68	1138,89	1068,21
J	1210,71	1198,68		1064,10	1094,34	1005,37	1010,16
K	1320,77	1259,48	1149,40	1214,98	1197,13	1203,55	1229,91
L	1466,98	1480,64	1265,63	1489,90	1402,21	1367,93	1352,40

Table 12: ETP [nM×min]; mean, standard deviation (STD), p-value

Sampling point	Mean	STD	P-value
BDC-5	1353,265	136,059	
HDT+2	1302,187	164,316	0,2236
HDT+7	1270,683	132,438	0,0300
HDT+14	1253,828	128,821	0,0070
HDT+21	1252,163	106,103	0,0035
R 0	1205,136	141,084	0,0006
R+2	1201,522	128,112	0,0014

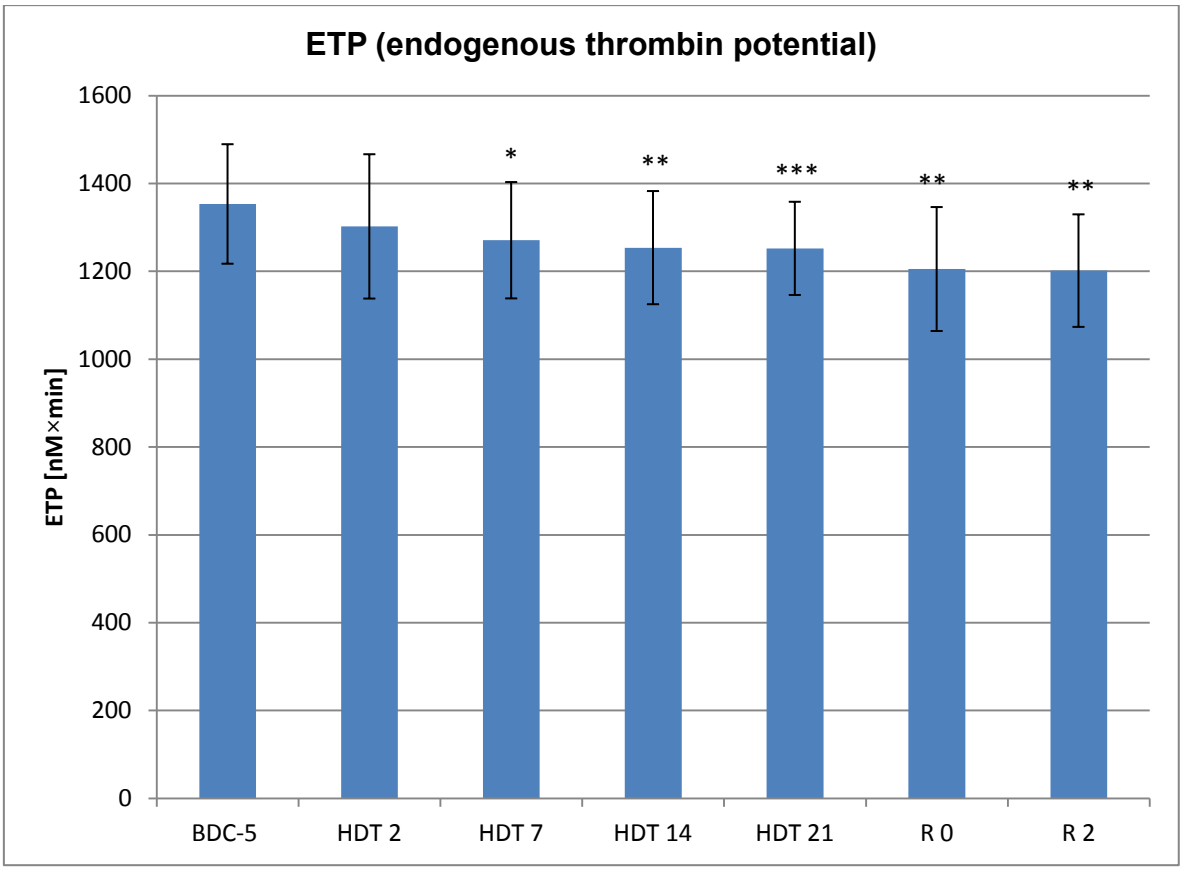

Figure 19: ETP [nM×min]

4.4.2 Thrombin peak

Thrombin peak (TP) reflects the maximum thrombin activity (nmol/l) and can be referred to as the maximum concentration of thrombin reached during a clotting process.

Only results from day R 0 (225,463 ± 39,058 nM; *P* = 0,037) showed statistically significant results. Every other sampling point showed no significant effect. Nevertheless on the second day of bed rest TP drops by about 10%, but continuously rises during the whole bed rest until recovery day two, when TP almost returns back to baseline.

Table 13 displays the raw results from the TP measurements. Mean, standard deviation and p-value are listed in Table 14 and graphically displayed in Figure 20.

Table 13: Thrombin peak [nM] results

Subject	BDC-5	HDT+2	HDT+7	HDT+14	HDT+21	R 0	R+2
A	225,69	267,71	218,19	199,89	209,35	236,16	204,13
B	207,05	154,86	196,91	179,65	202,9	258,48	185,84
C	197,45	173,84	166,57	164,16	224,16	187,48	154,51
D	175,44	153,1	164,41	214,33	235,56	251,93	211,24
E	217,58	105,42	142,88	169,91	196,28	230,74	196,51
F	115,84	135,67	153,5	158,6	190,46	224,99	213,41
G	197,52	208,26	213,29	203,48	208,34	252,05	213,02
H	194,85	184,88	191,44	216,76	241,16	215,29	221,67
I	195,28	166,17		156,6	160,95	166,87	175,03
J	178,35	144,83		144,64	157,82	155,1	162,42
K	219,57	194,46	184,2	228,65	229,25	237,19	249,27
L	245,48	248,17	231,01	285,48	271,27	289,28	293,16

Table 14: Thrombin peak [nM]; mean, standard deviation (STD), p-value

Sampling point	Mean	STD	P-value
BDC-5	197,508	32,490	
HDT+2	178,114	46,396	0,114
HDT+7	186,240	29,283	0,193
HDT+14	193,513	39,758	0,692
HDT+21	210,625	32,609	0,216
R 0	225,463	39,058	0,037
R+2	206,684	37,960	0,446

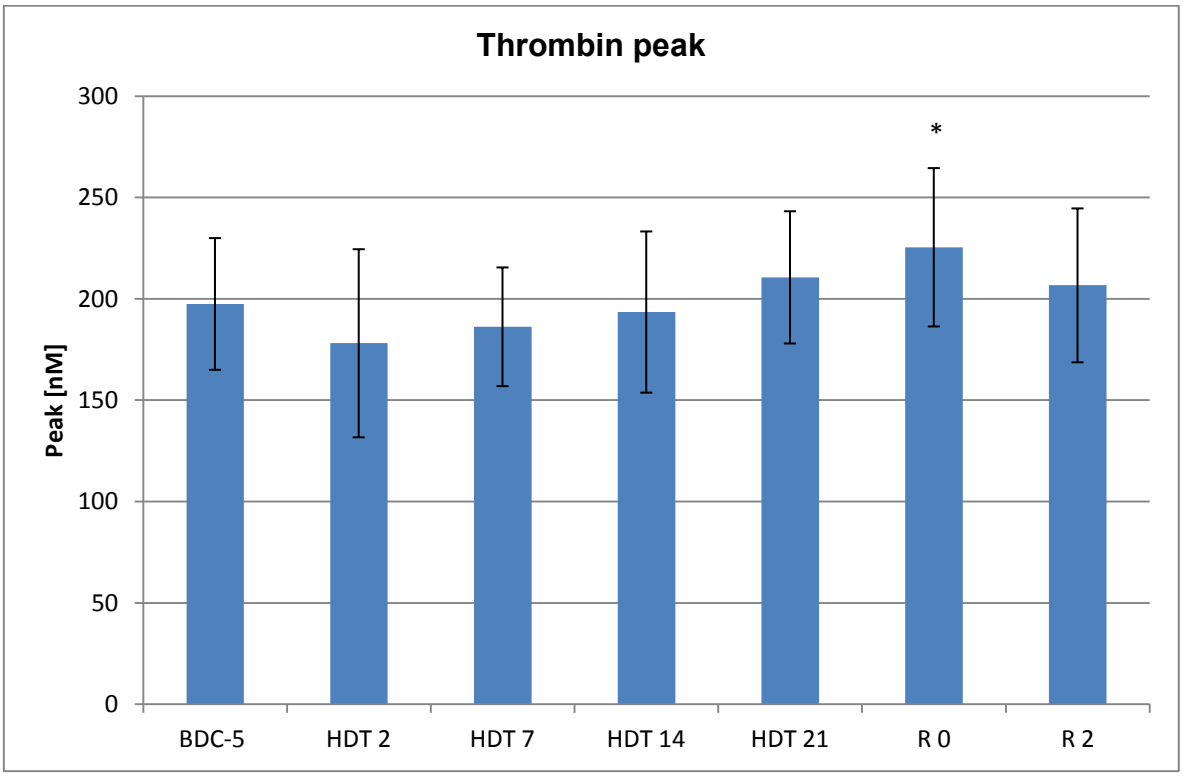

Figure 20: Thrombin peak [nM]

4.4.3 Time to peak

Time to peak (TTP) is influenced by lag time and represents the time between adding tissue factor and reaching the thrombin peak.

Table 15 shows TTP results. Mean, standard deviation and p-value are listed in Table 14 and graphically displayed in Figure 21.

Only at the end of the bed rest period significant changes occur regarding TTP. On HDT+21 TTP was 6,169 ± 0,812 min (*P* = 0,007). One day later it further decreases to 5,820 ± 0,837 min (*P* = 0,002). The second day of recovery shows a tendency to return back to baseline values (6,021 ± 0,862 min; *P* = 0,0019).

Table 15: Time to peak [min] results

Subject	BDC-5	HDT+2	HDT+7	HDT+14	HDT+21	R 0	R+2
A	6,69	6,13	6,69	7,03	6,80	6,02	6,24
B	6,80	7,70	6,58	7,14	6,58	5,13	6,36
C	7,33	8,00	7,67	7,67	6,67	7,33	7,67
D	7,67	8,11	6,78	5,78	5,44	5,00	5,67
E	7,49	10,39	8,27	7,16	6,49	5,60	6,27
F	8,94	8,83	7,94	7,60	6,6	6,04	6,27
G	7,00	7,00	6,89	6,67	6,22	5,89	6,56
H	6,00	5,67	5,89	5,67	4,89	5,22	5,00
I	7,11	7,67		7,00	7,22	7,17	6,44
J	6,67	7,56		7,00	6,78	6,44	6,22
K	5,33	5,78	5,56	4,78	4,67	4,67	4,33
L	6,33	6,11	5,78	5,33	5,67	5,33	5,22

Table 16: Time to peak [min]; mean, standard deviation (STD), p-value

Sampling point	Mean	STD	P-value
BDC-5	6,947	0,906	
HDT+2	7,413	1,385	0,1028
HDT+7	6,805	0,924	0,4009
HDT+14	6,569	0,940	0,0980
HDT+21	6,169	0,812	0,0074
R 0	5,820	0,837	0,0021
R+2	6,021	0,862	0,0019

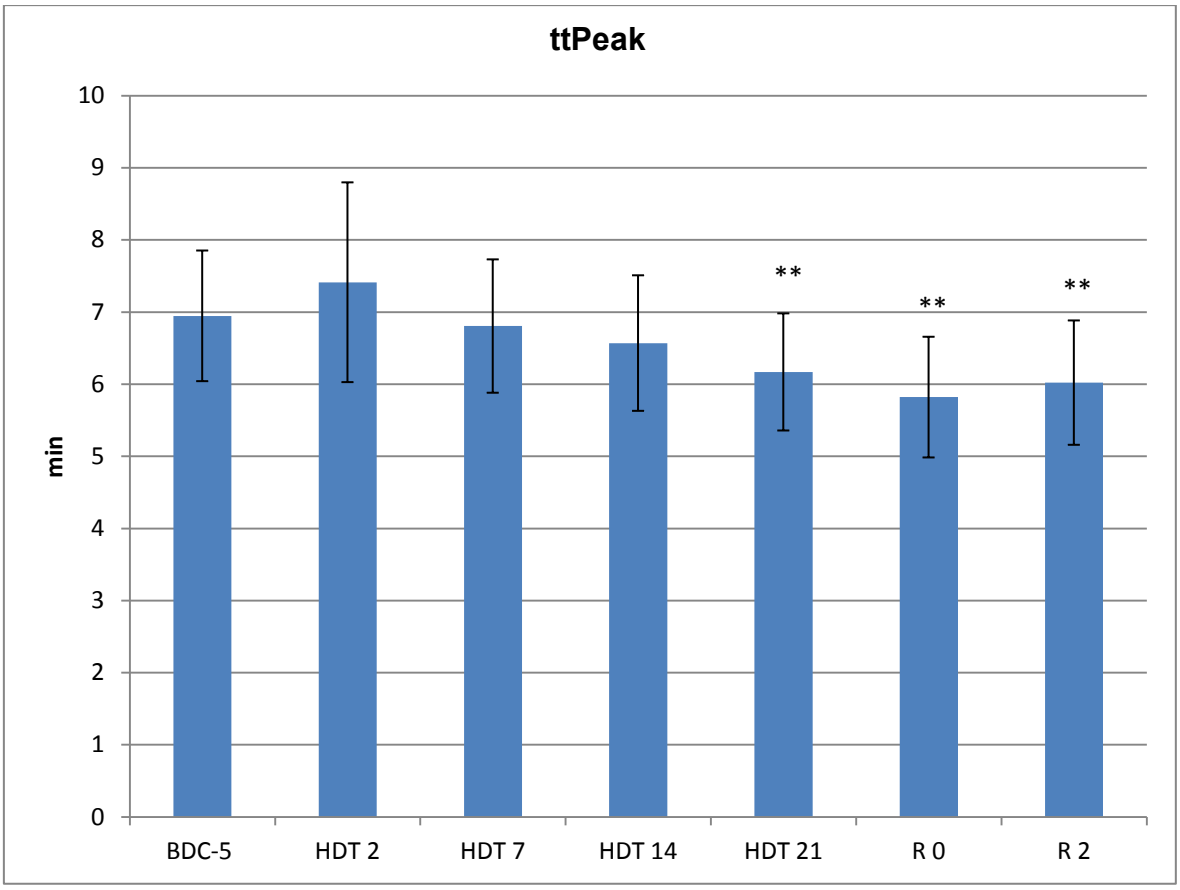

Figure 21: Time to peak [min]

4.4.4 Lag Time

The lag time is equivalent to CT which correlates with APTT and PT and describes the time from inducing coagulation to the actual building of a clot.

Table 17 shows the lag time results.

Table 17: Lag time [min] results

Subject	BDC-5	HDT+2	HDT+7	HDT+14	HDT+21	R 0	R+2
A	2,45	2,67	2,45	2,67	2,67	2,45	2,34
B	2,67	2,67	2,67	3,01	3,01	2,67	2,79
C	3,00	3,22	3,33	3,33	3,22	3,33	3,33
D	2,33	2,33	2,00	2,33	2,33	2,11	2,33
E	3,26	3,60	2,93	2,60	2,48	2,26	2,60
F	2,60	2,82	2,60	2,60	2,60	2,60	2,60
G	2,44	2,67	2,67	2,67	2,56	2,67	2,67
H	2,33	2,33	2,33	2,44	2,33	2,33	2,33
I	3,00	2,78		2,67	2,89	3,00	2,56
J	2,67	2,78		2,78	2,78	2,67	2,67
K	2,00	2,00	2,11	2,00	2,00	2,00	1,67
L	2,44	2,56	2,33	2,33	2,33	2,56	2,44

Mean, standard deviation and p-value are listed in Table 18 and graphically displayed in Figure 22.

Table 18: Lag time [min]; mean, standard deviation (STD), p-value

Sampling point	Mean	STD	P-value
BDC-5	2,599	0,350	
HDT+2	2,703	0,415	0,0401
HDT+7	2,542	0,392	0,8845
HDT+14	2,619	0,340	0,8133
HDT+21	2,600	0,340	0,9920
R 0	2,554	0,369	0,6462
R+2	2,528	0,384	0,3949

The values for lag time seem to oscillate slightly around the baseline. Nevertheless on day two of bed rest lag time is increased by 4% from 2,599 ± 0,350 min to 2,703 ± 0,415 min (*P* = 0,0401).

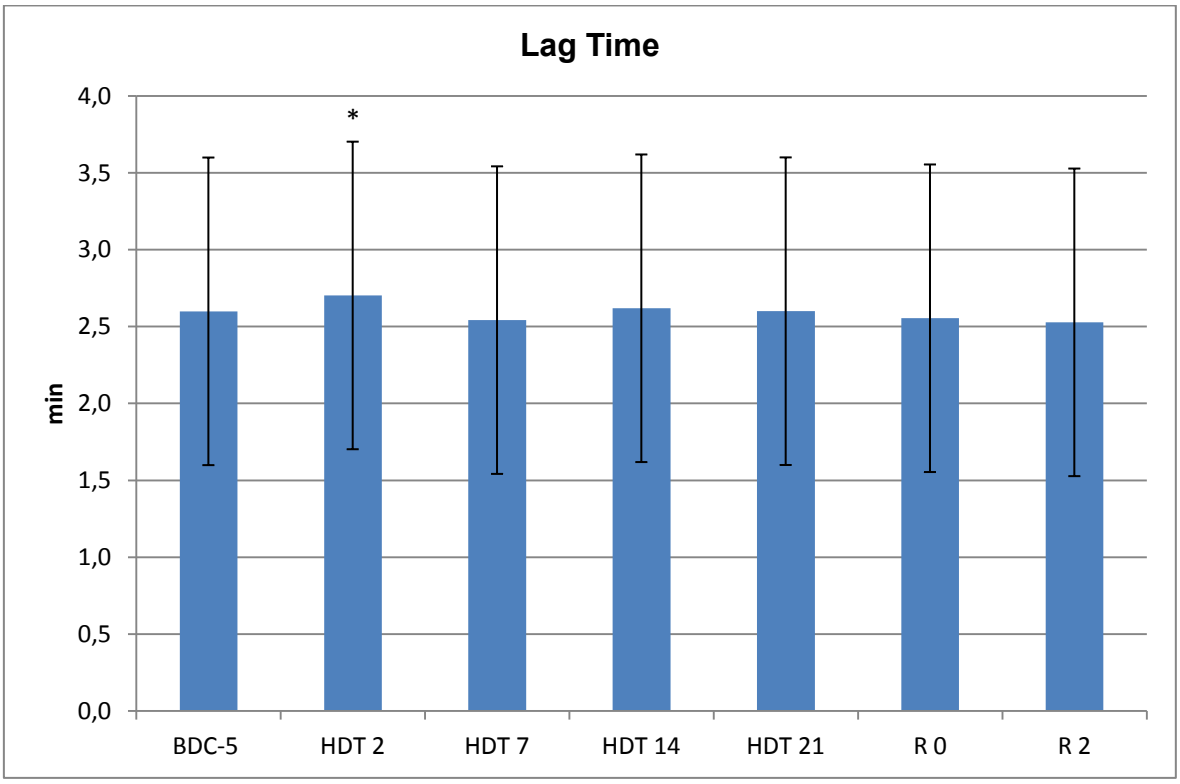

Figure 22: Lag time [min]

4.4.5 Slope

The slope is the build-up speed of the ascending curve.

Table 19 shows the Slope results. Mean, standard deviation and p-value are listed in Table 20 and graphically displayed in Figure 23.

The values on HDT+21 are significantly higher than baseline (61,832 ± 18,828 nM/min; *P* = 0,0175) and peak on R 0 (72,412 ± 22,260 nM/min; P = 0,0023). Even on the second day of recovery (R+2) the values do not return to baseline.

Table 19: Slope [nM/min] results

Subject	BDC-5	HDT+2	HDT+7	HDT+14	HDT+21	R 0	R+2
A	53,30	77,58	51,54	46,33	50,82	66,25	52,85
B	50,26	30,84	50,57	43,61	57,02	105,74	52,30
C	45,57	36,43	38,44	37,88	65,19	46,87	35,66
D	32,90	26,71	34,48	62,37	76,07	87,40	63,37
E	51,60	15,54	26,88	37,31	48,99	69,11	54,06
F	18,26	22,58	28,74	31,82	47,54	65,37	58,11
G	43,52	48,06	50,58	50,87	57,26	78,63	54,89
H	53,14	55,46	54,16	67,37	94,85	74,87	83,12
I	47,57	34,05		36,14	37,44	40,14	45,92
J	44,59	30,33		34,3	39,45	41,15	45,85
K	65,87	51,56	53,8	83,82	85,97	88,94	93,48
L	63,16	69,93	67,35	95,16	81,38	104,47	105,94

Table 20: Slope [nM/min]; mean, standard deviation (STD), p-value

Sampling point	Mean	STD	P-value
BDC-5	47,478	12,680	
HDT+2	41,589	19,115	0,2158
HDT+7	45,654	12,944	0,5328
HDT+14	52,248	20,691	0,3366
HDT+21	61,832	18,828	0,0175
R 0	72,412	22,260	0,0023
R+2	62,129	21,082	0,0180

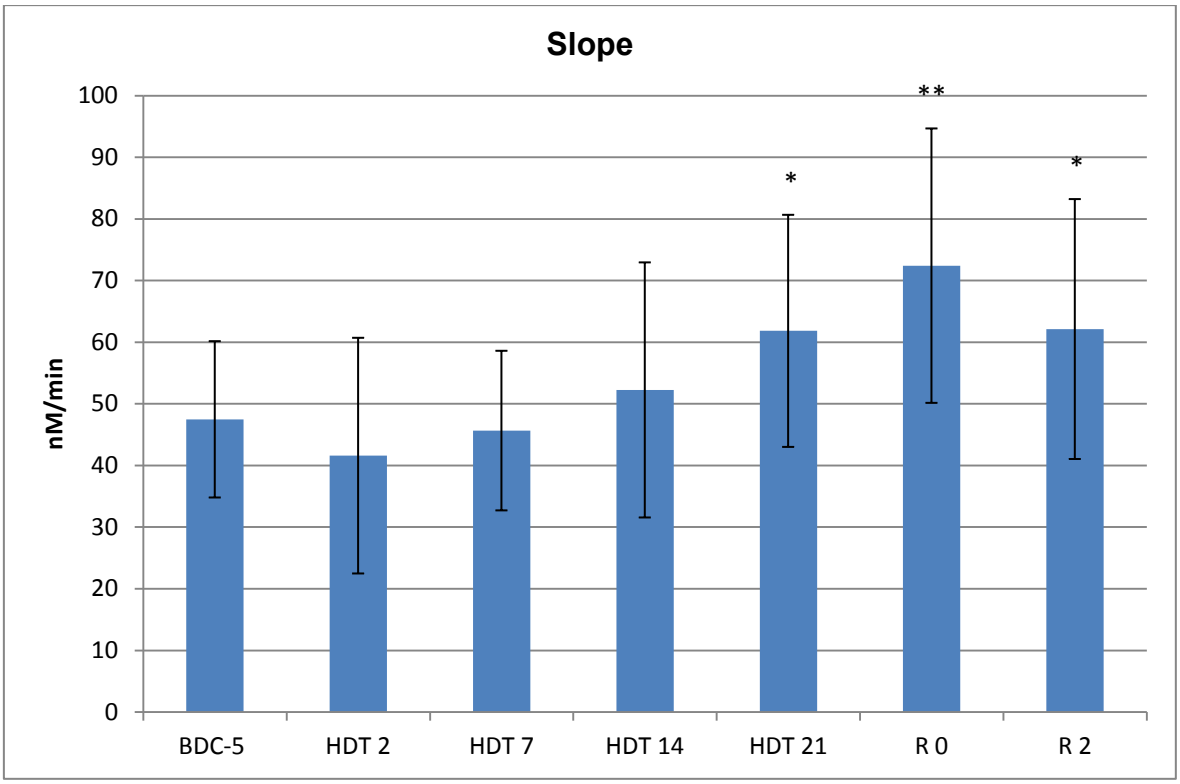

Figure 23: Slope [nM/min]

4.4.6 Start Tail

In the tail phase no free thrombin is available in the plasma. The start of the tail defines the time point from which onwards no free thrombin is available for a reaction.

Table 21 shows start tail results. Mean, standard deviation (STD) and p-value are listed in Table 22 and graphically displayed in Figure 24.

The start tail shows a significant time effect on the 21st day (HDT+21) (21,972 ± 1,417 min; *P* = 0,0160) and the first day of recovery (R 0) (20,770 ± 1,532 min; *P* = 0,0023). On the second day of recovery (R+2) the value is showing a tendency to return to baseline (21,608 ± 1,711; *P* = 0,0053).

Table 21: Start tail [min] results

Subject	BDC-5	HDT+2	HDT+7	HDT+14	HDT+21	R 0	R+2
A	23,43	21,87	22,87	23,99	23,32	21,31	22,42
B	22,76	23,54	21,98	22,54	21,98	18,41	21,75
C	24,67	26,44	25,89	25,89	21,44	23,44	25,22
D	25,33	25,89	23,44	21,44	20,78	19,11	20,56
E	23,96	29,08	26,97	23,18	22,85	20,96	22,18
F	28,97	27,75	25,07	23,96	23,07	20,18	22,29
G	23,22	23,44	23,33	23,67	22,56	21,89	23,11
H	20,67	20,33	20,33	19,89	19,33	19,33	19,00
I	23,33	24,33		23,78	24,00	22,50	21,33
J	23,00	25,22		23,56	23,11	22,11	22,00
K	21,78	22,00	21,89	20,67	20,89	20,44	20,00
L	21,89	21,89	20,56	20,56	20,33	19,56	19,44

Table 22: Start tail [min]; mean, standard deviation (STD), p-value

Sampling point	Mean	STD	P-value
BDC-5	23,584	2,116	
HDT+2	24,315	2,642	0,1773
HDT+7	23,233	2,199	0,4724
HDT+14	22,761	1,775	0,1575
HDT+21	21,972	1,417	0,0160
R 0	20,770	1,532	0,0023
R+2	21,608	1,711	0,0053

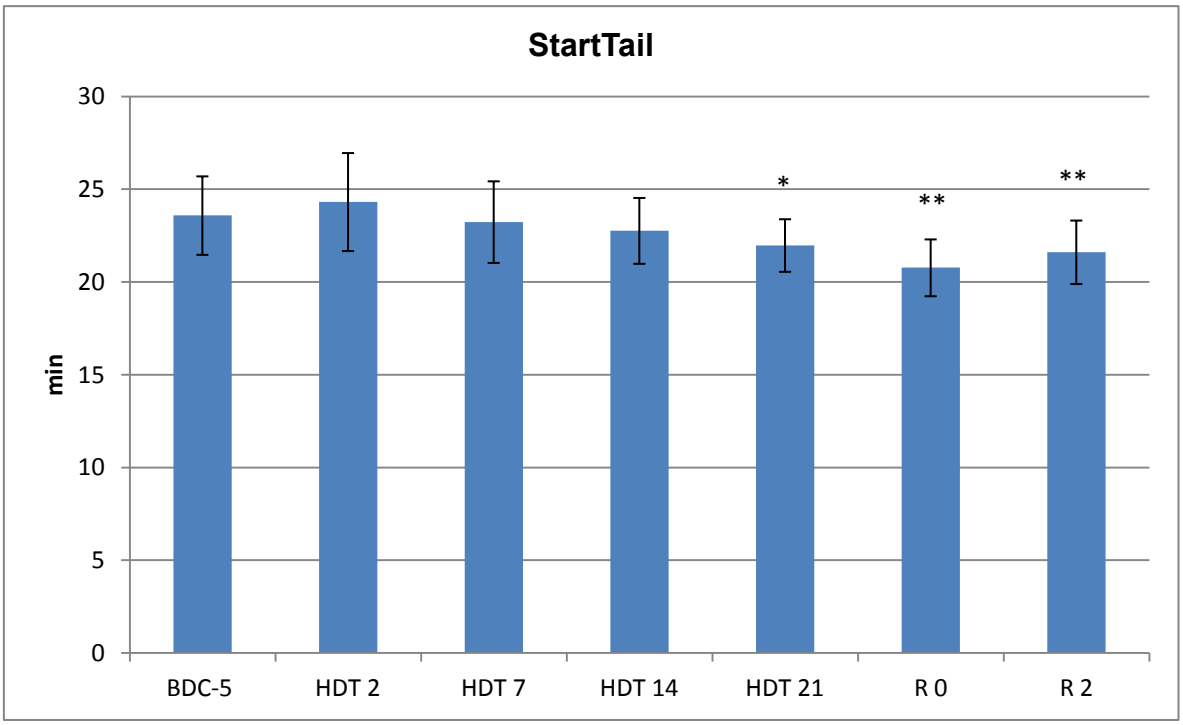

Figure 24: Start tail [min]

4.5 One-stage PT and APTT for single factor determination

Factor II, Factor VII and Factor VIII were measured. While factor VII showed no change, the alteration of factor II and factor VIII is described below.

4.5.1 Factor II plasma concentration

Prothrombin (factor II) is vitamin K dependent and is split into thrombin.

There seems to be no significant alteration during the whole bed rest phase. On the second day of recovery (R+2) the factor II plasma concentration is decreased by 10 % (123,917 ± 19,416 %; *P* = 0,040)

Table 23 shows factor II plasma concentration [%] results.

Table 23: Factor II plasma concentration [%] results

Subject	BDC-5	HDT+2	HDT+7	HDT+14	HDT+21	R 0	R+2
A	168	130	125	138	116	113	138
B	142	123	118	120	120	121	125
C	135	133	135	128	130	135	125
D	160	135	125	133	135	120	128
E	125	135	135	120	100	100	93
F	148	128	123	115	90	120	108
G	88	120	128	130	122	114	114
H	116	114	118	118	128	118	116
I	150	138		130	150	154	130
J	120	132		124	130	135	128
K	122	122	108	120	126	106	110
L	170	178	154	172	174	176	172

Mean, standard deviation and p-value are listed in Table 24 and graphically displayed in Figure 25.

Table 24: Factor II plasma concentration [%]; mean, standard deviation (STD), p-value

Sampling point	Mean	STD	P-value
BDC-5	137,000	24,117	
HDT+2	132,333	16,064	0,418
HDT+7	126,900	12,494	0,206
HDT+14	129,000	15,178	0,207
HDT+21	126,750	21,562	0,215
R 0	126,000	21,312	0,138
R+2	123,917	19,416	0,040

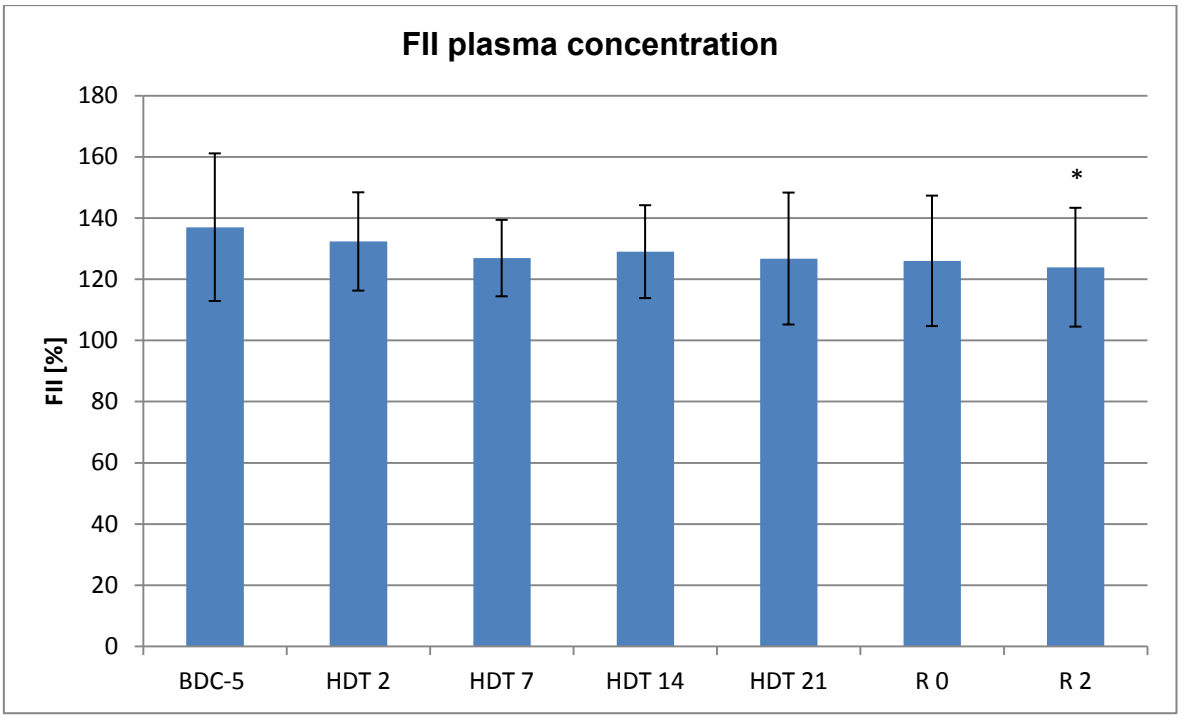

Figure 25: Factor II plasma concentration [%]

4.5.2 Factor VIII plasma concentration

Factor VIII is a Co-factor for factor IXa, needed to activate factor X. In turn factor Xa activates prothrombin to thrombin.

Factor VIII plasma concentration is elevated at the end of the bed rest (R 0) by 13,7 % compared to baseline (113,167 ± 35,061; *P* = 0,015).

Table 25 shows the factor VIII plasma concentration results. Mean, standard deviation (STD) and p-value are listed in Table 26 and graphically displayed in Figure 26.

Table 25: Factor VIII plasma concentration [%] results

Subject	BDC-5	HDT+2	HDT+7	HDT+14	HDT+21	R 0	R+2
A	148	228	168	165	193	175	130
B	100	105	110	110	120	118	120
C	88	85	90	75	70	90	80
D	80	78	73	83	93	88	93
E	108	120	100	113	100	90	98
F	62	73	65	68	57	63	63
G	70	114	124	130	130	118	136
H	96	100	106	106	110	110	106
I	118	118		116	132	128	114
J	84	96		92	98	98	100
K	88	80	62	94	98	98	92
L	152	122	114	160	146	182	184

Table 26: Factor VIII plasma concentration [%]; mean, standard deviation (STD), p-value

Sampling point	Mean	STD	P-value
BDC-5	99,500	28,118	
HDT+2	109,917	40,995	0,2198
HDT+7	101,200	31,650	0,8076
HDT+14	109,333	30,568	0,0763
HDT+21	112,250	35,922	0,0712
R 0	113,167	35,061	0,0153
R+2	109,667	31,114	0,1464

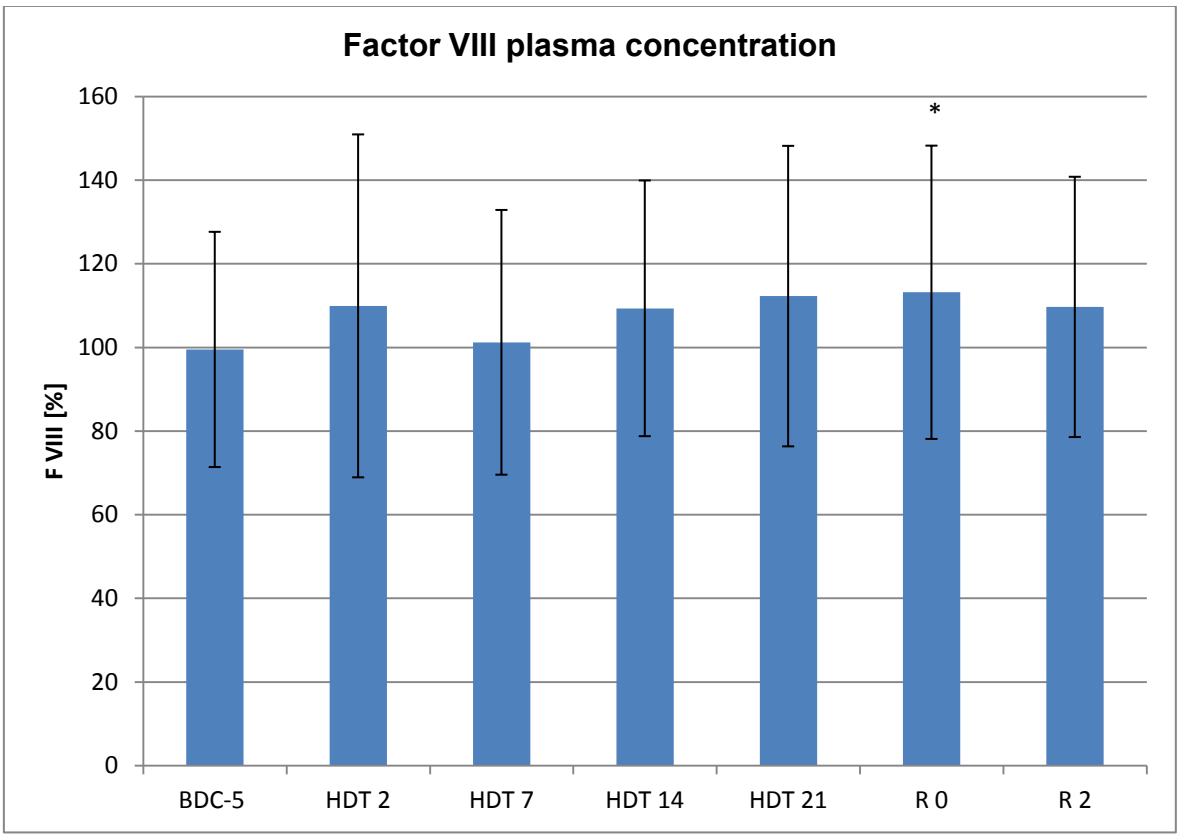

Figure 26: Factor VIII plasma concentration [%]

4.6 ELISA

ELISA was used to gather data on thrombin-antithrombin-complex (TAT), tissue plasminogen activator (t-PA) and prothrombin fragment 1+2 (F1+2). TAT and t-PA results were inconclusive and showed no statistically significant changes.

4.6.1 Prothrombin fragment 1+2

One prothrombin splits into one thrombin and one prothrombin fragment 1+2. During bed rest the values drop by 20 % to 95,439 ± 36,581 pM/L (*P* = 0,011) on HDT+14 and 95,907 ± 33,080 pM/L (*P* = 0,010) on HDT+21. Table 27 shows the plasma concentration of prothrombin fragment 1+2 [pM/L]. Mean, standard deviation and p-value are listed in Table 28 and graphically displayed in Figure 27.

Table 27: Prothrombin fragment 1+2 [pM/L] results

Subject	BDC-5	HDT+2	HDT+7	HDT+14	HDT+21	R 0	R+2
A	145,94	248,16	140,57	130,53	131,61	146,62	229,98
B	72,772	83,716	80,319	85,546	110,18	274,55	112,17
C	134,31	242,52	119,48	91,64	96,922	116,91	108,71
D	154,32	100,13	121,39	130,52	119,39	255,75	170,19
E	189,65	119,48	92,084	121,22	155,37	147,68	183,44
F	127,05	99,015	104,09	118,89	111,61	150,32	302,96
G	169,26	134,87	121,31	117,41	98,102	98,752	153,19
H	131,48	116,66	107,07	127,67	94,327	89,699	120,79
I	87,4	71,085		67,357	64,024	107,33	112,98
J	106,63	66,039		70,95	64,188	62,771	91,7
K	69,195	59,835	40,979	48,434	67,402	73,393	92,644
L	47,765	52,363	27,1	200,92	37,755	36,825	65,603

Table 28: Prothrombin fragment 1+2 [pM/L]; mean, standard deviation (STD), p-value

Sampling point	Mean	STD	P-value
BDC-5	119,648	43,468	
HDT+2	116,156	65,465	0,832
HDT+7	95,439	36,581	0,011
HDT+14	109,257	40,162	0,534
HDT+21	95,907	33,080	0,010
R 0	130,050	72,238	0,639
R+2	145,363	67,583	0,141

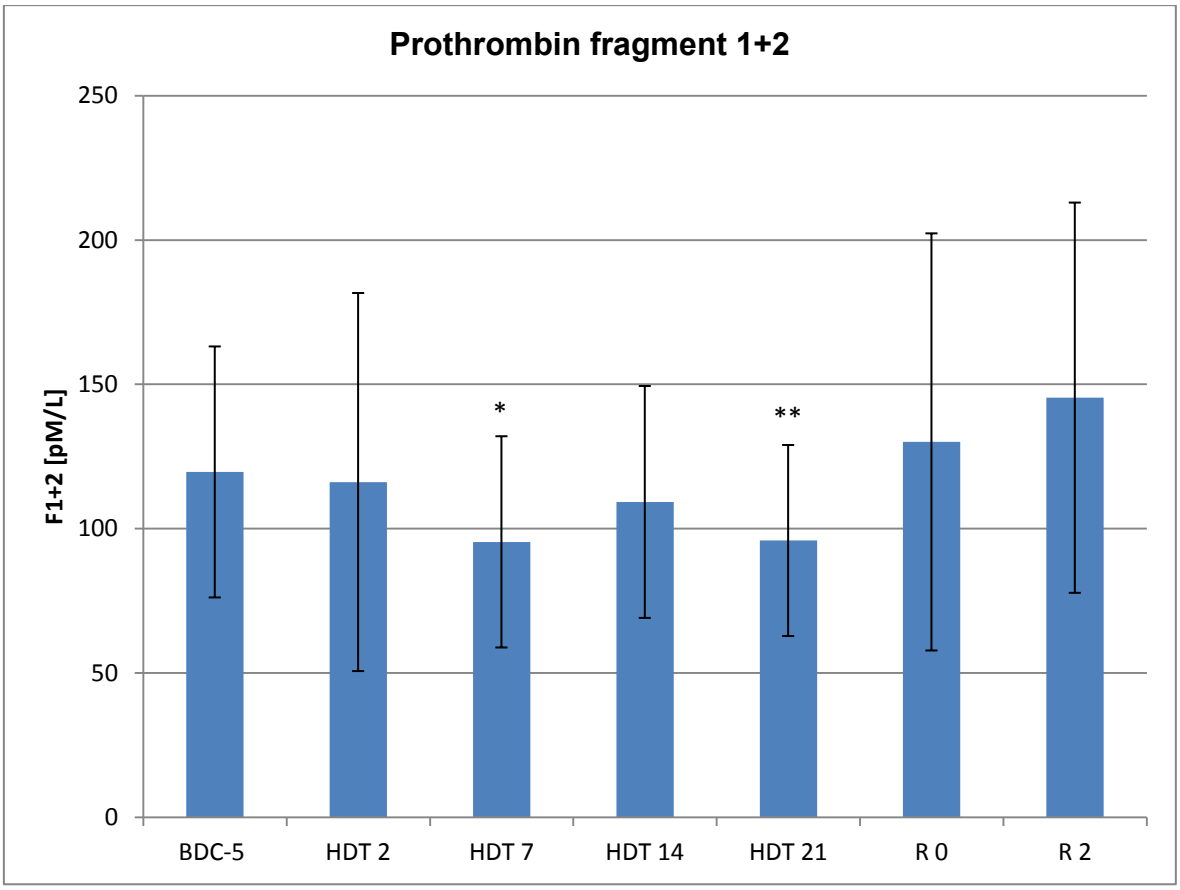

Figure 27: Prothrombin fragment 1+2 [pM/L]

5 Discussion

According to the results of this study there is no pro-coagulant effect in healthy subjects undergoing 21 days of strict 6 degrees head-down bed rest.

Trauma and surgery increase risk for thromboembolic events (67,68). Cancer and pregnancy are also known to cause hypercoagulable states (69-71). Even non-surgical hospitalized medically ill patients are at risk to develop venous thromboembolism (VTE) (67). It is clinical practice to mobilize bedridden patients as soon as possible during hospitalization. Out of all contributing factors to developing VTE, immobilization is the center of attention. Whether immobilization is a main cause or just a contributing factor for thromboembolic events can only be studied in healthy subjects.

While in a clinical context, long-term immobility by itself or together with trauma is a well known risk for venous thrombosis and/or embolism, the underlying mechanisms which shift the hemostatic system towards hypercoagulability, are not fully understood.

A rather new physiological effect on the human body to be studied is microgravity or pure weightlessness, which can be considered having equal effects related to immobilization on the human body. Because carrying out studies related to life science in space is always limited to a low number of subjects and technically and logistically hardly feasible, an excellent model to study the physiology of humans in space is the simulation of microgravity for the cardiovascular system applied by a 6 degrees head-down tilt bed rest campaign (19). Haider et al. were the first who just recently provided data of 24 males who completed a 60 day bed rest study using a similar protocol but focusing on countermeasures (72).

However our study was conducted to analyze hemostatic changes in twelve healthy subjects participating in a medium-term bed rest campaign of 21 days. The subjects lay 6 degree head-down for the whole campaign. Antecubital blood was collected during morning hours, centrifuged and frozen for later analysis. The analysis involved measurement of clotting time (CT), α-angle (α), endogenous thrombin potential (ETP), thrombin peak (TP), time to peak (TTP), lag time, slope (velocity index), start tail, factor II and factor VIII plasma concentration and prothrombin fragment 1+2 (F1+2). To get a detailed picture of the alteration during bed rest and the progression of each parameter, blood was collected almost

weekly before, during and after bed rest. Baseline data collection was done five days before the bed rest (BDC-5). The other sample points were on the 2nd (HDT+2), the 7th (HDT+7), the 14th (HDT+14), the 21st (HDT+21), the 1st day (R 0) after the bed rest and the 2nd day of recovery (R+2). Blood collection on R 0 was done just before the subjects got up from bed rest and before they were tilted supine.

Throughout the whole study the clotting time (CT) is significantly increased. The CT prolongation is statistically significant. The high standard deviation throughout the clotting time measurements is explained by the procedure of the thrombelastometry itself (73). Although the measurements were carried out by just one person to rule out interpersonal differences, the ROTEM® results show a high standard deviation even within each subject, which is due to the soft activation by using small amounts of tissue factor.

The α-angle values were reduced in general compared to baseline, indicating a lesser tendency to form clots. This could be associated with reduced concentration of fibrinogen, but which was not measured due to limited volume. Both thrombelastometry results reveal a rather hypocoagulable state and show no evidence for hypercoagulability.

The endogenous thrombin potential (ETP) is continuously dropping throughout the whole campaign. From the ETP values, which are calculated by the area under the thrombin formation curve, one can derive, that the amount of formed thrombin is reduced. Possible influences on low ETP values are low factor II (FII), factor V, factor X and factor XI plasma concentrations (61). Our findings however, show no change in FII plasma concentration to that extent. Only one FII sample point showed a significant reduction (R+2).

There was no statistically significant difference in thrombin peak (TP) results during the study, except for an increase of about 10% on R 0.

The FVIII plasma concentration showed no significant change, except for an increase of about 13% on R 0.

While the lag time presents only one slightly elevated time point (HDT+2) with significance, the time to peak (TTP) was decreased on HDT+21, R 0 and R+2.

AfteR+21 days of bed rest the thrombin burst is accelerated (slope is markedly increased) with a higher maximum thrombin concentration on R 0 and the end of the thrombin generation occurs earlier, shown by the decreased start tail.

Prothrombin fragment 1 + 2, an important parameter for measuring hypercoagulability, showed no change on most sample points and was significantly decreased on HDT+7 and HDT+21.

However, most of the parameters appear to be within the reference range and show no pathological signs of a hyper- or hypocoagulable state.

The plasma volume changes which occur during bed rest and when exposed to low gravity, were not monitored and therefore the results were not related to the plasma volume shifts.

The low number of subjects for this study had to be accepted, but could have influenced some results which were not statistically significant and showed no change.

It has to be stated that in vitro analysis of the coagulation process using platelet poor plasma is different from the physiological process in a human being. The influence of blood vessels and thrombocytes cannot be displayed adequately using this method. Also some of the results could be biased by the handling of the samples, i.e. freezing, shipment on ice and thawing, although the samples were handled with tremendous caution. The amount of blood was limited, therefore the sampling points could not be carried out predominantly to serve scientific concerns, but had to be arranged according to feasibility and to treat the subjects with consideration. Therefore it was not possible to find out when the parameters returned back to baseline during recovery.

Nevertheless the findings from this study queue in with the recent conclusions of Haider et al. who reported results from a 60 day bed rest study in 2007 and a not yet published 21 day bed rest study in 2012 conducted by Cvirn et al (see Figure 28). Both studies showed no change regarding coagulation during medium-term and long-term bed rest in healthy subjects.

These findings support the evidence-based clinical practice guidelines by the American College of Chest Physicians from 2012, that “for acutely ill hospitalized medical patients at low risk of thrombosis, we recommend against the use of pharmacologic prophylaxis or mechanical prophylaxis” (74).

6 Conclusion

Medium-term bed rest of 21 days does not shift the coagulation towards hypercoagulability in healthy volunteers. Unlike the broadly acknowledged belief, that immobilization by itself increases the tendency to clot during bed rest, very interestingly, there was no evidence found. In the clinical context the reasonable question arises whether all patients staying in bed for certain periods of time need to be unexceptionally treated with anticoagulants to prevent thromboembolic events. It is perhaps those subjects who have imbalance in the anti- and pro-coagulatory system that tip towards increased clotting during immobilization. Concerning space missions, it is evidently unnecessary to provide astronauts with anti-coagulatory therapies during missions when exposed to low gravity.

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8 Appendix

8.1 Appendix A: Raw Data sorted by subject

Table 29: Subject A results; raw data

Subject A	BDC-5	HDT+2	HDT+7	HDT+14	HDT+21	R 0	R+2
CT [s]	202	227	206	487	484	211	572
CFT [s]	354	558	1996	1511		1367	
MCF [mm]	25	26	21	22	19	23	16
α [°]	59	51	43	33	21	44	16
Lagtime_(min)	2,45	2,67	2,45	2,67	2,67	2,45	2,34
ETP_(nM•min)	1491,5	1524,1	1421,99	1397,08	1403,26	1309,12	1305,73
Peak_(nM)	225,69	267,71	218,19	199,89	209,35	236,16	204,13
ttPeak_(min)	6,69	6,13	6,69	7,03	6,8	6,02	6,24
VelIndex_(nM/min)	53,3	77,58	51,54	46,33	50,82	66,25	52,85
StartTail_(min)	23,43	21,87	22,87	23,99	23,32	21,31	22,42
FII [%]	168	130	125	138	116	113	138
FVII [%]	164	123	130	125	135	125	130
FVIII [%]	148	228	168	165	193	175	130
F1+F2 [pmol/L]	145,94	248,16	140,57	130,53	131,61	146,62	229,98
TAT [µg/L]	2,311	17,15	2,8	2,198	24,4	1,8	2,227
t-PA [ng/mL]	9,077	7,23	11,81	13,057	9,305	7,988	9,478

Table 30: Subject B results; raw data

Subject B	BDC-5	HDT+2	HDT+7	HDT+14	HDT+21	R 0	R+2
CT [s]	258	374	374	656	752	322	453
CFT [s]				2174		1870	
MCF [mm]	19	19	18	22	17	22	19
α [°]	41	25	22	27	14	35	34
Lagtime_(min)	2,67	2,67	2,67	3,01	3,01	2,67	2,79
ETP_(nM•min)	1318,82	1147,8	1188,82	1170,12	1188,71	1016,95	1101,59
Peak_(nM)	207,05	154,86	196,91	179,65	202,9	258,48	185,84
ttPeak_(min)	6,8	7,7	6,58	7,14	6,58	5,13	6,36
VelIndex_(nM/min)	50,26	30,84	50,57	43,61	57,02	105,74	52,3
StartTail_(min)	22,76	23,54	21,98	22,54	21,98	18,41	21,75
FII [%]	142	123	118	120	120	121	125
FVII [%]	90	83	90	120	98	93	95
FVIII [%]	100	105	110	110	120	118	120
F1+F2 [pmol/L]	72,772	83,716	80,319	85,546	110,18	274,55	112,17
TAT [µg/L]	5,441	1,716	2,148	2,115	5,709	19,84	1,937
t-PA [ng/mL]	3,108	2,267	2,294	2,999	2,853	3,345	3,159

Table 31: Subject C results; raw data

Subject C	BDC-5	HDT+2	HDT+7	HDT+14	HDT+21	R 0	R+2
CT [s]	251	551	457	1864	462	401	306
CFT [s]						2567	
MCF [mm]	14	12		12	13	21	16
α [°]	18	6		5	16		
Lagtime_(min)	3	3,22	3,33	3,33	3,22	3,33	3,33
ETP_(nM•min)	1398,26	1426,95	1285,87	1275,06	1175,6	1224,22	1153,37
Peak_(nM)	197,45	173,84	166,57	164,16	224,16	187,48	154,51
ttPeak_(min)	7,33	8	7,67	7,67	6,67	7,33	7,67
VelIndex_(nM/min)	45,57	36,43	38,44	37,88	65,19	46,87	35,66
StartTail_(min)	24,67	26,44	25,89	25,89	21,44	23,44	25,22
FII [%]	135	133	135	128	130	135	125
FVII [%]	133	118	123	110	115	125	133
FVIII [%]	88	85	90	75	70	90	80
F1+F2 [pmol/L]	134,31	242,52	119,48	91,64	96,922	116,91	108,71
TAT [µg/L]	3,868	22,641	3,632	2,066	2,467	2,653	1,871
t-PA [ng/mL]	21,014	21,054	23,755	19,936	25,804	23,528	19,031

Table 32: Subject D results; raw data

Subject D	BDC-5	HDT+2	HDT+7	HDT+14	HDT+21	R 0	R+2
CT [s]	323	380	917	939	557	605	721
CFT [s]	970						
MCF [mm]	24	19	18	14	20	14	18
α [°]	34	17	12		27	10	18
Lagtime_(min)	2,33	2,33	2	2,33	2,33	2,11	2,33
ETP_(nM•min)	1493,97	1384,76	1233,43	1290,36	1335,92	1245,43	1212,31
Peak_(nM)	175,44	153,1	164,41	214,33	235,56	251,93	211,24
ttPeak_(min)	7,67	8,11	6,78	5,78	5,44	5	5,67
VelIndex_(nM/min)	32,9	26,71	34,48	62,37	76,07	87,4	63,37
StartTail_(min)	25,33	25,89	23,44	21,44	20,78	19,11	20,56
FII [%]	160	135	125	133	135	120	128
FVII [%]	100	100	95	103	105	100	108
FVIII [%]	80	78	73	83	93	88	93
F1+F2 [pmol/L]	154,32	100,13	121,39	130,52	119,39	255,75	170,19
TAT [µg/L]	2,088	2,635	1,866	1,728	2,514	20,545	3,312
t-PA [ng/mL]	7,057	7,908	8,121	8,547	9,651	8,348	8,095

Table 33: Subject E results; raw data

Subject E	BDC-5	HDT+2	HDT+7	HDT+14	HDT+21	R 0	R+2
CT [s]	350	788	803	588	530	429	583
CFT [s]							2426
MCF [mm]	16	19	15	17	17	18	21
α [°]	27	16		12	22	39	20
Lagtime_(min)	3,26	3,6	2,93	2,6	2,48	2,26	2,6
ETP_(nM•min)	1504,11	1093,87	1306,42	1208,8	1284,11	1239,26	1230,02
Peak_(nM)	217,58	105,42	142,88	169,91	196,28	230,74	196,51
ttPeak_(min)	7,49	10,39	8,27	7,16	6,49	5,6	6,27
VelIndex_(nM/min)	51,6	15,54	26,88	37,31	48,99	69,11	54,06
StartTail_(min)	23,96	29,08	26,97	23,18	22,85	20,96	22,18
FII [%]	125	135	135	120	100	100	93
FVII [%]	108	113	98	103	83	88	90
FVIII [%]	108	120	100	113	100	90	98
F1+F2 [pmol/L]	189,65	119,48	92,084	121,22	155,37	147,68	183,44
TAT [µg/L]	4,16	1,814	1,978	1,468	5,228	3,165	4,867
t-PA [ng/mL]	32,93	1,389	32,93	19,125	32,93	32,93	30,391

Table 34: Subject F results; raw data

Subject F	BDC-5	HDT+2	HDT+7	HDT+14	HDT+21	R 0	R+2
CT [s]		772	711	487	766	699	448
CFT [s]							
MCF [mm]		15	15	14	13	13	6
α [°]		11	5	10	9	12	7
Lagtime_(min)	2,6	2,82	2,6	2,6	2,6	2,6	2,6
ETP_(nM•min)	1176,79	1295,5	1307,24	1248,36	1299,79	1228,75	1313,02
Peak_(nM)	115,84	135,67	153,5	158,6	190,46	224,99	213,41
ttPeak_(min)	8,94	8,83	7,94	7,6	6,6	6,04	6,27
VelIndex_(nM/min)	18,26	22,58	28,74	31,82	47,54	65,37	58,11
StartTail_(min)	28,97	27,75	25,07	23,96	23,07	20,18	22,29
FII [%]	148	128	123	115	90	120	108
FVII [%]	78	78	75	80	72	98	86
FVIII [%]	62	73	65	68	57	63	63
F1+F2 [pmol/L]	127,05	99,015	104,09	118,89	111,61	150,32	302,96
TAT [µg/L]	4,157	3,44	1,346	2,748	2,837	3,622	15,722
t-PA [ng/mL]	3,806	28,664	4,276	4,01	4,609	6,139	4,17

Table 35: Subject G results; raw data

Subject G	BDC-5	HDT+2	HDT+7	HDT+14	HDT+21	R 0	R+2
CT [s]	482	729	1122	859	786	665	900
CFT [s]							
MCF [mm]	12	13	19	12	18	15	13
α [°]	16	12	18	12	15	17	17
Lagtime_(min)	2,44	2,67	2,67	2,67	2,56	2,67	2,67
ETP_(nM•min)	1438,45	1490,67	1507,76	1419,46	1336,31	1459,75	1401,48
Peak_(nM)	197,52	208,26	213,29	203,48	208,34	252,05	213,02
ttPeak_(min)	7	7	6,89	6,67	6,22	5,89	6,56
VelIndex_(nM/min)	43,52	48,06	50,58	50,87	57,26	78,63	54,89
StartTail_(min)	23,22	23,44	23,33	23,67	22,56	21,89	23,11
FII [%]	88	120	128	130	122	114	114
FVII [%]	63	80	86	86	82	78	92
FVIII [%]	70	114	124	130	130	118	136
F1+F2 [pmol/L]	169,26	134,87	121,31	117,41	98,102	98,752	153,19
TAT [µg/L]	1,675	4,4	4	3,47	4,179	3,082	3,46
t-PA [ng/mL]	4,516	5,1	5,54	2,579	0,349	3,626	4,809

Table 36: Subject H results; raw data

Subject H	BDC-5	HDT+2	HDT+7	HDT+14	HDT+21	R 0	R+2
CT [s]	279	491	650	506	622	556	604
CFT [s]							
MCF [mm]	7	10	8			9	8
α [°]		12					
Lagtime_(min)	2,33	2,33	2,33	2,44	2,33	2,33	2,33
ETP_(nM•min)	1098,36	1022,58	1040,27	1104,54	1108,89	1022,41	1040,06
Peak_(nM)	194,85	184,88	191,44	216,76	241,16	215,29	221,67
ttPeak_(min)	6	5,67	5,89	5,67	4,89	5,22	5
VelIndex_(nM/min)	53,14	55,46	54,16	67,37	94,85	74,87	83,12
StartTail_(min)	20,67	20,33	20,33	19,89	19,33	19,33	19
FII [%]	116	114	118	118	128	118	116
FVII [%]	72	64	68	72	76	76	82
FVIII [%]	96	100	106	106	110	110	106
F1+F2 [pmol/L]	131,48	116,66	107,07	127,67	94,327	89,699	120,79
TAT [µg/L]	1,967	2,323	2,729	3,085	2,958	2,537	2,114
t-PA [ng/mL]	2,815	2,387	3,288	3,198	2,86	2,883	2,568

Table 37: Subject I results; raw data

Subject I	BDC-5	HDT+2	HDT+7	HDT+14	HDT+21	R 0	R+2
CT [s]	216	539		593	505	336	1331
CFT [s]				2094			
MCF [mm]	17	14		20	18	14	14
α [°]	38	12		18	16	30	13
Lagtime_(min)	3	2,78		2,67	2,89	3	2,56
ETP_(nM•min)	1320,46	1301,21		1163,18	1199,68	1138,89	1068,21
Peak_(nM)	195,28	166,17		156,6	160,95	166,87	175,03
ttPeak_(min)	7,11	7,67		7	7,22	7,17	6,44
VelIndex_(nM/min)	47,57	34,05		36,14	37,44	40,14	45,92
StartTail_(min)	23,33	24,33		23,78	24	22,5	21,33
FII [%]	150	138		130	150	154	130
FVII [%]	100	102		96	110	100	100
FVIII [%]	118	118		116	132	128	114
F1+F2 [pmol/L]	87,4	71,085		67,357	64,024	107,33	112,98
TAT [µg/L]	1,543	2,421		1,431	2,408	3,397	5,349
t-PA [ng/mL]	24,718	25,439		24,324	31,115	30,87	19,55

Table 38: Subject J results; raw data

Subject J	BDC-5	HDT+2	HDT+7	HDT+14	HDT+21	R 0	R+2
CT [s]	518	723		1073	742	730	794
CFT [s]							
MCF [mm]	15	16		16	15	16	16
α [°]	17	18		10		17	18
Lagtime_(min)	2,67	2,78		2,78	2,78	2,67	2,67
ETP_(nM•min)	1210,71	1198,68		1064,1	1094,34	1005,37	1010,16
Peak_(nM)	178,35	144,83		144,64	157,82	155,1	162,42
ttPeak_(min)	6,67	7,56		7	6,78	6,44	6,22
VelIndex_(nM/min)	44,59	30,33		34,3	39,45	41,15	45,85
StartTail_(min)	23	25,22		23,56	23,11	22,11	22
FII [%]	120	132		124	130	135	128
FVII [%]	88	80		92	88	88	90
FVIII [%]	84	96		92	98	98	100
F1+F2 [pmol/L]	106,63	66,039		70,95	64,188	62,771	91,7
TAT [µg/L]	4,126	2,178		1,914	2,178	2,112	1,682
t-PA [ng/mL]	2,196	2,534		2,613	2,736	2,624	2,928

Table 39: Subject K results; raw data

Subject K	BDC-5	HDT+2	HDT+7	HDT+14	HDT+21	R 0	R+2
CT [s]	668	1171	884	1139	1038	1546	812
CFT [s]							
MCF [mm]	13	12	8	11	9	14	14
α [°]	15	8	20	9		10	10
Lagtime_(min)	2	2	2,11	2	2	2	1,67
ETP_(nM•min)	1320,77	1259,48	1149,4	1214,98	1197,13	1203,55	1229,91
Peak_(nM)	219,57	194,46	184,2	228,65	229,25	237,19	249,27
ttPeak_(min)	5,33	5,78	5,56	4,78	4,67	4,67	4,33
VelIndex_(nM/min)	65,87	51,56	53,8	83,82	85,97	88,94	93,48
StartTail_(min)	21,78	22	21,89	20,67	20,89	20,44	20
FII [%]	122	122	108	120	126	106	110
FVII [%]	88	94	80	80	100	94	94
FVIII [%]	88	80	62	94	98	98	92
F1+F2 [pmol/L]	69,195	59,835	40,979	48,434	67,402	73,393	92,644
TAT [µg/L]	1,939	3,64	2,099	2,009	1,935	3,291	2,666
t-PA [ng/mL]	3,142	3,468	3,322	3,176	3,919	3,435	3,243

Table 40: Subject L results; raw data

Subject L	BDC-5	HDT+2	HDT+7	HDT+14	HDT+21	R 0	R+2
CT [s]	390	736	1055	731	815	545	787
CFT [s]							
MCF [mm]	18	16	10	15	14	20	15
α [°]	31	13		15	12	27	14
Lagtime_(min)	2,44	2,56	2,33	2,33	2,33	2,56	2,44
ETP_(nM•min)	1466,98	1480,64	1265,63	1489,9	1402,21	1367,93	1352,4
Peak_(nM)	245,48	248,17	231,01	285,48	271,27	289,28	293,16
ttPeak_(min)	6,33	6,11	5,78	5,33	5,67	5,33	5,22
VelIndex_(nM/min)	63,16	69,93	67,35	95,16	81,38	104,47	105,94
StartTail_(min)	21,89	21,89	20,56	20,56	20,33	19,56	19,44
FII [%]	170	178	154	172	174	176	172
FVII [%]	80	80	80	94	94	96	106
FVIII [%]	152	122	114	160	146	182	184
F1+F2 [pmol/L]	47,765	52,363	27,1	200,92	37,755	36,825	65,603
TAT [µg/L]	4,011	4,336	1,78	37,248	3,063	1,701	2,748
t-PA [ng/mL]	5,191	5,18	4,73	5,856	14,426	6,227	4,223

8.2 Appendix B: Poster presentation "Effects of Long Term Bed Rest on the Coagulation System as a Model for Post Surgical Immobilization"

Medical University of Graz

Effects of Long Term Bed Rest on the Coagulation System as a Model for Post Surgical Immobilization

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Introduction

Although there is no direct evidence, it is generally believed that bed rest shifts the haemostatic system towards hypercoagulability. Therefore, bed rested and immobilized patients are commonly treated with anticoagulants to prevent thrombotic events and hyper-clotting. It is still unclear which exact endothelial and haemostatic effects occur during long-term bed rest over time. We examined whether long-term bed rest leads to anti-/pro-coagulatory changes (Fig. 1) in healthy subjects using the possibility of a bed rest campaign (Fig. 2) at the Deutsches Zentrum für Luft- und Raumfahrt, Cologne, Germany.

Methods

The blood samples were collected from 8 healthy male (aged: 20-45) subjects undergoing 21 days of strict bed rest. All test persons received a comprehensive medical examination prior to participation. None of the participants suffered from any pathological disorder or were under any treatment. Blood collection was before and immediately after bed rest. We recorded clot development curves (Fig. 3) and measured markers of thrombin formation (prothrombin fragment 1+2, F 1+2), fibrinolysis (tissue plasminogen activator, t-PA) and endogenous thrombin potential.

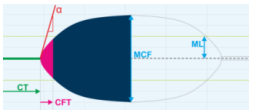

Figure 3: Thrombelastometry

http://www.rotemimc.com/site/index.php?option=com_content&view=article&id=28&Itemid=8

Results

Table 1: Coagulation values prior and after 21 days of bed rest

	Pre-bedrest	Post-bedrest	Significance
Prothrombin Fragment 1+2 [mmol/L]	164.6 (82.9 - 230.2)	128.7 (82.8 - 443.6)	n.s.
Endogenous thrombin Potential [nmol/L . min]	1572.9 ± 211.2	1396.0 ± 129.6	n.s.
Tissue Plasminogen activator [ng/mL]	4.18 ± 0.83	3.70 ± 0.80	n.s.
Thrombelastometry values:			
Coagulation time [s]	271.1 ± 28.8	302.4 ± 46.4	n.s.
Maximum clot firmness [mm]	21.0 (16 - 25)	20.0 (18 - 29)	n.s.
Alpha angle [°]	57.3 ± 6.2	56.5 ± 3.7	n.s.

Bed rest did apparently not cause hypercoagulability. Thrombelastometry values and parameters of thrombin generation and fibrinolysis were essentially the same before and after 21 days of strict bed rest, shown in Table 1.

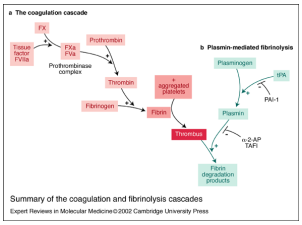

Figure 1: Coagulation cascade

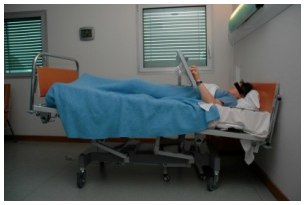

Figure 2: Bed Rest Campaign

http://spaceinimages.esa.int/var/esa/storage/images/esa_multimedia/images/2012/11/bedrest/12097643-2-eng-GB/Bedrest.jpg

Conclusion

This study shows that long-term bed rest has no effect on the coagulation system. Neither pro-coagulatory nor anti-coagulatory values increase during bed rest. Unlike the broadly acknowledged belief, that immobilization increases the tendency to clot during bed rest, very interestingly, there is no evidence found. In the clinical context the reasonable question arises whether all patients undergoing surgery or suffer from physical restriction and stay in bed for certain periods of time need to be unexceptionally treated with anticoagulants to prevent thromboembolic events.

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Figure 28: Poster presentation "Effects of Long Term Bed Rest on the Coagulation System as a Model for Post Surgical Immobilization"