**Diplomarbeit**

# Immobilization and Hormonal Changes


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unter der Anleitung von

**Prof. Dr. Nandu Goswami**

Graz, 12. Februar 2015

# Eidesstattliche Erklärung

*Ich erkläre ehrenwörtlich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst habe, andere als die angegebenen Quellen nicht verwendet habe und die den benutzten Quellen wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.*

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# Vorwort

Diese Arbeit widmet sich den Einflüssen der Hormone Adrenomedullin und Galanin auf das Herz-Kreislaufsystem unter Schwerelosigkeit. Diese Arbeit gab mir einen interessanten Zugang den komplexen Regelkreis des menschlichen Hormonhaushaltes näher zu untersuchen. Von besonderem Interesse war hier die Umwandlung von physikalischen Reizen wie der Druckverteilung im Kreislaufsystem auf die Konzentration von Hormonen. Eine Analogie zu technischen Regelkreisen ist deshalb nicht von der Hand zu weisen.

# Danksagungen

Ich möchte mich bei allen Unterstützern der Arbeit bedanken. Ganz besonderer Dank gebührt Herrn Professor Goswami, der durch seine weitsichtige Unterstützung und Geduld diese Arbeit erst ermöglicht hat.
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# Zusammenfassung


## *Hintergrund und Ziel der Studie*

Länger andauernde durch Krankheiten hervorgerufene Bettlägerigkeit hat Einfluss auf das kardiovaskuläre System. Adrenomedullin und Galanin sind zwei Hormone, die in die Regulation von Blut- und Herzkreislaufsystem involviert sind. In dieser Studie haben wir die Anpassung der Adrenomedullin und Galanin – Expression während einer länger andauernden Bettlägerigkeit mit einer Kopftiefneigung von 6° (Head down tilt HDT 6°), die eine Simulation der Schwerelosigkeit darstellt, untersucht.

## *Methoden und Materialien*

Die randomisierte Crossover-Studie wurde an 12 gesunden männlichen Probanden in der MEDES in Toulouse durchgeführt. Die Testpersonen lagen 21 Tage mit einer Kopftief-neigung von 6° im Bett um die durch den Einfluss der Schwerelosigkeit hervorgerufenen Flüssigkeitsverschiebungen hervorzurufen. Blutproben wurden vor Beginn, während und nach der Studie entnommen. Die Adrenomedullin- und Galaninkonzentrationen in den Proben wurden an der Medizinischen Universität Graz ermittelt.

## *Ergebnisse*

Alle Studienteilnehmer zeigten einen Anstieg der Adrenomedullin- und Galaninwerte. Die Konzentration von Adrenomedullin stieg um +29 % von Beginn der Studie bis zum Ende, allerdings fiel dieser Wert zwei Tage nach der Studie auf +19 %. Im gleichen Zeitraum stieg der Galaninwert um 49 %. Zusätzlich war eine Steigerung der Konzentration zwei Tage nach der Studie auf 56 % feststellbar.

## *Diskussion*

Wir haben einen Konzentrationsanstieg sowohl von Adrenomedullin als auch von Galanin ab dem 14.Tag der Studie beobachtet. Wir nehmen an, dass die Veränderungen der hydrostatischen Volumenverteilung und der Flüssigkeitsverschiebungen in den Blutgefäßen und Herzkammern der Grund für die Konzentrationsanstiege sind. Eine Hypothese für den Anstieg von Adrenomedullin könnte die kardio- und gewebeprotektive Wirkung dieses Hormons während einer Bettruhe sein. Der Anstieg der Galaninwerte könnte von der stärkeren Sympathikusaktivität abhängen. Der Sympathikus wird bei einer langfristigen Bettruhe stärker aktiviert (Christensen, et al.2005)

# Abstract

## *Background and aim of the study*

Long-term bed rest caused by several diseases has effects on the cardiovascular system. Adrenomedullin and Galanin are two of several hormones, which are involved in the regulation of blood and heart system. In this study we investigated the adjustment of the Adrenomedullin and Galanin expression during a long-term bed rest with a head down tilt position of 6 ° (HDT 6°) simulating microgravity.

## *Methods and Materials*

The randomized cross over study was carried out 12 healthy men at MEDES, Toulouse. The subjects stayed in bed rest for 21 days with a head down tilt position of 6 ° (HDT 6°), so as to simulate the effects of microgravity induced fluid shifts. Blood samples were taken before, during and after the long-term bed rest. Blood samples were analyzed for Adrenomedullin and Galanin concentrations at the Medical University of Graz.

## *Results*

All participants showed an increase in Adrenomedullin and Galanin values. The concentration of Adrenomedullin rose +29 % from the beginning until the end of the study however two days after the end of the study it was only +19 %. For the same duration the Galanin concentration increased by 49 % and kept on increasing two days after completion of the study and reached values of 56 %.

## *Discussion*

We observed significant increases of the concentration of Adrenomedullin and Galanin from the 14$^{th}$ day of the study. Our assumption is that an alteration of hydrostatical load distribution and fluid shift within the blood vessels and the heart chambers causes the increased concentration. One hypothesis for the increase of ADM is the tissue- and cardio-protective function of this hormone during immobilization.

The rise of the Galanin values could depend on greater effects of the sympathetic nervous system of the subjects in long-term bed rest (Christensen, et al. 2005). By a higher function of the sympathetic nervous system Galanin is released in an extended way like we could have seen in our study.

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# List of Abbreviations

## A

| | | | |
|---|---|---|---|
| AC | Angiotensin Converting Enzyme | ANP | Atrial Natriuretic Peptide |
| ACTH | Adrenocorticotrophic hormone | Arg | Arginin |
| AD | Adrenaline | ASAT | Aspartate aminotransferase |
| ADH | Anti Diuretic Hormone | Asp | Asparagine acid |
| ADM | Adrenomedullin | AVP | Arginin-Vasopressin |
| ALAT | Alanine aminotransferase | | |

## C

| | | | |
|---|---|---|---|
| cAMP | Cyclic adenosine monophosphate | COMT | Catechol – O – Methyltransferase |
| CBC | Cell blood count | CONT | Standard bed rest |
| CGRP | Calcitonin gene-related peptide | | |

## D

| | | | |
|---|---|---|---|
| DEXA | Dual –energy X-ray absorptiometry | DOPEG | Dihydroxyphenylglycol |
| DHMA | Dihydroxmandelic acid | | |

## H

| | | | |
|---|---|---|---|
| HCO3 | Bicarbonate | His | Histidin |
| HDT | Head Down Tilt | HUT | Head Up Tilt |
| HIF | Hypoxia inducible factor 1 alpha | | |

## I

| | | | |
|---|---|---|---|
| Ile | Isoleucine | | |

## L

| | | | |
|---|---|---|---|
| LBNP | Low Body Negative Pressure | Leu | Leucine |

## M

| | | | |
|---|---|---|---|
| MAO | Monoamine oxidases | MDA | Malondialdehyde |
| MAPK | Mitogen-activated protein kinase | | |

## N

| | | | |
|---|---|---|---|
| NA | Noradrenalin | NO | Nitric oxide |
| NEX | Study design with exercise with special nutrition | | |

## P

| | | | |
|---|---|---|---|
| PAMP | Proadrenomedullin-N-terminal-Peptide | PT | Prothrombin Tim |
| Phe | Phenylalanine | PTT | Partial Thromboplastin Time |
| PNMT | Phenyl Ethanolamine- N-methyltransferase | PVN | Paraventricular nucleus |
| Pro | Prolin | | |

# R

| | | | |
|---|---|---|---|
| RVE | Study design with exercise | | |

# S

| | | | |
|---|---|---|---|
| SOD | Superoxide dismutase | SOP | Supraoptic nucleus |

# T

| | | | |
|---|---|---|---|
| TF | Tissue Factor | Tyr | Tyrosine |

# V

| | | | |
|---|---|---|---|
| Val | Valin | | |

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# 1 Introduction

Since the beginning of mankind, men pay tribute to the stars. Cultures and religions developed calendars and navigational instruments with the help of the stars. And from the start there was always the dream to travel to the seat of the gods.
Travelling to space has also a long tradition in literature, when Jules Verne published his novel „From the earth to the moon“ (*De la Terre à la Lune)* he described the possibility of spaceflight.
The beginning of the real space age was in the roaring twenties, when the Austrian physicist Herman Oberth and the American physicist Robert Hutchins Goddard were the first ones that specified the theoretical fundamentals as well as the practical knowledge of rocketry and astronautics. They build the first rockets in 1923 and 1926. In World War II the Germans pushed the research in rocket science for developing weapons. After world war II, the Soviet Union and the United States of America started to develop space crafts. The Soviets shocked the western world on October 4th 1957 when they placed the first manmade object into the orbit. The first mammal sent to space was the dog Laika in the year of 1957, again the Soviets were the leading nation.
Then US president John F. Kennedy started the program to conquer to land on the moon, which was successful in 1969. Decades after decades many astronauts orbited the space but there is still enough to explore and study. Countless tests and studies into physiological alterations through space travel have made great advances in medical research possible.

Spaceflight is known to have short term and long-term effects. The figure below summarizes the physiological effects of spaceflight (Figure 1).

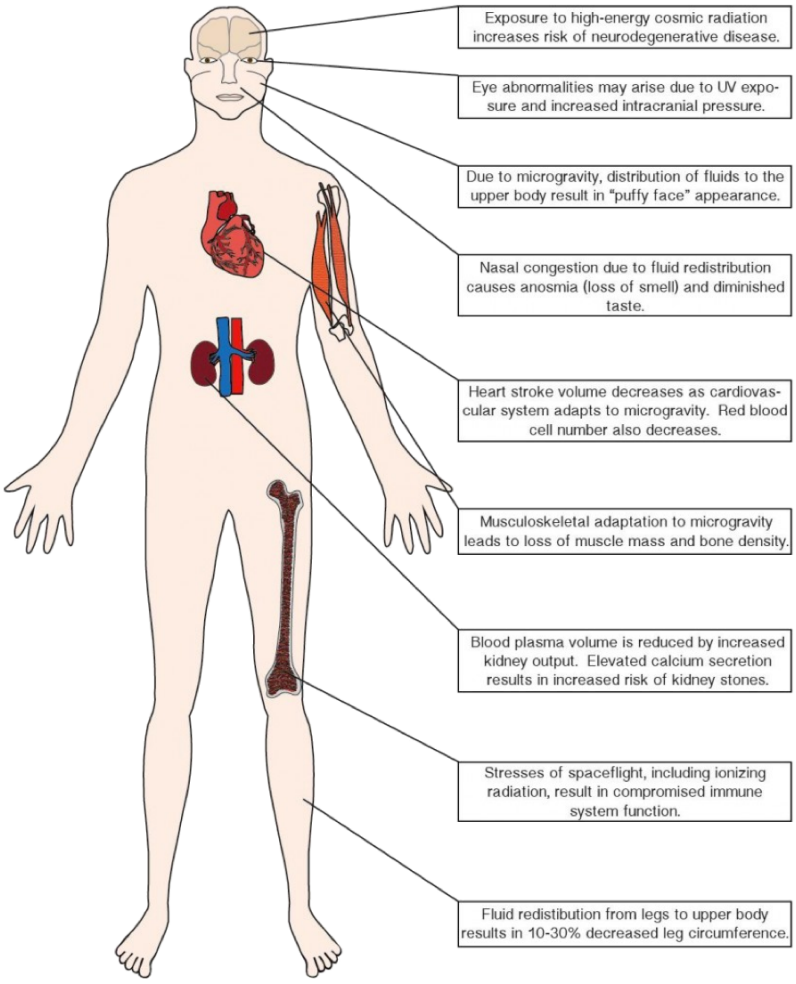



**Figure 1: Physiology of spaceflight induced deconditioning (Illustration by Mark Springel, edited by Hannah Somhegyi)[1]**

After spaceflight, astronauts still have signs and symptoms like the bone and muscle atrophy because of the lack of gravity, higher cranial pressure, retinal damages and alterations of the cardiovascular systems, leading to orthostatic intolerance.

As missions' numbers are limited and astronauts cost a lot of money, scientists try regularly to simulate space flight like conditions (e.g. microgravity) to enable them to understand, control and avoid the influence and impact of long-term spaceflight on the human body. Two models that are commonly used to simulate the effects of spaceflight are bed rest and water immersion (both wet and dry). In this diploma thesis the effects of

[1] Source: The human body in space: Distinguishing fact from fiction http://sitn.hms.harvard.edu/flash/2013/space-human-body/

microgravity was simulated using bed rest immobilization for 21 days (see methodology). I examined specifically how immobilization affects Adrenomedullin and Galanin, both of which are novel neuropeptides.

This diploma thesis is organized into the following sections:

- Orthostatic challenge
- Cardiovascular effects
- Hormonal effects
- Simulated Orthostatic Stress
- Head up tilt (HUT)
- Head down tilt (HDT)
- Lower body negative pressure (LBNP)
- Bed Rest Study
- Cardiovascular responses to bed rest

## *1.1 Physiology of Orthostasis*

What happens if we stand up from the lying into the upright position? Which physiological effects arise at this moment and sustain the blood circulation? This chapter explains the effects of orthostasis more in detail with a division to the different ranges like cardiovascular and hormonal effects.

### 1.1.1 Orthostatic challenge

A challenge our organism has to manage is the dynamic change of arterial and venous blood pressure to avoid inadequate blood supply when changing the position (Klinke, et al. 2005). To maintain the cardiovascular functions and a controlled blood pressure the organism must be able to react very quickly. So negative effects to the organism like insufficient blood circulation to the brain are suppressed and syncope is avoided.
The most significant impact could be seen when the body is moved from a horizontal to a vertical position, which means from lying to standing. This issue is also interesting for long term space flights, where the very low gravity, nearly weightlessness, leads to a kind of virtual bed rest.
The organism has several mechanisms to compensate pressure drops in the cardiovascular system to keep the blood circulation stable. These are:

- Rising of blood pressure by activation of the sympathetic nervous system
- Changing of the diameter of the periphery venous system
- Changing of the flow resistance
- Changing of the heart rate

### 1.1.2 Cardiovascular effects

Lying supine in horizontal position, the pressure load to the organism is nearly constant. The slight pressure drop between the heart, the head and lower extremities is based on the flow resistance of the blood vessels. The behavior of the blood vessels their self has only little influence to the system.

But when the position is changed to vertical, the pressure distribution changes totally. The arteries, which have a more rigid structure, do not change their volume capacity. Due to their elasticity the veins are able to perform this change in volume. More blood is stored in the lower extremities then in the head. The pressure in the arteries shows an increase at the lower extremities and a decrease in the head, depending on the distance from the indifferent plateau. The level where the pressure is constant and independent from position changes defines this plateau, which is close to the diaphragm. To guarantee the blood supply far from the heart, the veins have to change their volume. They collapse above the indifferent plateau and extend below.

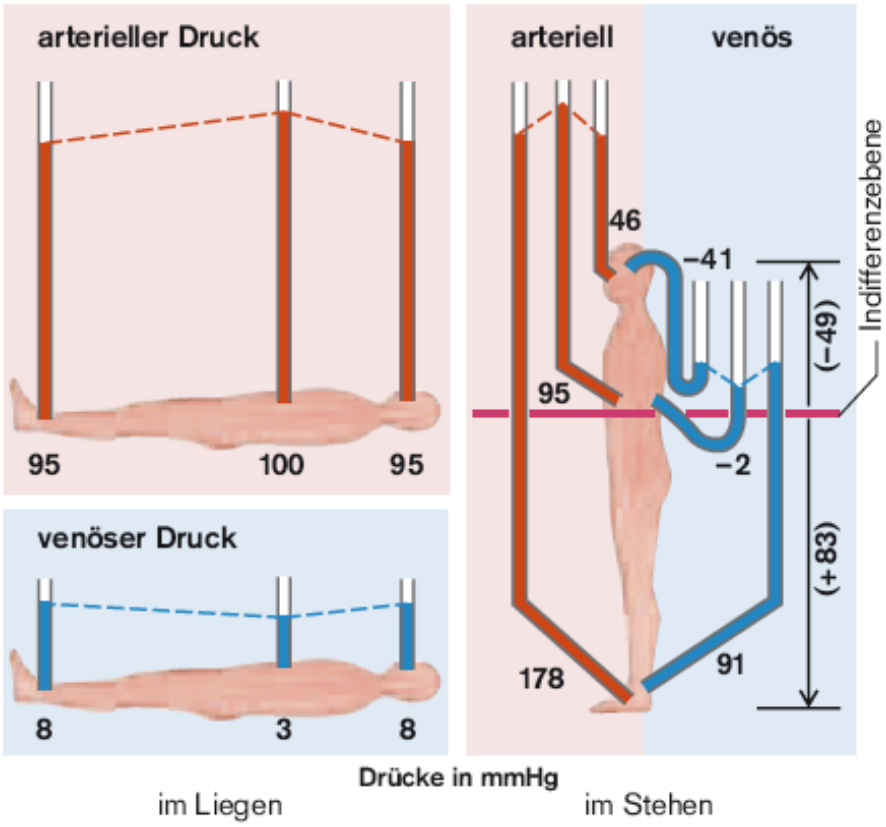



**Figure 2: Pressure Distribution**[2]

Strongly simplified the system can be explained by the Bernoulli equation.

[2] source: (Klinke, et al. 2005) page 219

$$p + \rho \cdot g \cdot h + \rho \cdot \frac{v^2}{2} + \xi \cdot \rho \cdot \frac{v^2}{2} = Constant$$

$p = \text{Pressure } [Pa]$
$\rho = \text{Density } [\frac{kg}{m^3}] \text{ (blood ca. } 1060 \frac{kg}{m^3})$
$g = \text{Gravity } [\frac{m}{s^2}] \ (9.81 \frac{m}{s^2})$
$v = \text{Velocity } [\frac{m}{s}]$
$h = \text{Height } [m]$
$\xi = \text{Flow Resistance [-]}$

**Equation 1: Bernoulli Formula**

As an example for this behavior the veins of the arm collapse if you bring them above the indifferent plateau and are fully filled if the arm is situated below this plane. The logical improvement conclusion of this theory leads to a more detailed simulation model of the cardiovascular response of orthostatic stress (Heldt, et al. 2002).

### 1.1.3 Hormonal effects

The adaptivity of the vessels, the heart and all other components of the body's circulation system requires numerous physiological processes. These interactions guarantee the ability of adjusting by keeping the blood circulation in every part of the body constant and adapt blood pressure, stroke volume and heart rate to the changed physiological alteration within a second to keep the blood and oxygen supply of essential organs alive. Several hormones are activated to create a feedback loop, which regulates the impact on the blood vessels, blood volume and the heart. In this section, the effects, regulation and influence of these hormones are explained more detail.

#### 1.1.3.1 Epinephrine / Norepinephrine

The hormones Epinephrine and Norepinephrine are derivatives of amino acids, similar to Serotonin, Melatonin, Thyroxin and Triiodothyronine. They belong to the catecholamines, which are based on Tyrosine like L-DOPA and Dopamine.

The Greek “epi-nephros” means “on the kidney” and explains the origin of this hormones. In detail they are formed:

- DOPA → Dopamine (Dopamine-Neurons)
- DOPA → Dopamine → Noradrenaline (Noradrenalin-Neurons)
- DOPA → Dopamine → Noradrenaline → Adrenalin (chrome-affine adrenal medulla cells)

The first two transformations take place in the cytosol of the cell with Tyrosine-Hydroxylase as the characteristic enzyme. Its activity controls the amount of produced noradrenaline and adrenaline. Dopamine is actively transported into the vesicles and only there the dopamine β-hydroxylase is active. They appear in the noradrenergic neurons and in the adrenal medulla.
The last enzyme of the Catecholamine - biosynthesis, the phenyl ethanolamine- N-methyltransferase (PNMT) is the characteristic enzyme of the chrome-affine cells of the adrenal medulla and is stimulated by ACTH. It’s the enzyme, which converts Noradrenalin to Adrenalin.
As the result of the different enzyme equipment there are neurons, which produce dopamine (Kleine and Rossmanith 2007)
Neurons, which only produce Dopamine have not got the Dopamine ß –hydroxylase and those who release Noradrenalin and Adrenalin possess the enzyme PNMT.
Noradrenalin has two different ways of function, the first one is to react as a neurotransmitter via neurons, which directly have an effect on the target cell like muscle cells and the second function is to effect further distant cells through the bloodstream. In contrast to Noradrenalin Adrenalin only acts in the endocrine way and gets to the receptors via blood. There are ubiquitous receptors for Adrenalin on the nerve cells, too. Fact is that Noradrenalin is released almost exclusively from the nerve endings, while Adrenalin is released of the adrenal medulla. Recent findings show that adrenalin is produced also in the brain, but there are no further studies about that. The effect of both hormones on the myocytes is the same, but Adrenalin gets to these cells via blood circulation, released from the adrenal medulla and NA via nerve cells. The receptors of AD are also sensitive for NA, but less than for AD.
The adrenal mark contains the chrome-affine cells, which include NA or AD, the ones with AD inside have a brighter granule than the ones with NA. These chrome-affine cells are connected with each other by cell fibers. The cells of the medulla are originally derivatives

of the sympathetic nerve, that means they are neuro-secretory nerves and so they act like these cells by storing the hormones in vesicles and release them by activation of potassium (Kleine and Rossmanith 2007). The metabolism of AD and NA differs from each other, while AD is mostly taken up from the liver, NA is absorbed back in the axons. NA is transformed from the MAO to DOPEG or DHMA. AD on the other hand is a circulating catecholamine and is eliminated by COMT to metanephrines and normetanephrines, after that there is a conjugation with water-soluble groups of the liver.
One of the main functions of NA and AD is the impact on the heart and the cardiovascular system. Involved in the sympathetic nervous system NA works as a neurotransmitter directly released from the nerve endings to the cardiac cells. The blood transports AD to its target cells.
The effect of AD and NA depends on the different types of receptors and the biochemical affinity to them. There are five types of receptors having different localization and roles, $\alpha_1$, $\alpha_2$, $\beta_1$, $\beta_2$, $\beta_3$.. All of them are sensitive for NA and AD in a varying extent. The strongest affinity for AD shows $\beta_2$ and $\beta_3$ of the smooth muscle cells e.g. in the liver, pancreas, in the brain and gastrointestinal tract.

#### 1.1.3.2 RAAS

Angiotensine and renin-angiotensine are released through renin from the angiotensinogen precursor. These proteins are produced in the liver. The so called juxtaglomerular cells of the kidneys are able to measure the blood pressure and if the pressure drops or the osmolarity (salt concentration in the blood) increases, these cells release the enzyme renin. The only known substrate of renin is angiotensinogen. Renin takes from the precursor the angiotensin I: Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu. Angiotensin I circulates in the blood and is modified in the lungs by the angiotensin-converting enzyme AC to angiotensin II: Asp-Arg-Val-Tyr-Ile-His-Pro-Phe. Angiotensin II stimulates the creation of the mineralocorticoid aldosterone in the adrenal glands. Angiotensin II could take a special role in the brain. Here mRNA is found for angiotensinogen, for renin, for ace and for angiotensin-receptors. This represents the full functional angiotensin spectrum, so angiotensin II could be produced and activated in the brain.
ACTH and angiotensin II control the formation and release of aldosterone. A drop in blood pressure, decreasing potassium and sodium concentration releases renin in the kidneys.

Similar to the prohormone convertase, renin is a so called split enzyme. It releases angiotensin I from angiotensinogen, which is primarily reduced to the angiotensin II with angiotensin converting enzymes in the lungs. By angiotensinase the angiotensin II could be reduced to angiotensin III. Similar to ACTH both angiotensine increase the aldosterone-synthesis (CYP11B2) in the adrenal glands.
Round or horseshoe shaped nests of bright cells are found in the glomerular zone. These cells produce and release mineralocorticoids, primary aldosterone. Angiotensin I is released from angiotensinogen by renin from the juxtaglomerular apparatus of the kidneys, which is converted to angiotensin II by angiotensin-convertase (ACE). This stimulates the aldosterone synthesis in the adrenal gland and their release.

### 1.1.3.3 Aldosterone

Aldosterone is produced in the adrenal cortex. It is one of the so-called "C 21 steroids". More precisely it is a member of mineralocorticoid family.

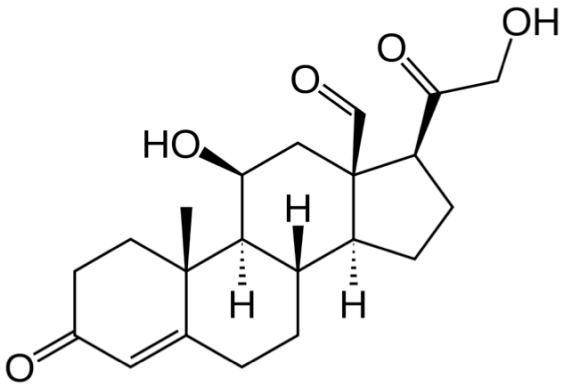



**Figure 3: Structure of Aldosterone**[3]

First studies of Morbus Addison, Tait and Simpson delivered the final proof of a mineralocorticoid substance in 1952. Two years later, in 1954, Jerome Conn described a disease caused by an aldosterone erythraea. The symptoms of the disease are hypertension, potassium loss over the urine and adrenal tumors (Pumberger 2014).
The outer section of the adrenal cortex in the glomerular zone produces aldosterone. The detailed biosynthesis is shown in Figure 4.

[3] source: http://en.wikipedia.org/wiki/Aldosterone

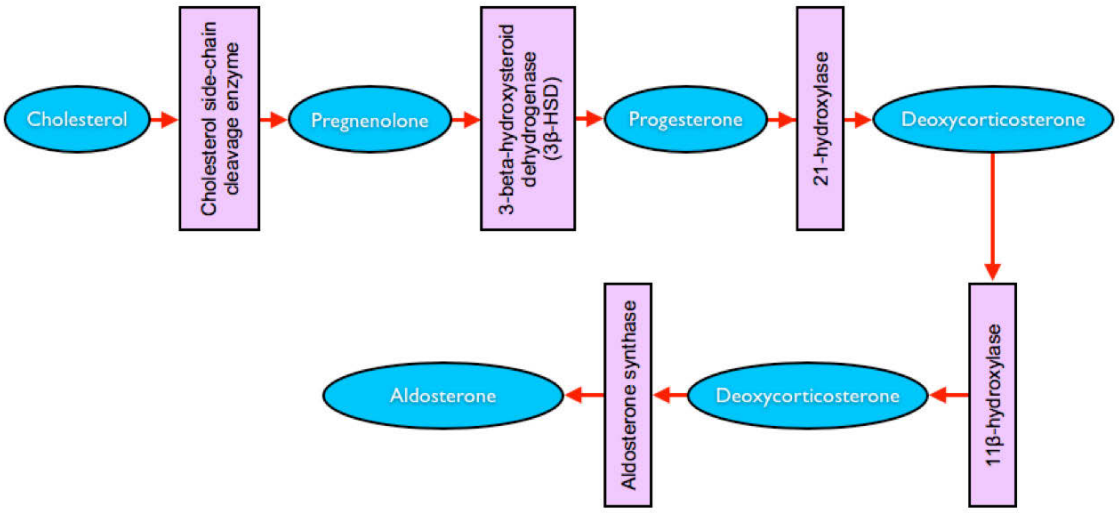



**Figure 4: Biosynthesis of Aldosterone**

The adrenal gland of an adult man produces 0,100 – 0,175 mg Aldosterone in average per day. The half-life time is about 20 to 30 minutes and much shorter than of cortisol. Passing the liver, about 75% of the circulating hormone is inactivated.

Most of aldosterone is converted in the adrenal gland to tetrahydro-glucuronide-derivate, less in liver and kidneys into 18-glucuronide. The secretion of aldosterone is via urine where still 5 % of the aldosterone is present (Althenne 2007).

The mineralocorticoids effects of aldosterone are the regulation of the electrolyte balance and the intravascular volume. Major effects are at the distal tubules and minor effects are on the cortical of the collecting channels. It increases the sodium-renewed resorption from perspiration and gastric juice. The sodium-renewed resorption is done by passive diffusion in apical membranes.

Additionally aldosterone induces the synthesis of the $Na^+$-/$K^+$ - ATPase in the laterobasal cell membranes. The enzyme creates a diffusion influencing electro-mechanical gradient. With an intercellular receptor, aldosterone effects the cellular level (mineralocorticoid-receptor). Another possibility is the linking to membrane-bound receptors. But not all effects of aldosterone can be explained with by concept.

The secretion of aldosterone is mainly influenced by several control systems, the most relevant are:

- The renin-angiotensin-system
- The sodium level
- The intravascular volume
- ACTH

It is also influenced by several stimuli where the glucocorticoid secretion is increased :

- Surgical treatment
- Fear
- Trauma
- Hemorrhage

And by some without the changing of glucocorticoid secretion:

- High supply of potassium
- Low sodium intake
- Compression of the vena cava inferior
- Standing
- Secondary hyperaldosteronism

The renin-angiotensin system is the classical way to control the Aldosterone level. Here, Angiotensin is involved in regulation; angiotensin II acts synergistically with potassium and indirectly by decreasing the blood flow through the liver and kidneys.
For a sustained production of Aldosterone, persistent calcium entry through calcium channels is required.
ACTH effects some stimulations of the secretion of aldosterone by stimulating the precursor desoxycorticosteron. It is increased by blood loss and other physical exertion.
Other regulators are the function of sympathetic nerves which influences the carotid artery pressure, baroreceptors in the vessel walls of the arteries in the thorax which adjust the blood pressure by changing the sodium and water retention. The Aldosterone release is also an inverse function of the sodium intake.

### 1.1.3.4 Vasopressin

This hormone also called anti diuretic hormone ADH or arginine – vasopressin AVP is stored and released in the posterior pituitary, but is produced in the paraventricular nucleus and supraoptic nucleus by the neuroendocrine cells in the hypothalamus. It is a peptide consisting of 9 amino acids. The main function of ADH is the vasoconstrictive effect and beside that the anti diuretic effect on the water balance.

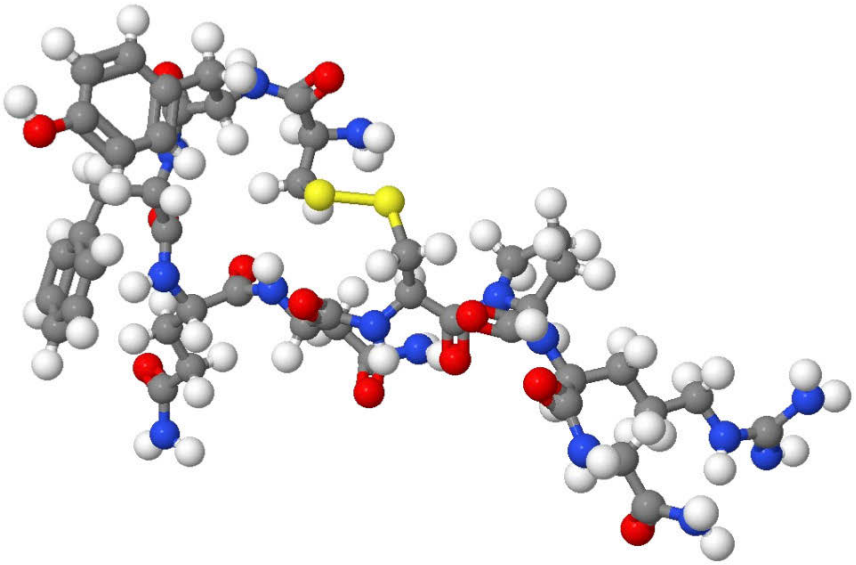


**Figure 5: Vasopressin**[4]

A complex network of hormones controls the water balance of the human body. The release of vasopressin is controlled by the osmolarity of blood by osmoreceptors. Vasopressin causes, by the increase of the reabsorption of water the release of renin, which leads to the synthesis of aldosterone. Thereby the expression of aquaporin and sodium transporters is controlled in the kidneys.
Central neuronal networks containing pressure sensors in the carotid arteries, in the aortic bow and atrial myocytes control the water balance. It also controls the vessel volume or the release of the atrial natriuretic peptide, an opponent of vasopressin. In fact it is the thirst that makes us drink and the osmolarity that controls our thirst.

[4] source:
http://www.chemgapedia.de/vsengine/vlu/vsc/de/ch/26/fmp/niere_msw/niere.vlu/Page/vsc/de/ch/26/fmp/niere_msw/niere_vasopressin.vscml.html

Adrenomedullin

The neuropeptide adrenomedullin (ADM) is a peptide with 52 amino acids, which was isolated of a humane pheochromocytoma in 1993. It is similar to other neuropeptides like Calcitonin-gene-related peptide (CGRP), Calcitonin and Amylin (Eto and Kitamura 2001). Among position 16 and 2 there is only one disulfide bond in the ADM-molecule. At the c-terminal end there is thyrosine with an amino-group (Kitamura, Kangawa und Kawamoto, et al. 1993). Both of these structures are important for the biological activity of the molecule. An ADM without this special intramolecular ring has an antagonistic effect by binding at the ADM-receptor (S. Eguchi, et al. 1994). After synthesis ADM undergoes a maturing process. It is synthesized as a part of a larger precursor-protein called pre-proadrenomedullin; this molecule comprises 185 amino acids. It possesses a signal peptide with 21 amino acids at the N -terminal end and a 20 amino acids long amidated peptide named proadrenomedullin – N-terminal peptide (PAMP), (Kitamura, et al. 1994). The gene for the pre- proadrenomedullin is located on the chromosome 11 (the gene of ADM composes of 4 exons and 3 introns with TATA, CAAT and GC boxes in the 5´-flanking region (Hinson, et al. 2000). Furthermore there are binding sites for the activator protein-2 (AP-2) and a cAMP-regulated enhancer element (Hinson, et al. 2000). In addition to these there are nuclear factor-$_K$B sites on the promoter of the adrenomedullin gene (Hinson, et al. 2000)

ADM is expressed in most of the tissue cells, primarily in vascular endothelial cells, that revises the assumption that ADM is mostly produced in the adrenal medulla (Hinson, et al. 2000). The storage of ADM is in the secretory granule of the pancreas (Hinson, et al. 2000). There is a carboxy-terminal glycine extended peptide form of ADM, which is the most common circulating form of it. This intermediate form is driven off the pre-proadrenomedullin. ADM and PAMP are split of from this peptide by enzymatic amidation. This ADM is the intermediate form of the hormone and circulates in the plasma in 85 %.

It has been shown that ADM has effect on the cardiovascular system. It is not only expressed in the vascular endothelial cells but also in the myocytes, ventricles and smooth vascular muscle cells. Although it is suggested that the release of this autacoid does not

implicitly show any cardiovascular effects (Endocrine regulation of orthostatic stress), many studies have revealed the contrary (Andreis , et al. 1998).

The effect of ADM on the cardiovascular system consists of vasodilatory and natriuretic effects, greater quantity of glomerular filtration and diuresis. It causes a hypotension by reducing the peripheral resistance. It is hypothesized that the vasodilatory effect is caused by an inhibition of different vasoconstrictors, decreases of calcium sensitivity of contractile areas, release of NO and activation of the cAMP pathway. The precursor of ADM, Proadrenomedullin has the same releasing area like catecholamine, the adrenal medulla, but the vasodilatory effect of it seems to be less than ADM, however, lowers the body temperature and the oxygen consumption, inhibits the ACTH, catecholamine and angiotensin II stimulated aldosterone secretion and reduces the blood glucose (Endocrine regulation of orthostatic stress (Andreis , et al. 1998)), thereby the effect of the direct inhibition of aldosterone release on the adrenal cortex is much stronger than of ADM (Endocrine regulation of orthostatic stress (S. Eguchi, et al. 1994) (Feng, et al. 1994)). The most common receptors in the vascular endothelial cells called the CGRP1-receptors. These ADM - effects can be suspended by CGRP8 - 37, a special CGRP1 - receptor antagonist. In addition, the binding to a specific ADM - receptor can be inhibited by ADM 22-52 (Geissler, et al. 2010).

The enzyme nitric oxide synthase can be activated in rabbit cardiomyocytes and endothelial cells,  it can stimulate the mitogen – activated protein kinase in the smooth vascular endothelial cells and to inhibit the MAPK in the mesangial cells. Besides, it is able to activate ATP sensitive K+ in the smooth muscle cells of the vessels (Sakai, Saito and Ishizuka 1998).

There is a possible correlation between the single stages of heart failure according to he New York Heart Association (stages I – IV) and the increased levels of ADM but without specification about the stage and a connective ADM-Level (Hinson, Kapas and Smith 2000). Studies about exercises and change of altitude from low to high also may change the ADM levels with an increase; furthermore there is a relationship of increasing ADM and blood pressure.
The fact that the increase of atrial natriuretic peptide leads to an increase of ADM shows that ADM „follows“ ANP by enhancing the effects of this hormone relaxing vascular

smooth muscle cells, inhibiting vasoconstrictor release (endocrine regulation during orthostatic stress) shown in a study with a control and a test group of healthy subjects, where the test subjects were given an infusion of atrial natriuretic peptide; the elevation of plasma ADM was only present for the 60 minute duration of the infusion (Hinson, et al. 2000).

Another interesting correlation between ADM and catecholamine is shown in a study with an epinephrine infusion during head up tilt and supine position and changing ADM levels. It is recognized that head up tilt increases ADM levels, but not whether epinephrine shows any changes in ADM distribution while HUT. In this study healthy subjects were given an epinephrine infusion during HUT and supine position. Normally short - term HUT increases ADM, in this case by administering epinephrine in the supine position and HUT the usual effects of epinephrine occurred, but ADM increases to a lesser extent; supine levels of ADM increase with epinephrine much more than HUT induced ADM levels by an infusion of epinephrine (Rössler, et al. 2011). There is a correlation between cardiac diseases and the ADM plasma level. It is assumed that in this case ADM reduces the blood pressure.

#### 1.1.3.5 Galanin

Tatemoto and Mutt have discovered this hormone over 20 years ago in 1978 (Tatemoto and Mutt 1978). In 1983 further research results have been released (Tatemoto, et al. 1983) and Galanin got its name. The typical structure with the N-terminal glycine and the C-terminal alanine lead to the name Galanin (GAL).
Galanin is synthesized and stored mainly in the hypothalamus including the preoptic area, paraventricular nucleus (PVN), supraoptic nucleus (SON) and median eminence (Baranowska-Bik, et al. 2005) and in the pituitary gland. It has the function of a neurotransmitter in the central and peripheral nervous system. GAL was also found in the solitary nucleus and the dorsal motor nucleus of the vagus nerve of the rats andthe medius subnucleus of the dorsal motor nucleus of the vagus nerve (Härfstrand, et al. 1987). The nervous system distributes this hormone throughout the body to the target organs as well as into the blood stream (Dunning, et al. 1990). The half-life of Galanin in the blood stream is about three to four minutes and thus rather short (Carrey, et al. 1993) (Holst, et al. 1993).

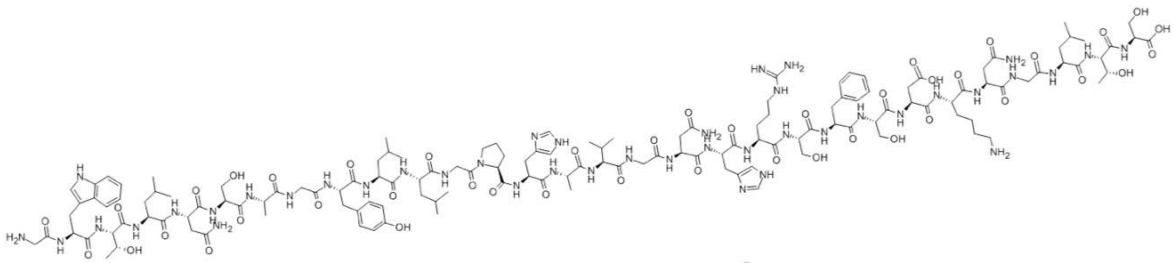


**Figure 6: Galanin (Human)**[5]

The first research results are based upon porcine Galanin. But this differs in its design (Holst, et al. 1993) in the way, that the human galanin consists of 30 amino acids without a α-carboxy-amidation and the porcine GAL possesses 29 amino acids. There is one peptide more in the human GAL than in the porcine Galanin Figure 6 shows the structure of Galanin.

There are three different Galanin receptors distributed in different areas GALR 1-3 with different functions in the neuroendocrine system. Only recently the functions of these receptors were detected by using subtype specific agonists and antagonists and GALR-1 and GALR-2 gene deletion strains.
The occurrences of GAL released of the parvicellular neurons of the hypothalamus strengthens the assumption that GAL influences the hypophysical system. These neurons are located in the medial septum, paraventricular nucleus, arcuate nucleus, preoptic area and anterior periventricular nucleus. In addition to those, there are neurons in the magnocellular system, which release GAL for the function of peripheral areas. In the pituitary gland the neurons also release GAL, in females from the lactrotrophs, in males from the thyreotrophs, corticotrophs and somatrotrophs. It has been investigated that the maturation and development of the milk glands is depending on GAL. In a study with GAL gene defected mice the female mice were unable to breastfeed their offspring (Kleine and Rossmanith 2007). It has been proven that females have lower concentrations of Gal, but the concentration increases with age.

GAL has many functions, e.g. modulating and transmitting of processes, nociception, cognition, sleep-wake cycle, acting as a growth factor which is manifested in the different localization (Hökfelt and Tatemoto 2010)

[5] source: http://www.chemicalbook.com/ProductChemicalPropertiesCB5234914_EN.htm

Possessing receptors in the myocardia it has the ability to lower the stress induced rise of catecholamines in the periphery and to trigger vasodepressive mechanisms. By an increase of the sympathetic function GAL is released in a higher concentration and reduces the parasympathetic neurotransmission.

## *1.2 Simulated Orthostatic Stress*

Orthostatic stress means the disturbance of the orthostatic system. Hereby, different methods are used e.g. head up tilt (HUT), head down tilt (HDT) and lower body and negative pressure (LBNP). These methods are explained in this section in more in detail.

### 1.2.1 Head up tilt (HUT)

Head up tilt, see Figure 7, is one of the methods in physiology to investigate the flow of the blood circulation processes in each species during change from lying into standing position. This test was originally used for recording blood pressure, heart rate and the beginning of feeling dizziness by patients with syncope symptoms.

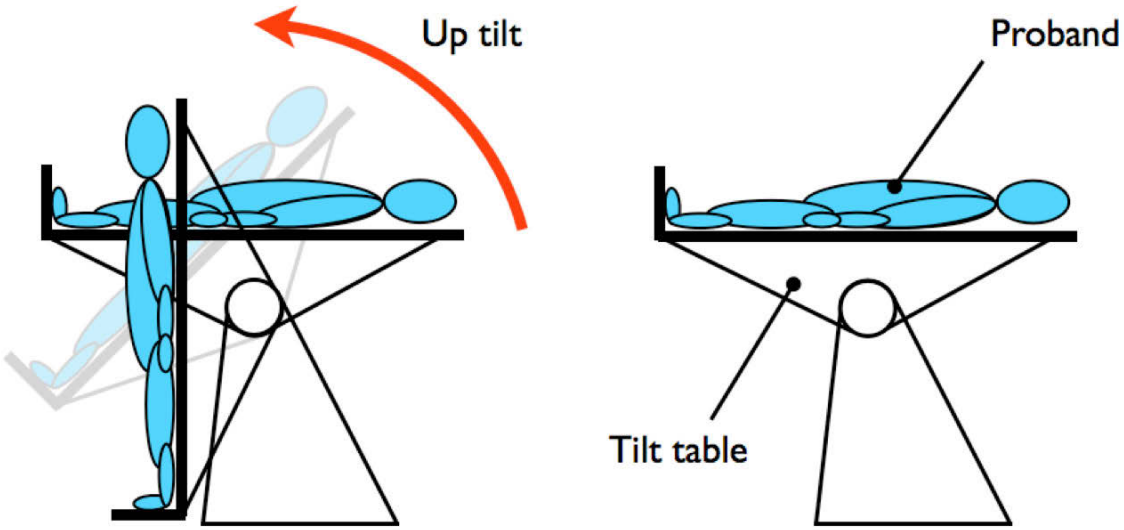



**Figure 7: Head up tilt**

The procedure in this study is performed on a special table called tilt table with many tilt angles, in the area of the head it can be tilted up and down and can also be completely lifted or taken into a the horizontal position. Another possibility is the HDT position. This

position has different effects on the blood and heart circulation, which will be described in a special chapter.

With several devices, connected to the test person the scientist is able to check and record different parameters. The most important physiological effect resulting from the HUT position is the decrease of the cardiac filling caused by gravitational force. The decrease of the preload leads to several alterations and readjustments of the blood circulation system. The immediate reply to this alteration is the central hypovolemia, a reduction of the cardiac output because of the lower preload and the shift of fluid from the intra-vascular to the interstitial area (Goswami, Loeppky and Hinghofer-Szalkay 2008).
Because of a lower preload and the reduced baroreceptor reflex during HUT the pressure in the diastole decreases or declines. Due to the decrease of the preload the right heart is not able to eject the same volume as before the HUT so the low pressure system gets a commensurate blood volume. Furthermore the passive HUT increases the vascular volume and the pressure in the lower extremities (Hinghofer-Szalkay and Cvirn 2013). Because of the higher hydrostatic pressure in the lower limbs the flow from the intravascular to the extravascular system is increased. This is the reason for the considerably elevated hematocrit, plasma protein levels and blood viscosity. It is assumed that the increased viscosity is the origin for the higher coagulation risk (Hinghofer-Szalkay and Cvirn 2013). The splanchnic volume increases during HUT because of the vasodilatation of the veins (Goswami, Loeppky and Hinghofer-Szalkay 2008). HUT induces shear stress in all of the vascular compartments, the same result can be observed by HDT. This shear stress induces the expression of TF, which is produced by the endothelial cells of the vascular system (Masoud, et al. 2008). The TF, also called F III or tissue thromboplastic is a membrane protein, which can be found in the fibroblasts of the Adventitia (Fleck, et al. 1990) and also can be expressed on the surface of macrophages, monocytes and endothelial cells (Schwartz, Gajdusek and Selden 1981). It transforms prothrombin into thrombin together with Ca2 ions and thrombin again transforms fibrinogen to fibrin (Löffler, Petrides and Heinrich 2006). This effect leads to the assumption that HUT and also HDT leads to a higher risk of thrombosis. The hematocrit value of the lower limbs is higher than that of the upper limbs by an upright position caused of the gravitational force, which pulls the intravascular fluid in earth direction and presses the plasma into the extravascular space.

### 1.2.2 Head down tilt (HDT)

In 1694 the Dutch physician Abraham Cyprianus invented the head down tilt position test, shown in Figure 8. This HDT position is still used in the intensive medical care during the procedure of setting a central venous catheter to avoid air embolism and getting a better preload of the right atrium. 170 years later the German surgeon Trendelenburg used this position and since then it is known as the Trendelenburg position (Kompanje, van Genderen and Ince 2012).

Some of the effects of HDT are contrary to the one of HUT and some are similar. While HUT leads to a decrease of the preload, HDT causes an increase and therefore a larger cardiac output (Nixon, et al. 1979). A -6° HDT position is used for simulating microgravity with a good cardiovascular response but is a poor model of the effects on pulmonary ventilation (Prisk and West 2002)

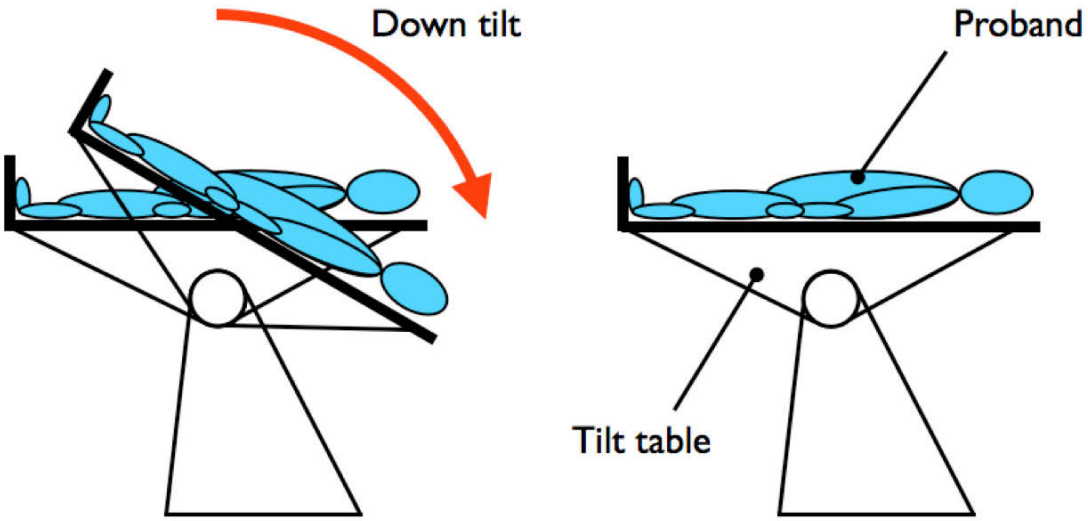



**Figure 8: Head down tilt**

### 1.2.3 Lower body negative pressure (LBNP)

Lower body negative pressure is used to place stress on the cardiovascular system. It is a well-established and important technique and often used to simulate gravity load. Due to applying negative pressure at the lower abdomen and lower extremities the orthostatic stress can be simulated very precise. Additional tilting is possible.

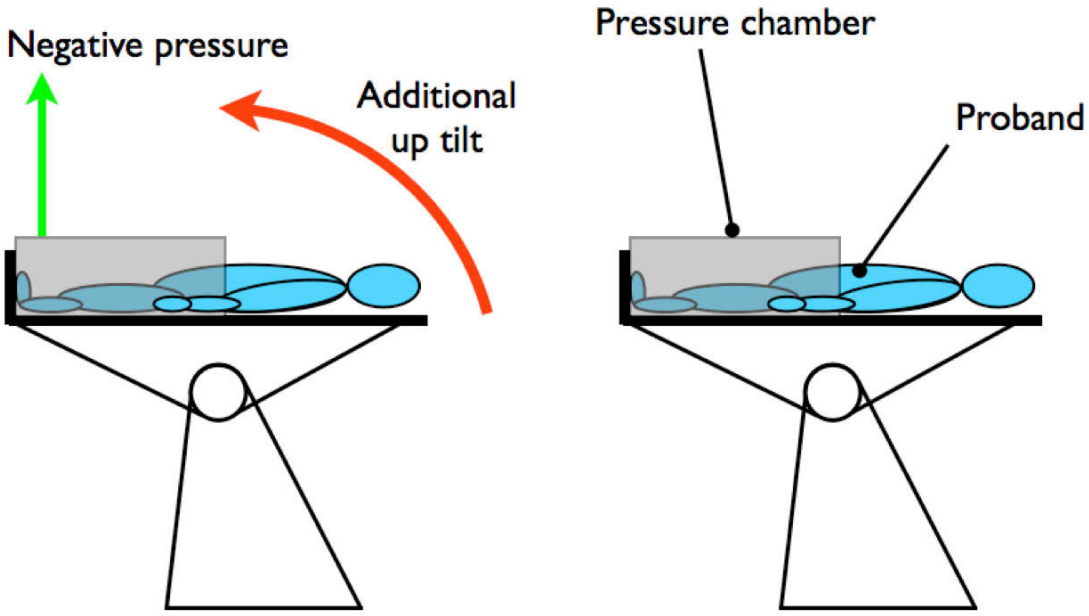



**Figure 9: Lower body negative pressure**

## *1.3 Bed Rest Study*

Bed rest studies are used to identify effects to the human physiology under microgravity conditions. They help to develop countermeasures to these effects on long space flights. But not only astronauts benefit from this research but also bedridden people on earth.

### 1.3.1 Cardiovascular responses to bed rest

It might be reasonably assumed that the effects and influence of a short - and long - term bed rest appears to a different extent.

What happens if the test subject lies for maximal 48 hours in bed? First of all, the hydrostatic fluid gradients drops and results in a cascade of alterations in the different parts of the body. The response of the changed hydrostatic fluid gradient is the movement of the fluid from the lower areas of the body head wards. The perfusion of the lower body decreases and in contrast to that the thoracic fluid volume and the cranial pressure rises. Because of that the thirst of the test subject decreases and the plasma volume is reduced too. The orthostasis might only function when there is sufficient fluid and in this case the quantity is not adequate, therefor causing a dysfunction of orthostasis.

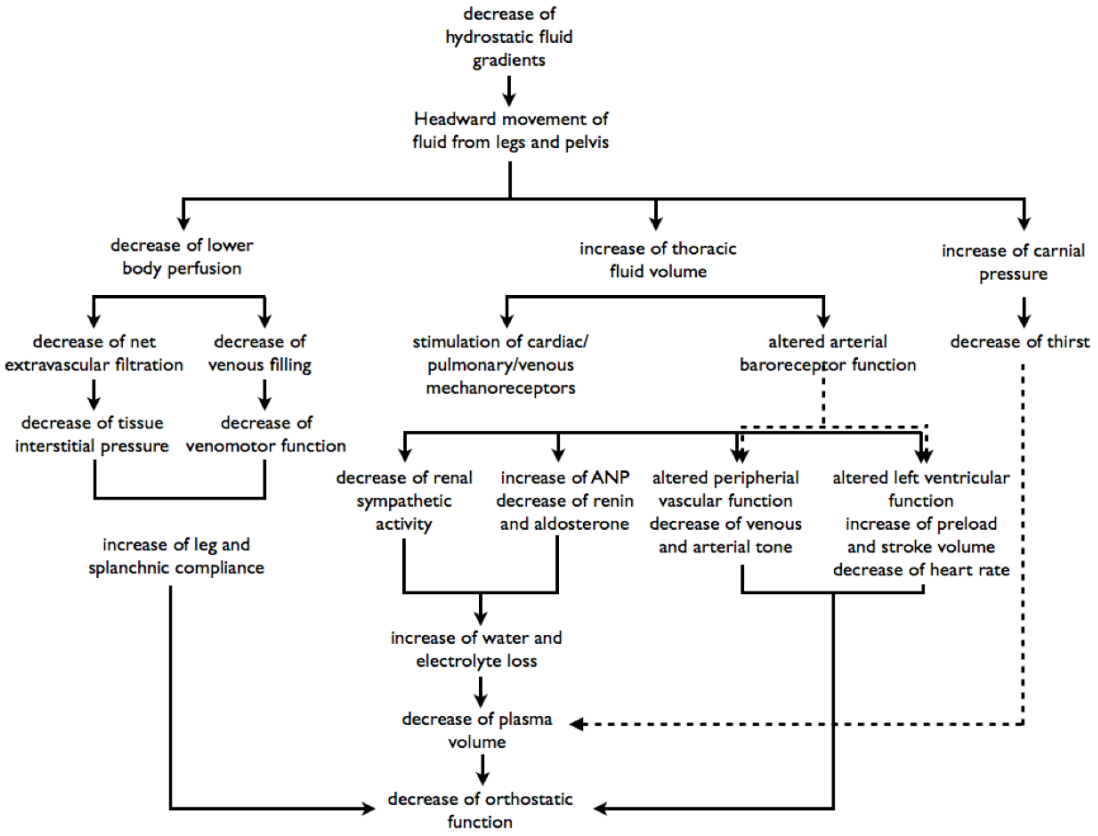
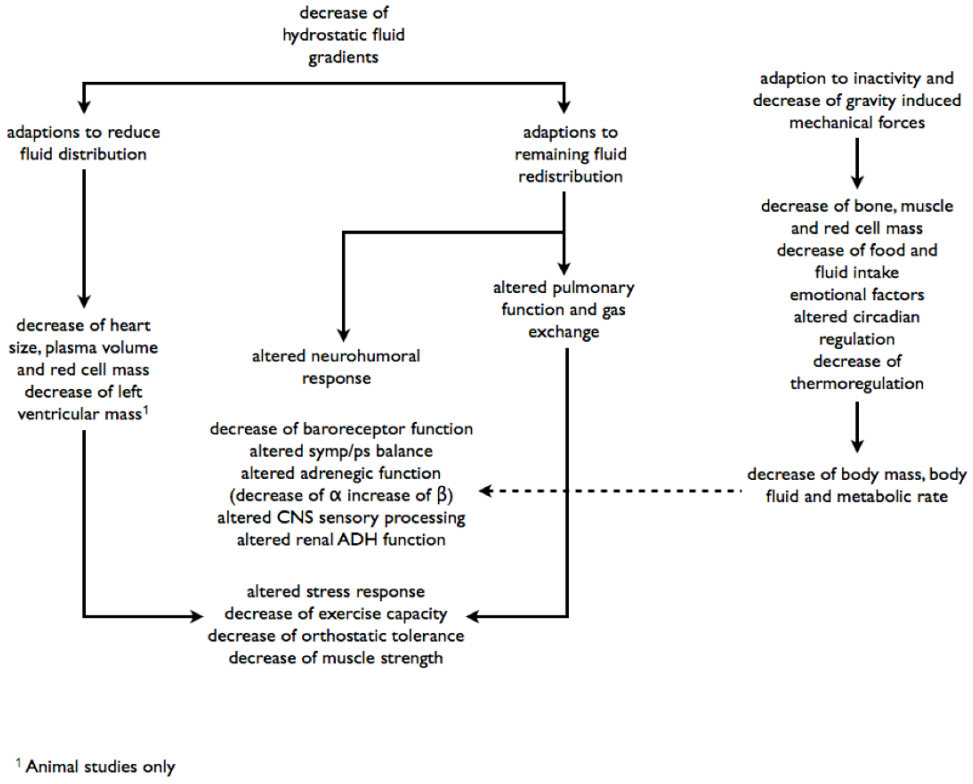



**Figure 10: Acute effects (0-48hours) to the cardiovascular response to bed rest (Fortney, Schneider and Greenleaf 1996)**




[1] Animal studies only

**Figure 11: Long-term effects (days, weeks, month) to the cardiovascular response to bed rest (Fortney, et al. 1996)**

# 2 Aims and Objectives

This study investigated the alteration of novel neuropeptides during HDT of 6°. The focus of this study is the analysis of the hormones ADM and GAL. They are one of the main hormones for the control of the heart and blood pressure regulation.

The study is performed with 12 healthy men with 25 to 45 years of age in the ESA medical center MEDES in Touluse, France. The test subjects are in a 6° HDT position for 21 days. Before starting the test procedure, blood samples were taken from the participants. Besides the hormonal level other blood parameters have been checked, too. With a randomized procedure the subjects are divided into three groups. One of these groups was to stay in bed in the 6° position. Another group was to practice some exercises and the last group was not only to perform the exercise but also to take some special nutrition.

The goal of the 6° HDT is to simulate the microgravity occurring during spaceflights. The 6° position is used because it combines the microgravity indicated load to the blood circulation and the comfort of the test persons best.

The information taken out of the behavior of different parameters influencing the cardiovascular system, lead to a better understanding of the changes and their influence to the human body.
The results of this study can improve medical care of people who are bedridden and therefor in a similar situation. But most of all, it will alter the understanding and impact of spaceflight and microgravity on the human body.

To join the study, the subjects had to fulfill high application criteria and had to pass a comprehensive test. During the study the test was repeated, with the same test subjects, three times with a break of 4 month in between.

The question asked, how does microgravity influence the hormonal concentration in long-term space flights. Due to the reaction of the blood circulation and pressure it is comparable to a long bed rest. The aim of this study is to determine the situation of GAL and ADM after a 21-day head down tilt procedure and the impact on the cardiovascular system.

# 3 Methodology

This chapter describes the methodology of the study and the analysis more in detail.

## *3.1 Procedure of the study preliminary*

The bed rest study oft the Medical University of Graz, Institute of Physiology and ESA, the European Space Agency was carried out in the MEDES. MEDES is based in Toulouse, France with a staff of 10-50 people. The aim and mission of this institute is research manned spaceflight and the physiological impacts on human beings. Both the preparation and the investigation were carried out in MEDES.

Approximately 70 volunteers were pre selected, of whom 12 test subjects and 2 back ups were finally chosen depending on the test results. It had to be ensured that every volunteer has read the information and consent document precisely and any information about the study is clear to everyone. After consent to the study every participant had got the right to leave the study in any phase and time without making a declaration.

Inclusion criteria were

- Healthy male volunteer with age between 20 – 45
- No overweight nor excessive thinness with BMI between 20 – 26
- Height between 158 – 190 cm
- No personal nor family past record of chronic or acute disease or psychological disturbances which could effect the physiological data and / or create a risk fort he subject during the experiment
- Fitness level assessment:
    - If age < 35 years: 35 ml / min. / kg < VO2max < 60 ml / min. / kg
    - If age > 35 years: 30 ml / min. / kg < VO2max < 60 ml / 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

And Non-inclusion criteria like

- 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 results for lower limbs echo-doppler
- History 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 giving blood (more than 8 ml / 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 oft he following tests: HAV IgM (Hepatitis A), HBs antigen (Hepatitis B), anti-HCV 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
- Subject who, in the judgment of the investigator, is likely non-compliant during the study or unable to cooperate because of a language problem or poor mental development
- Subject who has received more than 4500 Euros within the last 12 months for being a research subject
- Subject under guardianship or trusteeship
-

There is a detailed selection including a medical test and a physiological screening, both carried out from MEDES. The medical examination consist of a medical and surgery history including life habits, consuming nicotine, alcohol, caffeine and prior medication, a

clinical examination, taking measurements oft he systolic and diastolic blood pressure, the heart rate in supine and standing position after 3 and 10 minutes. Further the volunteers were under examination of 12 leads electrocardiogram, of their maximal oxygen consumption (VO2, max test), a DEXA measurement of their bone density, an alcohol breath test and several tests of the blood in a biological screening. This screening includes a biochemistry test with the examining of fasting glucose, sodium, potassium, chloride, calcium, HCO3, total protein, albumin, total cholesterol, HDL and LDL cholesterol, triglycerides, urea, creatinine, total bilirubin, aspartate aminotransferase (ASAT / SGOT), alanine aminotransferase (ALAT / SGPT), alkaline phosphatase, gamma glutamyl transferase (GGT), uric acid, C-reactive protein (CRP), TSH.

The hematology test includes cell blood count (CBC), platelets, reticulocytes, fibrinogen, PTT, PT, phlebitis markers (anti thrombin III, S-protein, C-protein), molecular screening, in which the mutation of Factor V Leiden and the mutation 20210 of the prothrombin gene is examined. If the test person has one of these mutations, he would be informed about this disease. Testing the vitamin and mineral status the fat-soluble vitamins like retinol, retinyl palmitate, beta-carotene, alpha-carotene, serum phylloquinone, alpha-tocopherol and gamma-tocopherol are controlled and also the water-soluble vitamin status like erythrocyte glutathione reductase, vitamin B6, red cell folate, vitamin C, vitamin B12, 25 OHD (vitamin D) and also serum iron, ferritin, transferrin saturation, transferrin receptor, selenium, copper and zinc. In addition to that the serology test with the hepatitis markers A, B, C and serological HIV are controlled, too.

All these tests were completed at the “Laboratoire Biopole France” : the analysis of the urine drug screen for detecting the consume of nicotine, barbiturates, benzodiazepines, opiates and cannabis, furthermore the urine analysis checking for haematuria, leucocyturia, glucose, ketonuria, proteinuria, bilirubin, urobilinogen, nitrites, pH and density. In the specialized departments Rangueil and Larrey Hospitals in Toulouse the volunteers have been examined in an Echo-Doppler of the lower limbs to eliminate any venous deficiencies, chest radiography of the front and side and a cardiac US scan test. Finally a psychologist went through several tests with the subjects to detect psychological imbalances and also to find the ideal volunteers who can take part in the study. (source „Selection of volunteers“)

**Table 1: Test person details**

| Letter | Date of Birth | AGE | Height (m) | Weight (Kg) |
|---|---|---|---|---|
| A | 23.02.68 | 44 | 1,75 | 76,3 |
| B | 03.03.72 | 40 | 1,69 | 61,1 |
| C | 09.09.69 | 42 | 1,77 | 78,5 |
| D | 26.05.76 | 36 | 1,9 | 80,7 |
| E | 11.09.71 | 41 | 1,72 | 59 |
| F | 01.12.70 | 41 | 1,69 | 62,8 |
| G | 31.12.71 | 40 | 1,77 | 71,2 |
| H | 29.04.92 | 20 | 1,77 | 65 |
| I* | 07.08.88 | 24 | 1,75 | 64,2 |
| J | 04.10.87 | 25 | 1,74 | 71,8 |
| K | 30.10.83 | 29 | 1,84 | 81,8 |
| L | 11.08.83 | 29 | 1,75 | 65,1 |

I* withdrawal from the study on campaign 2

In the described study, the participants have to stay in bed for 21 days with a head down tilt of 6°. Twelve healthy, non-medicated male volunteers are divided in three groups participating in different studies (see Figure 12).

The twelve test subjects chosen for this study, healthy men between the age of 20 and 44 years, have been anonymised (see Table 1) and each volunteer was given a letter for identification, A, B, C, D, E, F, G, H, I, J, K, L. The members of the three different groups, shown in Figure 12, have different study designs.

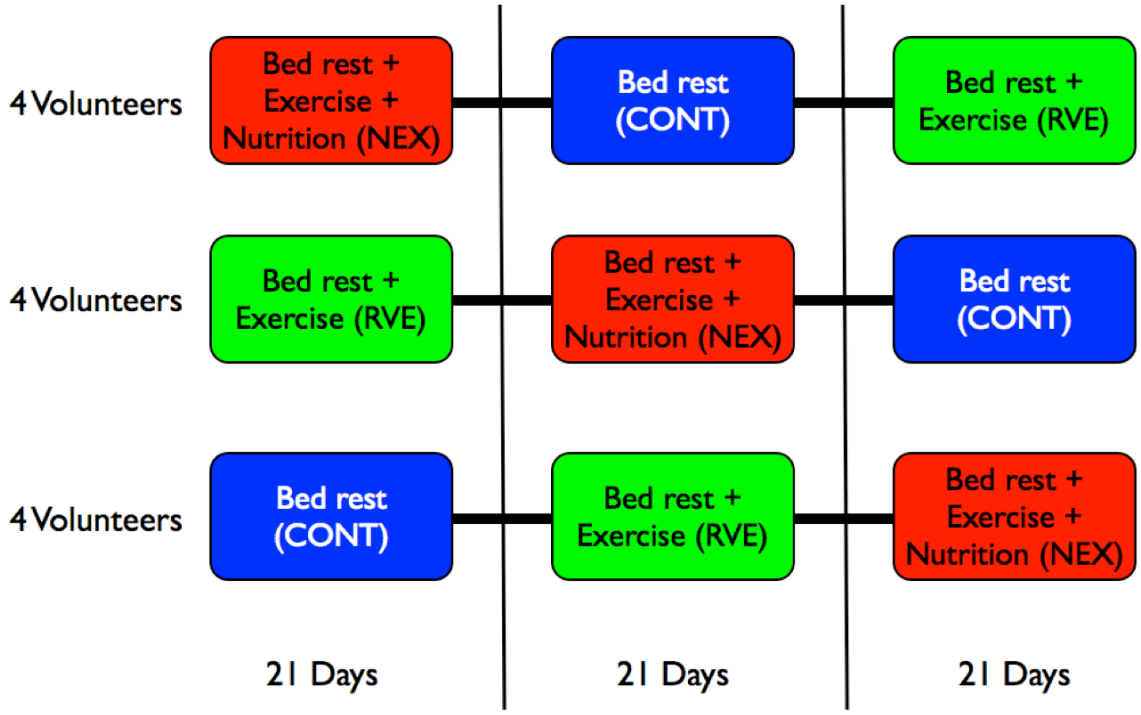



**Figure 12: Study design**

Group 1 subjects participate in 21 days bed rest without any special nutrition and bed exercise, group 2 does the bed rest with an additional special protein nutrition and the third group 3 the test includes the bed rest, the special protein nutrition and bed exercise. Data used in the results section of this diploma thesis are from immobilization protocol only.

**Table 2: Study group details**

| | C1 | C2 | C3 |
|---|---|---|---|
| A | RVE | NeX | CONT |
| B | CONT | RVE | NeX |
| C | RVE | NeX | CONT |
| D | NeX | CONT | RVE |
| E | NeX | CONT | RVE |
| F | CONT | RVE | NeX |
| G | NeX | CONT | RVE |
| H | CONT | RVE | NeX |
| I* | RVE | ~~NeX~~ | ~~CONT~~ |
| J | RVE | NeX | CONT |
| K | NeX | CONT | RVE |
| L | CONT | RVE | NeX |

Since there are three sets of the study with an interval of four month in between, every volunteer changes the group and takes part in every different study design. The typical exercise is shown in Figure 13.

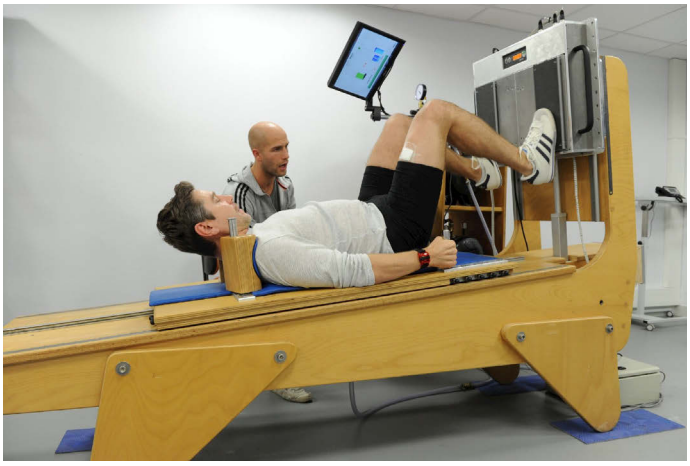


**Figure 13: Head down tilt exercise (courtesy of ESA and MEDES, Toulouse)**

The question asked is the influence of microgravity to the hormonal concentration in long-term space flights. Due to the reaction of the blood circulation and pressure it is comparable to a long bed rest. The aim of this study is to determine the situation of GAL and ADM after a 21-day head down tilt procedure.

## 3.2 Measurements

The tests are performed as a part of the European Program for Life and Physical Sciences at the MEDES clinical research facility in Toulouse, France.

Before the blood is analyzed, 10ml are given in an EDTA- aprotinin tube and three times carefully swiveled. The tubes are rotated with 3000g for 15 minutes in the centrifuge (Figure 14).

**Figure 14: Blood Centrifuge**

**Figure 15: Preparing blood samples**

After the centrifugation the blood plasma is taken out of the tubes and filled in five Eppendorf tubes with 0.1 ml, shown in Figure 15.


**Figure 16: Storage of blood samples in low temperature refrigerator**

The Eppendorf tubes are stored at -70° C in a low temperature freezer.
The following procedure of measurements contains the ELISA technique, which is carried out in one of the laboratories of the Uscn Life Science Inc. The assays made in this institute are for in vitro and research use only, diagnostic and therapeutic researches are not allowed.
The reagents and materials used for the assay are listed below:

- 1 Precoated ready to use 96-well strip plate
- 2 standard (lyophilized)
- 1x120 µl detection reagent A (green)
- 1x120 µl detection Reagent B (red)
- 1x9 ml TMB substrate
- 1x20 ml wash buffer (30 x concentrate)
- 4 plate sealer for 96 wells
- 1x20 ml standard diluent
- 1x6 ml assay diluent A (2x concentrate)
- 1x6 ml assay diluent B (2x concentrate)
- 1x6 ml stop solution
- 1 manual instruction

All of the materials are included in the kit for the assay. The storage of the kits is depending in the state, if they are unopened or opened. The standard, detection, reagent A and B and the 96-well-strip plate should be stored at -20° C after receiving, the rest of the materials should be stocked at 4° C.

## *3.3 Statistic Analysis*

### 3.3.1 Description of the "Box-Whisker"

The analysis is presented in this chapter. First the data is shown in tables. The first two figures deal with the results of each subject so the behavior of each person can be identified clearly. The third plot is a so-called "Box-Whisker" plot, which is used to display statistical results in a compact form. This type of plot is explained a little more in detail.

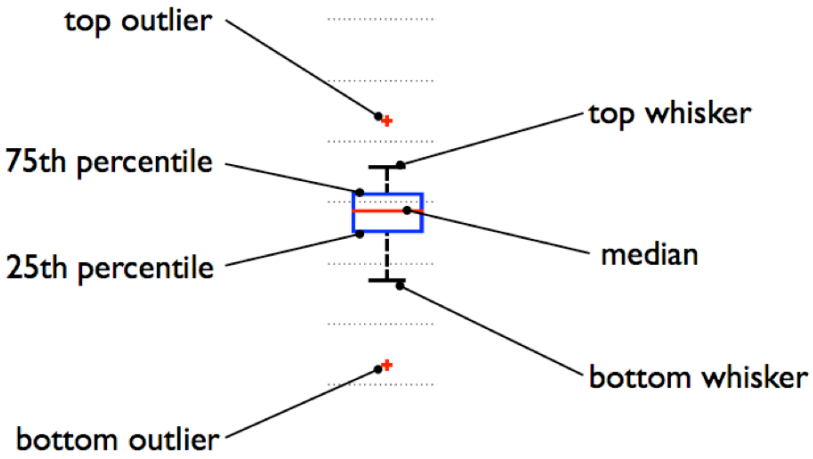



**Figure 17: Box-Whisker plot**

The box-whisker plot, see Figure 17, consist of the median of the analyzed data. The median divides a number of samples into two halves where one half is equal or smaller than the median value and the other half is equal or bigger. The limits of the box are the 25% and 75% percentile. Here either 25% or 75% are equal or smaller than the sample point. The whiskers are the minimum and maximum values but they have an allowed maximum length. If a sample point is out of this range, it is an outliner. The maximum and minimum whisker lengths are defined by 1.5 times the difference between the 75% and

25% percentile. The limit itself is the sample point, which fulfills this limit (BOXPLOT 2009).

### 3.3.2 MATLAB post processing of the results

The measurement results are provided in an excel sheet. The structure of the file made it difficult to analyze the data. To guarantee a good quality of the plotted figures, the software tool MATLAB has been chosen as analysis tool. In cooperation with tectos, Graz, a simple script has been written which plots the data and saves the figures to png´s.

The beginning of the script shows two vectors, which include the axes and allow the correlation between the different data sets. The first vector with 7 values includes the different time steps when the test probes are taken, from BDC to R2. The second vector includes all the 12 subjects and the mean value. Therefore this vector has a total of 13 values.

The next step in the script is a sorting of the matrices of all measurement values. The columns of these matrices are linked to the test person and the rows to the time stamps. This leads to a matrix size of 12x7. From the provided data 4 matrices are extracted, Galanin, Galanin 450 nm, ADM and ADM 450 nm.

The analysis of each of these 4 matrices was done separately. The results are plotted in 3 figures per matrix. The first plot shows the statistical analysis in a „boxplot“. The other two figures show the results from each subject. Due to the number of test subjects they are splitted in two figures, linked with the mean value of all subjects.

Plotting the statistical analysis a figure is opened and named after the shown data. The results are plotted with the Matlab function „boxplot “. To format and save the figures, the tectos own scripts „formatFigures“ and „figure2png“ are used.

The figure of the different test subjects, the rows of the according matrix is plotted. The first figure shows the first 6 subjects, from A1 to F1. The second figure shows subjects G1 to L1. The last curve in both figures is the mean value, which is calculated by the row of the matrix divided by the number of rows.

# 4 Results

This chapter summaries the statistical results of ADM and Galanin concentrations in the blood of each test person from those who did only the immobilization protocol. The concentration is measured by the ELISA method.

## *4.1 ADM*

**Table 3: ADM data subjects A-F**

| | | | A1 | B1 | C1 | D1 | E1 | F1 |
|---|---|---|---|---|---|---|---|---|
| BDC | Conc. | [pg/ml] | 24,106 | 18,563 | 20,103 | 11,389 | 22,246 | 17,899 |
| | Abs 450 nm | [-] | 0,614 | 0,753 | 0,711 | 0,990 | 0,657 | 0,772 |
| HDT2 | Conc. | [pg/ml] | 25,213 | 25,166 | 21,031 | 21,469 | 23,793 | 25,642 |
| | Abs 450 nm | [-] | 0,590 | 0,591 | 0,687 | 0,676 | 0,621 | 0,581 |
| HDT7 | Conc. | [pg/ml] | 25,450 | 23,660 | 19,431 | 18,885 | 31,649 | 28,725 |
| | Abs 450 nm | [-] | 0,585 | 0,624 | 0,729 | 0,744 | 0,471 | 0,521 |
| HDT14 | Conc. | [pg/ml] | 25,594 | 28,075 | 25,835 | 25,642 | 28,835 | 32,474 |
| | Abs 450 nm | [-] | 0,582 | 0,533 | 0,577 | 0,581 | 0,519 | 0,458 |
| HDT21 | Conc. | [pg/ml] | 27,030 | 27,599 | 25,981 | 22,921 | 30,317 | 28,344 |
| | Abs 450 nm | [-] | 0,553 | 0,542 | 0,574 | 0,641 | 0,493 | 0,528 |
| R0 | Conc. | [pg/ml] | 30,025 | 31,095 | 31,525 | 28,452 | 32,026 | 36,176 |
| | Abs 450 nm | [-] | 0,498 | 0,480 | 0,473 | 0,526 | 0,465 | 0,405 |
| R2 | Conc. | [pg/ml] | 30,435 | 28,946 | 26,276 | 5,938 | 34,638 | 27,235 |
| | Abs 450 nm | [-] | 0,491 | 0,517 | 0,568 | 1,226 | 0,426 | 0,549 |

**Table 4: ADM data subjects G-L**

| | | | G1 | H1 | I1 | J1 | K1 | L1 |
|---|---|---|---|---|---|---|---|---|
| BDC | Conc. | [pg/ml] | 28,075 | 26,625 | 30,613 | 32,538 | 26,877 | 33,195 |
| | Abs 450 nm | [-] | 0,533 | 0,561 | 0,488 | 0,457 | 0,556 | 0,447 |
| HDT2 | Conc. | [pg/ml] | 26,525 | 26,177 | 27,287 | 36,635 | 29,394 | 32,281 |
| | Abs 450 nm | [-] | 0,563 | 0,570 | 0,548 | 0,399 | 0,509 | 0,461 |
| HDT7 | Conc. | [pg/ml] | 26,877 | 26,726 | 29,621 | 35,726 | 30,083 | 30,317 |
| | Abs 450 nm | [-] | 0,556 | 0,559 | 0,505 | 0,411 | 0,497 | 0,493 |
| HDT14 | Conc. | [pg/ml] | 27,339 | 28,780 | 33,396 | 33,598 | 35,726 | 34,780 |
| | Abs 450 nm | [-] | 0,547 | 0,520 | 0,444 | 0,441 | 0,411 | 0,424 |
| HDT21 | Conc. | [pg/ml] | 28,398 | 30,673 | 35,726 | 35,285 | 37,823 | 32,733 |
| | Abs 450 nm | [-] | 0,527 | 0,487 | 0,411 | 0,417 | 0,384 | 0,454 |
| R0 | Conc. | [pg/ml] | 31,774 | 40,582 | 37,500 | 34,851 | 39,772 | 40,309 |
| | Abs 450 nm | [-] | 0,469 | 0,352 | 0,388 | 0,423 | 0,361 | 0,355 |
| R2 | Conc. | [pg/ml] | 31,034 | 30,025 | 39,334 | 32,026 | 34,078 | 30,613 |
| | Abs 450 nm | [-] | 0,481 | 0,498 | 0,366 | 0,465 | 0,434 | 0,488 |

### 4.1.1 ADM statistical plots

Comparing the results of all 12 subjects (Table 3) a clear divergence between the samples of subject D and all others is identified. The results of GAL of these test subjects are unremarkable.

The statistical analysis in Figure 18 shows an increase of the median until the end of the study. Two days after standing up the ADM level decreases. Two samples are outliners.

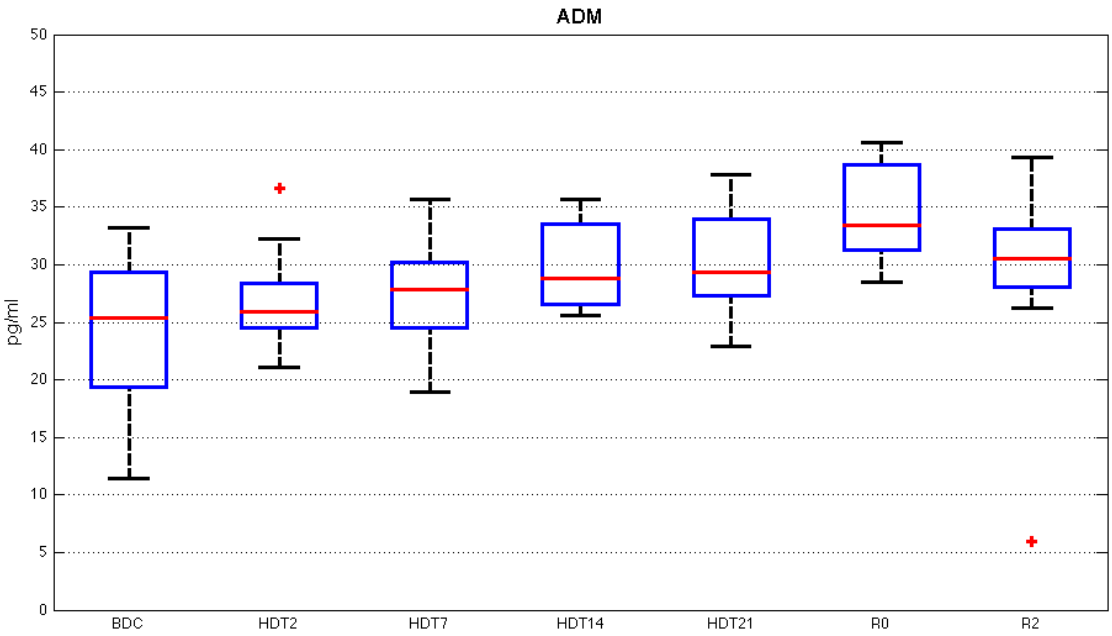



**Figure 18: ADM results in boxplot view**

**Table 5: Results ADM (Tukey's Multiple Comparison Test)**

| | Mean Diff. | q | Significant? P < 0.05? | Summary |
|---|---|---|---|---|
| BDC vs. HDT2 | -2.365 | 2.487 | No | ns |
| BDC vs. HDT7 | -2.910 | 3.060 | No | ns |
| BDC vs. HDT14 | -5.654 | 5.945 | Yes | ** |
| BDC vs. HDT21 | -5.883 | 6.187 | Yes | *** |
| BDC vs. R0 | -10.15 | 10.68 | Yes | *** |
| BDC vs. R2 | -4.862 | 5.113 | Yes | * |

## 4.2 Galanin

Galanin is the second hormone, which was investigated intensively in this study because of its effect on the heart rate and the blood pressure regulation among many other impacts, which will be discussed in this chapter.

**Table 6: Galanin data subjects A-F**

| | | | A1 | B1 | C1 | D1 | E1 | F1 |
|---|---|---|---|---|---|---|---|---|
| BDC | Conc. | [pg/ml] | 100,281 | 81,753 | 93,316 | 78,232 | 105,891 | 81,753 |
| | Abs 450 nm | [pg/ml] | 0,441 | 0,557 | 0,480 | 0,584 | 0,413 | 0,557 |
| HDT2 | Conc. | [pg/ml] | 109,824 | 111,199 | 97,135 | 84,951 | 125,100 | 113,088 |
| | Abs 450 nm | [pg/ml] | 0,395 | 0,389 | 0,458 | 0,534 | 0,336 | 0,381 |
| HDT7 | Conc. | [pg/ml] | 108,925 | 98,225 | 91,180 | 86,405 | 143,638 | 132,069 |
| | Abs 450 nm | [pg/ml] | 0,399 | 0,452 | 0,493 | 0,524 | 0,283 | 0,314 |
| HDT14 | Conc. | [pg/ml] | 111,665 | 113,570 | 113,570 | 120,527 | 137,370 | 133,781 |
| | Abs 450 nm | [pg/ml] | 0,387 | 0,379 | 0,379 | 0,352 | 0,299 | 0,309 |
| HDT21 | Conc. | [pg/ml] | 126,917 | 114,795 | 119,981 | 106,314 | 143,225 | 147,055 |
| | Abs 450 nm | [pg/ml] | 0,330 | 0,374 | 0,354 | 0,411 | 0,284 | 0,275 |
| R0 | Conc. | [pg/ml] | 128,476 | 126,305 | 138,117 | 122,199 | 138,873 | 177,960 |
| | Abs 450 nm | [pg/ml] | 0,325 | 0,332 | 0,297 | 0,346 | 0,295 | 0,221 |
| R2 | Conc. | [pg/ml] | 160,974 | 143,638 | 130,081 | 159,857 | 159,307 | 162,692 |
| | Abs 450 nm | [pg/ml] | 0,247 | 0,283 | 0,320 | 0,249 | 0,250 | 0,244 |

**Table 7: Galanin data subjects G-L**

| | | | G1 | H1 | I1 | J1 | K1 | L1 |
|---|---|---|---|---|---|---|---|---|
| BDC | Conc. | [pg/ml] | 105,054 | 128,793 | 137,742 | 134,130 | 124,803 | 150,694 |
| | Abs 450 nm | [pg/ml] | 0,417 | 0,324 | 0,298 | 0,308 | 0,337 | 0,267 |
| HDT2 | Conc. | [pg/ml] | 104,434 | 113,813 | 106,955 | 130,407 | 116,047 | 148,845 |
| | Abs 450 nm | [pg/ml] | 0,420 | 0,378 | 0,408 | 0,319 | 0,369 | 0,271 |
| HDT7 | Conc. | [pg/ml] | 108,925 | 112,135 | 109,824 | 143,225 | 123,922 | 155,089 |
| | Abs 450 nm | [pg/ml] | 0,399 | 0,385 | 0,395 | 0,284 | 0,340 | 0,258 |
| HDT14 | Conc. | [pg/ml] | 115,543 | 114,302 | 124,508 | 158,762 | 154,082 | 152,121 |
| | Abs 450 nm | [pg/ml] | 0,371 | 0,376 | 0,338 | 0,251 | 0,260 | 0,264 |
| HDT21 | Conc. | [pg/ml] | 126,305 | 117,587 | 153,093 | 159,857 | 137,370 | 158,223 |
| | Abs 450 nm | [pg/ml] | 0,332 | 0,363 | 0,262 | 0,249 | 0,299 | 0,252 |
| R0 | Conc. | [pg/ml] | 146,617 | 156,634 | 158,762 | 193,691 | 166,917 | 174,990 |
| | Abs 450 nm | [pg/ml] | 0,276 | 0,255 | 0,251 | 0,203 | 0,237 | 0,225 |
| R2 | Conc. | [pg/ml] | 152,605 | 138,873 | 195,744 | 193,691 | 158,762 | 177,203 |
| | Abs 450 nm | [pg/ml] | 0,263 | 0,295 | 0,201 | 0,203 | 0,251 | 0,222 |

### 4.2.1 Galanin statistical plots

At the beginning of the study the GAL values were between 80 to 150 pg/ml. Until the 7$^{th}$ day of the study the GAL level kept nearly constant. Then an increase of the levels could be detected. After ending the study the values increased only slightly as the statistical analysis of the data, Figure 19 shows.

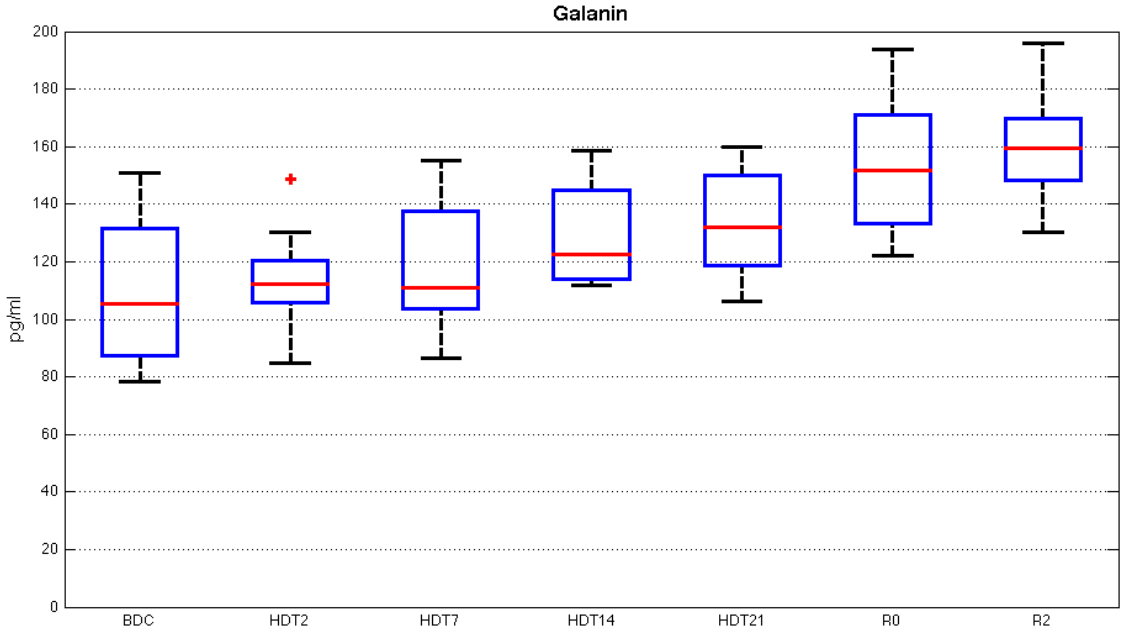



**Figure 19: GAL results in boxplot view**

**Table 8: Results Galanin (Tukey's Multiple Comparison Test)**

| | Mean Diff. | q | Significant? P < 0.05? | Summary |
|---|---|---|---|---|
| BDC vs. HDT2 | -3.280 | 0.9628 | No | ns |
| BDC vs. HDT7 | -7.593 | 2.229 | No | ns |
| BDC vs. HDT14 | -18.95 | 5.562 | Yes | ** |
| BDC vs. HDT21 | -24.02 | 7.052 | Yes | *** |
| BDC vs. R0 | -42.26 | 12.41 | Yes | *** |
| BDC vs. R2 | -50.92 | 14.95 | Yes | *** |

# 5 Discussion

We investigated the hormonal changes of Adrenomedullin and Galanin during bed rest in 12 healthy men lying for 21 days in head down tilt of 6°. The result of this study shows a significant change of these hormone concentrations during bed rest. This study specifically focused on the novel hormones Adrenomedullin and Galanin, as they are known to affect the cardiovascular system during space flight and/or immobilization.

Our results are particularly important, as the human body shows several changes and adaptations under simulated microgravity such as cardiac and neurovestibular alterations, loss of bone density, muscle atrophy and fluid shift from intravasal to interstitial space (Sutton 2005). The role of hormones, during immobilization or simulated gravity, however, has not yet been clearly elucidated. ADM has previously been investigated in several non-bed rest studies. Examined previously in these studies were the synthesis, structure, function and metabolism of ADM. For example, the widespread function of ADM was shown in the study of Kirisci (Kirisci, et al. 2013). They investigated the effects of ADM on rat muscle cells after ischemia and reperfusion and found out that ADM has a tissue-protective function. If we compare this fact with the ADM levels during bed rest in our study we can find a correlation with the tissue –protective effect of ADM and a significant increase of it from the 14$^{th}$ day of bed rest. During immobilization, the increases of ADM could have a protective function and prevent further muscle tissue damage.

In the year 2006 a study was performed with subjects staying immersed in water for 41 hours (Loder, et al. 2006). Water immersion is a good physiological model, which is widely used to simulate physiological conditions like microgravity. Specifically, changes of ADM were investigated in the Loder et al. study. The results for ADM showed an increase from 7,9 -+ 0,9 to 12,5 (c) -+2,3 pg / ml during water submersion. In our study of bed rested men we did not observe ADM increases during the first two days of the study, but from the 14$^{th}$ day there was a significant increase. It is possible that the hemoconcentration / plasma volume loss that occurs during immersion or during later stages of immobilization could have contributed to the increases in adrenomedullin (see Loder et al. 2006). The fact that plasma volume losses could be related to the increases in Adrenomedullin during bed rest or immersion also are supported by data from head up tilt studies, during which there is plasma volume loss and accompanied by a corresponding increase in Adrenomedullin concentration (Rössler 1999).

At orthostasis a vasodilatation arises in the peripheral vessels below the indifference plane. The increasing hydrostatic pressure causes this, which is a reaction of the gravity following column of liquid. The liquid settles down in the veins and even more if they are able to store more volume in varicose veins. The body's countermeasure is an increasing activation of the sympathetic nerve. This forces a rise of the vessels resistance and of heart frequency. An increase of the renin distribution leads to an increase of the aldosterone activation and therefore to a vasoconstriction and hypertension. ADM reacts to a vasoconstriction forced by angiotensin II, norepinephrine and endothelin-1 with a reactive vasodilatation. This reaction is also induced in studies associated with physical stress (Kitamura and Eto 1997), (Hinson, et al. 2000), (Benditt and Chen 2012), (Luodonpää, et al. 2003), (Haehling, et al. 2010). That means that ADM has an additional regulative function by counteracting if high levels of vasoconstrictors, vascular resistance and hypertension occur. In other words, ADM opposes the renin angiotensin system compensatorily. It is also possible that the observed increases of ADM during bed rest immobilization also contributed to minimizing the effects of the activated RAAS system, which occurs under bed rest. The hormone could, therefore, due to its increased release under vasoconstriction and hypertension, be considered as having a cardio-protective function.

In this study the microgravity is simulated with a HDT position of 6°. This leads to a decrease of the heart rate comparable to microgravity and weightlessness. Under this conditions the blood volume in the lower veins increase and 20 % of plasma shifts from intravasal to interstitial space within 24 hours and consequently the central venous pressure is reduced from 4-8 mm Hg to 0-2 mm Hg. The fluid distribution shifts in direction of the head so the neck and face of the test subjects swells.

Not only was ADM analyzed in this study but also the role of GAL was investigated. We have detected a significant increase of GAL from the $14^{th}$ day of head down bed rest. As we can see in Figure 11 one of the effects of long-term immobilization is its effect on baroreceptors and sympathetic activity. As Galanin also has sympatho-modulatory effects, the Gal - increases during immobilization could be related to the accompanying sympathetic changes that occur during bed rest.

*Limitations:*

Our study was performed only with male subjects therefore the ADM and Gal concentrations in female subjects needs to be studied.

*Summary and Conclusions*

ADM and GAL increased during immobilization. The ADM and GAL concentrations could be related to the hydrostatical fluid distribution in the blood vessels and their effects on the pressure receptors. Finally, ADM decreased following the termination of bed rest while the levels of GAL were still maintained during recovery.

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# 7 Apendix

MATLAB Script for generating the result plots

```
function TestResultsPlots
% Creating Box Plots from test results.
%
%% Galanin Data
close all
clear all

Data.Time{1} = 'BDC';
Data.Time{2} = 'HDT2';
Data.Time{3} = 'HDT7';
Data.Time{4} = 'HDT14';
Data.Time{5} = 'HDT21';
Data.Time{6} = 'R0';
Data.Time{7} = 'R2';

Data.Proband{1} = 'A1';
Data.Proband{2} = 'B1';
Data.Proband{3} = 'C1';
Data.Proband{4} = 'D1';
Data.Proband{5} = 'E1';
Data.Proband{6} = 'F1';
Data.Proband{7} = 'G1';
Data.Proband{8} = 'H1';
Data.Proband{9} = 'I1';
Data.Proband{10} = 'J1';
Data.Proband{11} = 'K1';
Data.Proband{12} = 'L1';
Data.Proband{13} = 'Mean Value';

Data.GAL = [100.281   109.824 108.925 111.665 126.917 128.476 160.974
81.753  111.199 98.225  113.570 114.795 126.305 143.638
93.316  97.135  91.180  113.570 119.981 138.117 130.081
78.232  84.951  86.405  120.527 106.314 122.199 159.857
105.891 125.100 143.638 137.370 143.225 138.873 159.307
81.753  113.088 132.069 133.781 147.055 177.960 162.692
105.054 104.434 108.925 115.543 126.305 146.617 152.605
128.793 113.813 112.135 114.302 117.587 156.634 138.873
137.742 106.955 109.824 124.508 153.093 158.762 195.744
134.130 130.407 143.225 158.762 159.857 193.691 193.691
124.803 116.047 123.922 154.082 137.370 166.917 158.762
150.694 148.845 155.089 152.121 158.223 174.990 177.203];
```

```
Data.GAL450 = [0.441  0.395  0.399  0.387  0.330  0.325  0.247
0.557  0.389  0.452  0.379  0.374  0.332  0.283
0.480  0.458  0.493  0.379  0.354  0.297  0.320
0.584  0.534  0.524  0.352  0.411  0.346  0.249
0.413  0.336  0.283  0.299  0.284  0.295  0.250
0.557  0.381  0.314  0.309  0.275  0.221  0.244
0.417  0.420  0.399  0.371  0.332  0.276  0.263
0.324  0.378  0.385  0.376  0.363  0.255  0.295
0.298  0.408  0.395  0.338  0.262  0.251  0.201
0.308  0.319  0.284  0.251  0.249  0.203  0.203
0.337  0.369  0.340  0.260  0.299  0.237  0.251
0.267  0.271  0.258  0.264  0.252  0.225  0.222];

Data.ADM = [24.106    25.213 25.450 25.594 27.030 30.025 30.435
18.563 25.166 23.660 28.075 27.599 31.095 28.946
20.103 21.031 19.431 25.835 25.981 31.525 26.276
11.389 21.469 18.885 25.642 22.921 28.452 5.938
22.246 23.793 31.649 28.835 30.317 32.026 34.638
17.899 25.642 28.725 32.474 28.344 36.176 27.235
28.075 26.525 26.877 27.339 28.398 31.774 31.034
26.625 26.177 26.726 28.780 30.673 40.582 30.025
30.613 27.287 29.621 33.396 35.726 37.500 39.334
32.538 36.635 35.726 33.598 35.285 34.851 32.026
26.877 29.394 30.083 35.726 37.823 39.772 34.078
33.195 32.281 30.317 34.780 32.733 40.309 30.613];

Data.ADM450 = [0.614  0.590  0.585  0.582  0.553  0.498  0.491
0.753  0.591  0.624  0.533  0.542  0.480  0.517
0.711  0.687  0.729  0.577  0.574  0.473  0.568
0.990  0.676  0.744  0.581  0.641  0.526  1.226
0.657  0.621  0.471  0.519  0.493  0.465  0.426
0.772  0.581  0.521  0.458  0.528  0.405  0.549
0.533  0.563  0.556  0.547  0.527  0.469  0.481
0.561  0.570  0.559  0.520  0.487  0.352  0.498
0.488  0.548  0.505  0.444  0.411  0.388  0.366
0.457  0.399  0.411  0.441  0.417  0.423  0.465
0.556  0.509  0.497  0.411  0.384  0.361  0.434
0.447  0.461  0.493  0.424  0.454  0.355  0.488];

%% GAL
figure('Name','Galanin');
boxplot(Data.GAL,Data.Time)
set(get(get(gca,'Children'),'Children'),'Linewidth',1.5)
yLim([0 200]);
formatFigures('Galanin','','pg/ml')
pause;
%fig2png;
```

```
figure('Name','Galanin A-F')
hold on
plot(Data.GAL(1,:),'-r','Linewidth',1.5)
plot(Data.GAL(2,:),'-g','Linewidth',1.5)
plot(Data.GAL(3,:),'-b','Linewidth',1.5)
plot(Data.GAL(4,:),'-m','Linewidth',1.5)
plot(Data.GAL(5,:),'-c','Linewidth',1.5)
plot(Data.GAL(6,:),'-k','Linewidth',1.5)
plot(sum(Data.GAL,1)/size(Data.GAL,1),'--r','Linewidth',1.5)
hold off
legend(Data.Proband{1:6},Data.Proband{13},'Location','SouthEast')
set(gca,'xTickLabel',Data.Time)
yLim([0 200]);
formatFigures('Galanin  Proband A-F','','pg/ml')
pause;
%fig2png;

figure('Name','Galanin G-L')
hold on
plot(Data.GAL(1+6,:),'-r','Linewidth',1.5)
plot(Data.GAL(2+6,:),'-g','Linewidth',1.5)
plot(Data.GAL(3+6,:),'-b','Linewidth',1.5)
plot(Data.GAL(4+6,:),'-m','Linewidth',1.5)
plot(Data.GAL(5+6,:),'-c','Linewidth',1.5)
plot(Data.GAL(6+6,:),'-k','Linewidth',1.5)
plot(sum(Data.GAL,1)/size(Data.GAL,1),'--r','Linewidth',1.5)
hold off
legend(Data.Proband{7:12},Data.Proband{13},'Location','SouthEast')
set(gca,'xTickLabel',Data.Time)
yLim([0 200]);
formatFigures('Galanin Proband G-L','','pg/ml')
pause;
%fig2png;

%% GAL450
figure('Name','Galanin Abs 450 nm');
boxplot(Data.GAL450,Data.Time)
set(get(get(gca,'Children'),'Children'),'Linewidth',1.5)
yLim([0 1]);
formatFigures('Galanin Abs 450 nm','','pg/ml')
pause;
%fig2png;

figure('Name','Galanin Abs 450 nm A-F')
hold on
plot(Data.GAL450(1,:),'-r','Linewidth',1.5)
plot(Data.GAL450(2,:),'-g','Linewidth',1.5)
plot(Data.GAL450(3,:),'-b','Linewidth',1.5)
plot(Data.GAL450(4,:),'-m','Linewidth',1.5)
plot(Data.GAL450(5,:),'-c','Linewidth',1.5)
plot(Data.GAL450(6,:),'-k','Linewidth',1.5)
```

```
plot(sum(Data.GAL450,1)/size(Data.GAL450,1),'--r','Linewidth',1.5)
hold off
legend(Data.Proband{1:6},Data.Proband{13},'Location','NorthEast')
set(gca,'xTickLabel',Data.Time)
yLim([0 1]);
formatFigures('Galanin Abs 450 nm Proband A-F','','pg/ml')
pause;
%fig2png;

figure('Name','Galanin Abs 450 nm G-L')
hold on
plot(Data.GAL450(1+6,:),'-r','Linewidth',1.5)
plot(Data.GAL450(2+6,:),'-g','Linewidth',1.5)
plot(Data.GAL450(3+6,:),'-b','Linewidth',1.5)
plot(Data.GAL450(4+6,:),'-m','Linewidth',1.5)
plot(Data.GAL450(5+6,:),'-c','Linewidth',1.5)
plot(Data.GAL450(6+6,:),'-k','Linewidth',1.5)
plot(sum(Data.GAL450,1)/size(Data.GAL450,1),'--r','Linewidth',1.5)
hold off
legend(Data.Proband{7:12},Data.Proband{13},'Location','NorthEast')
set(gca,'xTickLabel',Data.Time)
yLim([0 1]);
formatFigures('Galanin Abs 450 nm Proband G-L','','pg/ml')
pause;
%fig2png;

%% ADM
figure('Name','ADM');
boxplot(Data.ADM,Data.Time)
set(get(get(gca,'Children'),'Children'),'Linewidth',1.5)
yLim([0 50]);
formatFigures('ADM','','pg/ml')
pause;
%fig2png;

figure('Name','ADM A-F')
hold on
plot(Data.ADM(1,:),'-r','Linewidth',1.5)
plot(Data.ADM(2,:),'-g','Linewidth',1.5)
plot(Data.ADM(3,:),'-b','Linewidth',1.5)
plot(Data.ADM(4,:),'-m','Linewidth',1.5)
plot(Data.ADM(5,:),'-c','Linewidth',1.5)
plot(Data.ADM(6,:),'-k','Linewidth',1.5)
plot(sum(Data.ADM,1)/size(Data.ADM,1),'--r','Linewidth',1.5)
hold off
legend(Data.Proband{1:6},Data.Proband{13},'Location','SouthEast')
set(gca,'xTickLabel',Data.Time)
yLim([0 50]);
formatFigures('ADM  Proband A-F','','pg/ml')
pause;
%fig2png;
```

```
figure('Name','ADM G-L')
hold on
plot(Data.ADM(1+6,:),'-r','Linewidth',1.5)
plot(Data.ADM(2+6,:),'-g','Linewidth',1.5)
plot(Data.ADM(3+6,:),'-b','Linewidth',1.5)
plot(Data.ADM(4+6,:),'-m','Linewidth',1.5)
plot(Data.ADM(5+6,:),'-c','Linewidth',1.5)
plot(Data.ADM(6+6,:),'-k','Linewidth',1.5)
plot(sum(Data.ADM,1)/size(Data.ADM,1),'--r','Linewidth',1.5)
hold off
legend(Data.Proband{7:12},Data.Proband{13},'Location','SouthEast')
set(gca,'xTickLabel',Data.Time)
yLim([0 50]);
formatFigures('ADM Proband G-L','','pg/ml')
pause;
%fig2png;

%% ADM450
figure('Name','ADM Abs 450 nm');
boxplot(Data.ADM450,Data.Time)
set(get(get(gca,'Children'),'Children'),'Linewidth',1.5)
yLim([0 1.3]);
formatFigures('ADM Abs 450 nm','','pg/ml')
pause;
fig2png;

figure('Name','ADM Abs 450 nm A-F')
hold on
plot(Data.ADM450(1,:),'-r','Linewidth',1.5)
plot(Data.ADM450(2,:),'-g','Linewidth',1.5)
plot(Data.ADM450(3,:),'-b','Linewidth',1.5)
plot(Data.ADM450(4,:),'-m','Linewidth',1.5)
plot(Data.ADM450(5,:),'-c','Linewidth',1.5)
plot(Data.ADM450(6,:),'-k','Linewidth',1.5)
plot(sum(Data.ADM450,1)/size(Data.ADM450,1),'--r','Linewidth',1.5)
hold off
legend(Data.Proband{1:6},Data.Proband{13},'Location','NorthEast')
set(gca,'xTickLabel',Data.Time)
yLim([0 1.3]);
formatFigures('ADM Abs 450 nm Proband A-F','','pg/ml')
pause;
%fig2png;

figure('Name','ADM Abs 450 nm G-L')
hold on
plot(Data.ADM450(1+6,:),'-r','Linewidth',1.5)
plot(Data.ADM450(2+6,:),'-g','Linewidth',1.5)
plot(Data.ADM450(3+6,:),'-b','Linewidth',1.5)
plot(Data.ADM450(4+6,:),'-m','Linewidth',1.5)
plot(Data.ADM450(5+6,:),'-c','Linewidth',1.5)
```

```
plot(Data.ADM450(6+6,:),'-k','Linewidth',1.5)
plot(sum(Data.ADM450,1)/size(Data.ADM450,1),'--r','Linewidth',1.5)
hold off
legend(Data.Proband{7:12},Data.Proband{13},'Location','NorthEast')
set(gca,'xTickLabel',Data.Time)
yLim([0 1.3]);
formatFigures('ADM Abs 450 nm Proband G-L','','pg/ml')
pause;
%fig2png;


function formatFigures(inpTitle,inpXlabel,inpYlabel)
%FORMATFIGURES Formats figures.
%
%   FORMATFIGURES('Title','X- Axis Label','Y- Axis Label')
% --------------------------------------------------------------------------
% File:      formatFigures.m
% Author(s): DH
% Date:
% Version:   01
%---------------------------------------------------------------------------
% Copyright (c) 2004-2010 tectos gmbh
%---------------------------------------------------------------------------
% History:
%
%---------------------------------------------------------------------------
% Size should always be 650 x 500 pixels
%
%---------------------------------------------------------------------------
% bdwidth = 200;
% topbdwidth = -340;
% set(0,'Units','pixels');
% scnsize = get(0,'ScreenSize');
% fig_pos = [bdwidth,...
%   0 + bdwidth/2,...
%   scnsize(3) - 2*bdwidth,...
%   scnsize(4)/2 - (topbdwidth + bdwidth)];
% set(gcf,'Position',fig_pos);
% title(inpTitle,'FontSize',12,'Fontweight','bold');
% grid on;
% xlabel(inpXlabel);
% ylabel(inpYlabel);

xsize = 950;  %800;
ysize = 600;
set(0,'Units','pixels');
scnsize = get(0,'ScreenSize');
fig_pos = [scnsize(3)/2-xsize/2,...
           scnsize(4)/2-ysize/2,...
           xsize,...
           ysize];
```

```
set(gcf,'Color','w')
units = get(gcf,'Units');
set(gcf,'Units','pixels');
set(gcf,'Position',fig_pos);
set(gcf,'Units',units);
title(inpTitle,'FontSize',14,'Fontweight','bold');
grid on;
xlabel(inpXlabel,'FontSize',12);
ylabel(inpYlabel,'FontSize',12);
```