Diplomarbeit

Vasopressin and Aquaporin Changes in Young Healthy Volunteers Undergoing Orthostatic Challenge

eingereicht von

Johannes Reichmuth

zur Erlangung des akademischen Grades

Doktor der gesamten Heilkunde

(Dr. med. univ.)

an der

Medizinischen Universität Graz

ausgeführt am

Institut für Physiologie

unter der Anleitung von

Assoz.-Prof. Priv.-Doz. Dr.med. Nandu Goswami, PhD

ao.Univ.-Prof. Mag. Dr.rer.nat. Andreas Rössler

Graz, am 09.04.2016

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.

Graz, am 09.04.2016 ***Johannes Reichmuth eh***

Acknowledgements

First of all I would like to express my gratitude to my primary supervisor, Assoz.-Prof. Priv.-Doz. Dr.med. Nandu Goswami, PhD. for his continuous assistance and support through all stages of this thesis and for giving me an insight into the fascinating world of science and aerospace physiology. Also, discussing life in Nigeria and how to respond to spam emails with him greatly helped to raise my spirits during the time we worked together.

Secondly I want to thank my assistance supervisor ao.Univ.-Prof. Mag. Dr.rer.nat. Andreas Rössler for his assistance, providing literature and lending support during the experiments.

Furthermore I am very grateful to Prof. Ivana Valenti and Grazia Tamma, Ph.D. from the university of Bari, Italy for analyzing the urine samples and providing the aquaporin-2 graphs in this thesis.

Another big thanks goes to Andreas Jantscher and Rebecca Ruedl of the Institute of Physiology in Graz who were also a great help and pleasant company during the time we worked together.

I am highly grateful to the Medical University of Graz for the possibility of studying medicine here and for supplying access to databases, its library and other ressources when writing my thesis.

Last but not least I wish to express my thanks to my girlfriend, the love of my life for her love and understanding and for being very patient during the time I was busy working on this thesis.

Table of Contents

Acknowledgements....iii
List of Abbreviations....vi
List of Figures....viii
List of Tables....ix
Abstract....x
Zusammenfassung....xi
1. Introduction....1
1.1 The Cardiovascular System....1
1.2 The Role of the Vascular System in Maintaining Blood Pressure....2
1.3 Role of Blood Volume and the Relationship Between Venous and Arterial System....3
1.4 Cardiovascular Regulation....4
1.5 Neurohumoral Short-Term Regulation....4
1.5.1 The Frank-Starling Mechanism....4
1.5.2 How Do We Compensate a Drop in Preload?....5
1.5.3 Central Role of the Baroreceptor Reflex in Cardiovascular Regulation....6
1.5.4 The Role of Norepinephrine, TPR and the Venous Reservoir....6
1.6 Hormonal Long-Term Regulation....8
1.6.1 The Renin-Angiotensin-Aldosterone System (RAAS)....8
1.6.2 The Role of Arginin-Vasopressin (AVP)....9
1.6.2.1 Synthesis and Chemical Structure....10
1.6.2.2 Release and Secretorial Stimuli....10
1.6.2.3 Circadian Rhythmicity....12
1.6.2.4 Types of AVP Receptors....12
1.6.2.5 Effects on Blood Vessels....13
1.6.2.6 Renal Effects....14
1.6.2.7 Aquaporins....16
1.6.2.8 Interaction of AVP and the Baroreceptor Reflex....18
1.7 Cerebral Autoregulation....19
1.8 Upright Posture and the Cardiovascular System....20
1.9 LBNP as a Model for Orthostatic Stress....21
1.10 AVP and Aging....26
1.11 Sex Differences in AVP Secretion and Effects....27
1.12 Does the Menstrual Cycle Affect AVP Secretion?....28

1.13 Copeptin – a Novel Surrogate Marker for AVP....29
1.13.1 AVP and Copeptin Properties and Measurement....31
2. Aims and Goals....34
3. Methodology....36
3.1 Experimental Protocol....37
3.2 Procedure for Blood Extraction and Analysis....39
3.3 Procedure for Urine Analysis....40
3.4 Statistical Methods....41
4. Results....42
4.1 Copeptin Results....42
4.2 Aquaporin Results....47
4.3 Correlation of uAQP2 and Copeptin....48
5. Discussion....49
5.1 Observations....49
5.2 Alternative Methods to Assess Responses to Alterations of Orthostatic Loadings....49
5.3 What is the Exact Role of AVP in Orthostasis?....50
5.4 Does LBNP Affect AVP Release?....55
5.5 Gender Differences in Copeptin....57
5.6 Does Copeptin Correlate with Urinary AQP2 Output?....57
5.7 AVP and Presyncope....59
5.8 Limitations....60
5.9 Conclusion....61
6. Conflict of Interest....62
Appendix A - Overview of AVP receptors and physiological effects....63
Appendix B - Subject recruitment poster....66
Appendix C - Aquaporins, Vasopressin and Aging - Tamma, Goswami, Reichmuth....67
Bibliography....79

List of Abbreviations

ACE	Angiotensin Converting Enzyme
ACTH	Adrenocorticotropic Hormone
ADH	Antidiuretic Hormone = AVP
ANG II	Angiotensin II
ANOVA	Analysis of Variance
AQP	Aquaporin
AT1	Angiotensin II-Receptor subtype 1
ATP	Adenosine Triphosphate
AVP	Arginine-Vasopressin
BMI	Body Mass Index
BP	Blood Pressure
cAMP	Cyclic Adenosine Monophosphate
CHF	Chronic Heart Failure
CO	Cardiac Ouput
CVP	Central Venous Pressure
DDAVP	1-Desamino 8-D-Arginine Vasopressin short: Desmopressin
ENaC	Endothelial Natrium Channel
G-LOC	G-Force-Induced Loss Of Consciousness
HDBR	Head-Down Bed Rest
HDT	Head-Down Tilt
HR	Heart Rate
HUT	Head-Up Tilt
IP_3	Inositol Triphosphate
LAD	Left Atrial Diameter
LBNP	Lower Body Negative Pressure
MAP	Mean Arterial Pressure
NO	Nitric Oxide
NPV	Nucleus Paraventricularis
NSO	Nucleus Supraopticus
NTS	Nucleus Tractus Solitarii
OVLT	Organum Vasculosum Laminae Terminalis
PKA	Protein Kinase A

pCO_2	CO_2 partial pressure
pO_2	O_2 partial pressure
PP	Pulse Pressure
PRA	Plasma Renin Activity
R	Resistance
RAAS	Renin Angiotensin Aldosterone System
SIADH	Syndrome of Inadequate release of Antidiuretic Hormone
SV	Stroke Volume
TPR	Total Peripheral Resistance
uAQP2	Urinary Aquaporin 2
UT A1 (2,3,4 etc.)	Urea Transporter A1 (2,3,4, etc.)

List of Figures

Figure 1:	AVP and BP	9
Figure 2:	Main Stimuli of AVP Release	12
Figure 3:	V1a Receptor Action in Vascular Smooth Muscle Cells	14
Figure 4:	AVP Actions	15
Figure 5:	V2 Receptor Action and Aquaporin 2	16
Figure 6:	Working Principle of a LBNP Device	22
Figure 7:	Copeptin and AVP Precursor Molecule	30
Figure 8:	AVP and Copeptin Release	31
Figure 9:	Aminoacid Sequence of AVP	32
Figure 10:	Experimental Protocol	37
Figure 11:	Male Test Subject on LBNP Table During Our Experiment	38
Figure 12:	Cardiovascular Response to LBNP	39
Figure 13:	Copeptin in Men and Women	44
Figure 14:	Copeptin in Men and Women Combined	44
Figure 15:	Female uAQP2	47
Figure 16:	Male uAQP2	47
Figure 17:	uAQP2 Gender Comparison	47
Figure 18:	uAQP2 Correlation With Copeptin of Females	48
Figure 19:	uAQP2 Correlation With Copeptin of Males	48

List of Tables

Table 1:	Subjects' Characteristics	42
Table 2:	Copeptin Results	43
Table 3:	uAQP2 Results	47
Table 4:	Correlation of uAQP2 With Copeptin	48

Abstract

Introduction: Orthostatic syncope, caused by an inability of the cardiovascular system to compensate a reduction in venous return to the heart, is a major source of fall in the elderly often leading to injury and hospitalisation. Furthermore, women are known to be particularly prone to orthostatic syncope, for reasons which are still not clearly understood. In a recent review (see Appendix C), we have discussed the effect of aging on the vasoconstrictor hormone Arginine-Vasopressin (AVP). AVP regulates plasma osmolality and volume mainly by regulating the presence of a water channel called aquaporin 2 (AQP2) in the apical membrane of the principal cells of the renal collecting ducts, which is also excreted in the urine where its concentration correlates with plasma AVP. In recent years copeptin, a larger peptide released from the same precursor molecule as AVP has been established as a surrogate marker of plasma AVP. Its measurement is much cheaper and simpler and it is much more stable even at room temperature; thus, it offers considerable advantages over direct measurement of AVP. While it is known that AVP increases massively if syncope occurs, its role in conditions of orthostatic stress without syncope is still poorly understood.
Aims & Goals: In the present study we assessed the effect of Lower Body Negative Pressure (LBNP) which simulates orthostatic challenge-induced central hypovolemia both on plasma copeptin and urinary AQP2 (uAQP2). We hypothesized that copeptin and uAQP2 increase upon graded LBNP and that there are gender differences in this response.
Methodology: 38 (21 female and 17 male) healthy young volunteers were recruited for this study. Our experimental protocol consisted of a 30-minute supine baseline period followed by 20 min of LBNP, increasing every five min by -10 mmHg up to -40 mmHg followed by 10 min of supine rest. Blood was sampled at the end of baseline, at the end of the LBNP and at the end of the recovery period. 24-hours urine samples were collected as baseline before the experiment and another urine sample was collected immediately after the end of the experimental protocol.
Results: Contrary to our expectations, copeptin and uAQP2 failed to increase upon 20 minutes of increasing LBNP; instead a significant drop in copeptin took place. Males had higher copeptin than females, a difference that became more pronounced after LBNP. There were no significant alterations of uAQP2 and no significant correlation between copeptin and uAQP2 could be found.
Discussion: The observation that the difference between men and women in copeptin was more pronounced at the end of the experiments supports the notion of gender-specific kinetics and thus partly confirms our hypothesis. We consider the observed drop in copeptin to be most likely an effect of a slower elimination of copeptin compared to AVP in conjunction with the posture change into supine position at the beginning of the baseline period, 30 minutes before LBNP application.

Zusammenfassung

Orthostatische Synkopen sind eine der häufigsten Sturzursachen im gehobenen Lebensalter und ziehen oft Verletzungen und Verlust an Mobilität nach sich. Aus bisher unzureichend erforschten Gründen sind Frauen für solche Sturzereignisse besonders anfällig. Vor kurzem haben wir in einem Review die Auswirkungen des Alterns auf das Hormon Arginine-Vasopressin (AVP) diskutiert (siehe Appendix C). AVP reguliert sowohl Plasmaosmolarität als auch -volumen vorwiegend über einen transmembranösen Wasserkanal names Aquaporin 2 (AQP2) in der apikalen Membran der Hauptzellen des renalen Sammelrohrs. AQP2 findet sich auch im Urin, wo seine Konzentration von der AVP-Wirkung abhängig ist.

Copeptin, ein Peptid, das aus einem gemeinsamen Vorläufermolekül mit AVP abgespalten wird, kann als Surrogatmarker für AVP verwendet werden. Seine Messung ist deutlich einfacher, billiger und genauer als die von AVP, so dass sich aus seiner Verwendung Vorteile für die Forschung ergeben.

AVP steigt im Rahmen einer orthostatischen Synkope massiv an, allerdings ist sein Verhalten bei orthostatischer Belastung ohne Synkope bisher unzureichend erforscht.

Für die vorliegende Studie habe wir die Auswirkungen von Lower Body Negative Pressure (LBNP), einer Methode zur Simulation orthostatischer Belastungen auf den AVP-Spiegel im Blut und die Exkretion von AQP2 im Urin untersucht, um die Hypothese zu überprüfen, dass diese zu einer Erhöhung von beidem führen und dass es dabei geschlechtsspezifische Unterschiede gibt.

17 Probandinnen und 21 Probanden zwischen 18 und 35 nahmen an der Studie teil. Das Versuchsprotokoll bestand aus 30 min. ruhig liegen gefolgt von Applikation von LBNP für 20 min., dabei alle 5 min. Erhöhung des LBNP um -10 mmHg bis -40 mmHg, wiederum gefolgt von 10 min. Ruhephase. Am Ende jeder der 3 Versuchsphasen erfolgte eine Blutabnahme, Urin wurde am Ende des Protokolls abgegeben und eine 24-h Urinprobe vom Vortag wurde als Referenz verwendet.

Entgegen unserer Erwartungen stieg das Copeptin nicht, sondern fiel ab. Männer hatten dabei eine deutlich höhere Konzentration als Frauen, was sich nach LBNP-Applikation noch verstärkte. Es ließ sich keine signifikante Veränderung des AQP2 im Urin und auch keine nennenswerte Korrelation zwischen Copeptin und AQP2 feststellen.

Die Beobachtung, dass die Unterschiede zwischen den Geschlechtern sich nach LBNP Anwendung verstärkten, bestätigt unsere Hypothese zum Teil. Vermutlich liegt das Absinken des Copeptins dabei an einer im Vergleich zu AVP langsameren Kinetik in Verbindung mit dem Wechsel der Probanden von stehender zu liegender Position am Beginn des Experiments.

1. Introduction

1.1 The Cardiovascular System

Human tissue, like all biological tissue, requires oxygen and nutrients as well as a means to dispose of its waste products. Unicellular organisms can take up nutrients from and remove waste products to their surroundings by simple diffusion; however, diffusion can, in a sensible amount of time, move molecules only over very short distances due to the law of Fick which states that diffusion speed is always proportional to concentration gradients. Therefore, to keep concentration gradients between intra- and extracellular space big enough to maintain fast diffusion, complex life form require a transportation system which, by means of a continuous flow, quickly removes waste products and delivers nutrients towards the various cells of a body. In Humans, this transportation system is called the cardiovascular system. It relies on a pump – the heart – to maintain a steady output of a liquid transportation medium – the blood – along a network of channels – the vascular system – towards all the different capillary beds in the human body to keep the tissues of the various organs which constitute the body alive.

The Cardiac Output (CO) of the human heart is determined by Stroke Volume (SV) and Heart Rate (HR) according to the following formula:

$$CO = SV \times HR \qquad (1)$$

Naturally, the output needs to meet the demands of the different organs regarding oxygen and nutrients. These demands vary depending on the different types of activity of the organs: a tissue that is “working”, that means: under stress, requires more energy than when resting. The need therefore to adjust the perfusion of the various tissues in order to meet the changing needs of the different organs is one of the central aspects towards understanding the physiology of humans as well as other mammals.

1.2 The Role of the Vascular System in Maintaining Blood Pressure

A highly important factor to keep in mind when observing the relationship between the blood vessels and CO is that, with the exception of only the small capillaries, all blood vessels, especially the arteries, but also the veins, possess a muscular hull, enabling them to exercise pressure on the liquid in them and to change their diameter. As liquids are practically incompressible, the total volume of liquid in the body stays the same, while the arterial and venous pressure shifts liquid between intra- and extravasal spaces and regulates the perfusion of the various capillary beds in comparison to each other.

Every vessel offers to a liquid moving through it a certain resistance, and of course this applies to the blood vessels as well. According to the law of Hagen-Poiseuille the Resistance R of a pipe depends on its diameter d, its length l and the dynamic viscosity η of the fluid:

$$R = \frac{8\,\eta l}{\pi d^4} \qquad (2)$$

The most important conclusion one has to draw from this formula is the massive effect even of small vasoconstriction or -dilatation on vascular resistance, because the overall resistance is inversely proportional to the fourth power of the vascular diameter.

For the blood stream to reach even remote capillary beds, the heart needs to build up a certain pressure against the combined resistance of all blood vessels, called Total Peripheric Resistance (TPR). This follows approximately the formula for newtonian fluids regarding the relationship between resistance R, flow, or in that case more precisely Cardiac Output (CO) and Mean Arterial Blood Pressure (MAP):

$$MAP = CO\ x\ TPR \qquad (3)$$

Since the blood pressure decreases with increasing distance from the heart, it needs to be high enough that the blood pressure ist still positive even in remote regions of the human body and most importantly in the brain even against the pressure of the water column from heart to head when standing. This is the reason why giraffes have an mean arterial blood pressure of 200-330 mmHg at heart level, because they need to maintain cerebral perfusion against the pressure of the water

column caused by their very long neck in order to achieve, at the level of the head, blood pressure levels similar to ours[1]. It is important to highlight at this point that for the heart, the peripheric resistance is more than a mere obstacle in the circulation. To the contrary, a too low peripheric resistance will prevent the heart from building a pressure high enough for adequate perfusion of the brain, even though the CO itself would be sufficient, resulting in syncope due to lack of oxygen in the cerebral tissue.

1.3 Role of Blood Volume and the Relationship Between Venous and Arterial System

The human vascular system falls apart in a high pressure system – the arteries and left ventricle of the heart during systole and a low pressure system – the veins, the right heart and the pulmonary circulation. Between these two systems are the capillary beds where the actual exchange of nutrients and waste products from blood to tissue takes place.

Mean pressure in the high pressure system, is usually around 60 to 100 mm Hg while in the low pressure system the mean pressure usually does not exceed 20 mmHg, while on the other hand it usually contains almost 85% of our blood volume [2, p. 582ff.].

The walls of the arteries possess a thick layer of smooth muscles which, by very small changes in vascular diameter, in line with the law of Hagen Poiseuille – formula (2) above – can greatly alter their vascular resistance, especially in the peripheral arteries which are therefore also known as resistance vessels [2, p. 581f.].

The venous system possesses a much higher compliance than the arteries, which means that veins can expand greatly when the blood volume increases, while upon a decrease in blood volume they don't maintain their circular diameter, but instead collapse into a figure-8 shape which offers very little vascular resistance [2, p. 583]. This means that without a sufficient filling and due to the low pressure in the venous system, the veins by themselves will be unable to maintain venous return to the heart.

At the same time, the resistance vessels cannot contract beyond a certain point [2, p. 578], which means they also need a minimum blood volume inside them to maintain blood pressure, which is important in order to understand how hormones regulating blood volume affect the cardiovascular system.

1.4 Cardiovascular Regulation

The question is now by which mechanisms blood perfusion throughout the whole human body and especially in the brain is controlled and maintained. To understand the regulatory mechanisms of the cardiovascular system, it is useful to have a look at the time in which – upon an alteration of the physiological conditions – they kick in, thus establishing a sequence in which they take effect. We can thus categorize those into short-term regulatory mechanisms which are needed to adjust to rapid changes, for example when standing up or lying down, and long-term mechanisms which are needed to adjust to prolonged periods of standing or walking around.

1.5 Neurohumoral Short-Term Regulation

1.5.1 The Frank-Starling Mechanism

The most simple mechanism to alter the cardiac output is the so-called Frank-Starling mechanism or more precisely length-tension relationship of the heart muscle [2, p. 543ff.]. It refers to the fact that the more stretched a muscle fiber is at the beginning of a contraction, the greater is the force the muscle fiber can generate. As to the heart, this means that the greater the pressure in the ventricle is when the heart muscle is relaxed before the beginning of the systole, the greater is the force the ventricle can exercise on the blood in it and hence, the bigger the volume of the blood it can pump out during each stroke. A higher amount of ventricular fluid at the end of the diastole means an increased pressure in the ventricle and thus greater stretch of the heart muscle and increased tension of the heart muscle when relaxed. This tension is what we call **Preload,** while the tension of the muscle fibers in the walls of the ventricle during systole when actively contracting is called **Afterload** [2, p. 547f.]**.** These two factors determine SV as well as systolic blood pressure in the ventricles; they are key aspects in explaining changes in the working cycle of the heart. How high the afterload is, depends largely on the pressure generated by contraction of the smooth muscles aortic walls; how high preload is depends mostly on the venous return to the heart and is expressed by enddiastolic ventricular pressure, which in turn depends largely on the central venous pressure (CVP) [2, p. 548ff.]. An increased afterload will lead to an increase in blood pressure along with a reduction in SV, while an increase in preload will lead to an increase in stroke volume.

The Frank Starling mechanism is not neurally or humorally mediated, but an adaptation of each heart muscle fiber to alterations in pre- or afterload taking place within one heartbeat [2, p. 542] and is essential in understanding why under orthostatic stress hypotension and syncope occur: when a subject stands up, central venous pressure will drop because blood will pool in the leg veins [3, p.

87]. The drop in preload will, through the Frank-Starling mechanism, reduce cardiac output, which will lead to hypotension, which will mean that the heart will not be able to pump up blood to the brain against the force of the water column when standing. Without some sort of compensation mechanism this would cause an immediate syncope.

1.5.2 How Do We Compensate a Drop in Preload?

To maintain cardiac output in the face of a reduction of preload, the heart has to maintain cardiac output, to which end it can, according to formula (1), adjust HR or SV. These two parameters are influenced by the autonomic nervous system which falls apart in the sympathetic and the parasympathetic nervous system [2, p. 404].

While the Frank-Starling mechanism affects the SV, it has no effect on the heart rate. The most rapid adjustment of the heart rate is mediated by a sudden decrease in vagal activity which takes place within one beat and affects only the heart rate, but has no inotropic effects on the heart muscle [2, p. 543ff.].

The sympathetic response takes a little bit longer, needing about 5 to 10 seconds, but, contrary to the vagal response, it also has an effect on the contractile force of the heart due to the effects of epinephrine and norepinephrine which are released upon sympathetic activation from the adrenal medulla and the nerve endings and which take their effect on the heart through β_1-Receptors situated in atriae and ventricles [2, p. 407f.].

This means that if there is a sudden reduction in preload, for example due to fluid shifting into lower body when moving from supine to upright body position, in order to maintain cerebral perfusion, the heart has to rely for a few seconds solely on the increased heart rate triggered by a reduction of vagal activity until the positive inotropic effects of the symathetic system take effect. It is enabled to do so mainly by depleting the reservoir constituted by the blood still in the pulmonary circulation, lasting only a few heartbeats since the pulmonary circulation can contain only a few 100 mL of blood [4].

To reduce the fluid shift due to postural changes, blood vessels in the limbs possess venous valves that prevent blood in the veins from flowing backwards. In order to maintain CVP when in upright position the body has a mechanism that is called the muscle pump: contractions of the muscles in the legs compress the veins and since the fluid, due to the venous valves, cannot move backwards inside of the leg veins, it has to move forward towards the heart. This mechanism is of great help if we have to stand for extended periods of time and its inactivation when we are not able or allowed to move is the reason why soldiers standing at attention have a tendency to faint [5, p. 75] [2, p.

585].

1.5.3 Central Role of the Baroreceptor Reflex in Cardiovascular Regulation

So how is the vagal and sympathetic response to a drop in preload triggered? The human body possesses in the carotic sinus as well as in the aortic arch so-called baroreceptor cells that detect a change in pressure and who project to the Nucleus Tractus Solitarii (NTS) in the medulla oblongata where the vagal and sympathetic response is triggered accordingly [6, p. 205ff.]. This reflex is called **Baroreceptor Reflex** and it is what prevents unconsciousness during a shift into the upright body position [2, p. 608f.] [7, p. 161ff.]. It plays an important role in both long and short-term regulation of blood pressure and volume [8]. In this context, it is essential to note that there is a resetting of the baroreceptors, meaning that during prolonged change of Mean Arterial Pressure (MAP) the parameters that trigger the baroreceptor reflex are adjusted to the effect that the operating point of the baroreceptor reflex is moved into the direction of the change in MAP, leading to a stabilization of a new MAP within hours to days [2, p. 610]. This is important during exercise because otherwise, it would be impossible to achieve the simultaneous increase in HR and MAP necessary to meet the increased muscular oxygen demand [9], while at the same time it is harmful during pathologically changed MAP because it stabilizes a pathological MAP. Rapid resetting of the baroreceptors can take place within a timespan as short as 5 to 10 minutes [10, p. 62ff.].

1.5.4 The Role of Norepinephrine, TPR and the Venous Reservoir

In order to quickly adjust to a drop in preload, and since a change in vagal activity is purely chronotropic and can thus maintain cerebral circulation only for a few seconds until the reservoir constituted by the pulmonary vessels is depleted, the body requires activation of the sympathetic nervous system which exercises its effect through release of catecholamines and norepinephrine in particular. Norepinephrine is a catecholamine that is synthetized from dopamine in the postganglionic sympathetic nerve endings, from where it is released upon sympathetic activation. A small quantity of norepinephrine is synthetized in the adrenal medulla which produces mostly epinephrine; however, the concentration of norepinephrine in the blood is almost always at least 3 times higher than epinephrine, due to a spillover of locally produced norepinephrine from the sympathetic neurons into the blood stream. In comparison to epinephrine, norepinephrine binds especially to α_1, α_2 and β_1-receptors, but shows a lesser affinity to β_2-receptors.

While the parasympathetic system does have a inotropic effect only on the myocardial cells of the

atriae, the sympathetic system also has an inotropic effect on the myocard of the ventricles. When, due to orthostasis, the walls of the lower body veins expand as a result of the increased pressure of the water (or, in that case: blood) column, blood will pool in the legs and the venous return to the heart will decrease. The reduction of venous return will of course reduce preload with, according to the Frank-Starling mechanism, subsequent reduction of stroke volume. The drop in baroreceptor activity due to decreased cardiac output will immediately lead to a reduction in vagal activity and a rise in heart rate that will within a few seconds deplete the reservoir constituted by the pulmonary vessels. After a few seconds the sympathetic reaction will kick in and cause an inotropic effect through activation of β_1-receptors in the myocard to which epinephrine and norepinephrine will bind. The increased contractility of the myocard that will partially compensate the Frank-Starling mediated loss in cardiac output. However, an increase in cardiac output has the property of further depleting the venous reservoir thus leading to a reduction in preload that would rapidly offset the gain in cardiac output via a further increase of the Frank-Starling effect and quickly lead to syncope due to a lack of cerebral perfusion. In order to prevent this, the body relies on the second, extremely important property of the sympathetic nervous system: in contrast to the parasympathetic system, which innervates only very few blood vessels, the wall of human blood vessels are densely equipped with sympathetic nerve endings and α_1-receptors to which norepinephrine released from the sympathetic nerve endings will bind and trigger a vasoconstriction that will increase vascular resistance. This effect is particulary prominent in small arteries and arterioles which are accordingly called resistance vessels because they serve to regulate TPR in particular. The rise of TPR due to the effect of norepinephrine in the walls will, due to the relationship between afterload and blood pressure, lead to a rise in blood pressure that maintains cerebral perfusion under orthostatic conditions against the force of the water column from heart up to brain, but it will also further reduce stroke volume which would exacerbate the problem of reduced cardiac output under conditions of orthostasis. However one must look at the properties of the vascular system as a whole: due to liquids being incompressible, a volume reduction of the arteries due to vasoconstriction must lead to a shift of fluid into the venous system due to the veins possessing a very high compliance, thus increasing central venous pressure. The aorta on the other hand possesses a low resistance due to its high diameter, in accordance to the law of Hagen-Poiseuille (formula (2) mentioned above), but due to its strong muscular wall, it shows a very low compliance, so blood pooling in the aorta is not possible. Veins on the other hand possess only thin muscular layers, but the low resistance due to its high diameter – keep in mind that the resistance falls indirectly proportional to the fourth power of the diameter – and high compliance makes shifting blood into the big vein stems require only low forces that are easily achieved by the heart in

conjunction with contraction of the albeit weak venous walls. This effect of blood shifting is especially important in the splanchnic veins which are richly packed with α_1-receptors. Sympathetic activation through vasoconstriction of resistance vessels massively reduces inflow of blood in this region allowing the smooth muscle tissue in the walls of splanchnic veins to contract and shift their blood into the large abdominal vein stem, thus increasing venous return to the heart. It is this contraction of arteries in conjunction with increased tonus of the vein walls which serves to maintain preload and thus to maintain cardiac output under orthostatic conditions [2, p. 406ff] [11, p. 360f.] [12, p. 99] [10, pp. 39–52].

1.6 Hormonal Long-Term Regulation

To understand the mechanisms that determine the adaptation of the body to fluid shifts that last longer than a few seconds, it is essential to have a look at the function of the kidneys regarding blood circulation. While the body can change and maintain blood pressure by active constriction of muscle in the heart and the walls of its blood vessels, this process naturally consumes energy in the form of ATP, so over longer periods of time, it is economically necessary to do so by changing the volume of blood in the body. Since every organism needs to excrete waste products in form of fluids removed from its blood, it is only logical that by changing the amount of fluid taken from the body, it can also change the pressures within the body. Because the kidney is the organ responsible for the filtering and excretion of waste products from the blood it naturally is the most important organ for changing the blood volume itself.

Renal responses to fluid shifts in the body are mainly regulated by two hormonal systems: the renin-angiotensin-aldosterone system and the hormone vasopressin or more precisely Arginin-Vasopressin (AVP) which is the form that occurs in humans [2, p. 672]. Both affect the kidney as well as the blood vessels and thus contribute to maintaining the perfusion of the vital organs necessary to keep the body alive and conscious.

1.6.1 The Renin-Angiotensin-Aldosterone System (RAAS)

This system consists of an enzyme, renin, and two hormones, angiotensin and aldosterone which are activated in a cascade pattern [2, p. 657f.]: first, renin is secreted in the juxtaglomerular apparatus in the kidney and triggers the conversion of angiotensinogen which is circulating in the bloodstream to angiotensin I which, in a second step, is converted by the Angiotensin Converting Enzyme (ACE) to angiotensin II which in turn is responsible for most of the effects on the body. These consist mainly

of an increased reabsorption of natrium in the kidney, partly mediated directly by angiotensin II and partly via an excretion of aldosterone from the cortex of the adrenal gland. The resulting increase in plasma osmolality leads to an increase in blood volume and therefore blood pressure [2, p. 657f.]. Furthermore, angiotensin II triggers a vasoconstriction of blood vessels which, according to formula (3) increases vascular resistance and thus blood pressure [13, p. 32]. Also, angiotensin II causes thirst and stimulates the release of vasopressin from the posterior pituitary [2, pp. 673f., 657].

The release of renin itself is triggered by the baroreceptor reflex via the sympathetic activation through release of catecholamines and by a drop of NaCl concentration at the macula densa, a cluster of cells in the wall of the distal tubule in the loop of henle and part of the juxtaglomerular apparatus from which renin is released [13, p. 29].

1.6.2 The Role of Arginin-Vasopressin (AVP)

The hormone AVP is usually known as the hormone which maintains plasma osmolality constant; however, it also contributes to blood pressure maintenance in the face of plasma volume shifts. It is able to do so by two different mechanisms, one by increasing the reabsorption of water in the kidneys and thereby directly altering plasma volume and osmolality within the body, the other by triggering a constriction of blood vessels, thus increasing TPR and thereby helping to maintain MAP at a necessary level. Both these effects as well as the controlling mechanisms of AVP secretion and its physiological significance will now be discussed in detail.

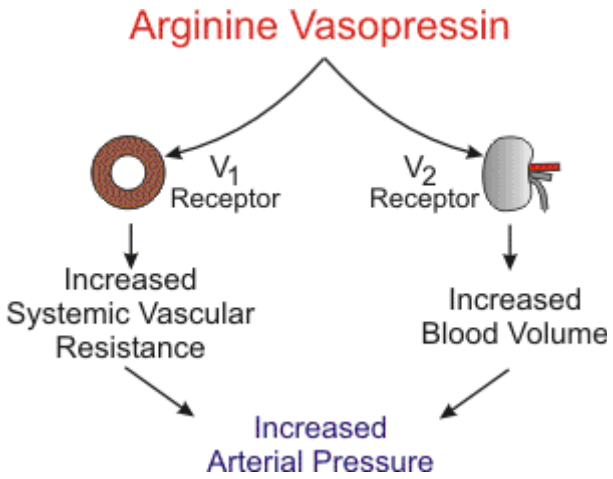


Figure 1: AVP and BP

Source: http://www.cvpharmacology.com/vasoconstrictor/AVP **30.08.2015**

1.6.2.1 Synthesis and Chemical Structure

Vasopressin, also called Adiuretin, is synthetized by magnocellular neurons in the Nucleus Paraventricularis (NPV) and Nucleus Supraopticus (NSO) in the hypothalamus, enclosed in vesicles that are transported within the axons into the posterior pituitary and stored there close to the nerve terminals until release [14].

A second class of so-called parvocellular neurons synthetize and release AVP into the superior hypophysial artery, which leads to the anterior pituitary where AVP causes a release of ACTH (see below) [14].

Chemically, it is a peptide consisting of nine aminoacids separated from a much larger precursor hormone of 164 aminoacids called preprovasopressin during the transport along the axon along with the molecules neurophysin II and copeptin, which will be discussed in detail below [15] [2, p. 445] [16, p. 46f.]. During this process, at first a signal molecule is cleaved from preprovasopressin thus creating a new molecule called provasopressin, 144 aminoacids long. Within the endoplasmatic reticulum, eight disulfide bonds are formed, the carboxy domain (part of the later copeptin) is glycosylated, the molecule is packed into vesicles and transported down the axons which lead into the posterior lobe of the pituitary. It is during this transport that provasopressin is cleaved into its final products AVP, neurophysin II and copeptin. Of these products, neurophysin II is important for the correct storage and release into the bloodstream [16, p. 46], while the biological function of copeptin once released into the bloodstream is still unknown, although it seems to play a role as a chaperone in the correct maturation of the AVP molecule [15] [17]. What is known is that copeptin appears to be secreted along with AVP and there is strong evidence that its concentration mimick AVP levels in the blood stream [18] [19] [20] and indeed, it has been shown that it can be used as biomarker in conditions such as myocardial infarction, respiratory tract infections and septic shock, each of them conditions which show alterations of AVP levels [17] [21] [22].

1.6.2.2 Release and Secretorial Stimuli

The release of AVP from the synapses is triggered by action potentials of the neurons that cause a rise in cellular Ca^{2+} concentration by opening voltage-sensitive Calcium channels leading to a fusion of the vesicles with the cell membrane and thus the release of AVP into the extracellular space and then by way of the veins of the posterior pituitary into the bloodstream [2, p. 445] [23, p. 323] .

The release of vasopressin is triggered as far as is currently known by four mechanisms:

- a change in plasma osmolality that is detected by receptor cells in the Organum Vasculosum Laminae Terminalis (OVLT), an organ situated at the front of the third ventricle in the hypothalamus [23, p. 332f.]. From there, efferent nerve fibers run to the NSO and NPV mentioned above, where AVP is synthetized. Osmoreceptor cells that trigger the release of AVP are found in the liver as well [2, p. 429f.]. The actual stimulus that triggers the secretion of AVP from the posterior pituitary is thought to be a shrinking of the sensor cells due to hyperosmolality of the extracellular fluid. Situated in the membrane of the sensor cells are a class of so called stretch-inhibited non-selective Ion channels that are inhibited by the tension of the cell membrane [2, p. 445]. Shrinking reduces membrane tension, activates the Ion channels and causes depolarization of the cell with subsequent AVP release [2, p. 445]. AVP release is triggered even by a small change of osmolality of 1 percent, which makes the osmolality-control of AVP secretion much tighter than the control via blood pressure related systems, which is supported by the fact that plasma osmolality does not seem to change significantly upon water immersion or orthostasis [10, p. 103] [2, p. 671]. This system is affected by estrogen levels; therefore, although AVP levels stay roughly the same, the osmolality levels which trigger an AVP release in women change during the menstrual cycle [2, p. 671];
- a reduction in blood pressure detected by the same arterial baroreceptors in the aortic arch and the carotic sinus which trigger the baroreceptor reflex [24]. The afferents which trigger this reaction run via the cerebral nerves IX (for the receptors in the carotid sinus) and X (for the ones in the aortic arch) to the Nucleus Tractus Solitarii (NTS) [2, p. 420]; from there neurons project directly to the NPV via the longitudinal dorsal fascicle [23, p. 338];
- furthermore there are also tension receptors in the wall of the right atrium and the adjacent part of the vena cava [2, p. 671] which have a negative feedback on AVP secretion. The inhibition of vasopressin secretion due to an increased tension of the vascular walls of the right atrium and the vena cava due to of an increase in CVP is called Henry-Gauer Reflex or **cardiorenal reflex**. It is caused by an increased central blood volume, for example through water immersion of the body or during spaceflight [2, p. 671] [25];
- as mentioned above, angiotensin II (ANG II) triggers a release of AVP as well. It does so via the Subfornical Organ (SFO) and also the OVLT mentioned above where ANG II – sensitive receptors are found. These organs which are made up from cerebral ependyma possess a

reduced blood-brain barrier which allows ANG II from the blood to come into contact with the receptors there [23, p. 332f.] [2, p. 429f.].

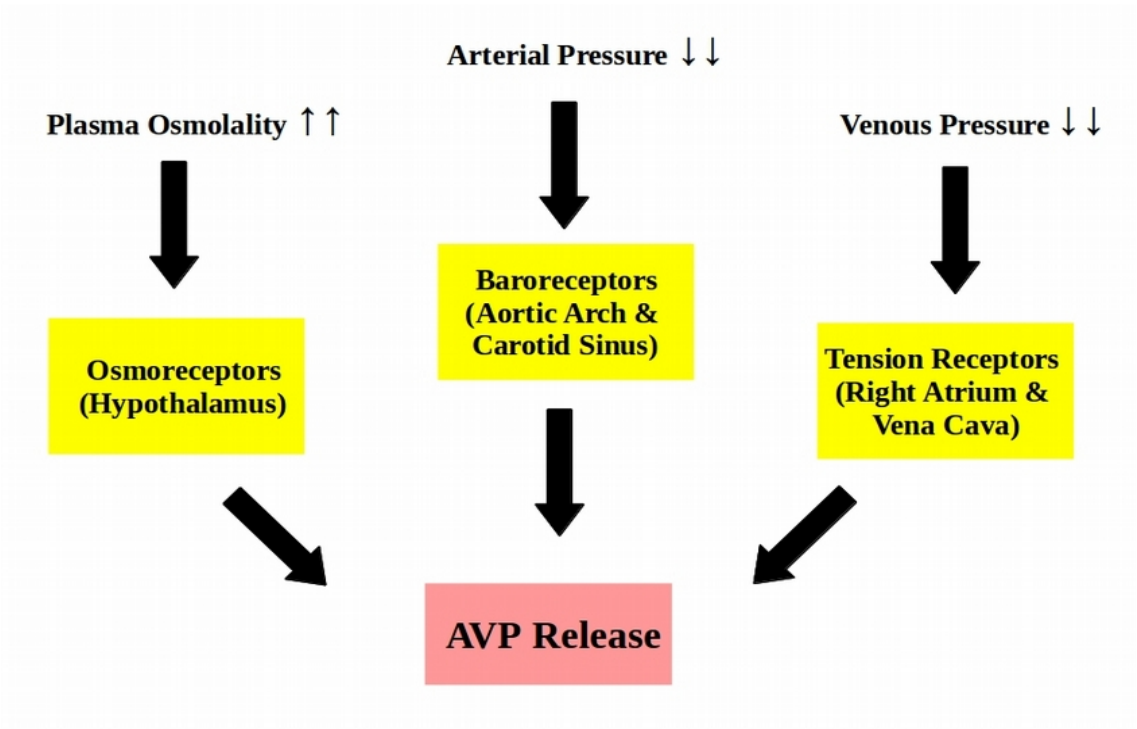


Figure 2: Main Stimuli of AVP Release

1.6.2.3 Circadian Rhythmicity

It is important to mention that AVP secretion in young, healthy individuals underlies a marked circadian rhythmicity with peak concentrations during the night. This rhythmicity of AVP release is governed by the suprachiasmatic nucleus und is gradually lost with aging, leading to a high incidence of nocturnal polyuria in the elderly [26] [27] [28] [29] [30] [31].

1.6.2.4 Types of AVP Receptors

Once circulating in the bloodstream, AVP causes its effect almost exclusively through 3 G-Protein coupled receptors named V1a, V1b and V2 receptors [14] [16, p. 100 ff.]. These are transmembrane proteins from 371 to 424 aminoacids in size [16, p. 71] (for a detailed overview of AVP receptors and their effects see Appendix A).

- The V1a receptors are situated mainly in the wall of smooth muscle cells and trigger activation of a G-Protein which activates phospholipase C. Phospholipase in turn cleaves certain phospholipids thus producing Inositol-Triphosphate (IP_3) which mediates a release

of Ca^{2+} Ions from the endoplasmatic reticulum and through that a contraction of the muscle cells [14] [16, p. 71] [11, p. 344].

- The V1b receptors are found predominantly in the anterior pituitary where they trigger a release of Adrenocorticotrope Releasing Hormone ACTH [14]. Structurally they are quite similar to the V1a receptor; they also transmit into the cell through a G-Protein and IP_3 mediated mechanism rising the intracellular Calcium level [14].
- The V2 receptors are situated mainly in the wall of the collecting tubes of the kidney and take their effect through an adenylate cyclase mediated surge of intracellular cyclic adenosine monophasphate (cAMP) generated from ATP. They are responsible for most of the renal effects of AVP, including the trafficking of AQP 2 to the apical membrane of the collecting ducts' principal cells and therefore will be discussed in detail below [32, p. 133].

1.6.2.5 Effects on Blood Vessels

As the name vasopressin indicates, AVP is capable of raising the blood pressure via vasoconstriction, although under everyday circumstances the antidiuretic effect is much more prominent and AVP-mediated vasoconstriction is negligible. AVP leads to a marked vasoconstriction only in very high concentrations of more than twice what is needed to maximize its antidiuretic activity [11, p. 389] [2, p. 445] [14], thus making the name antidiuretic hormone that is sometimes used instead of vasopressin somewhat more fitting since, under normal circumstances the vasoconstrictory effect of AVP is of minor importance. Nevertheless, under situations of hypotension for example due to hemorrhage AVP has been observed to play a significant role in maintaining blood pressure making it somewhat of an “emergency hormone” when the autonomic nervous system along with the RAAS and the effects of the renal V2 receptors fails to uphold necessary blood pressure [11, p. 389] [2, p. 445] [14]. Additionally, AVP increases the influence of the Baroreceptor Reflex on the heart rate thus exerting a stabilizing effect on blood pressure in situations of hypotension [14].

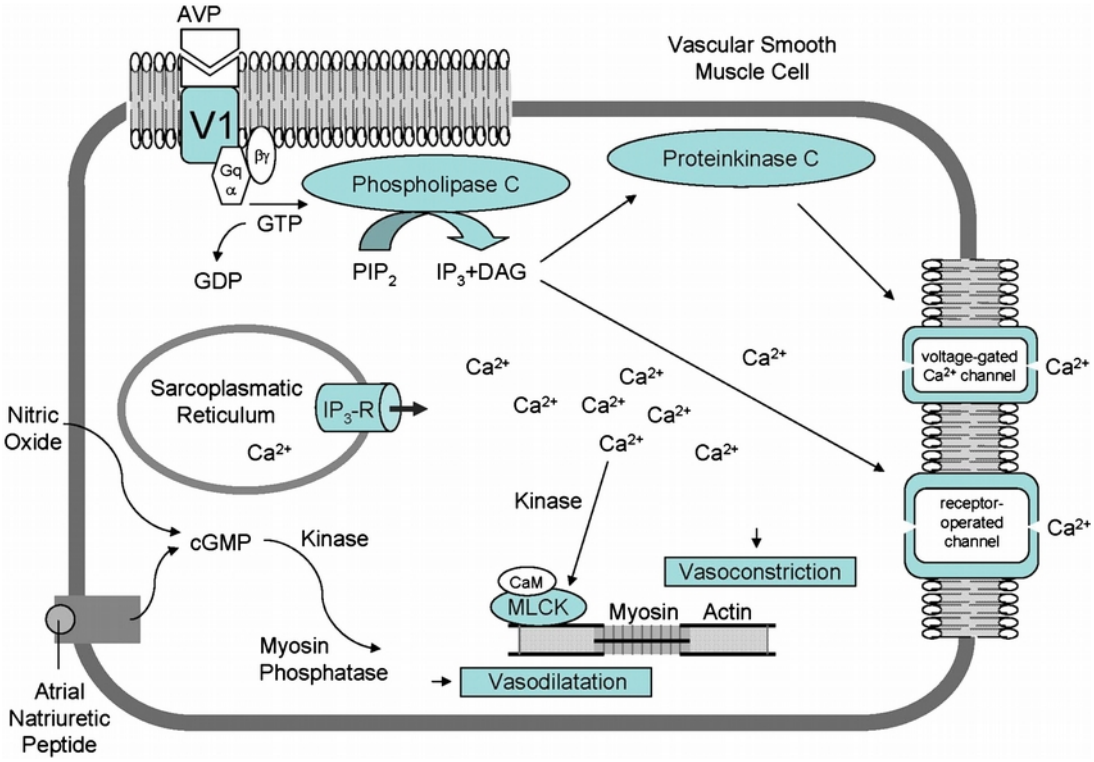


Figure 3: V1a Receptor Action in Vascular Smooth Muscle Cells

Source: https://vasopressin.files.wordpress.com/2011/09/vasopressin-receptor.jpg **Retrieved 29.08.2015**

The vasoconstriction via AVP is mediated by V1a receptors in the walls of blood vessels with the exception of the pulmonary artery where V1a was found to have a vasodilatory effect, while the V2 receptor, which is found on endothelial cells, causes vasodilatation through NO release [14], thus acting as a counterweight to V1a-mediated TPR increase. Application of DDAVP, a V2 selective receptor agonist, has a powerful vasodilatory effect [33]. With apical V1a in the Nephron inhibiting basolateral V2 receptor activity it is interesting to note that in the kidney as well as the blood vessels the actions of the V1a and V2 receptors are of an opposing nature to each other.

1.6.2.6 Renal Effects

The best known effect of AVP is the enhancement of antidiuresis which is achieved mostly through aquaporin 2 (AQP 2) which will be discussed in detail below. Aquaporins in general are enzymes which serve as water channels through the cellular membrane [11, p. 388] [2, p. 646] [12, p. 461]. By increasing the amount of AQP2 in the wall of the connecting tubule and the collecting duct particulary in the medulla of the kidney, more water can be reabsorbed following the osmotic

gradient between intra and extravasal space [2, p. 646]. As already mentioned, the main function of the aquaporins is to regulate blood osmolality with the RAAS System being the main mechanism for regulation of plasma volume [13, pp. 285ff., 258ff.]. Nevertheless, increased reabsorption of water automatically increases plasma volume, a fact which the body has to compensate mainly by means of the RAAS system if blood pressure is not to rise. Furthermore, AVP also affects a number of other transmembrane transport proteins such as the Urea transporter UT-A1 and the Endothelial Natrium Channel (ENaC) which is also strongly affected by aldosterone and there appears to be a synergism between AVP and aldosterone action here [34] [35]. AVP action in the kidney through V1a receptors also promotes renin secretion and aldosterone action, all of which work towards an increase in blood pressure [14] (for a detailed overview of AVP action please see the table in the appendix). In summary, the renal and vascular effects of AVP work together towards an increase in MAP.

Besides the ones mentioned there are a number of effects of AVP regarding very different aspects of our lives such as hemostasis, behaviour, vasoconstriction ans -dilatation, different aspects of renal urine concentration, insuline, catecholamine and ACTH secretion as well as glycogenolysis and muscular proliferation (for a detailed overview of AVP effects see Appendix A).

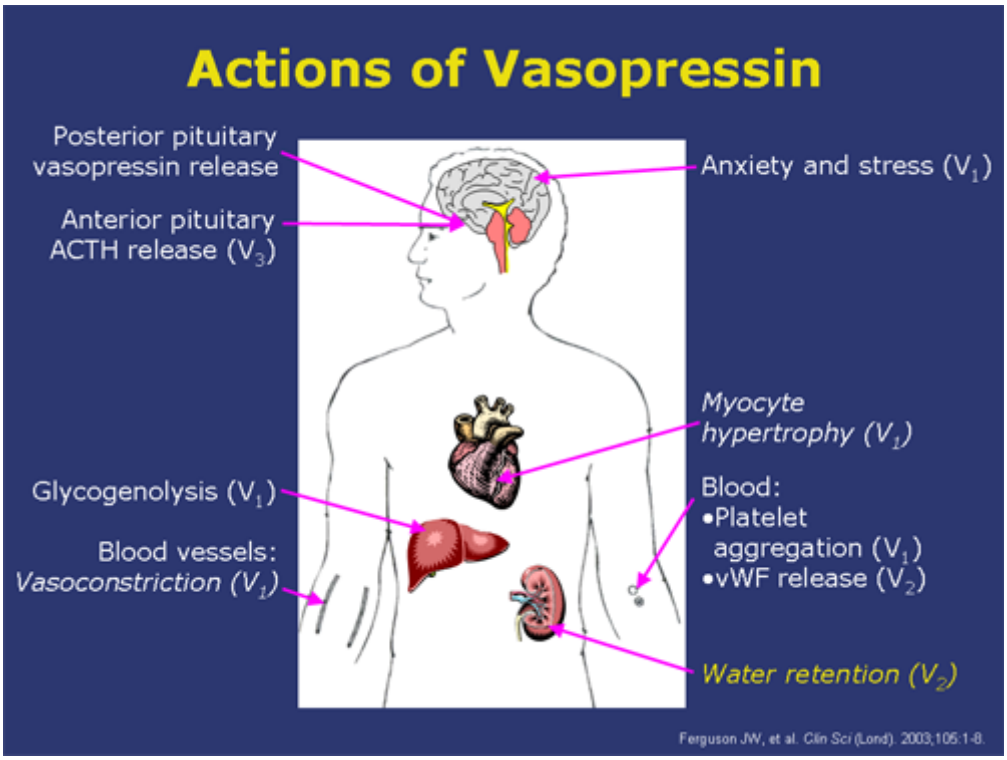


Figure 4: AVP Actions

Source:http://img.medscape.com/fullsize/migrated/editorial/cmecircle/2008/17762/images/massie/slide12.png **retrieved: 29.08.2015**

1.6.2.7 Aquaporins

The diuretic effects of AVP are mainly caused by AQP 2 in the renal collecting duct, allowing H_2O molecules to shift from the collecting duct back into the bloodstream following the osmotic gradient in the renal medulla [11, p. 388] [2, p. 646] [12, p. 461]. In general the term aquaporins refers to a family of proteins that serve as channels for H_2O molecules in cell membranes of which in the kidney there are at least 7 types of aquaporins, AQP 2 being the one through which AVP causes most of its renal effects [36] [12, p. 461]. Stimulation of the V2 receptors situated in the cell membrane of the principal cells of the collecting duct activates heterotrimeric G-Proteins that in turn activate the enzyme adenylat cyclase that is situated on the interior side of the cell membrane. Adenylat cyclase catalyzes the transformation of Adenosin Triphosphate to cAMP which by binding to its regulatory subunits activates the cytosolic enzyme Protein Kinase A (PKA) [36]. While other AQP types are permanently present within the cell membrane, AQP 2, in the absence of AVP, is stored in the membrane of intracellular vesicles but upon phosphorylation of AQP 2 by PKA, it is shifted towards the lumen of the collecting duct by fusion of the vesicles with the apical membrane and thus is able to exert its function as a water channel. Recent research has shown that the phosphorylation by PKA not only triggers the integration of AQP into the cell membrane, but also increases the permeability of AQP2 for H_2O[37]. The antidiuretic effect of AQP 2 is achieved in conjunction with AQP 3 and 4 which are permanently (i.e. independently of AVP)

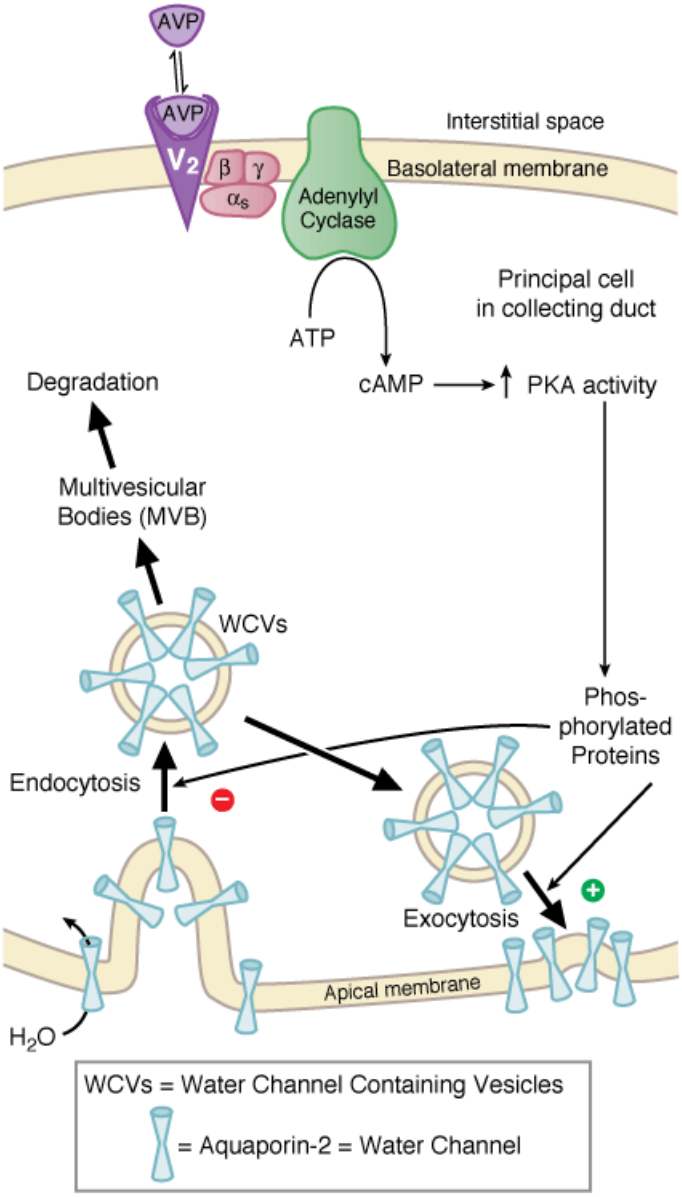


Figure 5: V2 Receptor Action and Aquaporin 2

located in the basolateral membrane of the principal cells and which enable the further shift of water into the interstitial fluid and from there into the blood vessels to return it into the circulation. Note that the tight junctions between the principal cells are impenetrable to water and therefore hinder the exchange of water between intercellular fluid and the lumen of the collecting duct [12, p. 460f.].

It is established since the 90s that AQP2 is secreted in the Urine and that this secretion is not due to shedding of the whole principal cells [38] but rather because there are exosomes released from the principal cells in which AQP2 can be found [39]. AQP 2 levels in the urine are dependent on vasopressin action [32, p. 96ff.], a fact not surprising considering that vasopressin causes an increased presence of AQP 2 in the cellular membrane, which in turn forms the exosomes in the urine [39] [38]. This fact makes AQP useful towards understanding a number of renal diseases with pathologies affecting the AVP/AQP2 system and since its discovery, it has been successfully used to that effect [39] [40] [41] [42].

Recent research found that AQP 2 excretion in rats is not only influenced by AVP levels, but also by urine pH [43].

Recent research by Li et al. [44] has found that not only AVP but also ANG II is capable of stimulating a release of AQP2 into the urine. It does so via activation of the AT1-receptor with both calcium and calmodulin as well as cAMP and PKA as second messenger systems. Furthermore there seems to be some kind of interaction between V2 and AT1 receptors because a V2 receptor blocker prevented the ANGII-induced rise in AQP2 secretion while a AT1-blocker attenuated the rise in urinary AQP2 induced by Desmopressin, a V2 agonist [45] [44].

In addition to the short-term regulation of water permeability of the cellular membrane by AVP through membrane trafficking of AQP2 already present in the cell as described above, there is also a long term regulation of the overall abundance of AQP2 which takes place within hours to days upon change of AVP levels. Whether this effect is due to increased gene transcription, decreased excretion of AQP2 into the urine or an inhibited degradation of AQP2 is currently not known [46].

Regarding the magnitude of AVP influence on AQP2 activity current evidence strongly indicates that AVP is essential for a significant activity of AQP2 despite a possible influence of other factors such as ANG II. Research on a breed of rats who naturally lack endogenous AVP called Brattleboro rats has provided evidence that these show only minimal AQP2 mRNA as well as protein, while upon AVP infusion AQP2 in collecting duct cells massively increased, a fact which highlights that without AVP there is next to no AQP2 activity as well as yielding evidence that AVP does affect AQP2 gene transcription [46].

1.6.2.8 Interaction of AVP and the Baroreceptor Reflex

Besides being released in response to hypotension, AVP has been shown to influence baroreceptor-mediated sympathetic control of the blood pressure [47].

It is important to note at this point that although AVP has the capacity to raise blood pressure through a constrictive effect on the blood vessels as well as through its antidiuretic properties, patients who suffer from SIADH – and thus have elevated AVP levels – usually are not hypertensive while patients with diabetes insipidus, which is characterized by a lack of AVP usually do not suffer from hypotension [16, p. 192]. This signifies that there are mechanisms who compensate for pathological alterations of AVP concentrations. Cowley et al. found as early as 1974 that while vasopressin injection in dogs usually caused only a moderate increase in blood pressure, baroreceptor denervation multiplied this increase in blood pressure by a factor of about 6 to 7 (33mmHg increase in comparison to 5 mmHg to a physiological dose of AVP) along with an elevenfold increase in threshold sensitivity and notably an absence of baroreceptor-mediated rise of HR [48]. These results show that under normal conditions, the baroreceptor can exercise a powerful attenuating effect on AVP-mediated increases in blood pressure. Interestingly this attenuating effect appeared to be much stronger that in norepinephrine, which yielded only a moderate boost in its hypertensive effect after baroreceptor denervation. From an evolutionary point of view, this makes insofar sense as AVP's main physiological task is tight control of plasma osmolality, meaning that there is a need to prevent hypertension caused by a drop in plasma osmolality while blood pressure regulation through vasoconstrictionis is the main task for norepinephrine. Therefore, a more powerful compensatory action of the baroreceptor reflex makes perfect sense. However, it appears that not only the baroreflex attenuates AVP vasoconstrictive action, because Cowley in the same 1974 study observed that the effects of a physiological dose of AVP in decapitated, spinal dogs were even more powerful than in the ones who had only their baroreceptors denervated [48], therefore there seems to also be a mechanism within the central nervous system activated by AVP which contributes to suppress AVP-mediated rises in blood pressure, a fact which is now considered firmly established [47] [16, p. 192]. Without these influences, it appears that sensitivity of blood pressure to AVP is greatly enhanced.

Later research established the presence of V1a receptors in the area postrema of the brain, where the blood-brain barrier is permeable and thus plasma AVP can come into contact with receptors in the central nervous system. From the area postrema, afferent fibers run to the NTS, an important centre of blood pressure regulation where the afferents from the arterial and sinusoidal baroreceptors end as well. Stimulation of these receptors by AVP, with adrenergic α_2-receptors playing a vital role in the processing of these stimuli, increases baroreceptor sensitivity and thus helps stabilizing blood

pressure in the face of increased AVP levels [14] [47].

However, the view that AVP is not involved in hypertension has been challenged with one study by Zhang finding that AVP is increased in hypertensive subjects with low renin activity [49]. Whether AVP does play a role in essential hypertension is as of now unclear [16].

1.7 Cerebral Autoregulation

Under normal conditions, perfusion of the human brain is maintained at about 50 to 65 mL per 100 g of brain tissue and per minute. For the whole human brain this amounts to about 750 to 900 mL per minute, which is about 15 percent of the CO for an organ which contains roughly 2 percent of our whole body mass. The disproportionally high perfusion of our brain in comparison to the rest of the body highlights the enormous importance of this organ as well as its high need for oxygen and nutrients. Remarkably, this steady perfusion of the brain is maintained over a wide range of mean arterial blood pressures, from about 60 mmHg up to 140 mmHg.

The regulator by which cerebral perfusion is adjusted is, as in other organs, the resistance vessels, that is, the small arteries right in front of the capillary beds, which can greatly alter their vascular resistance with comparatively small changes in vascular diameter, in accordance to the law of Hagen-Poiseuille mentioned above [50, p. 27ff.]. Additionally, the larger arteries leading to the brain play more significant role in blood flow regulation than they do in other organs. This is especially the case in hypertonia, where constriction of large arteries protect the brain from cerebral hemorrhage [50, p. 30]. The variables that control perfusion adjustment are mainly:

- the CO_2-partial pressure (pCO_2) in the arteries supplying the brain with an increase in pCO_2 by a factor of about 1.7 leading to a twofold increase in cerebral blood flow, the setpoint being roughly at 40 mmHg of pCO_2. Similarily, alterations of hydrogen ions affect cerebral perfusion;
- the oxygen consumption of the cerebral tissue is controlled in very narrow parameters between 3.3 to 3.7 mL per 100 mg brain tissue per minute and the arterial oxygen partial pressure (pO_2) is usually maintained at over 30 mm Hg. If it falls below that point cerebral blood flow is rapidly increased to maintain oxygen supply. This is of high importance because at a (pO_2) below 20 mmHg brain functions quickly collapse, possibly leading to unconscioussness and coma as a result;
- substances released from astrocytes upon a rise of intracellular calcium and possibly to a lesser extent from neurons appear to mediate cerebral blood flow by regulating vasoconstriction by releasing vasoactive substances. However the exact nature of these

mediators is as yet unknown, with substances such as NO and prostaglandins having been suggested. Triggering the release are excitatory glutaminergic neurons, however the type of stimulus that causes their activity is currently unknown [50, p. 30f.] [6, p. 744].

The role of the sympathetic nervous sytem in cerebral autoregulation appears to be a minor one. It is only when high levels of hypertonia are reached – for example during exercise – that sympathetic nerve activity causes vasoconstriction in the large and intermediate cerebral arteries, thus protecting cerebral tissue from hemorrhage and potentially fatal consequences [6, p. 745].
The outstanding capabilities of the cerebro-vascular system to maintain an equal perfusion when confronted with changing blood pressures, are necessary to cope with the changes in MAP, for example during exercise or posture changes.

1.8 Upright Posture and the Cardiovascular System

The central factor in understanding the peculiar physiology of human circulation is the one that sets the human body aside from most animals: while in almost all quadrupeds – the giraffe being a notable exception – the heart is situated at roughly the same level as the brain and all other important organs are on a similar height above the ground, the human brain – due to the humans bipedal posture – is situated a good deal above the heart which thus needs to pump the oxygen-rich arterial blood upward against gravity while at the same time putting a lot of stress on the walls of the blood vessels in the legs due to the pressure of the high water column when standing. This problem is complicated by the fact that the human body isn't always standing in the upright position: when sitting or lying down, the height of the water column in the body is considerably reduced and the pressure the heart has to work against is much lower, thus reducing the stress on the heart muscle and giving an opportunity to relax and regenerate. However, with the body shifting again to the upright posture, the heart needs to adapt rapidly to maintain the perfusion of the brain against the sudden increase of gravity and preventing sudden unconsciousness resulting in fall and possibly injury or even death. It has been shown that the mean arterial pressure in the cranial arteries can drop by 20-45 mmHg, which means that average arterial pressure at the level of the skull can drop to as little as 50 mmHg when standing, making the powerful capabilities to maintain steady cerebral perfusion mentioned above highly necessary. This challenge constitutes a fundamental disadvantage of the upright bipedal posture of the homo sapiens in comparison to the quadrupedal position of most land animals. While naturally, all walking animals have to overcome our planets' gravitation when standing up, due to our upright posture with the brain situated

considerably above the heart when standing the burden placed by upright posture upon the cardiovascular system is uniquely challenging. It is this burden we call orthostatic stress, the word orthostasis coming from greek orthos – upright and stasis –standing [51], thus meaning stress that is caused by upright standing.

Any motion away from Earth's center of gravity will place an orthostatic loading – however minor – upon the body, not only when standing up, but for example when jumping, climbing, walking or crawling up a ladder or stairway, being inside an elevator moving upwards as well as taking off with an aircraft or rocket. The same goes, of course, for any change when in motion, due to the blood being subjected to the physical laws of inertia. This is of special importance for example in fighter pilots who undergo inertial forces which are up to several times stronger than Earth's gravity [52] [53] when flying tight turns and who are therefore prone to what is called G-Force-Induced Loss Of Consciousness (G-LOC) with first incidents being reported as early as World War I [52]. In the general population however, orthostatic challenge is also often accompanied by syncope and subsequent fall; subsequent injuries occur frequently, especially in the elderly among which their prevalence is as high as 7 % in non-risk groups with up to 55% in groups which have a disposition to the syndrome [54]. Therefore a failure to handle orthostatic loadings is a major health problem in our modern society.

1.9 LBNP as a Model for Orthostatic Stress

In order to better understand the effects of a drop in central venous pressure, Lower Body Negative Pressure (LBNP), also called lower body suction, has long been used in particular in space medicine where it has a role as a substitute for Earth's gravity in preparing astronauts after long missions for their return to Earth – or possibly, in the future for a landing on Mars. It is known that during extended stays in space the human body undergoes a number of adaptions to microgravity environment such as loss of muscle mass, bone demineralization and cardiovascular deconditioning, leading to a significant reduction of orthostatic tolerance upon return to normal gravity. LBNP was invented as a method to partially reverse the effects of microgravity on the cardiovascular system with the additional benefit that the effects of gravity could be studied in a controlled environment [55, p. 166ff.]. The same principle can be used on Earth to simulate the effects of gravitational loads on the body without the body having to undergo the actual gravitational forces. This is for example interesting in the case of fighter pilots who have to undergo very high inertial forces during maneuvering, which can otherwise be researched in a controlled environment only by means of a

centrifuge which is difficult to build and maintain [56]. In these applications LBNP has the purpose to simulate the effects of gravity or, in the case of pilots, inertial forces on the human body without having to travel to space to obtain the necessary baseline values first or having to board a plane and to conduct risky flight maneuvers which naturally would upset the whole experimental setup. Furthermore, in an LBNP set-up, the strength of the applied forces can be controlled from only a slight decompression to simulate very light gravitational loadings to a decompression in excess of a 100 mmHg to simulate the high inertial loadings experienced by fighter pilots [57].

So how does LBNP work? The LBNP device consists of an airtight chamber to which a suction device – usually a regular vacuum cleaner – is attached. In some of the devices used in spaceflight, the chamber is actually an airtight trouser, allowing the astronaut to exercise while undergoing LBNP. Some LBNP devices can be uprighted to move the subject into a standing position while undergoing LBNP, thus making it possible to compare the effects of upright posture and LBNP and to examine a combination of both [58]. Typically, the seal of the LBNP chamber is closed around the iliac crest, since the moving of the abdominal wall while breathing would prevent an airtight fit if the seal was in the vicinity of the navel. Even if airtight, the distance of the seal from the heart has been shown to affect the experimental results [59], therefore care must be taken to place the seal in the same location for each subject of a study.

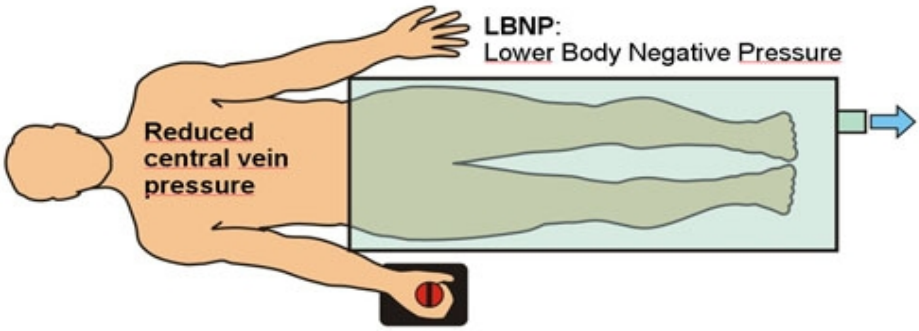


Figure 6: Working Principle of a LBNP Device

Showed in the right hand is the emergency button enabling the subject to terminate the LBNP by himself in case of presyncope.

Source: http://www.strteknikk.no/Previous%20products/lbnp1.jpg **retrieved: 30.08.2015**

The application of LBNP leads to a pooling of venous blood in the lower extremities, the sucking force of the decompression chamber replacing the gravitational or inertial pull along the body axis for which LBNP serves as a model. The shifting of fluid into the legs reduces blood volume in the right atrium and the venae cavae causing a reduction of preload and thus, via the Frank-Starling mechanism a reduction of stroke volume and, according to formula (1), a reduction in cardiac output. Now in line with formula (3), a reduction in cardiac output would lead, if the peripheral resistance stays the same, to a reduction in blood pressure with the result of reduced cerebral

perfusion with the consequence of the subject losing consciousness. The reason this does not happen is the baroreceptor reflex described above: pressure receptors in the wall of the aortic arch and the carotid sinus notice even the slightest decrease in blood pressure and will, at first trigger, via a reduction of vagal activity an increase in heart rate and then, after a few seconds, a release of norepinephrine which will cause vasoconstriction especially of the middle and small arteries, thus causing an increase in total peripheral resistance (TPR) [10, pp. 119, 82ff.] [2, pp. 616ff., 598]. This increased TPR, together with the increased heart rate, will in line with formula (3) help to maintain perfusion and to prevent loss of consciousness.

However, the increase in HR and TPR does not last forever: at some point, depending on the elapsed time and the level of LBNP, the cardiac output, due to a sudden reduction in HR, TPR or a combination of both will sharply drop, leading to hypotension and a subsequent loss of consciousness. The sudden reduction in cardiac output is called presyncope, while the loss of consciousness itself is termed syncope [60] [61]. A drop of mean blood pressure <80 mm Hg, followed by bradycardia is the most common symptom which indicates presyncope [62] [53] [10, p. 133ff.] [63, p. 87], meaning that fainting is imminent. Other symptoms may or may not include nausea, dizziness, a sensation of lightheadedness, blurred vision, abdominal discomfort, sudden headache or a sudden increase in sweating [64, p. 52].

Besides norepinephrine, the RAAS is also involved in the maintenance of blood pressure during orthostatic stress, renin as well as angiotensin II and aldosterone showing a significant increase upon presyncope as well as in subjects undergoing LBNP without presyncope [65] [10, p. 136] [66]. Angiotensin II is a very powerful vasoconstrictor, its potency being about 40 times higher than an equimolar amount of norepinephrin [10, p. 98], and aldosterone has the effect of increasing the total circulating volume by raising natrium reabsorption in the kidney, which boosts osmotic water retention leading to an increase in extracellular fluid [13, p. 278ff.] [2, p. 458f.]. AVP also plays a role in this context, because by shifting aquaporins into the basal membrane it increases the permeability for water in the distal collecting duct and thus facilitates the osmotic shift of water back into the bloodstream. This effect is provoked by the shift in blood osmolality caused by aldosterone action [13, p. 278ff.]. The larger amount of extracellular fluid will result in an increased cardiac filling, thus helping to maintain the minimum preload necessary for sufficient cardiac action. The effect of retaining water through the kidneys might be of special importance, since Hinghofer in 2011 has shown that LBNP with presyncope causes a significant decrease in plasma volume, possibly through leaking from the blood vessels into the interstitial space, an effect

which could be attenuated through aldosterone and AVP action.

The RAAS is known to be activated during LBNP and it has been shown that a reduced RAAS activation is associated with an increased incidence of presyncope during LBNP [10, p. 136] [67] [68]. AVP on the other hand has been shown to increase during LBNP if a marked reduction in blood pressure occurs (which usually signifies presyncope); however there are observations that AVP is also stimulated during LBNP without MAP reduction, although until now it is not clear whether this is caused by a narrowing of the pulse pressure (PP) or a change in left atrial diameter or some other factor [69] [24] [70] [67] [71] [72]. In general, observations on AVP are less compelling than on the RAAS. In 2001, Hinghofer observed a significant rise with an alpha of 0,01 in plasma AVP levels during orthostatic load by 53° Head-Up-Tilt (HUT), a procedure that creates physiological reactions similar to LBNP [58] [73], while in 2011, Roessler noticed during repeated LBNP bouts in one group and HUT in a second group, both with intermitting supine resting periods, that AVP levels were not significantly different between LBNP and HUT; at the end of the resting period following the second LBNP or respectively HUT bout, a significant augmentation of plasma AVP in comparison with the baseline was observed. On the whole, current evidence gives the impression that there is a sequence in which the different physiological systems are activated during LBNP: the immediate response upon reduction of venous return is a decrease in vagal activity, leading to a rise in heart rate, followed a few seconds later by sympathetic activation triggering peripheral vasoconstriction and increase in TPR, partly directly through innervation, partly through a release of norepinephrine from the nerve endings [10, pp. 88, 94]. The RAAS system is also activated, while AVP appears to increase, although the exact nature of its change is still controversial, something that will be discussed in detail below.

The time each subject can spend in the LBNP device without fainting depends on the strength of the decompression inside the LBNP chamber, but also on several other factors. Endurance athletes for example have a higher incidence of presyncope under LBNP [74], a fact that is as of now not yet fully understood. Furthermore women in comparison with men are known to have a lower orthostatic tolerance during LBNP as well as under post-spaceflight conditions[75] [76] [77] [78] [79] [80] and the general female population also has a higher incidence of vasovagal syncope as a disease [63, p. 45]. The reasons why this is the case are currently the subject of much scientific interest. A study by Jarvis in 2009 found that during LBNP women show smaller splanchnic vasoconstriction with increased splanchnic blood pooling, possibly being a contributing factor due to a decreased venous return [81]. Russomano in 2015 analyzed the cardiovascular responses in

men and women during LBNP and found that TPR was higher among the males [82].

An interesting question regarding gender-related differences in orthostatic tolerance is whether the female menstrual cycle affects the ability of women to withstand orthostatic stress. Meendering in 2005 reported that the incidence of presyncope during HUT in a heated environment did not differ between early follicular, ovulatory or mid-luteal phase and Russomano in 2015 confirmed these results with LBNP at room temperature [82] [76].

Despite the observation that the female menstrual cycle appears not to affect orthostatic tolerance, there is evidence that estrogen affects neurovascular control: in 1996 Tagawa reported that in postmenopausal women, estrogen treatment augmented forearm blood flow mediated by Nitric Oxide [83] and Tanaka in 2003 reported that baroreflex sensitivity assessed by Valsalva maneuver as well as by pressor and depressor tests using phenylephrine and nitroprusside correlated significantly with plasma estradiol [84]. A study by Minson in 2000 observed that muscular sympathetic nerve activity and plasma norepinephrine were higher during the mid-luteal phase, apparently due to an observed higher baroreceptor sensitivity during this phase, but these changes appeared not to affect peripheral resistance because vascular transduction showed no significant difference [85]. In Minson's study the cardiovagal baroreflex, i.e. the reduction in vagal activity which raises the heart rate upon reduction of mean blood pressure, was observed to not be affected by the menstrual cycle [85], thus contradicting the findings by Tanaka mentioned above. Research done by Hartwich and published in 2012 on the effect of the early and late follicular phase on heart rate, baroreflex sensitivity during exercise and blood pressure found no difference between the two phases, indicating that estrogen, which is low during early and high during late follicular phase, while progesteron is low during the whole follicular phase, has no significant influence on these parameters [86], supporting Minson's findings.

In summary, current evidence indicates that the menstrual cycle has at most very slight, if any, influence on orthostatic tolerance, although there is some evidence of interaction between gonadal steroids and cardiovascular regulation. Regarding the question whether the human baroreceptor reflex is influenced by gonadal steroids, there are conflicting findings, but still yielding the overall impression that the female menstrual cycle has at most a minor effect on baroreflex-mediated alterations of the heart rate. Nevertheless, there is a need for further research and assessment in the future.

1.10 AVP and Aging

In a previous review (see Appendix C), we have discussed the role that AVP plays during the human aging process. It has been shown in this context that both baseline AVP as well as AVP release upon osmotic stimulation is elevated in the elderly, while at the same time urine concentrating ability is gradually lost, indicating a decrease of renal sensitivity to AVP [87] [88] [89] [90] [91]. Orthostatic intolerance on the other hand, often accompanied by syncope – especially after standing up – and subsequently serious injury is an recurrent problem in the elderly [92]. Since AVP has been found to be drastically elevated after incidents of syncope, it is of high interest to get a deeper knowledge of how it is involved in cardiovascular regulation during orthostatic stress [65]. Therefore, it is of crucial importance to improve our understanding of the effects of central hypovolemia such as induced by orthostatic stress on plasma AVP on healthy young volunteers of both sexes, with the hope of providing a basis for further research with the goal of treating orthostatic hypotension in aged people.

Studies in rats have shown that AQP 2 abundance is reduced in aged people, along with AQP 3, EnaC and the AVP-regulated Urea-Transporters [93] [94], further supporting the notion of a decreasing AVP sensitivity of the kidney during aging and the importance of the role played by AQP 2 in this. However, hyponatremia, a condition that might be caused by AVP action, is also very frequent during aging. A possible explanation for this could be that the increase in AVP in the elderly compensates not only for the reduction in renal sensitivity to AVP, but also for other renal reabsorption defects in order to maintain sufficient blood volume, leading to an excess of AVP activity in comparison to other renal reabsorption mechanisms. In recent years, a new class of AVP receptor antagonist called vaptans have been experimented upon in order to assess their value in treating hyponatremia of different etiologies with so far promising results [95] [96] [97].

The circadian rhythmicity of AVP secretion tends also to be lost during aging [26] [28], a fact which contributes to the nocturia frequently observed in the elderly [30] and desmopressin, an AVP analogon can be used in treating this condition [98].

Another possibly harmful effect of the elevated AVP levels in the elderly might be an increased risk of thromboembolic events due to its stimulating effect on hemostasis, as well as a possible increase of afterload due to V1a-receptor-mediated vasoconstriction; indeed AVP has been shown to be increased in patients with chronic heart failure (CHF) [99]. However, treatment of CHF patients with tolvaptan, a selective V2 receptor antagonist, lead to no significant change of long-term mortality [100] and the role of AVP regarding thromboembolic events in the elderly remains to be assessed.

One important conclusion to draw from these findings is that experiments assessing AVP secretion

must control age as a variable in order to avoid a bias due to the subjects' age.

1.11 Sex Differences in AVP Secretion and Effects

Men are known to possess higher plasma levels as well as higher urinary excretion of AVP than women [101] [102] with their plasma levels being about 40 percent higher [18], which was recently confirmed by a study by Roussel in 437 healthy subjects stratified along BMI, age, water as well as intake of alcohol and sweet beverages. This is largely consistent with the observation made bei Perucca et al. in a metaanalysis in 2007 that while urine output is roughly the same in men and women, the urine osmolality however is higher in men [103] [104], which is consistent with a higher AVP activity in males and a lower urine concentrating ability in females. In rats, where AVP plasma levels are also higher in males than females, gonadectomy was found to enhance AVP levels in females and reduce them in males, an effect which could be partially reversed by testosterone in males and oestrogen in female rats [105]. In postmenopausal women, three weeks of estrogen therapy was found by Punnonen in 1982 to reduce AVP levels, whereas Forsling in 1982 in a similar study found estrogen to boost AVP plasma concentration while a combination of oestrogen and progesteron did reduce AVP plasma levels [106]. Progesterone alone did not have an effect on AVP concentrations [107]. Stachenfeld in 1998 found a 14-day estrogen treatment to shift basal AVP concentration upwards, thus giving support to the findings by Forsling [108].

It is known that there are distinctive AVP-induced vasoconstriction patterns in males and females [109, p. 42f.].

An interesting finding was made by Edgell in 2012, that after a 4-hour period of 6° Head-Down Bed Rest (HDBR) AVP was significantly higher in men compared to baseline and lower in women compared to baseline [66]. The experimental protocol used by Edgell consisted of increasing LBNP in supine position with 5 min baseline, 3 min at -10 mmHg, 5 min at -20, 3 min at -30 and 5 min at -40mmHg. As a control, 4 hours of being seated in a comfortable chair instead of the 4-hour HDBR was used, taking place within 48 hours of each other with both experiments being in the follicular phase of the menstrual cycle. The seated control resulted in an equally significant increase in AVP in men and women during LBNP, but no difference before and after the 4 h seated period, while after the 4 hours of HDBR, baseline values did not change, but LBNP led to a rise of AVP in men and a decrease in women. At the same time, women had lower pelvic impedance, suggesting blood pooling in the pelvic region and men higher pelvic impedance after HDBR, indicating a reduction of blood volume in the pelvic region [66]. This observation suggests that there might be some differences in the underlying mechanisms of AVP release in response to periods of altered

orthostatic loading, although the majority of parameters in Edgell's study was quite similar between the sexes. The relationship between AVP and pelvic blood pooling remains to be examined thouroughly. It is of interest in this respect, that the pelvic region is the place where both sexes anatomically differ the most from each other, a fact that might have something to do with the different perfusion patterns observed in Edgell's study.

1.12 Does the Menstrual Cycle Affect AVP Secretion?

During the 1980s the plasma level of AVP as well as plasma osmolality during the course of the menstrual cycle were examined by a number of scientists. In 1981 Forsling et al. found AVP plasma concentrations to be significantly changing over the course of the menstrual cycle with the maximum during the early luteal phase and the minimum at the onset of menstruation, the peak concentration being a little more than twice as high as the lowest concentration [110]. However, Forsling did not observe a significant correlation of AVP levels with either estrogen or progesteron [110]. Other studies by Vokes [111], Punnonen [112] and Spruce [113] on the other hand did not report significant alterations of plasmatic AVP concentration during the menstrual cycle, although Punnonen reported a tendency to increase during day 11 to 13 of the cycle. Basal plasma osmolality was found to be reduced during the luteal phase by Spruce [113]. Vokes in 1988 also found plasma osmolality to be significantly lower in the luteal phase in fed as well as fasted women. Oral water intake of 20mL/kg or infusion of 0.1mL per kg and minute of a 3% (and thus hypertonic) saline infusion for two hours did alter AVP levels and plasma osmolality in a similar fashion during al menstrual phases. However, the osmotic threshold for AVP release was found to be lower during the luteal phase and likewise, the plasma osmolality level at which AVP was maximally suppressed was also found to be lower during the luteal phase. Vokes et al. concluded that during the luteal phase, the thresholds for AVP release and inhibition are reset at a lower value [111]. This is consistent with the observation by Forsling and Stachenfeld that a combination of progesterone and estrogen – both hormones present in high concentrations during the luteal phase [114, p. 87] – lowers AVP levels while estrogen alone – which is dominant around the ovulation – has an increasing effect on basal AVP [107] [108] [114, p. 96ff.].

In summary, despite advancements in our understanding of the sexual dimorphism of the regulation of plasma osmolality through the hormone AVP, there is still a need for much more research in order to achieve a thorough understanding of the characteristics of the synthesis, secretion and effects of AVP which are peculiar to the female sex in humans.

1.13 Copeptin – a Novel Surrogate Marker for AVP

In recent years there has been an emerging interest in a molecule named copeptin, a molecule first described in 1972 by Holwerda [115] [116], which is part of the same precursor molecule as AVP and appears to be cleaved from that molecule simultaneously with AVP and neurophysin 2 [117] [116]. While AVP consists of 9 aminoacids, copeptin, with 39 aminoacids and a sugar moiety with a molecular weight of about 5000 Dalton, is much larger than AVP with its approximately 1000 Dalton [17]. The actual physiological function of copeptin – if any – beside acting as a chaperone for the formation of AVP inside of the neuron is still unknown [118]. However, it shows some properties which might render it useful as a substitute for AVP when measuring its plasma levels. First, it appears to be secreted in an equimolar quantity to AVP since it is released from the same precursor molecule [116]. Second, it is much more stable ex vivo than AVP, lasting several days even at room temperature and much longer when cooled [19]. Third, its measurement takes much less effort and is somewhat cheaper than AVP [22]. There have been in recent years a number of studies dealing with the possible use of copeptin as a diagnostic marker in conditions such as SIADH, acute and post-acute myocardial infarction as well as a number of other conditions which affect AVP hormone levels or peripheral actions [22] [116]. It is however usually present in higher concentrations in the plasma than AVP, suggesting a longer half-life than AVP [18] [119]. A very recent study by Koch and Schnyder in 2015 suggested its half-life to be at about 40 minutes, between eight and 1,7 times the half-life of AVP, depending which calculation is used as a reference [120] [121].

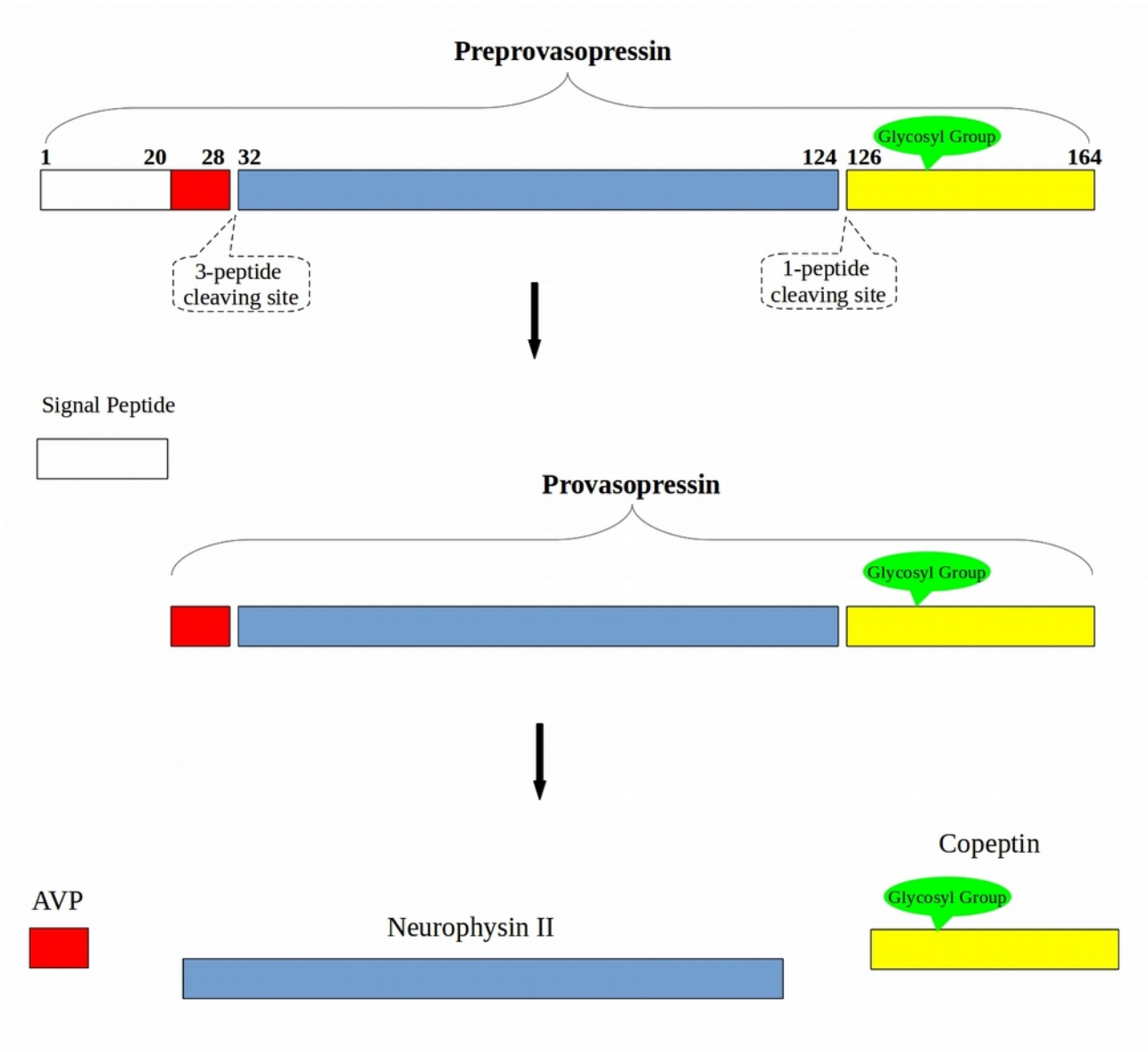


Figure 7: Copeptin and AVP Precursor Molecule

Copeptin concentrations, similarly to AVP plasma concentrations, were found to be higher in men than in women with osmotic stimuli of AVP or changes in plasma volume appearing to not significantly change their relative concentrations to each other [18] [122]. Therefore, it is widely believed that copeptin closely mirrors AVP plasma concentrations.

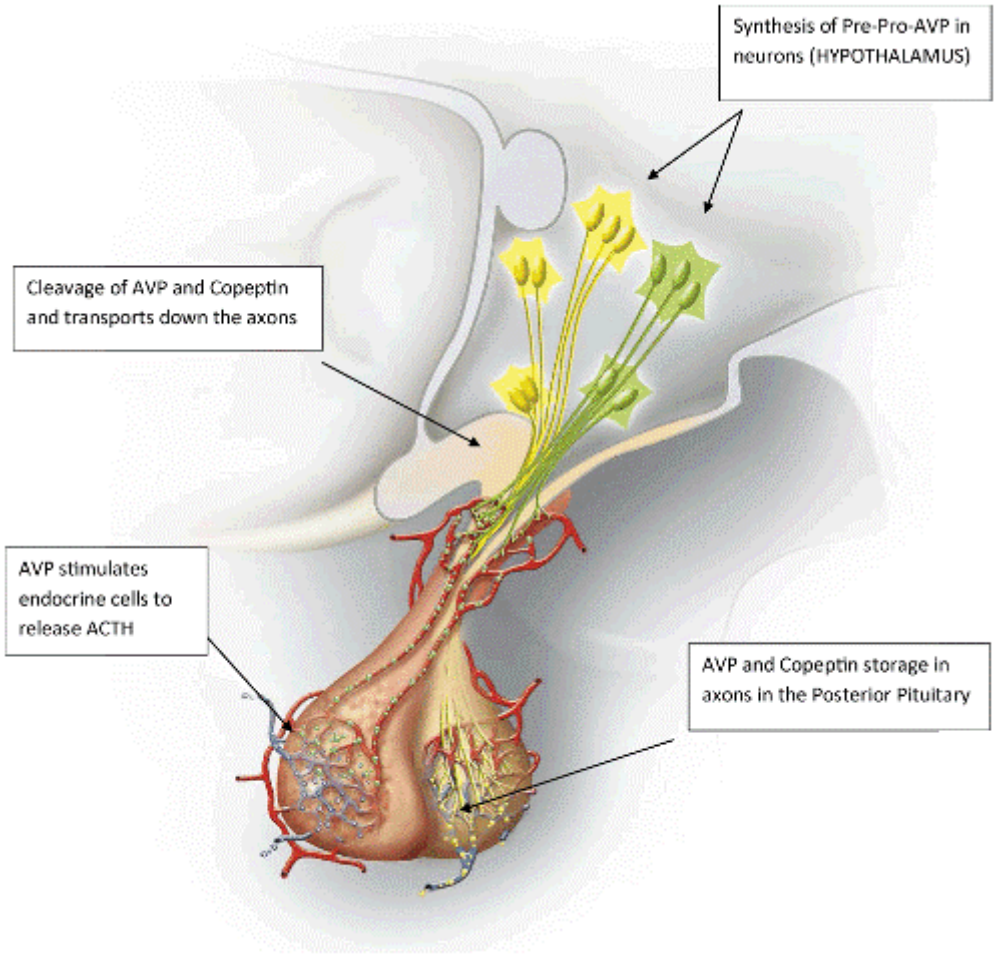


Figure 8: AVP and Copetin Release

Source: http://www.omicsgroup.org/journals/EGMimages/2165-7548-4-186-g001.gif
retrieved: 29.08.2015

1.13.1 AVP and Copeptin Properties and Measurement

AVP is a peptide hormone consisting of 9 aminoacids; The order of their position in the peptide chain is (from N to C terminus) Cystein, Tyrosin, Phenylalanine, Glutamin, Asparagin, Cystein, Prolin, Arginine, Glycine. The two cysteins form a disulfide bond so that the first 6 aminoacids form a ring, while the remaining three form an attached chain. The C-Terminal hydroxy group is replaced by an aminogroup, thus forming a carboxamide [15].

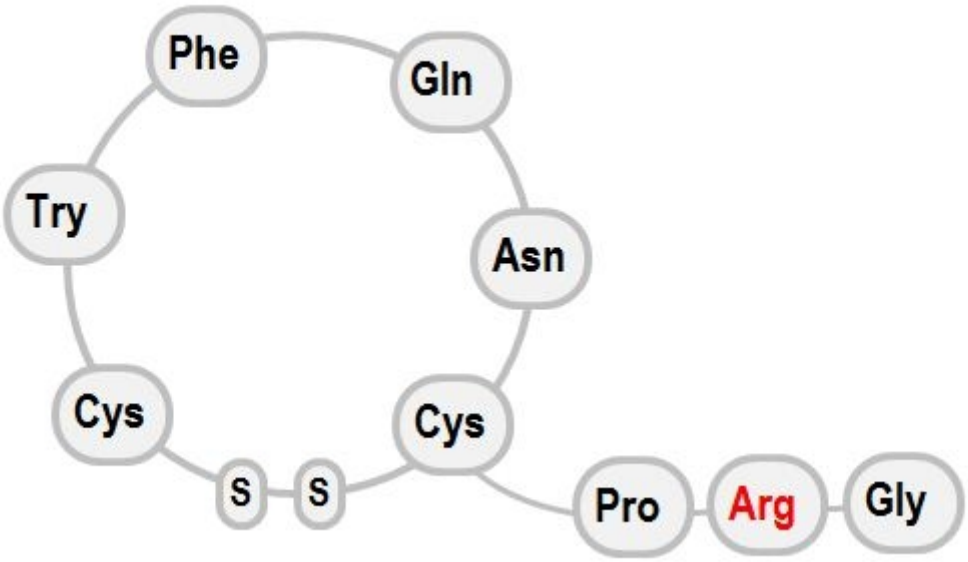


Figure 9: Aminoacid Sequence of AVP

Source: http://www.derangedphysiology.com/php/Drugs-of-interest/vasopressin.php **retrieved 30.08.2015**

The plasma concentration of AVP is measured by means of a radioimmunoassay since sandwich immunoassays are unable to determine AVP concentrations with a sufficient precision due to the hormones' small molecular size [21] [22]. This requires a quantity of at least 2mL EDTA plasma and about 20-30 minutes of careful preparation per sample involving radio labelling and extraction of the hormone from the plasma [123] [21] [124]. The current price is at about 450 € for a kit which can do 65 measurements and additionally about 500 € of material costs per kit for the handling vacuum gas manifold used during the process for the separation of plasma lipids, as well as the petroleum ether and acetone used in the protocol from the AVP containing plasma. Furthermore, a nitrogen blower and a fume hood are required which many small laboratories do not possess. An even bigger handicap is that AVP is quite unstable ex vivo, even when stored at -20 degree celsius and that in vivo 90% of the AVP in the bloodstream is bound to platelets, meaning that there is a constant risk of alteration of AVP levels by release of platelet-bound AVP. Sensitivity is also limited with a lower detection limit of about 0.4 pmol/L [18]. These factors make the measurement expensive and time-consuming, which is the reason why AVP measurement is as of now not a widely established clinical method.

Copeptin on the other hand is a much larger peptide consisting of 39 aminoacids which facilitates detection by sandwich immunoassays. It can therefore be measured by means of a chemiluminescence sandwich immunoassay in a much simpler process than AVP which requires less time, is considerably cheaper (650 € for 96 measurements without any significant additional

costs) and can be conducted without the expensive laboratory equipment mentioned above[19] [22]. Furthermore, in contrast to AVP, copeptin, at room temperature, is stable for up to 14 days in EDTA plasma and 7 days in heparin plasma and it is not bound to platelets, making measurement much easier and precise, even after a period of storage of the samples [22]. For these reasons it is not surprising that there is a strong interest in the possibility of using copeptin as an indirect marker for AVP plasma concentrations, especially considering the possibility of establishing it as a marker for a variety of medical conditions which are known or suspected to affect AVP plasma levels in a manner relevant for the patient's outcome.

2. Aims and Goals

The population we aim to analyze are young men and women without cardiovascular, neurological or psychiatric conditions. It is hoped that our study can provide a basis for further research, especially regarding the influence of aging on AVP activity as we have already discussed in our 2014 minireview about aging and AVP. For this we aspire to provide reference values in young, healthy people to gain a solid base for cross-referencing further research.

In this study we try to evaluate the following things:

1. the influence of LBNP on plasma copeptin concentrations as a surrogate marker for plasma AVP concentrations;

2. whether there is a difference between males and females in plasma copeptin levels;

3. whether the relative plasma copeptin levels of men and women change during or after LBNP;

4. whether there is a correlation between plasma copeptin and urinary AQP2.

To this end we hypothesize the following:

(A) that there will be a difference between men and women in copeptin levels reflecting the differences in AVP plasma concentrations observed in past studies;

(B) that LBNP application will lead to an increase in plasma copeptin as an indicator for an increase AVP with which it is released in an equimolar fashion;

(C) considering some recent evidence which indicates that there are different patterns of AVP release in men and women under certain conditions of orthostatic stress [66] [84] while there are also results that indicate that regulation by AVP might affect orthostatic tolerance [75] which is know to be lower in women [77] [82] [75] [76] [78], we hypothesize that there is a distinctive pattern in AVP release in men and women, meaning

that the relative levels of plasma AVP will behave differently during LBNP;

(D) that urinary AQP 2 will reflect alterations of copeptin due to AVP being the main regulator for AQP 2 trafficking to the apical membrane of the principal cells of the collecting duct.

3. Methodology

Criteria for the recruitment of both male and female subjects were as follows.

Inclusion criteria:

- Age between 18 and 35
- Sex male or female
- 160 to 175 cm of height for females, 170 to 185 cm for males

Exclusion criteria:

- Smoking
- Regular practice of endurance sports, due to reduced orthostatic tolerance of this population [74]
- History of orthostatic intolerance
- Cardiovascular or renal disorders
- Intake of any medication affecting the cardiovascular system such as diuretics, antiarrhythmic or antihypertensive drugs

Pregnant women were excluded from our study. The phase of the menstrual cycle of the women at the time of the experiment was asked and written down in order to be able to consider the phases of the menstual cycle separately. In order to clearly distinguish between the luteal and follicular phase, female subjects were examined only in the days 4-10 or 21-27 of their cycle. The day of the cycle was determined from the first day of the last menstruation.

Subjects were recruited via poster at the blackboard in the Medical University of Graz and received a 40 € compensation for taking part in the study (for the recruitment poster, see Appendix B).

Both alcohol and caffeine are known to affect orthostatic tolerance [125] [126], therefore care was taken to instruct each subject to abstain from either of them at least 24 hours before the beginning of the experiment in order to avoid a possible bias in our study.

The actual measurements were conducted at the Institute of Physiology of the Medical University of Graz from April to May 2015. All of the measurements were started between 08:16 (earliest) and

12:42 (latest) with the majority of the experiments taking place between 9:00 and 12:00. This was done in order to minimize the influence of circadian variation of hormone levels as observed by Forsling in 1998.

3.1 Experimental Protocol

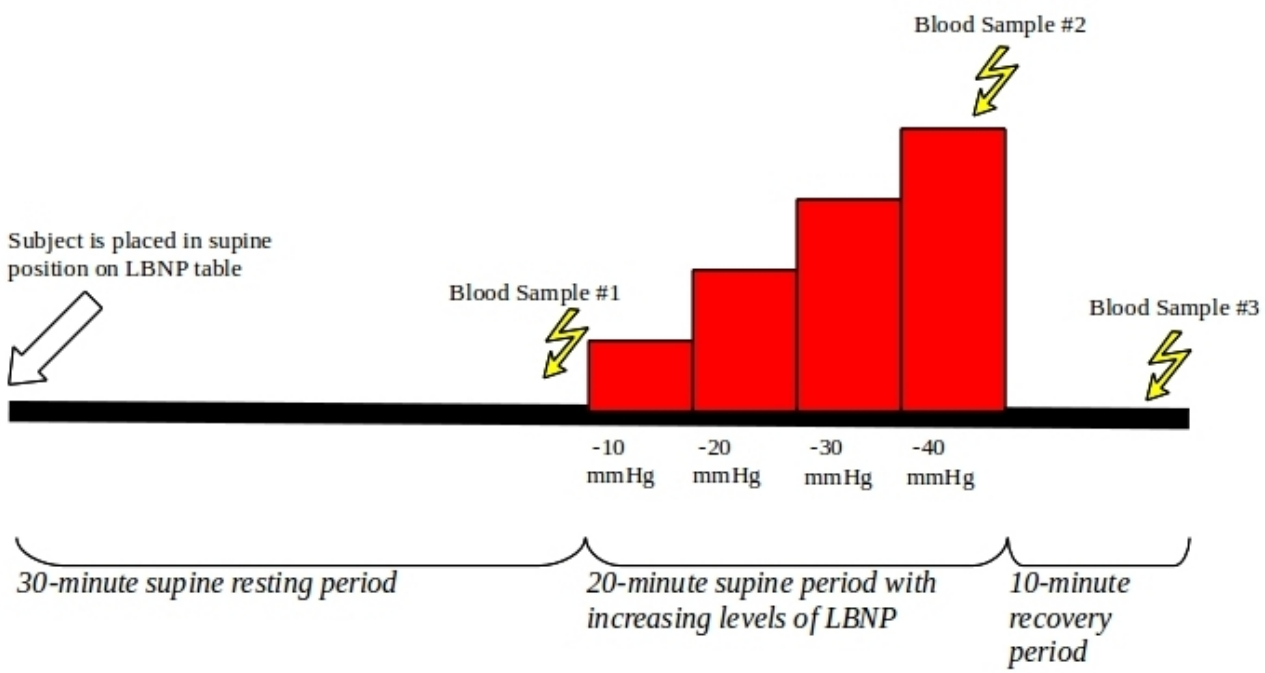


Figure 10: Experimental Protocol

The actual experimental protocol lasted 1 hour in total and consisted of a 30-min supine rest period without any LBNP during which the monitoring equipment was installed. At minute 30, 10 mmHg of LBNP was applied and increased by a further 10 mmHg every 5 minutes until value of 40 mmHg was reached and held for five minutes, thus totalling to a period of 20 minutes of increasing LBNP. After that, LBNP was brought to zero and the subjects were submitted to a further 10-minute rest period.

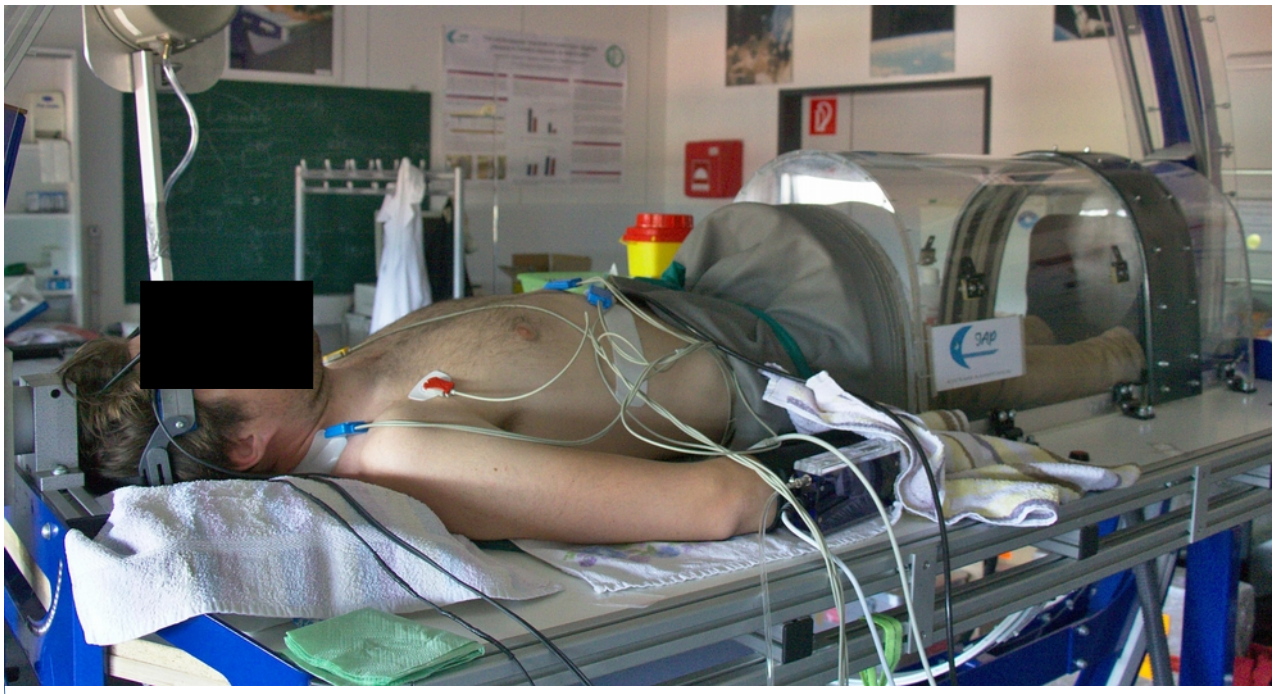


Figure 11: Male Test Subject on LBNP Table During Our Experiment

Test subject on LBNP table. Well visible are the electrodes used to monitor hemodynamic parameters as well as a device for continuous BP measurement on the right forearm. The feet are placed on an adjustable footrest to avoid downward movement of the subjects when LBNP is applied. Blood samples were taken from an antecubital vein on the left side (not visible).

During the experiment, BP and HR as well as the subjects themselves were closely monitored and the LBNP terminated if any of the following signs of presyncope occurred:

- drop of systolic BP under 80 [75] [64, p. 52] [53] [10, p. 133ff.] [63, p. 87];
- dizziness, a sudden dry mouth or nausea reported by the subject [75] [63, p. 87] [64, p. 52] [53];
- sudden increase in sweating, paleness or unconsciousness of the subject [75] [64, p. 52] [63, p. 87].

Subjects also had access to an emergency button allowing them to end LBNP by themselves in case of feeling unwell. Termination led to an earlier begin of the recovery period and thus a shorter experimental protocol in total. Data obtained from presyncopal subjects was analyzed separately from non-presyncopal subjects.

The measurements taken during the experimental protocol included an electrocardiogramm, thoracic impedance from which the stroke volume was continuously calculated – an established methode of monitoring stroke volume [127] – blood pressure continuously as well as intermittently, with the intermittent measurement providing the basis for error correction of the continuous monitor, as well as ultrasound monitoring of the middle cerebral artery by means of an ultrasound

headset whose probe was placed over the left temple. The device for monitoring hemodynamic parameters was a Task Force® Monitor produced by CN Systems, Graz, Austria. The effect of LBNP on cardiovascular parameters is not the subject of this thesis, however the image below illustrates the adaptation of BP, HR, CO, SV and TPR to gradually increasing LBNP in a non-syncopal subject during our experiment.

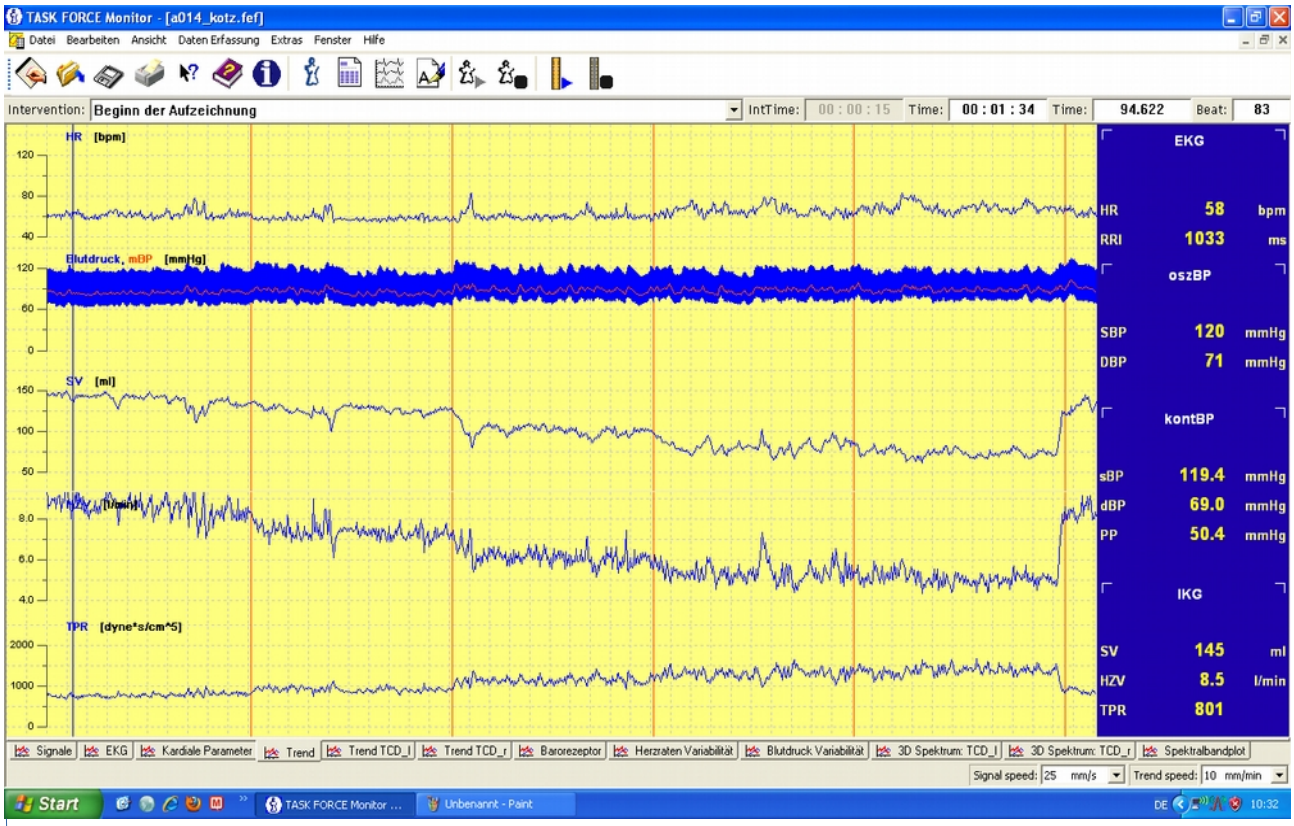


Figure 12: Cardiovascular Response to LBNP

Typical pattern of cardiovascular response during our LBNP protocol The first four red lines mark initiation of -10, -20,-30 and -40 mmHg of LBNP, the last line the end of LBNP. Well visible (in the blue curves from the bottom upwards) is the stepwise increase of TPR as well as the decrease of CO and SV. Also visible in the two top curves are the narrowing of the PP and the increase in HR.

3.2 Procedure for Blood Extraction and Analysis

A vein in the antecubital fossa, the back of the hand or on the forearm was canalized at minute 25 of the protocol, 5 minutes before the beginning of the LBNP using a 17G, 1·4 · 40 mm Teflon® cannula which was then attached to the skin with adhesive tape. Blood samples were taken at minute 28 (2 minutes before the beginning of LBNP), at minute 48 (2 minutes before the end of the LBNP) and at minute 58 (2 minutes before the end of the recovery period). The same cannula, which was left in place during the experiment was used for each sampling. Between the samplings 2mL of isotonic

natriumchloride solution were injected into the cannula to prevent clotting.

Blood was sampled into 3mL EDTA plasma vacutainers along with 500U/mL of aprotinin and the samples were centrifuged at 1000g for 15 minutes at 6°C immediately after the end of the experimental protocol. After centrifugation plasma was removed carefully with pipettes and the plasma samples were stored at -70°C. Hemolytic samples were not processed or analyzed due to possible alterations of the results.

Copeptin was analyzed with a special enzyme-linked immunosorbent assay (ELISA) kit designed by Cloud-Clone-Corp., Houston, USA and produced by Uscn Life Science Inc., Wuhan, People's Republic of China and the instruction manual contained in the kit was meticulously followed. The analysis took place within 4 months after the end of the experimentation period.

3.3 Procedure for Urine Analysis

In order to provide baseline values for urine measurements, each subject was asked to collect his urine for a duration of 24 hours before the experiments, beginning at 9:00 of the day before until 9:00 of the test day. The bottles to collect the urine samples were provided by our institute beforehand.

Immediately after the end of the protocol, the subjects were given a funnel and a bottle and asked to provide an urine sample.

1.2mL of urine, both from the 24-h sample and from the sample after the LBNP, were sampled into a 1.5mL microtube and stored at -70°C. The frozen samples were then shipped to Bari, Italy where analysis of the urinary AQP 2 took place.

The method chosen for analyzing uAQP2 was an immunological assay ELISA. Between 5 and 10µl of urine were diluted in a phosphate buffer solution with 0.01% of sodium dodecyl sulfate, spotted in 96 multiwell Maxisorp plates and incubated for 16 h at 4°Celsius. A standard curve was obtained in the same plate by using growing concentrations of a synthetic peptide reproducing the C-tail of human AQP2 (50, 100, 200, 300, 400, 500, 1000pg/ µL). The plate was then washed, underwent a blocking process to prevent non-specific antibody binding and afterwards incubated for 3 hours with 50 µL of a specific anti-AQP2 antibody obtained from rabbit immune cells. Afterwards, a specific anti-rabbit IgG conjugated with horseradish peroxidase and the samples were again incubated, this time with 50 µl of a special substrate (2,2 azino-bis(3 ethylbenzthiazoline-6-sulfonic acid)), under conditions of darkness for 30 minutes. Then the samples were examined in a plate reader set at an absorbance of 405 nm.

3.4 Statistical Methods

For the copeptin analysis, Shapiro-Francia's test was conducted on male and female samples at each stage of the measurement to test whether the data were normally distributed with a cumulative Alpha of 0.6. When it was found that they were not, Wilcoxon's signed rank test was used for the paired samples and Mann-Whitney's U-Test for testing the unpaired samples.

For the uAQP2 analysis, students t-test for paired samples was used for the comparison of the 24-h urine samples wih the samples from the end of the protocol, while for male-female comparison, Student's t-test was used. For the correlation of copeptin and uAQP2, Pearson's correlation coefficient was used.

For the necessary statistical calculations, the Linux-based program Gnumeric was used as well as the Windows-based Graphpad-Prism.

4. Results

Taking part in the study were 21 females and 17 males with the following characteristics. One female failed to produce urine after completion of the protocol and was thus excluded from the analysis.

Table 1: Subjects' Characteristics

	Age	**Weight**	**Height**
Males (n=17)	25.85 ± 3.47 years	79.35 ± 9.86 kg	181.40 ± 5.44 cm
Females (n=20)	24.28 ± 3.43 years	60.94 ± 8.61 kg	167.78 ± 5.29 cm

Of these 37 subjects, due to logistical and financial restraints, only the first 27 subjects' blood samples could be analyzed, while all of the urine samples were available for analysis.

4.1 Copeptin Results

Of the 27 subjects whose blood was analyzed, in 1 male and 1 female LBNP had to be terminated early due to symptoms of presyncope in the form of nausea and a sudden drop in blood pressure.
All in all, of the 27 subjects available for blood sample analysis, 25 subjects, 15 male and 10 female completed the experimental protocol without presyncope. In one of the female subjects the blood sample from the recovery period was hemolytic and therefore was excluded from the analysis for paired data, thus leaving 9 female and 15 male non-presyncopal subjects to yield complete data sets at all three measurements and one additional female subject to yield data only for basline and end of LBNP measurements.

Of the 9 females who did not experience presyncope and yielded complete data sets, five were in the follicular and four in the luteal phase of their menstrual cycle. The subject whose blood sample from the end of recovery period was hemolytic was in the follicular phase.

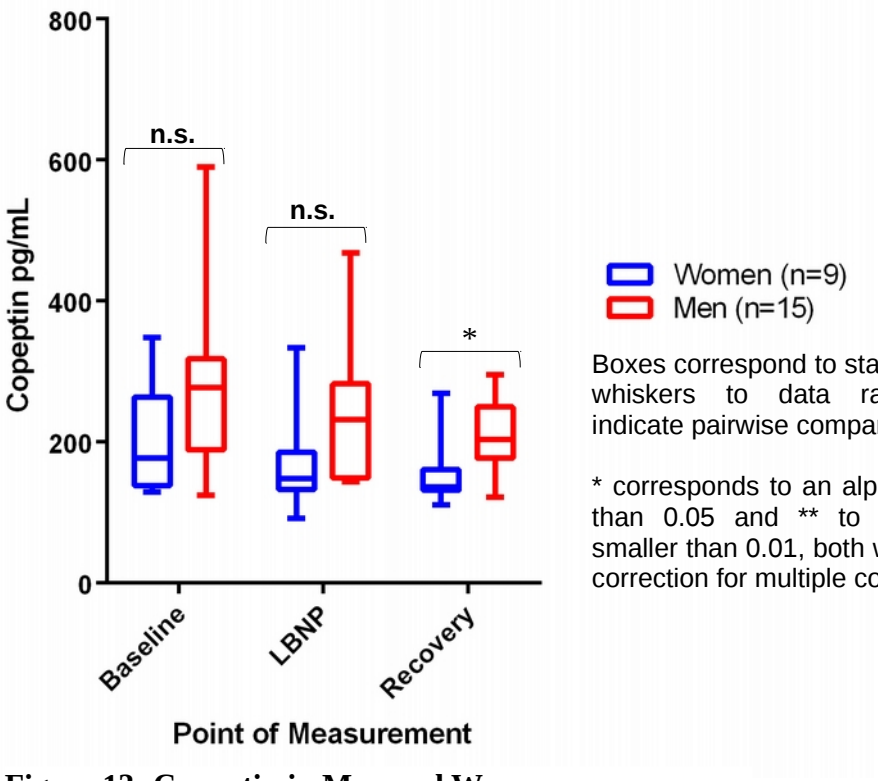
Statistical analysis of the data obtained yielded the following results: at all stages the males had higher copeptin mean and median levels than the women; furthermore, in both sexes, the means and medians were lower at the end of the LBNP phase than at baseline and even lower at the end of the recovery phase at the end of the experiment. The mean for the men at baseline was 284.03 pg/mL with a standard deviation of 112.70pg/mL, 236.58 pg/mL with 80.61 pg/mL standard deviation at

the end of LBNP and 226.69 pg/mL with 76.60 pg/mL standard deviation at the end of the recovery phase. For women, mean copeptin level at baseline was 199.48 pg/mL with 69.69 pg/mL standard deviation. At the end of LBNP the mean had decreased to 167.70 pg/mL with 61.36 pg/mL standard deviation while at the end of the recovery period, a further decrease had taken place to 157.83 pg/ml with 46.47 pg/mL standard deviation. Medians for the two groups showed a similar pattern with a baseline median of 277.78 pg/mL decreasing to 233.02 pg/mL at the end of LBNP and 209.73 pg/mL at the end of the recovery period for the men, while for the women, a baseline median of 179.17 pg/mL was measured decreasing to 160.49 pg/mL at the end of LBNP and 136.39 pg/mL at the end of the recovery period.

Table 2: Copeptin Results

Values (pg/mL)	**Baseline**		**End of LBNP**		**End of Recovery Period**	
	Arithmetic Mean	**Standard Deviation**	**Arithmetic Mean**	**Standard Deviation**	**Arithmetic Mean**	**Standard Deviation**
Male	284.03	±112.69	236.58	±80.61	226.69	±76.60
Female	199.48	±69.69	167.70	±61.37	156.99	±46.47

In the female subject who reached presyncope, the baseline copeptin was 211.68 pg/mL, dropping to 160.49 at the end of LBNP and rising again to 211.47 at the end of the recovery period. In the male subject who reached presyncope, baseline copeptin was 321.04 pg/mL, dropping to 242.87 at the end of LBNP and rising again to 449.27 at the end of the recovery period. The difference between the non-presyncopal groups and the presyncopal subjects was analyzed for men and women separately using Mann-Whitney's U-test, but found to be not significant.



Boxes correspond to standard deviation, whiskers to data range. Brackets indicate pairwise comparisons.

* corresponds to an alpha error smaller than 0.05 and ** to an alpha error smaller than 0.01, both with Bonferroni's correction for multiple comparisons

Figure 13: Copeptin in Men and Women

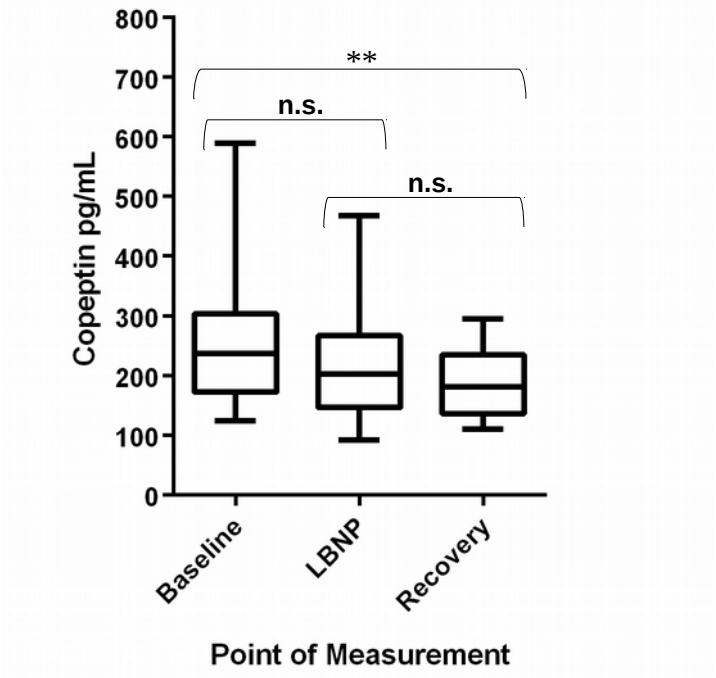


Figure 14: Copeptin in Men and Women Combined

To analyse whether the changes were statistically significant, it was decided to first determine whether the data was following the normal distribution to see whether it would be possible to run Student's t-test on the data, which requires the data to be normally distributed. To this end Shapiro-Francia's test of normal distribution was performed for each of the three consecutive measurements

for both sexes, therefore amounting to six tests in total. For this test, to keep the cumulative alpha error as low as possible, an alpha of 0.01 was chosen for each test, leading to a cumulative alpha of slightly under 0.06. The Shapiro-Francia test described 5 of the six samples as possibly sticking to the normal distribution, while the sample of the females at the end of the LBNP period was determined as not following a normal distribution. Taking into consideration the small and unequal size of our sample and in order to be able to analyse all of our data with the same test without having to worry about the data being normally distributed or not, it was chosen not to use an Analysis of Variance (ANOVA) for analysis, but to use Wilcoxon's signed rank test for the paired samples and Mann-Whitney's U-test for male-female comparison because these two are based on the median and do not require normativity.

In order to adjust the alpha errors for cumulative measurements, Bonferroni's correction was applied.

Using Mann-Whitney's U-test without Bonferroni's correction a significant difference between men and women was found at all three timepoints of the experiment with an alpha of 0.05 at the baseline and LBNP phase and an even more significant difference with an alpha of 0.01 at the end of the recovery period. With Bonferroni's correction, being taken into account only the difference in the recovery period still was significant on an alpha level of 0.05.

Combining the male and female data sets to analyze the differences between baseline, the end of LBNP and the end of the recovery period were analyzed using the Wilcoxon signed rank test. Between baseline and LBNP the decrease in copeptin was found to be significant on an alpha level of 0.05, but insignificant with Bonferroni's correction. Between the LBNP and end of recovery measurements the decrease was not significant even without the correction for cumulative alpha errors. However, assessing the decrease in copeptin from baseline to the end of recovery measurement, over the whole period of LBNP and the recovery phase, the fall in copeptin was found to be highly significant with $p=0.0015$, which was significant with an alpha of 0.01 even with Bonferroni's correction.

Still using Wilcoxon's signed-rank test, the differences within the separate male and female groups between the measurements were analysed. In both groups the difference between baseline and the end of the recovery period was found to be significant with an alpha of 0.05 for men and women, without Bonferroni's correction for cumulative alpha errors. With Bonferroni's correction included, this change was not significant. The difference of copeptin at the end of LBNP compared to its levels at baseline or at end of recovery was insignificant even without correction for cumulative alpha errors in both males and females, with copeptin values at the end of LBNP being intermediate between baseline and end of recovery levels, meaning that there was a constant

decrease of average copeptin during the course of the three measurements in both groups. However, with Bonferroni's correction taken into account, all this was on a non-significant level if considering the groups separately, while it was highly significant if combining men and women and considering the drop from the baseline to the end of the recovery period.

To see whether the reduction of copeptin happened at a constant rate, i.e. in a linear fashion, we proceeded to calculate Pearson's correlation coefficient for the relationship between time and AVP. The factor time was taken as an independent variable for the x axis with the time of the taking of baseline blood sample as a zero point and thus a value of 20 minutes for the measurement at the end of LBNP and 30 minutes for the end of the recovery period. Pearson's correlation coefficient was shown to be 0.30 for the females with $p=0.10$ and 0.27 for the males with $p=0.07$, thus showing only a weak correlation on a low significance level.

A two-way analysis of variance ANOVA for one factor with two independent treatments (gender) and one factor with three repeated measurements (the stages of the LBNP protocol) was also performed and yielded a significant effect for both gender ($p<0.05$) and LBNP ($p<0.01$). Tukey's post-hoc test however determined the differences between men and women as not significant before, during or after LBNP and the difference between the different measurements in the women as not significant as well; only in the men did it result in a significant difference only between baseline and end of recovery mesurements with $p< 0.01$, thus on a high significance level.

However, due to our small and unequal sample size and the prerequirement of normativity of all samples violated; furthermore, due to the very important condition of sphericity also being violated, the results of the ANOVA have to be considered as too unreliable as basis for a well-founded analysis.

4.2 Aquaporin Results

Regarding the urinary AQP2 (uAQP2), 17 females and 20 males were analyzed with the following results:

Table 3: uAQP2 Results

Values (fmol/mg creatinine)	Female (n=17)		Male (n=20)	
	Arithmetic Mean	Standard Deviation	Arithmetic Mean	Standard Deviation
Initial 24-h-sample	4939	±330.3	2660	±206.7
Final sample	5877	±1104	3101	±590.4

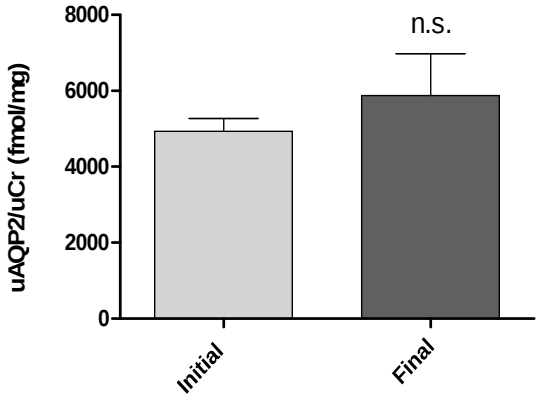


Figure 15: Female uAQP2 (n=17)

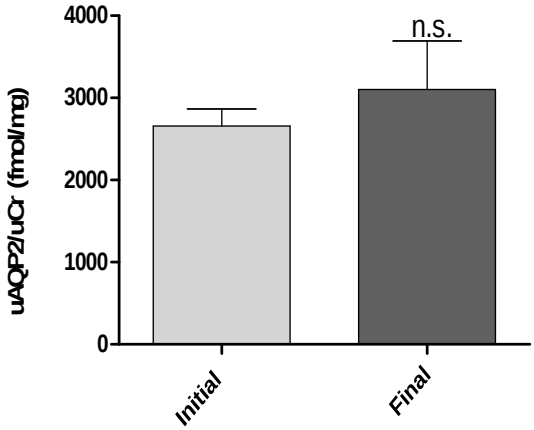


Figure 16: Male uAQP2 (n=20)

In the 24-h sample collected as baseline before the experiment, females had significantly higher uAQP 2 than the males ($p<0.001$). Compared to the end of the experiment, uAQP did increase in both sexes by a similar amount, however on a non-significant level.

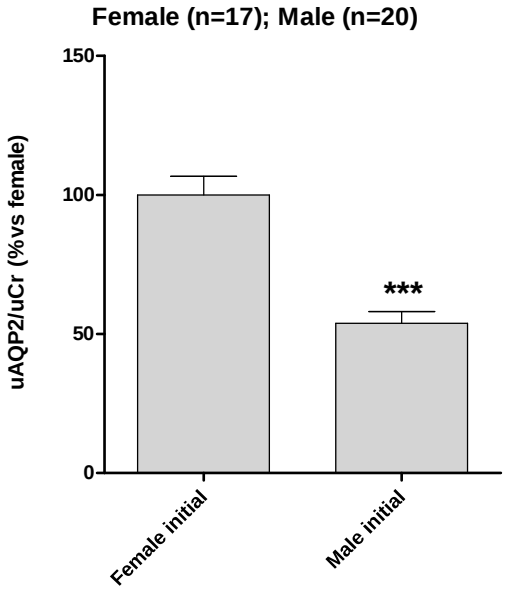


Figure 17: uAQP2 Gender Comparison

4.3 Correlation of uAQP2 and Copeptin

The change in urinary AQP2 was then correlated with the change in plasma copeptin from baseline to recovery, yielding a moderate correlation in the females (Pearson's correlation coefficient r=0.5042), but on a non-significant level (p=0.1373). In the males r was 0.1407 with p=0.6170.

Table 4: Correlation of uAQP2 With Copeptin

	Females (n=10)	**Males (n=15)**
Pearson's r	0.5042	0.1407
P value	0.1373	0.6170

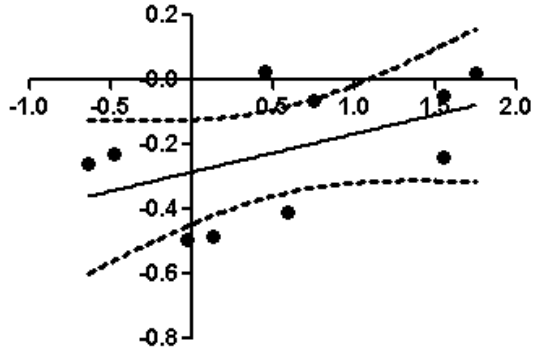


Figure 18:
uAQP2 Correlation With Copeptin of Females

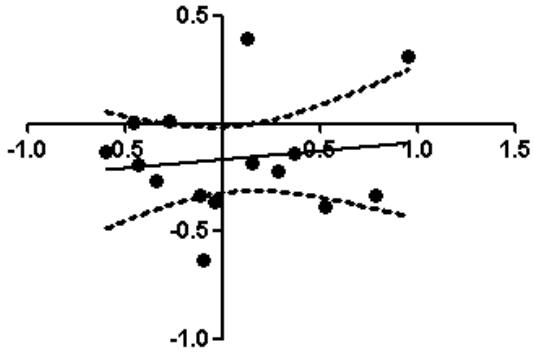


Figure 19:
uAQP2 Correlation With Copeptin of Males

5. Discussion

5.1 Observations

In our study, we observed the following:

- first, a rise in copeptin during or after LBNP did not occur. Instead, during our experiment, a drop in copeptin was observed at the end of LBNP, and even lower at the end of the recovery period;
- second, while men had at all stages higher copeptin than the women, the difference was more pronounced at the end of the recovery period;
- interestingly, the 2 subjects, one male and one female, who underwent presyncope only showed a quite moderate increase in copeptin.

In order to better understand the effect of orthostatic loadings on AVP and copeptin release, it is necessary to examine the different methods to assess these effects in a controlled fashion.

5.2 Alternative Methods to Assess Responses to Alterations of Orthostatic Loadings

Besides LBNP there are several other methods of provoking alterations of venous return and thus simulating the effect gravity and postural changes have on our bodies. The simplest form is simply letting the subject stand up or sit down to evaluate the physiological changes caused by posture. A problem with this type of method is the fact that many of the sensors placed on the subjects' body during the experiment can fall off or be otherwise affected by the sudden movement when standing up. Furthermore, when standing freely, small postural changes and the necessary muscular activity in the legs might affect the experimental results. Furthermore, in these experiments, the amount of gravitational force cannot be varied and thus only the effect of postural change within Earth's gravity can be analyzed, not the effect of the gravitational force itself. Head-Up-Tilt which is conducted with the subject strapped in supine position to a tilting table which is then uprighted can largely eliminate the muscular activity necessary for standing up and keeping the erect posture. In addition, the degree of the tilt can be varied, thus making it possible to assess the effects of the HUT with a ratio scale of measurement. Of course in HUT, too, the strength of the gravitational force itself can not be varied and the movement of the body can still affect the sensors placed on the subjects' body. In LBNP on the other hand, the strength of the negative pressure simulating gravity

can be varied, thus not only making it possible to use a ratio scale of measurement, but also to analyze forces exceeding Earth's gravity such as the inertial forces experienced by astronauts and pilots during lift-off or turning maneuvers. Using HUT and LBNP together is also a possibility, eliminating the supine position as a possible confounding factor but making the whole setup more complicated and making it difficult to assess whether effects are due to posture or due to LBNP. A different method to achieve this is by centrifuge, but this method has the disadvantage that the centrifuge apparatus is quite expensive to build and maintain, thus limiting it to very few research centers worldwide. Furthermore, it is impossoble to access the subject while the centrifuge is rotating, making it difficult to react in time to an eventual medical emergency and to correct the position of the sensors if necessary.

One method quite frequently used is the technique of water immersion as a model for weightlessness, either in the form of “wet” water immersion where the body is placed in supine, standing or sitting position in a swimming pool, or so-called dry water immersion where the subjects are placed in supine position on a water-filled cushion similar to a water bed, thus making it possible to connect electrodes and other devices which would be affected by direct contact with the water. However, both methods can act as models only for absence or reduction of gravity, and less for the effects of gravity itself. Still, they are of considerable value, especially since their results can be compared to the abovementioned methods of simulating an increase in gravitational force.

Naturally, the gold standard for experiments dealing with the absence of gravity and its physiological effects are experiments conducted in microgravity on a spacecraft, but these have the problem of very tiny numbers of probands due to the immense costs of sending people into space and maintaining them there.

5.3 What is the Exact Role of AVP in Orthostasis?

The main function of AVP is to maintain blood osmolality. While the vasoconstrictive and blood volume increasing effects of AVP have lent the hormone the name AVP, they are only a secondary function, a fact which has been firmly established in a widely-quoted study by Robertson and Athar in 1976. In their study entitled “The interaction of blood osmolality and blood volume in regulating plasma vasopressin in man”, they found a powerful linear correlation between AVP and plasma osmolality, however the increase of AVP due to a rise in osmolality was found to be smaller when standing upright, a fact that was attributed to the reduction in thoracic blood volume due to the upright posture [128]. Since then research has confirmed that osmolality changes as small as

1 or 2 % trigger an adaptation of AVP levels, thus controlling osmolality within a very narrow margin [13, p. 287]. It is obvious that with AVP levels being so strongly influenced by plasma osmolality, it is impossible for AVP to play a major role in everyday plasma volume and blood pressure regulation and that these function must be performed by other mechanisms such as the RAAS. On the other hand, Robertson and Athar did observe that altered central blood volume did have some effect, thus leading to the question how prominent this effect is and in which situations it might become more significant. Goldsmith in 1982 reported that during LBNP, only subjects who experienced arterial hypotension showed a very powerful AVP increase, with central venous pressure and norepinephrine release due to sympathetic activation showing no measurable influence on AVP plasma concentrations [69]. Leimbach in 1984 further examined the relationship between AVP release through osmolality changes and AVP release through baroreceptor stimulation and found that -15 mmHg of LBNP for 30 minutes did not increase AVP in either a high-osmolar (>294 mosm/kg) state or a low-osmolar (<294 mosm/kg) state. However, -40 mmHg for 10 minutes directly afterwards did significantly increase AVP, but only in a hyperosmolar state and without a significant fall in MAP, thus proving that AVP release can be affected without MAP alterations [129].

Further investigating the interaction of posture and AVP, Sato in 1995 in a study dealing with the influence of diabetic neuropathy on orthostasis observed no change of plasma AVP levels in healthy controls during 40 minutes of upright standing after 30 minutes of supine recumbence. It should be noted that Sato did not use a tilt table in this test, but had the subjects standing by means of their own muscular system [130]. Plasma osmolality was controlled in his study and did not change during supine or upright posture. Furthermore, Sato observed that in subjects who suffered orthostatic hypotension of 30mmHg or more, a significant increase in plasma AVP occurred, with a significant ($p<0.01$) correlation between MAP and AVP changes. This is consistent with Goldsmith's observation that during LBNP, only arterial hypotension provokes a marked AVP response[69].

Saad in 1988 in a study conducted on subjects with diabetes observed that injection of a V1 receptor antagonist led to a stronger decrease in blood pressure in subjects with a history of orthostatic hypotension, while it caused a more pronounced plasma renin activity (PRA) in subjects without a history of orthostatic hypotension [131], thus giving support to the theory that AVP plays a more pronounced role toward blood pressure maintenance in people who suffer from orthostatic intolerance due to impaired RAAS action. This theory attributes to AVP the role of an “emergency

blood pressure regulator" which jumps in if other regulators are unable to fulfill their function in upholding the blood pressure. This is largely consistent with the notion established by Goldsmith and Sato that only hypotension provokes AVP release, while reduction of central venous pressure or stroke volume alone do not have such an effect. In 1983 Goldsmith further observed that in patients with congestive heart failure, AVP levels are increased, a fact that might be connected to the function of AVP in blood pressure maintenance when normal regulation is unable to do so [132].

In 1999 Pump et al. examined whether a change from seated to supine posture has an effect on plasma AVP. It was found that during a 30-minute supine period after a 30-minute seated period, AVP fell significantly ($P<0.05$), an effect which was neutralized if LBNP was applied during the supine period in such a fashion that Left Atrial Diameter (LAD) remained unchanged from the seated period, thus varying the LBNP between -22 and -25 mmHg, while it was significantly increased if supine without LBNP. Furthermore, without LBNP, stroke volume changed significantly ($P<0.05$) from the seated period. Cardiac output on the other hand fell significantly ($p<0.05$) during LBNP but remained unchanged without LBNP. Mean arterial pressure fell when supine without LBNP but remained constant during LBNP, which means that the pressure at the level of the carotid baroreceptors was higher with LBNP than during the seated period and also higher than without LBNP. Arterial pulse pressure as well as stroke volume rose, also with $p<0.05$, during supine resting without LBNP, an effect which was largely due to a drop in diastolic blood pressure with systolic blood pressure largely unchanged. In this case, too, values for LBNP remained unaltered. Norepinephrine fell with a similar level of significance like AVP during supine recumbence without LBNP. Pump et al. concluded that the decrease in AVP was most likely to be caused by a combination of an increase in LAD and PP. Sodium was controlled by a fixed diet and a ban of other drinks than water for four days before the experiment, plasma osmolality was measured and remained unchanged throughout the whole experiment [133].

Laszlo in 2001 observed that 30° HUT led to a significant increase in AVP which was still observed 2 minutes after the end of HUT, but had returned to normal levels 50 minutes afterwards[72].

Roessler in 2011 found that during 3 30-minute runs of 55 mmHg of supine LBNP with 30-minute of supine rest between them, there was no significant increase in plasma AVP during the first 2 runs, but during the 3rd run a sharp increase ($p<0.001$) as compared to the preceding supine resting period occurred. A similar pattern showed when replacing the supine LBNP periods with 70° HUT runs using the same durations and the same 30-minute supine resting periods in between. The first

two HUT runs did not cause a significant AVP reaction, but during the 3rd run, a similar rise in AVP levels over the preceding resting period ocurred (this time with $p<0.01$) as in the LBNP protocol[67]. MAP was somewhat more affected by LBNP than by HUT, while stroke volume was affected roughly equally by LBNP and HUT. There was no correlation between MAP and AVP that could be observed. Loss of plasma volume was much higher ($p<0.001$) in HUT than in LBNP during all three runs.

A study by Tsuchihashi in 1989 found that 1-hour ambulation beginning at 08:00 in the morning increased plasma AVP levels with $p<0.01$ regardless of sodium depletion in female subjects, with baseline levels of AVP being higher during sodium depletion with $p<0.05$[134]. A significant correlation of AVP with MAP was found in the sodium-depleted, but not in the sodium-rich phase of the protocol. It should be highlighted that this particular study dealt with female subjects, which are considered to possess, as outlined above, less orthostatic tolerance than men [82] [81] and thus might, due to the aforementioned role of AVP in orthostatic failure, show a different pattern of AVP activation than men.

A study on professional saturation divers conducted by Claybaugh in 2007 found that under normal atmospheric pressure, plasma AVP showed a $p<0.05$ significant increase upon 10 minutes of 90° HUT after 15 minutes of supine resting, a reaction that was not present when conducted under hyperbaric conditions, except in two cases of syncope which showed a ten- to twentyfold increase in plasma AVP [135]. Cignarelli in 1986 found that supine-to-erect posture change caused an increase of 139% of plasma AVP 5 minutes after standing, which had increased to 275% 120 minutes after assuming standing posture, suggesting a gradual increase in plasma AVP over time while standing [136]. Interestingly, this reaction was abolished in a percentage of the subjects who suffered from diabetic neuropathy.

Another method to evaluate the effect of an altered intrathoracic blood volume on plasma AVP would be water immersion, which increases venous return to the heart through the pressure of the water column on the submerged parts of the body; indeed a number of studies have been conducted to that effect. Wolf in 1990 in a study dealing with the effect of head-out water immersion in supine position found that neither the controls who stayed supine out of the water for 60 minutes nor the subjects who stayed supine in a swimming pool from minute 20 to 40 experienced a significant change in mean AVP plasma concentration [137]. Viti in a study dealing with the influence of posture, water immersion and swimming found that neither moving from a relaxed standing to

supine position, nor water immersion, nor swimming significantly altered AVP levels[138]. These findings seemingly contradict the ones from Hammerum who found that water immersion suppressed AVP levels in the blood, which in turn lead to a significant increase in plasma osmolality and free water clearance[71]. LAD also was significantly higher during immersion, while the HR was significantly lower and blood pressure varied only insignificantly. Furthermore, Gabrielsen in 2000 conducted a study in which he also found that water immersion suppressed plasma AVP along with similar increases in arterial PP and LAD as observed by Pump and Hammerum [139]. However, until now, there is no evidence whether these changes in AVP were caused by LAD, PP, a mixture of both or a third factor. A comparison of 6° Head-Down-Tilt (HDT) with water immersion to the neck done by Shiraishi in 2002 found that both caused a similar suppression of AVP and similar change of LAD and stroke volume, although cardiac output was significantly higher during water immersion, while PP was higher and HR and MAP lower during HDT [140].

In summary, there is no doubt in the scientific community that hypotension, i.e. a reduction in MAP, does trigger AVP release via arterial baroreceptors. As to the question whether orthostatic stress without marked MAP reduction also causes plasma AVP to rise and if so by which mechanisms this is mediated, evidence is somewhat conflicting; however, the bulk of experiments as outlined above point to AVP being affected by orthostatic loading even without MAP alterations. However, it is not clear whether this is caused by a change in LAD or PP, because these two and AVP levels always seemed to change together, or another factor, for example through a change in CVP mediated through cardiopulmonary baroreceptors.
In a study designed to find out whether the response of AVP during syncope due to central hypovolemia was caused by a cardiorenal or a baroreceptor reflex, Lord in 1996 examined heart transplant recipients, whose vagal afferents have been cut and found that transplant recipients not only possess a greater tolerance to LBNP, but, if syncope occurs, also showed a still significant, but attenuated AVP response compared to the controls [141]. However, it is unclear whether this is due to an absent cardiorenal reflex due to the severed cardial nerves, which would mean that in healthy subjects a combination of baroreceptor and cardiorenal reflex affects AVP during syncope. The other possibility would be an attenuated cardiorenal reflex-mediated AVP release due to incomplete neural regrowth to the heart. What can however be said by considering Lord's findings is that the cardiorenal reflex does definitely play a role in orthostatic AVP regulation, in accordance with Egan's findings in 1984 that reduced central venous pressure affects AVP release [25].

Summarizing the current state of evidence, it is still impossible to determine exactly how changes in

AVP during orthostasis are mediated: through a combination of baroreceptor reflex and the cardiorenal reflex (also called Henry-Gauer reflex) or whether the cardiorenal reflex alone is responsible, because PP, LAD, and CVP in all studies changed together. However, it is clear that AVP is affected by central hypovolemia, meaning that a sufficiently strong orthostatic challenge should lead to a rise in plasma AVP and, due to its equimolar release copeptin levels. At the same time, there is sufficient evidence that a posture change from standing to supine does lead to a suppression of plasma AVP. Due to the in vivo plasma half-life of copeptin with about 40 minutes [120] [121] being much larger than the half-life of AVP, it must be assumed that a drop in copeptin will take place over a much longer period than the concomitant drop in AVP and that the decrease in copeptin observed was therefore most likely being caused by the posture change from standing to supine at the beginning of our experimental protocol.

5.4 Does LBNP affect AVP release?

Since we did not only not observe a significant rise in copeptin, but instead a decrease in both sexes throughout our measurements, the question is: could this decrease have been caused by LBNP application?

As outlined in the introduction, LBNP leads to a drop in central thoracic volume and thus a drop in CVP and from there via the Frank Starling mechanism to a decrease in MAP. These changes should normally, through the baroreceptor reflex and possibly the cardiorenal reflex, work toward a decrease in AVP and copeptin.

A substantial release of AVP was long thought to take place only after hypotension is provoked especially after Goldsmith's 1982 study which failed to find an increase of AVP upon LBNP administration for 20 minutes at increasing levels [69]. Goldsmith failed to report the exact strength of the LBNP used, although it was sufficient to reduce central venous pressure to 3.8 mmHg for the first 10 minutes and then with an increased LBNP as little as 1.0 mmHg for the second 10 minutes compared to an average of 7.2 mmHg at baseline. Taking a recent study regarding the interaction between central venous pressure, LBNP and hemorrhage as reference [142], this would amount to an LBNP under 15mmHg for the first 10 minutes and roughly 30 mmHg for the second 10 minutes. One subject underwent presyncope with marked hypotension and here a 30-fold increase in AVP occurred, which is completely in accordance with later studies regarding the effects of AVP and syncope [65] [70].

However, the view that AVP is not altered without substantial MAP decrease has been challenged by a number of studies by Roessler, Giannatasio, Norsk and Trimarco and others [67] [143] [144]

[145] [146] and indeed LBNP has been observed to cancel AVP alterations under conditions of stable MAP by postural changes alone [133]. As early as 1986, Trimarco in a study dealing with response to LBNP and hypertonia observed that in normotensive subjects, 20 minutes of LBNP at as little as -10 mmHg elicited a very slight but notable increase in AVP even without alteration of MAP and without presyncope. This change was more pronounced and ocurred in both normo- and hypertensive subjects if LBNP at -40 mmHg was used instead, also without MAP alteration and even in subjects who did not reach syncope [145]. Another study by Giannattasio in 1993 found that normal subjects produced a significant increase in AVP upon 20 minutes of 37,5 mmHg after 20 minutes of 15 mmHg, which by itself did not alter AVP levels [143]. Van Hoeyweghen in 2001 found that for young as well as elderly, upon a stepwise increase of LBNP to 50 mmHg over the course of 40 minutes, which was then maintained another 20 minutes, thus averaging 35 mmHg during a 60 minute-period, both groups showed a significant increase in AVP without fall in MAP [147]. In another study, Leimbach in 1984 found that 30 minutes of 15 mmHg failed to alter AVP, while 40 mmHg applied immediately afterwards for 10 minutes did so only in subjects who had a plasma osmolality over 294 mosm/kg [129].

In 1993 Norsk conducted a study on the effects of LBNP on AVP and found that 20 minutes of LBNP, the first 10 minutes with -20mmHg, the second 10 minutes with 50 mmHg failed to elicit a change in MAP, but did reduce central venous pressure and, during the 50 mmHg, phase did lower PP and increase AVP. They hypothesized that AVP release could be triggered by a reduction in PP via the arterial baroreceptors [24].

Therefore, from the studies mentioned, the view that there is no baroreceptor-mediated AVP release cannot be upheld, although it is unclear what exact parameter is associated with its release, with evidence from Norsk in 1993 and Gabrielsen in 2000 [24] [139] points to arterial PP being the underlying parameter. More importantly, there is little evidence as to the exact strength of the stimulus necessary to elicit a baroreceptor-triggered AVP release. In the studies we examined –with the exception of Trimarcos– low LBNP levels of approximately 15 or 20 mmHg failed to stimulate AVP, while higher levels of 35 to 50 mmHg applied for various periods of time did so. Still, in Roessler's 2011 study, AVP showed little reaction to LBNP with two 30-minute runs with a 30-minute break in between at a relatively high level of 55 mmHg of LBNP failing to stimulate AVP. Only on the third run a significant AVP increase was detected. Therefore it appears that only stimuli substantially stronger than our 20-minute run with an average of only 20 mmHg and reaching a maximum of 40 mmHg would elicit a noticeable AVP response because the study that used the LBNP protocol most similar to ours – Goldsmiths in 1982 – found no AVP response and even Roessler in 2011, who used a much stronger LBNP for longer periods than ours, found a response

only at the third 30-minute LBNP run [69] [67]. Therefore, we conclude that our level of LBNP was not strong enough to elicit an AVP response. Regarding the question, whether a drop in AVP and consequently copeptin could be caused by LBNP, we found absolutely no instance in any of the studies we mentioned above, in which LBNP caused a decrease in AVP, suggesting that the decrease in copeptin we observed was caused by a mechanism other than the application of LBNP.

5.5 Gender Differences in Copeptin

In our experiment, a significant difference between men and women in copeptin levels was found only at the recovery levels, with copeptin values at that stage being 44.4% higher in males compared to 42.4% at baseline and 41.1% at the end of LBNP. The two-factor ANOVA that we also conducted – as already described above – has to be considered with utmost caution due to the very important preconditions of sphericity and normativity in conjunction with a small and unequal sample size, which is why we chose to base our analysis on non-parametric tests.

As we have mentioned, baseline plasma AVP is known to be higher in men than in women by about 40% [101] [102] [18], which matches our results in copeptin very well and fits the common belief that copeptin mirrors AVP concentration. The fact that the difference in copeptin was larger at the end of the recovery period might either be due to a faster clearance of plasma copeptin in men than in women or because our application of LBNP did indeed cause a release of AVP and copeptin which was higher in men than in women and thus contributed to a larger difference between the sexes. In our view, the fact that the relative difference between both sexes in copeptin levels decreased during LBNP only to increase afterwards might be considered a hint that there might be different patterns of AVP release between the sexes; however, in our experiment, they were at a non-significant level and thus it would require a larger sample to assess whether there is a statistically significant effect.

5.6 Does Copeptin Correlate with Urinary AQP2 Output?

Although it is known that AVP induces AQP2 trafficking to the apical membrane of the principal cells of the collecting duct [148], and that AQP2 is released into the urine in exosomes [39], very few studies so far have dealt with the effect of orthostasis on urinary AQP2 output.

Valenti in 2006 conducted a study regarding the effect of water immersion on urinary AQP2 concentration and found that 6 hours of water immersion did indeed lead to an increase in urinary AQP2. At the same time, as part of the Gauer-Henry reflex described AVP is known to be

suppressed upon water immersion via stimulation of vagal afferents from the heart by an increase in diastolic filling which stimulates tension receptors in the wall of the atriae[2, p. 671]. Valenti's observations are in line with the ones made by Buemi in 2000, where a shorter water immersion period of 2 hours was sufficient to increase urinary AQP2 excretion [149]. Buemi, similarily to our study, gave the urinary AQP 2 concentrations as amount of AQP2 per mg creatinine, in order to avoid distortion by an alteration of water reabsorption in the tubules of the nephron and to give a value relative to the creatinine clearance as indicator of the glomerular filtration rate. However, creatinine clearance increased upon water immersion in Valenti's experiment, highlighting the importance of monitoring this parameter when assessing urinary AQP2 with this method. Nevertheless, the increase of urinary AQP2 per mg creatinine despite the possibly increased creatinine clearance adds significance to Buemi's findings.

The increase in urinary AQP2 upon water immersion despite a decrease in AVP plasma concentration gives rise to the notion that urinary AQP2 excretion is not merely a function of its presence in the apical membrane, because if that was be the case AQP2 in the urine should decrease upon a reduction of plasma AVP. A possible explanation could be that AQP 2 in the apical membrane, upon a sudden reduction of AVP action, is not reabsorbed in the form of exosomes but that the exosomes are rather released into the urine. In this model, AVP would not only trigger the fusion of AQP2 vesicles with the membrane, but also inhibit the release of AQP 2 containing exosomes into the urine. However, this could also be mediated by a different mechanism such as a part of the RAAS, which is also affected by intrathoracic volume changes [67].

In our study there was no overall effect on urinary AQP 2 observable. Both sexes did show a similar increase in urinary AQP 2 relative to creatinine, but not on a significant level. Furthermore, since we used a 24-h urine sample for comparison, even this increase could be related to the circadian rhythmicity of AVP release and thus not being caused by the application of LBNP. Correlation of the changes in urinary AQP2 with copeptin did show a moderate positive correlation in women, but not on a significant alpha level, while in men there was no correlation measurable. While this adds some support to the idea that mechanisms regulating AVP action are significantly different in men and women, it is not possible to draw a clear conclusion from this due to a statistically insignificant p level.

At first it appears odd that urinary AQP2 per mg creatinine is higher in women than in men, because men possess a higher AVP level and therefore might show a higher AQP2 activity in the apical membrane of the collecting duct. However, the daily excretion in AQP 2 is roughly the same in both sexes, while at the same time women possess a lower GFR and thus a lower creatinine clearance, leading to a low urinary creatinine concentration and thus a higher value of the amount of AQP2 per

mg creatinine [150].

In summary, our observations of urinary AQP 2 don't permit a clear conclusion due to absence of statistically significant changes in urinary AQP 2 in our experiment and due to our small sample size. What remains is therefore the question whether this was due to the LBNP level not being powerful enough to elicit a response or whether there is some mechanism that keeps the urinary AQP 2 stable even under conditions of reduced venous return due to LBNP. It remains to be explored whether a substantial rise in plasma copeptin is reflected in alterations of urinary AQP2 output.

5.7 AVP and Presyncope

So far we have concentrated on the effect of LBNP on AVP without presyncope. But what happens to hormone levels if syncope occurs? In our experiment 2 of the subjects, one male and one female, underwent presyncope. In these two subjects, copeptin fell during LBNP and rose again towards the end of the recovery period; however, both changes were non significant compared to the non-syncopal groups at that timepoint.

Hinghofer in 2011 reported that presyncope led to a significant increase in plasma AVP, which had returned to normal 20 minutes after presyncope in comparison to supine levels[65], thus confirming the role that AVP plays when it comes to failure of orthostatic regulation. The change observed by him was considerable: upon presyncope, plasma AVP increased roughly by a factor of 15. A similar response was observed by Goldsmith in 1982 [69] and Heath in 1977 [151].

It is remarkable that in our subjects, there was no similar increase in copeptin observed, although copeptin, if – as all abovementioned evidence indicates – released in an equimolar fashion with AVP, should show a similar massive increase in AVP. One reason for this effect might be the great importance we put on terminating the LBNP quickly if signs of presyncope occured. This was done in order to avoid for the subjects the extremely uncomfortable experience of actual syncope, especially since the goal of our experiment was not to evaluate the effects of presyncope, but only the non-presyncopal subjects. Since we took great care to react in time if signs of presyncope occurred, it is possible that we reacted prematurely in terminating the LBNP. However, while it is possible to mistake a feeling of unrelated non-wellbeing reported by subject for a sign of presyncope, we also observed a sharp drop in blood pressure to under 80 mmHg, which is typical for presyncope [152]. Another possibility is that for some reason copeptin release upon presyncope did not follow the equimolar pattern compared to AVP release. However, so far, in the literature used, we found no observation supporting this notion and with the measurement of the actual

plasma AVP itself not being part of this experiment, it is not possible to assess it.

5.8 Limitations

The present study has a number of limitations which reduce the impact of its result. First of all there is the small sample size, especially of the women with only 10 non-presyncopal subjects of whom one could not be measured due to technical reasons at the end of the recovery period. Furthermore, the sizes of the male and female samples were quite different with 15 male non-presyncopal subjects. For these reasons, the central limit theorem did not apply beforehand and thus the data could not be assumed to be normally distributed after the Shapiro-Francia test was found to be significant for non-normality in one of the female samples. Due to non-normality, an ANOVA could not be used for analysis and we had to resort to Wilcoxon's signed-rank test and Mann-Whitneys U-test, which yield a lower testing power in comparison. Remarkably, despite the lower power, there were still some significant results, lending additional support to our observations.

Another problem of the small sample size is that it inhibited us from stratifying the females along the phases of their menstrual cycle. As described in the introduction, the menstrual cycle is known to affect output of the hormone AVP, but understanding of its exact effects – especially regarding blood pressure and volume regulation – is as of now limited and thus controlling this variable would have been desirable.

Although the subjects were advised not to consume caffeine and alcohol 24 hours before the start of the experiment, we did not monitor food and water intake before the experiment. It cannot be completely ruled out that the sodium intake in particular has an effect on AVP secretion because, as outlined before, osmoregulation is the central physiological function of this hormone. Additionally, we did not measure plasma osmolality in our subjects, which might have been of importance due to this being the main stimulus for AVP release, thus a bias due to hypo- or hyperosmolality in some of our subjects cannot be ruled out.

Unfortunately, due to financial restraints, we were not able to directly measure AVP when this study was conducted. Direct comparison of copeptin with AVP would have been interesting and would probably have been of high value, but unfortunately was not possible. After the copeptin was found to behave in a much different way than expected (dropping instead of increasing), it was decided to perform direct AVP measurements as well, but at the point of completion of this thesis the results were not yet available.

Only two of our subjects experienced presyncope in our study and remarkably, these did not show the massive increase in copeptin which would have been expected due to AVP increasing

enormously (by a factor of 10 to 20) upon presyncope in other studies [65] [69]. A larger number of presyncopal subjects would have enabled us to better assess this observation on a statistically sound level. However, it would also have greatly impaired our analysis of non-presyncopal subjects due to our small sample size.

Another shortcoming of the present study is the size of the LBNP chamber which was used. While this particular chamber can accomodate people up to a body length of 185 cm, the seal of the chamber has a width of only about 45 cm, which led to one female subject having to be excluded from taking part due to her too wide hips. It has been shown that there is a connection between splanchnic pooling and orthostatic tolerance [81], therefore it might be possible that people with wider hips differ in terms of orthostatic tolerance in comparison with the average population due to a different volume of splanchnic blood vessels, possibly leading to a bias in our study.

5.9 Conclusion

Conclusively, three main observations can be drawn from our study:

1. LBNP of the strength which we used in our experiment did not lead to an elevated copeptin in men or women;
2. however, there was a decline in copeptin during the course of our experiment, a fact which in our opinion cannot be attributed to the LBNP, but rather to the change of posture when the subjects lied down at the beginning of our experimental protocol;
3. copeptin was higher in men than in women, a difference that in our experiment was significant only at recovery level, in contrary to the literature, where under normal (i.e. non-presyncopal) condition male AVP is significantly higher than in women. Possible reasons for this might be that copeptin was cleared at a higher rate in males than in females or maybe a higher release of copeptin in males in response to LBNP.

In summary, the results of our analysis do not support a rise in copeptin during or after LBNP, although a limited release cannot be ruled out. During the course of our experiment, copeptin showed a steady decrease between the first and the last of the three measurement points, with the intermediate measurement point being in between. This gives some support to the notion that postural changes affect copeptin (and therefore AVP), because the only explanation for the decrease in our view is the fact that the subjects had to lie down at the beginning of the protocol, thus leading to a decrease in AVP and copeptin, which is in concordance with the observations by Tsuchihashi in

1989 and Pump in 1999 [134] [133] that posture changes do affect AVP, but contradicts the findings by Viti and Wolf who did not find such evidence [137][138]. If there was a release of copeptin by LBNP application, it was masked by the observed decrease in copeptin.

It has to be noted that, due to the aforementioned instability of plasma AVP even in a cooled blood sample, it is possible that significant changes in some of the other studies did escape attention due to degradation after the sample was taken, especially if the samples were not frozen immediately. It would also be possible that minor hemolysis due to the release of AVP from the platelets led to an increase in AVP after the sample was taken, thus rendering neither of the studies 100% reliable.

Copeptin however as mentioned above is quite stable and has a longer half-life than AVP, therefore it is possible that the decrease in copeptin is caused by the posture change at the beginning of the protocol when the subjects placed themselves in supine position on the LBNP table. With copeptin having a much longer half-life than AVP, any drop in its plasma concentration caused by a decreased release of the common precursor molecule neurophysin II will take place over a longer period than the correspondent reduction of AVP levels. Thus upon any stimulus that inhibits AVP release, copeptin will take longer to reach its new baseline level. This would explain why the adaption of copeptin levels was still taking place during our LBNP period, while AVP levels itself were shown by Pump in 1999 to adapt very rapidly, within 10 minutes after a posture change from upright into supine position [133].

Interestingly in the 2 subjects who underwent presyncope copeptin did not show the marked increase which happens in AVP levels [65], but rather stayed at roughly the same level as in the other subjects. For this, we were unable to find a satisfactory explanation in the literature. In conjunction with the longer in vivo half-life of copeptin this leads us to the conclusion that in experimental settings such as ours dealing with rapid changes of AVP concentrations, copeptin is not a suitable marker for assessment of AVP plasma levels.

6. Conflict of Interest

The author declares that he has no conflict of interest.

Appendix A

Overview of AVP receptors and physiological effects

Receptor	**Location**	**Cellular mechanism of action**	**Effect**
V1a	Smooth muscle cells in the walls of blood vessels	Ca_{2+}-release from endoplasmatic reticulum	Vasoconstriction with subsequent rise in blood pressure, but only in considerably higher concentrations than for antidiuresis. **Main cardiovascular effect**
V1a	Adrenal cortex	Ca_{2+}-release from endoplasmatic reticulum	Increased synthesis of gluco- and mineralocorticoids
V1a	Pulmonary artery	Ca_{2+}-release from endoplasmatic reticulum	Vasodilatation
V1a	Platelet	Ca_{2+}-release from endoplasmatic reticulum	Calcium-dependent platelet adhesion
V1a	Cardiomyocyte	Ca_{2+}-release from endoplasmatic reticulum	Inotropic effect through proliferation of myocytes
V1a	Cardiomyocyte	Ca_{2+}-release from endoplasmatic reticulum	ANP secretion
V1a	Hepatocyte	Ca_{2+}-release from endoplasmatic reticulum	Glycogenolysis

V1a	Macula densa	Probably Ca_{2+} -mediated increase in eNOS and COX-2 expression with subsequent increase in NO and PGE_2 -levels	Stimulation by urinary AQP, promotion of renin activity
V1a	Intercalated cells of the renal collecting duct	Probably Ca_{2+} -mediated increase in mineralocorticoid receptor expression	Stimulation by urinary AQP, enhancement of aldosterone action on intercalated cells
V1a	Area postrema	Ca_{2+}-release from endoplasmatic reticulum	Increasing baroreceptor reflex sensitivity, leading to attenuation of AVP-mediated hypertension
V1b	Adrenal medulla	Ca_{2+}-release from endoplasmatic reticulum	Catecholamine secretion
V1b	Anterior pituitary	Ca_{2+}-release from endoplasmatic reticulum	ACTH release
V1b	B-cells of islets of Langerhans	Ca_{2+}-release from endoplasmatic reticulum	Insuline secretion
V2	Principal cells of the distal renal collecting duct	cAMP-pathway mediated fusion of AQP2 containing vesicles with the apical plasma membrane	Increased H_2O reabsorption within the collecting duct, leading to a reduction in plasma osmolality. Short- and long term effect. **Main antidiuretic effect**
V2	Principal cells of the inner medullary	CAMP-mediated accumulation of Urea-	Increased urea reabsorption

	collecting duct	Transporter UT-A1 in the apical membrane	
V2	Principal cells of the proximal renal collecting duct	cAMP-pathway mediated increase of action of Endothelial Natrium Channel (EnaC), mainly by fusion of EnaC-containing vessels with apical plasma membrane	Increased Na^+-reabsorption
V2, possibly also V1a	Loop of Henle	Stimulation of various transmembrane transport proteins	Increased Na^+- and Cl^--reabsorption
V2 (probably)	Medullary thick ascending limb of loop of Henle	Inhibition of N+/H+ exchange pump	Reduction of bicarbonate reabsorption
V2 (probably)	Collecting duct principal cells	Increased AQP 3 (and to a lesser extent AQP 4) presence in basolateral plasma membrane	Enhanced transcellular water permeability (in conjunction with AQP 2). **Long-term effect of AQP 2**
V2	Endothelial cells	NO-Synthesis	Vasodilatation
V2	Endothelial cells	Von-Willebrand-Factor release	Increased platelet adhesion

[16, pp. 72ff., 193, 190f.] [32, p. 98ff.] [12, p. 460f.] [14] [2, p. 646f.]

Appendix B

PROBANDEN GESUCHT!!!

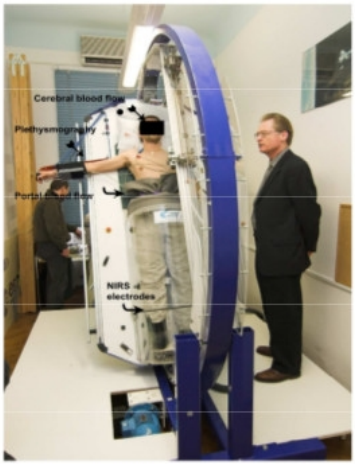
Wir suchen 30 weibliche Probandinnen und 30 männliche Probanden für das Forschungsprojekt

„VASCULAR STIMULUS RESPONSE IN HEALTH AND DISEASE: A LONGITUDINAL STUDY"

„Gefäßstimulation bei Gesunden und Kranken: Eine Längsschnittstudie"

- PILOTSTUDIE -

am Institut für Physiologie, MedUniGraz



KRITERIEN:

- **Gesunde, normalgewichtige ProbandInnen im Alter zwischen 18 und 35 Jahren**
- **Nichtraucher**
- **Körpergröße: Frauen 160 cm - 175 cm**
  Männer 170 cm - 185 cm

RAHMENBEDINGUNGEN:

- **Zeitaufwand: 4 x ca. 1 Stunde**
- **Zeitraum: Februar 2015 – Februar 2016**
- **Bezahlung: € 40,- pro Versuch**

INFO & ANMELDUNG
ab sofort per email an rebecca.ruedl@medunigraz.at

probandenaufruf/vers02 12012015

Appendix C

MINIREVIEW

Aquaporins, Vasopressin, and Aging: Current Perspectives

Grazia Tamma, Nandu Goswami, Johannes Reichmuth, Natale G. De Santo, and Giovanna Valenti

Department of Biosciences, Biotechnologies, and Biopharmaceutics (G.T., G.V.), University of Bari, 70125 Bari, Italy; Istituto Nazionale di Biostrutture e Biosistemi (G.T., G.V.), 00136 Roma, Italy; Gravitational Physiology and Medicine Research Unit (N.G., J.R.), Institute of Physiology, Medical University of Graz, 8036 Graz, Austria; Department of Medicine (N.G.D.S.), Second University of Naples, 80138 Naples, Italy; and Centro di Eccellenza di Genomica (G.V.) Campo Biomedico Ed Agrario, University of Bari, 70126 Bari, Italy

Functioning of the hypothalamic-neurohypophyseal-vasopressin axis is altered in aging, and the pathway may represent a plausible target to slow the process of aging. Arginine vasopressin, a nine-amino acid peptide that is secreted from the posterior pituitary in response to high plasma osmolality and hypotension, is central in this pathway. Vasopressin has important roles in circulatory and water homoeostasis mediated by vasopressin receptor subtypes V1a (vascular), V1b (pituitary), and V2 (vascular, renal). A dysfunction in this pathway as a result of aging can result in multiple abnormalities in several physiological systems. In addition, vasopressin plasma concentration is significantly higher in males than in females and vasopressin-mediated effects on renal and vascular targets are more pronounced in males than in females. These findings may be caused by sex differences in vasopressin secretion and action, making men more susceptible than females to diseases like hypertension, cardiovascular and chronic kidney diseases, and urolithiasis. Recently the availability of new, potent, orally active vasopressin receptor antagonists, the vaptans, has strongly increased the interest on vasopressin and its receptors as a new target for prevention of age-related diseases associated with its receptor-altered signaling. This review summarizes the recent literature in the field of vasopressin signaling in age-dependent abnormalities in kidney, cardiovascular function, and bone function. (***Endocrinology*** **156: 777–788, 2015)**

During normal aging, there is a decrease in maximal urine concentrating ability (1). When compared with younger age groups, individuals aged 60–79 years have an approximately 20% reduction in maximum urine osmolality, a 50% decrease in the ability to conserve solute, and a 100% increase in minimal urine flow rate.

Renal aging is a complex process. It is associated with compromise in the compensative and homeostatic regulatory mechanisms; these arise due to structural and functional changes, which accumulate over an individual's life span. Several factors including genetic background, gender, and exposure to endogenous inflammation cytokines or environmental xenobiotics, interact to create a complex scenario of effects. Indeed, renal phenotype of the elderly shows a high interindividual variability.

The elderly, due to alterations in plasma osmolality and fluid body volume, are at high risk of developing disturbances of water metabolism. Osmolality of body fluid is tightly controlled by the ratio of solutes to total body water content. Plasma osmolality is mainly dependent on the homeostatic control of water, rather than of sodium, which is the most abundant extracellular electrolyte regulating fluid body volume (2). Changes in water and sodium balance increase the frequency of hypo- or hyper-

ISSN Print 0013-7227 ISSN Online 1945-7170
Printed in U.S.A.

Received October 6, 2014. Accepted December 11, 2014.
First Published Online December 16, 2014

Abbreviations: AVP, arginine vasopressin; AQP, aquaporin; BP, blood pressure; CHF, congestive heart failure; GFR, glomerular filtration rate; miRNA, microRNA; NKCC2, Na-K-Cl cotransporter; NF-κB, nuclear factor kappa light-chain-enhancer of activated B cells; NOS, nitric oxide synthase; PKA, protein kinase A; ROS, reactive oxygen species; SCN, suprachiasmatic nucleus; SIRT, sirtuin; SON, supraoptic nucleus; TOR, target of rapamycin; UTA, Urea Transporter A; V2R, V2 receptor.

natremia associated with hypo- or hypervolemia during aging (3, 4). In 1949 Findley (5) hypothesized a positive association between age and alterations of the hypothalamic-neurohypophyseal-renal axis. This was based on the observation that arginine vasopressin (AVP) secretion may be augmented during normal aging in humans (5), despite aging being associated with a decrease of urinary concentrating ability (6, 7). On the other hand, dysfunctions in water metabolism during aging may arise due to several factors, including alterations of body composition, abnormal expression and trafficking of water channels (aquaporins) and solute (sodium and urea) transporters, altered thirst regulation, and AVP secretion. The level of many of the key transport proteins that contribute to urine concentrating ability is reduced in the kidney medulla of aged rats (8). The reduction in water, sodium, and urea transport protein levels, and their reduced response to water restriction, contribute to the reduced ability of aged rats to concentrate their urine and conserve water (8). It is likely that similar mechanisms occur in human kidneys, which would provide a molecular explanation for the reduced urine concentrating ability in aging and may provide opportunities for novel therapeutic approaches to improve urine-concentrating ability.

Materials and Methods

Vasopressin secretion and function in the elderly

Vasopressin has important roles in circulatory and water homoeostasis mediated by vasopressin receptor subtypes V1a (vascular), V1b (pituitary), and V2 (vascular, renal). A dysfunction in this pathway as a result of aging can result in multiple abnormalities in several physiological systems leading to several dysfunctions at organ, cellular, and molecular level (Figure 1).

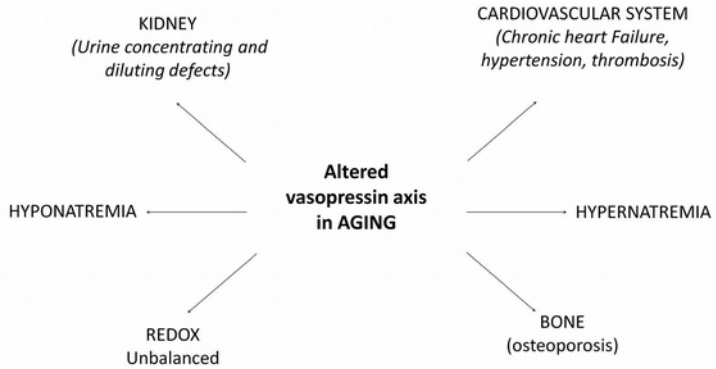


Figure 1. Major effects of altered vasopressin signaling occurring in aging.

AVP secretion and control of thirst play a major role in regulating water homeostasis in both the young and elderly. Vasopressin is synthesized by magnocellular neurons in the paraventricular and supraoptic (SON) nuclei in the hypothalamus, enclosed in vesicles that are transported within the axons into the posterior pituitary and stored there close to the nerve terminals until release (9). AVP is a peptide consisting of nine amino acids, which is separated (along with a number of other peptides) from a much larger precursor hormone during transport along the axon. A second class of so-called parvocellular neurons synthesize and release AVP into the superior hypophysial artery, which supplies the anterior pituitary. AVP, in turn, is associated with a release of ACTH (9).

The secretion of AVP is under the control of selective osmoreceptors. An individual-specific set point for AVP release exists; this is within a strict normal range (275–295 mOsm/kg H_2O). When plasma osmolality increases by 1%–2%, an increase in plasma AVP concentration of 1 pg/mL is also observed, which results in a significant decrease of free water excretion and reduce urine flow (10). Additionally, AVP release is also under baroreceptor control, even if the secretion stimulus only occur when arterial volume is decreased by 8%–10% (10). Therefore, the effective amount of the circulating AVP results from a precise and integrated functionality of osmoreceptors and baroreceptors.

AVP mainly targets its action on the V2 receptor (V2R) localized at the basolateral membrane of renal principal cells. Binding of AVP to its specific V2 receptor stimulates cAMP/protein kinase A (PKA) axis resulting in a significant increase of the apical expression of the vasopressin regulated water channel aquaporin (AQP)-2, thus regulating water permeability in the collecting duct (11, 12). AQP2 function and thus urine concentration is regulated by a variety of cell signaling mechanisms and posttranslational modifications allowing fine-tuning of AQP2 trafficking and expression and thus body water homeostasis (11).

In addition to AQP2, AVP increases the phosphorylation and the trafficking of the Na-K-Cl cotransporter (NKCC2) increasing sodium reabsorption in the medullary thick ascending limb (13, 14). AVP also increases urea permeability by modulating the expression and the trafficking of the urea transporters Urea Transporter A (UTA)-1 and UTA-3 (15).

In the elderly, a significant decrease of urinary concentrating abil-

ity has been described that cannot be explained only by a decrease of the glomerular filtration rate (GFR) or by the down-regulation of AVP release (which instead is generally elevated during aging) (16). Several studies have reported that baseline AVP is higher in the elderly. Furthermore, there is an increased AVP excretion in response to changes in osmolality (2). Therefore, a more likely explanation for the reduction of urinary concentrating ability may be the decrement in normal renal sensitivity to AVP. In this respect, in rat models of aging (F344BN rats), a significant decrease of V2R expression and/or decreased ability of AVP to bind its cognate receptor to stimulate cAMP/PKA signaling have been described. Studies in older F344/BN and Wag/Rij rats revealed a significant decrease of the protein abundance of NKCC2, and the epithelial sodium channel and the urea transporters UTA-1 and UTA-3. Under water restriction, renal expression of UTA-1, UTA-3, and NKCC2 increases (17, 18). However, these increases are much less when compared with the responses in younger animals. These findings strongly indicate that reductions of AVP-regulated aquaporins, renal urea, and sodium transporters all contribute to decreases the urinary concentrating ability of aged rats. Although a large degree of variation in renal responses may be due to animal traits, these observations may provide some explanations as to why the renal functions decline during aging in humans.

Interestingly, a very recent study in 2014 in elderly rats by Sauvant et al (19) showed a reduction in responsiveness to plasma hyperosmolarity of neurons in the SON, in which both AVP and apelin (an aquaretic peptide) are produced. This study demonstrated that plasma vasopressin concentrations were higher, but the plasma apelin concentrations lower, in aged rats as compared with younger adults (19). The fact that AVP concentrations in aged rats could be modified together with or independently from apelin showed that it is possible to influence AVP levels in aged subjects, which might be a basis for therapeutical strategies in the future.

Aquaporin expression in elderly

So far, 13 aquaporins have been described in mammals. Aquaporins mediate water transport with high velocity and specificity. The first cloned aquaporin, AQP1, originally called CHIP-28 (channel-forming integral protein) (20), is located at the apical and basolateral membranes of proximal tubules and the descending thin limb of Henle's loop and in vasa recta. *Aqp1* gene deletion in humans is associated with a relevant impairment in urinary concentrating ability, which becomes evident during water deprivation (21). AQP7 and AQP8 are also expressed in proximal tubules which contribute to water and glycerol reabsorption (22–24). AQP2 (AQP-CD) (25) is localized at the apical membrane and in intracellular vesicles of the principal cell of the collecting duct and is the main aquaporin that is vasopressin-regulated. AQP3 and AQP4 are both expressed at the basolateral membrane of renal principal cells and they mediate water transport towards the serosal side (26, 27). AQP6 (28) is expressed in intracellular vesicles, which are located in renal collecting duct intercalated cells type A.

The major mechanism by which AVP modulates renal collecting duct water reabsorption is by controlling the abundance of cell surface expressed AQP2. Specifically, binding of AVP to its cognate receptor (V2R) increases cAMP level resulting in the activation of PKA. PKA-dependent phosphorylation of the water channel AQP2, at S256, is essential to promote the translocation of AQP2-bearing vesicles from an intracellular pool to the apical plasma membrane (29, 30). Recently phosphoproteomic studies revealed that in addition to S256, vasopressin stimulation increases S264 and T269 but decreases the phosphorylation of S261 (31). Several studies have shown the role of phosphorylation at S261, S264, and T269 (32–36); the exact role of phosphorylation at these residues and particularly during aging, however, remains to be explored. It has also to be underlined that in the elderly some drugs can affect the regulation of AVP release or AQP regulation (37). However, in addition to these possibilities, several studies performed in animal models have shown that in aged Wag/Rij rats, there is a strong decrease in the level of AQP2 as well as its phosphorylated form at S256, which can contribute to the reduced renal concentrating abilities (8, 38). Furthermore, AQP3 is reduced in aged rats, but no change in the expression of AQP1 and AQP4 has been detected in aged rats. This distinction in the regulation of AQPs abundance may be related to the fact that only AQP2 and AQP3 expression are under control of vasopressin. Therefore, one possible therapy to overcome the decrease in AQP2 abundance in aging might be the administration of AVP. To explore the possibility that the decrease in AQP2 can be pharmacologically corrected, in a recent study desmopressin, a selective V2R agonist, was administered to 10- and 30-month-old Wag/Rij rats. Desmopressin administration led to a decreases in urine output in both rat groups and an increase in AQP2 and AQP3 abundance. These results suggest that a decrease in AQP2 and AQP3 expression levels partially account for the decrease in urinary concentrating ability in aging and can be corrected with AVP. However, those studies also demonstrated that older rats do not reach the maximal urinary osmolality as seen in younger rats indicating that factors other than AQP2 and AQP3 are involved in renal function.

In general, due to an impaired ability to conserve water, in the elderly there is a decrease in total body water content associated with a reduction of plasma volume (39, 40). These changes make the elderly much more sensitive to water overload or dehydration, resulting in abnormal movement of solutes and thereby increasing the possibility of developing hypo- or hypernatremia.

Circadian vasopressin rhythm alteration in aging

As discussed above, aging is accompanied by changes in AVP neuron activity, leading to high plasma AVP concentrations (19, 41). Aging may also affect the intrinsic properties of neurons and their reactivity to physiological stimuli such as dehydration.

Recent evidence suggests that renal physiological functions such as renal excretion of water and major electrolytes exhibits a significant circadian rhythm. A clear manifestation of circadian rhythmicity of renal function is a marked difference in the urine output between day and night. Dysfunction of renal excretory rhythms therefore might cause alteration of water conservation rhythms correlating with the development of nocturnal polyuria often seen in elderly (42).

In healthy young individuals the circadian rhythm in which AVP is released is governed by the suprachiasmatic nucleus (SCN) (43), with peak AVP concentrations developing about midnight and with a decrease during daytime toward a minimum in the afternoon (44) along with an attenuated AVP response upon water ingestion (44). In the elderly, however, the circadian oscillation of AVP plasma levels is lost (44, 45); this could be the main cause of nocturia in the elderly due to low AVP levels during the night (46–49).

The SCN serves as a master clock regulating our circadian rhythm, and although its volume and total cell number is not altered during aging (50), it undergoes a number of changes on the cellular level, such as alterations in electrical activity and expression of vasoactive intestinal peptide (51). AVPergic neurons, which are known to be present in the SCN, are decreased significantly in the number in aged rats but show increases in cell size (52). Similarly, the SON and paraventricular nucleus, in which AVP is synthesized, appear not to suffer a reduction in volume or total cell number, but the size of the AVP neurons in the SON and PVR appears to increase with aging (53–55).

In an elegant study, renal function was evaluated in *clock* (−/−) mice. These mice have a defect in urine concentrating ability and they excrete diluted urine with a significant reduction in renal RNA expression of V2R and AQP2 (56). Functionally, the loss of *clock* gene leads to a complex phenotype characterized by partial diabetes insipidus, deregulation of sodium excretion rhythms, and a significant decrease in blood pressure (BP). Collectively, this study uncovers a major role for the molecular clock in renal function. Whether a dysfunction of circadian AVP rhythm is involved in the alterations of water and solutes homeostasis in aging remains to be established.

Desmopressin is already used to treat nocturia in the elderly (48, 57). Additionally, because sleeping disorders of different etiologies are common in geriatric patients (58–60), recent findings that suggest the influence of AVP receptor activity on sleep might open new options regarding treatment of sleep-related disorders in these patients. Further research is necessary to verify additional benefits of drugs like vaptans, which interact with AVP receptors, for patients suffering from age related sleeping disorders.

Thirst in aging

The osmotic threshold for the thirst stimulus is higher than the one for AVP release. This difference is of physiological relevance because small variations in the individual osmotic set point results in AVP secretion and the consequent modulation of renal water reabsorption. Therefore, it appears that only elevated alterations in the individual osmotic set point determine thirst stimulation (2). It is indeed well established that relevant defect in thirst sensation occurs during aging. Specifically, compared with young subjects, the elderly drink less, even when they drink a similar equivalent of fluid for a given degree of thirst, suggesting that they have a higher osmotic set point for thirst, leading to a lower thirst sensitivity when compared with young subjects (61, 62).

Age-related risk of cardiorenal disease and gender

Cardiorenal syndrome refers to constellation of risk factors, such as hypertension, obesity, insulin resistance, microalbuminuria, and reduced renal function. In addition, family history, gender, and race contribute to cardiorenal syndrome (63). In humans and animal models, the age-related risk to develop cardiorenal disease is higher in males than in females (64). Castration of male normotensive rats slows the age-related glomerulosclerosis, which could be ameliorated by estrogens treatment (65, 66). Moreover, a relevant reduction of the age-related progression of hypertension, to levels measured in female spontaneously hypertensive rat (SHR), was found after castration, likely indicating the role of sexual hormones in regulating renal function (67). In addition, AVP plasma concentration is significantly higher in males than in females, and vasopressin-mediated effects on renal and vascular targets are more pronounced in males than in females (68, 69). Recent observations also show that urinary concentration does not exclusively rely on plasma vasopressin. In a study between genders and diurnal urine regula-

tion, Graugaard-Jensen et al (70) found that prostaglandin 17β-estradiol could be a mediator of the gender differences, not only as a mediator of the vasopressin response but also as an independent factor.

Role of genetic background in renal aging

Genetic profile plays a pivotal role in modulating renal aging. Genomic analysis of *Caenorhabditis elegans* and Drosophila melanogaster identified many genes associated with aging and also being involved in disparate cellular processes such as genome stability, mitochondrial regulation, insulin signaling, immune responses, and inflammation. These results suggest that a complex network of different signal transduction pathways could be involved in aging (71–73). In the healthy elderly population, *α-adducin* Gly460Trp gene polymorphism has been proposed as a predictor biomarker of renal functionality (74). Impaired autophagy, a process involved in degradation of damaged proteins is also described during aging (75). On the other hand, the specific role of microRNAs (miRNAs) is still not yet elucidated, even though four specific miRNAs have been found decreased during aging (76). Loss of three podocyte-selective miRNAs indeed rapidly alters the glomerular filtration barrier, thus affecting the diluting and concentrating ability of the tubules (77).

Four distinct genes have been found to play a role in renal aging: *IGF-1*, target of rapamycin (TOR), *sirtuins* (SIRTs), and *klotho* (78). IGF-1 signaling regulates protein synthesis, glucose metabolism, and life span in different animal models such as worms, flies, and mice. Different genetic variants have been found in genes participating in IGF-1 transduction pathways controlling the human life span (79). TOR regulates the lifespan by modulating food intake. Dietary restriction, by down-regulating TOR signaling, increases life expectancy (80, 81). In the rat kidney, TOR expression and phosphorylation increase during aging. Specifically, rapamycin reduce age-related renal features by inhibiting TOR signaling (82). Sirtuins (silent information regulator 2) control a wide range of cellular processes including DNA repair and recombination, chromosomal stability, and gene transcription. Mammals express seven different sirtuins, SIRT1-SIRT7. Among them, SIRT1, SIRT3, and SIRT6 modulate caloric restriction and thus are considered important antiaging proteins (83). Particularly, sirtuin-1 displays a cytoprotective effect and controls BP and sodium homeostasis (83). Deregulation in the stimulation of sirtuin-1 signaling plays a role in the onset of renal disease (84). The antiaging *klotho* gene was first described in 1997. Klotho knockout mice display short lifespan and develop a syndrome similar to human aging (85). Klotho is mainly expressed in the kidney and predominantly in distal and proximal convoluted tubules (86).

Two alternative splicing variants have been found: a single-pass transmembrane protein and the soluble form. The first variant functions as a coreceptor for fibroblast growth factor-23, resulting in renal phosphate excretion and a reduction in vitamin D synthesis. The soluble form exerts multiple nephroprotective functions because it decreases oxidative stress, controls renal ion transport, and modulates BP by inhibiting endotelin-1 signaling (86–88). In vitro studies have further confirmed that klotho deficiency directly promotes renal epithelial cell senescence (87). Fischer et al (89) (2010) observed in a study with Klotho-deficient mice fed a normal and a vitamin D-depleted diet that Klotho deficiency appeared to lead to a reduction in extracellular volume, which causes a rise in AVP. The volume depletion was considered by these researchers to be caused by triggering of the calcium-sensing receptor through hypercalcemia (due to high 1,25 dihydroxyvitamin D_3 levels) with the down-regulation of the Na-K-2Cl symport in the loop of Henle and subsequent volume loss. This mechanism is supported by the fact that vitamin D-depleted diet attenuates these effects.

Tang et al (90) found that dehydration causes a down-regulation of *Klotho* gene transcription as well as an increase in AVP and aldosterone. The fact that a deficiency of Klotho, which is associated with the aging process, leads to a rise in AVP, which, in turn, appears to play a role in a number of pathologies, associated with increasing biological age. Indeed, this might be an avenue through which Klotho exercises its effect on longevity. Much research is still needed to provide actual evidence that this is indeed the case.

Interestingly, the pathways activated by IGF-1, TOR, sirtuins, and klotho concur to activate nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling, which represents a key target in controlling renal aging (91, 92). Importantly, stimulation of the NF-κB signal pathway significantly decreases expression of the water channel AQP2 without affecting protein stability (34). Indeed, selective inhibition of the NF-κB ameliorates a sepsis-induced down-regulation of V2R and AQP2 expression (93). At the molecular level, these data underline the critical role of NF-κB signaling pathway in controlling AQP2-mediated water reabsorption in the collecting duct during aging.

Oxidative stress and reactive oxygen species (ROS) production in aging

Studies from several groups have shown that oxidative stress plays a role in modulating renal functionality including its diluting and concentrating ability during aging.

In healthy humans oxidative damage caused by the generation of free radicals increases with the life span (94). Oxidative stress increases the risk to develop several age-related diseases because ROS may alter cell signaling leading to inflammation, apoptosis and cellular senescence. In aging kidneys, significant increases of advanced glycosylation-end products and other oxidants (95, 96), known to reduce telomeres length and cell viability (97), have been detected. Aging kidneys particularly display high levels of advanced glycosylation-end products and asymmetric dimethylarginine, which are known inhibitors of nitric oxide synthase (NOS) that regulates renal blood circulation and function (24). Therefore, nitric oxide production decreases with the life span in the endothelium of peritubular capillaries, thus suggesting that aging-related renal injury might also be associated with oxidative stress-mediated NOS inhibition (65). Chronic inhibition of NOS regulates renal water balance by reducing the expression of AQP2 (98, 99). Importantly, oxidative stress is often associated with disorders linked to redox unbalance. At a cellular level, ROS can oxidize methionine and cysteine residue on target proteins. In this respect, S-glutathionylation has been described as a key posttranslational modification important to protect proteins against oxidative stress. Data from our group recently demonstrated that under oxidative stress AQP2 is subjected to S-glutathionylation (100). Whether S-gluthationylation of AQP2 plays a role during water unbalance observed during aging remains to be investigated.

Structural and functional renal alterations in the elderly

At the renal level, several structural and functional alterations are associated with aging (Figure 2). Kidney mass increases up to the fourth decade of life and then decreases at 10% per decade (78, 101, 102). Anatomical studies of aged kidneys show significant increases in parenchymal loss, calcification, and development of renal cysts. At the microscopic level, glomerulosclerosis, tubular alterations, and interstitial fibrosis have been also described (Figure 2). Importantly, such alterations have great impact during aging as several compensative mechanisms are often compromised resulting in significant unbalances in water and solute homeostasis. Normal adult human kidneys hold 617 000 glomeruli on average, having a mean size of 6.0 μm. A negative correlation between glomerular number and size to age has also been described (103).

These observations are fundamental for understanding the variations of GFR, which is significantly reduced with aging. Indeed, decrease of the GFR is considered as the main and earliest biomarker useful for monitoring renal function during aging as other functional renal parameters are associated with GFR decreases (104, 105). It has been estimated that after age 40 years GFR decreases approximately 1% per year (104). Decrease in GFR is paralleled by the activation of a tubuloglomerular feedback mechanism, which results in a significant increase in proximal renal tubular fluid absorption and a consequent decrease in tubular flux of free water to distal diluting segments of the nephron (106, 107). A consequence of this is a drastic decrease in excretion of free water load and loss of dilutional kidney ability, thus predisposing the elderly to dysnatremia (108). In addition to the loss of diluting capability, aging is often accompanied by a decrease in urine concentrating ability (109). The Baltimore Longitudinal Study of Aging demonstrated that individuals' aged 60–79 years display about 20% reduction in urine osmolality. By age of 80 years, maximal urinary concentrating ability (1100–1200 mOsm/kg H_2O) decreases to 400–500 mOsm/kg H_2O (7).

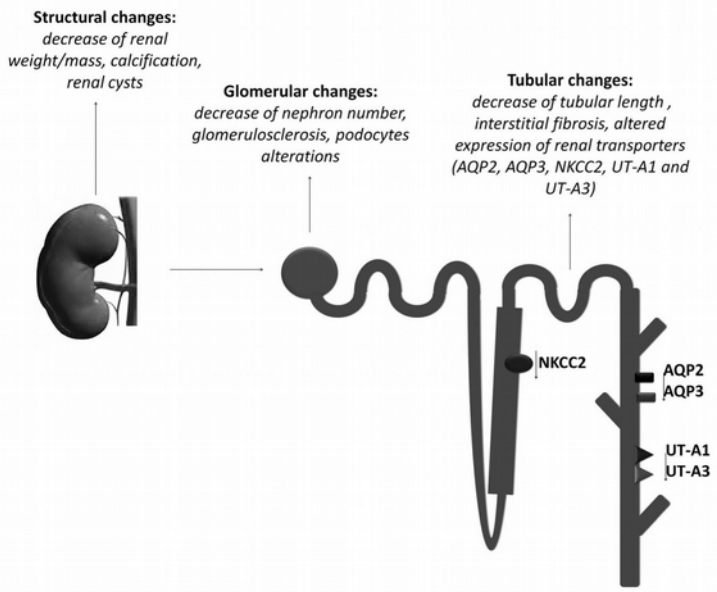


Figure 2. Abnormal AVP axis feedback and kidney dysfunction in aging.

Hyponatremia in aging

Hyponatremia (ie, blood sodium below 136 mM) is the most frequently encountered electrolyte disturbance in hospitals (1% inci-

dence). Hyponatremia incidence increases with age, and severe forms are associated with substantial morbidity and a 60-fold increased mortality (110–112). Hyponatremia is often associated with central nervous system disorders, pulmonary and kidney disease, cancer, congestive heart failure (CHF), liver cirrhosis, hypothyroidism, and syndrome of inappropriate antidiuretic hormone secretion (111). Clinical manifestations of hyponatremia range from mild symptoms such as headache, nausea, and vomiting to severe symptoms such as disorientation, disturbed consciousness, and seizures (113). Classical methods for diagnosis of hyponatremia include, in the first instance, the evaluation of plasma sodium level. In the second phase, assessed are the dosage of AVP and values of glycemia, which allow for classification into euvolemic/hypertonic hyponatremia (hyperglycemia and osmolality $>$ 275 mOsm/kg) or hypotonic hyponatremia (hyperglycemia or normal glycemia and osmolality $<$ 275 mOsm/kg). Recently copeptin, which originates from the same precursor as vasopressin, has been proposed as a new biomarker of hyponatremia (114).

Therapeutic approaches to correct hyponatremia are not specific and efficient and have many adverse effects. Therefore, the generation of new and more selective drugs such as vasopressin antagonists is fundamental to correct hyponatremia. Furthermore, the pathological involvement of vasopressin in most types of hyponatremia suggests that selective vasopressin antagonists could offer the opportunity for treatment. Several peptide vasopressin antagonists have been generated. However, because they are not selective antagonists, their use has been limited (115). Recently a new class of nonpeptide and orally available vasopressin antagonists, called vaptans, have been described (116–119). Vaptans have been found to improve hyponatremia in patients with mixed etiologies, and they can be considered to be a new tool in the treatment of euvolemic and hypervolemic hyponatremia. To date, two vaptans, ie, conivaptan and tolvaptan (OPC-41061), have been marketed in the United States for the treatment of euvolemic and hypervolemic hyponatremia, whereas in Europe the use of tolvaptan has been limited to euvolemic hyponatremia (116). Tolvaptan is generally well tolerated and has fewer side effects generally consistent with its physiological activity. However, to avoid overly rapid correction or overcorrection, caution should be used and the treatment should be strictly monitored (117, 120–122). Recent studies have demonstrated the efficacy of tolvaptan in influencing AQP2 signaling. Specifically, tolvaptan has been shown to abolish vasopressin effects on AQP2 phosphorylation (123). The molecular basis of this effect has, however, not yet been well clarified. Therefore, the role of vaptans for correcting age-associated hyponatremia needs to be further investigated.

Hyponatremia, osteoporosis, and aging

Hyponatremia is known to be associated with osteoporosis and a high fracture risk. The mechanism through which the bone loss occurs, however, remains unclear. As mentioned above, an increase in AVP levels is often found in elderly people, and this condition can cause water retention and hyponatremia but can also stimulate calcium release from bone, thus contributing to osteoporosis and risk to falls and fractures. In this regard a primary role for AVP signaling in bone mass regulation has been recently shown (124). This research suggests that regulation of bone remodeling by AVP through the V1a receptor explains the bone loss in patients who have elevated circulating AVP levels. Increased AVP is associated with hyponatremia in 7%–11% of healthy elderly people over 65 years. Interestingly, Tamma et al (125) recently showed that oxytocin, which is structurally very similar to AVP and synthetized in the same regions in the hypothalamus, has a promoting effect on bone formation. These findings suggest that despite the similarities between AVP and oxytocin, their effects regarding osteogenesis appear to be opposite to each other.

Together these data not only establish a primary role for AVP signaling in bone mass regulation but also highlight the importance of clinical studies examining skeletal actions of AVP inhibitors commonly used in hyponatremic patients. The observations related to the effects of AVP on bone physiology might lead to exciting new possibilities toward treatment of osteoporosis, particularly in patients with increased levels of AVP due to hyponatremia. Use of V1a or V2 receptor antagonists may be useful future therapeutic options in treatment of patients, which suffer from the effects of increased AVP levels on the bone.

Hypernatremia in aging

Hypernatremia (ie, blood sodium $>$ 145 mM) indicates a complex hypertonic and hyperosmolar scenario that often results in transient cellular dehydration (126). During aging, alteration in the thirst mechanism associated with diuretics drugs administration and decline of urinary concentrating ability, lead to the upsetting of the hypernatremia (37). Hypernatremia incidence increases with age and occurs in approximately 1% of hospitalized patients older than 60 years (126). Severe hypernatremia results in lethargy, weakness, stupor, seizure, and potentially in coma (127). Glucose concentration should be evaluated in hypernatremic patients to exclude osmotic diuresis caused by hyperglycemia. Chronic hypernatremia is associated with mild symptoms due to the activation of vol-

ume/ion compensative mechanisms. A correct diagnosis of hypernatremia includes the analysis of AVP-renal signaling. As stated above, hypernatremia might be caused by an age-related defect in thirst sensation. Alternatively, renal and extrarenal fluid loss might be the main cause of hypernatremia. Because elderly persons are prescribed many types and combinations of drugs, which can often lead to dysnatremia, such combinations need to be controlled in aged people (37). Loop diuretics might worsen hypotonic fluid loss, whereas use of mannitol results in osmotic diuresis (128). Hypernatremia has also been associated with hypokalemia in some aged patients (129). On the other hand, during aging, potassium secretion is often impaired due to alterations in tubular and ion transporters. In addition, potassium-sparing drugs, which affect the renin/aldosterone axis, are associated with a tendency to develop hyperkalemia (130). Hypokalemia is known to cause vasopressin-resistant polyuria due to a down-regulation of AQP2 expression (131). Therefore, the correction of hypokalemia is crucial to counterbalance the defect in water-concentrating ability.

The correct approach to treat hypernatremia is based on the identification of the underlying cause(s). Extracellular fluid loss could be counteracted by isotonic saline administration. Alternatively, based on the degree of hypernatremia, different hypotonic solutions can be also used considering that, at best, sodium concentration should be reduced by 10 mEq/L over a 24-hour period. A deep hypotonic solution should, however, be administrated very slowly because the risk of developing cerebral edema also increases with the volume of the used solution (110).

Altered vasopressin signaling and effects on cardiovascular system in aging

The chronically enhanced levels of AVP frequently found in the elderly can worsen some pathologies such as CHF, a serious condition that has a high prevalence in aged patients. As early as 1983, it was found by Goldsmith et al (132) that AVP levels in patients with CHF were elevated. Because AVP increases blood volume and promotes vasoconstriction, both of which are harmful in CHF, scientific interest soon arose concerning the underlying physiological mechanisms and whether could be exploited in new therapy strategies. In 1988, Lee et al (133) experimented on dogs and observed that AVP increases mean circulatory filling pressure and decreases cardiac output. Both of these could have harmful effects in a failing heart.

Of the three main vasopressin receptor types, V1a and V2 directly play a role in the cardiovascular system. The V1a receptors are situated mainly in the wall of smooth muscle cells. They trigger the release of Ca^{2+} ions from the endoplasmic reticulum via activation of the Gq/Inositol-triphosphate (IP_3) pathway, resulting in contraction of muscle cells. The production of nitric oxide, a powerful vasodilatory agent, appears also to be inhibited by V1a receptor activity (134). In the vascular beds of the brain and lungs, however, AVP enhances nitric oxide production through V2 receptors and thus causes vasodilation (135).

In addition to their immediate effects, V1a receptors enhance proliferation of myofilament in muscle cells (136). This suggests that chronically age-associated enhanced levels of AVP could lead to an increase in total peripheral resistance, thus increasing the afterload and promoting CHF. Moreover, reabsorption of water promoted by AVP through the V2 receptors increases plasma volume thus putting additional stress on the heart; this adversely affects existing CHF and hypertonia, which is highly prevalent in the elderly. Furthermore, AVP promotes hyponatremia, already a common problem in the elderly, which is also associated with a worsened outcome in CHF (134, 137, 138). The V1b receptors present in the adrenal medulla trigger a release of catecholamines. Locally produced AVP acts as a stimulus for AVP secretion in V1b-deficient mice, which show an attenuated epinephrine response under stress (9). Persistently elevated AVP levels in the elderly could lead to an increased epinephrine release during events such as acute coronary syndrome, which in turn may lead to an increase in morbidity and mortality.

The negative effects of AVP in CHF led to the idea that AVP antagonists might be beneficial in the treatment of CHF. The Efficacy of Vasopressin Antagonism in Heart Failure Outcome Study With Tolvaptan clinical study carried out in 2007 examined the effects of an addition of tolvaptan to standard therapy of patients hospitalized due to heart failure. In this study patients who received tolvaptan treatment showed a significant improvement of many of the symptoms associated with heart failure such as hyponatremia, edema, dyspnea, and fatigue without significant adverse effects (139). However, long-term mortality did not differ significantly between patients treated with tolvaptan and the controls (121).

In view of elevated AVP levels in the elderly, there might be an increased risk of thrombotic events. AVP is known to increase the plasma levels of the coagulation factor VIII as well as the von Willebrand factor, an aspect that is therapeutically used in treating patients suffering from von Willebrand disease, a disorder in which von Willebrand factor gene expression is insufficient with desmopressin, an AVP analog (140). Studies on patients with vasodilatory shock treated with AVP have shown no sig-

nificant changes in cardiovascular events due to increased coagulation (141, 142). Future studies need to investigate what effect chronically elevated AVP levels have on the coagulation parameters.

Conclusions

Over the last decade, exciting findings have been made proving the versatility of AVP as a hormone by showing its diverse effects on various organ systems and the consequences of altered AVP signaling often associated with aging. Furthermore, observations suggest that AVP plays a role in a number of age-related pathologies, and therefore, AVP receptors or its production sites could be therapeutical targets in treatment of these pathologies. Because the kidney is the chief target of AVP, of particular interest are consequences of aging in the renal structure and function leading to abnormal AVP axis feedback and kidney dysfunction mainly related to alteration of the expressions of many of the key proteins that contribute to urine concentrating ability including aquaporins, sodium, and urea transporters.

More clinical and theoretical research is needed to enhance our understanding of the underlying physiology, with the prospect of new therapeutical strategies in the treatment of age-related pathologies in the future.

Acknowledgments

Address all correspondence and requests for reprints to: Giovanna Valenti, PhD, Department of Bioscience, Biotechnology, and Biopharmaceutics, University of Bari, Via Amendola 165/A, 70125 Bari, Italy. E-mail: giovanna.valenti@uniba.it; or Nandu Goswami, Gravitational Physiology and Medicine Research Unit, Institute of Physiology, Medical University of Graz, 8036 Graz, Austria. E-mail: nandu.goswami@medunigraz.at.

This work was supported by Italian Space Agency Grant 2013-091-R.O and Telethon Grant GGP13227.

Disclosure Summary: The authors have nothing to disclose.

References

1. **Sands JM.** Urine concentrating and diluting ability during aging. *J Gerontol Series A Biol Sci Med Sci*. 2012;67:1352–1357.
2. **Cowen LE, Hodak SP, Verbalis JG.** Age-associated abnormalities of water homeostasis. *Endocrinol Metab Clin North Am*. 2013; 42:349–370.
3. **Carpenter CR.** Geriatric emergency medicine (preface). *Clin Geriatr Med*. 2013;29:xiii-xiv.
4. **Sherman FT.** Hyponatremia and SIADH in the elderly. Introducing the "water-offered test" for dehydration. *Geriatrics*. 2007;62:8–18.
5. **Findley T.** Role of the neurohypophysis in the pathogenesis of hypertension and some allied disorders associated with aging. *Am J Med*. 1949;7:70–84.
6. **O'Neill PA, McLean KA.** Water homeostasis and ageing. *Med Lab Sci*. 1992;49:291–298.
7. **Rowe JW, Shock NW, DeFronzo RA.** The influence of age on the renal response to water deprivation in man. *Nephron*. 1976;17: 270–278.
8. **Combet S, Gouraud S, Gobin R, et al.** Aquaporin-2 downregulation in kidney medulla of aging rats is posttranscriptional and is abolished by water deprivation. *Am J Physiol Renal Physiol*. 2008; 294:F1408–F1414.
9. **Koshimizu TA, Nakamura K, Egashira N, et al.** Vasopressin V1a and V1b receptors: from molecules to physiological systems. *Physiol Rev*. 2012;92:1813–1864.
10. **Wong LL, Verbalis JG.** Systemic diseases associated with disorders of water homeostasis. *Endocrinol Metab Clin North Am*. 2002; 31:121–140.
11. **Fenton RA, Pedersen CN, Moeller HB.** New insights into regulated aquaporin-2 function. *Curr Opin Nephrol Hypertens*. 2013;22(5): 551–558.
12. **Valenti G, Procino G, Tamma G, Carmosino M, Svelto M.** Minireview: aquaporin 2 trafficking. *Endocrinology*. 2005;146(12): 5063–5070.
13. **Ares GR, Caceres PS, Ortiz PA.** Molecular regulation of NKCC2 in the thick ascending limb. *Am J Physiol Renal Physiol*. 2011; 301:F1143–F1159.
14. **Gimenez I, Forbush B.** Short-term stimulation of the renal Na-K-Cl cotransporter (NKCC2) by vasopressin involves phosphorylation and membrane translocation of the protein. *J Biol Chem*. 2003; 278:26946–26951.
15. **Klein JD, Blount MA, Sands JM.** Urea transport in the kidney. *Compr Physiol*. 2011;1:699–729.
16. **Saito T.** Hyponatremia in elderly patients. *Intern Med*. 2001;40: 851.
17. **Combet S, Geffroy N, Berthonaud V, et al.** Correction of age-related polyuria by dDAVP: molecular analysis of aquaporins and urea transporters. *Am J Physiol Renal Physiol*. 2003;284:F199–F208.
18. **Tian Y, Riazi S, Khan O, et al.** Renal ENaC subunit, Na-K-2Cl and Na-Cl cotransporter abundances in aged, water-restricted F344 x Brown Norway rats. *Kidney Int*. 2006;69:304–312.
19. **Sauvant J, Delpech JC, Palin K, et al.** Mechanisms involved in dual vasopressin/apelin neuron dysfunction during aging. *PloS One*. 2014;9:e87421.
20. **Preston GM, Jung JS, Guggino WB, Agre P.** The mercury-sensitive residue at cysteine 189 in the CHIP28 water channel. *J Biol Chem*. 1993;268:17–20.
21. **King LS, Choi M, Fernandez PC, Cartron JP, Agre P.** Defective urinary-concentrating ability due to a complete deficiency of aquaporin-1. *N Engl J Med*. 2001;345:175–179.
22. **Elkjaer ML, Nejsum LN, Gresz V, et al.** Immunolocalization of aquaporin-8 in rat kidney, gastrointestinal tract, testis, and airways. *Am J Physiol Renal Physiol*. 2001;281:F1047–F1057.
23. **Ishibashi K, Kuwahara M, Kageyama Y, Tohsaka A, Marumo F, Sasaki S.** Cloning and functional expression of a second new aquaporin abundantly expressed in testis. *Biochem Biophys Res Commun*. 1997;237:714–718.
24. **Ishikawa K, Calzavacca P, Bellomo R, Bailey M, May CN.** Effect of selective inhibition of renal inducible nitric oxide synthase on renal blood flow and function in experimental hyperdynamic sepsis. *Critical Care Med*. 2012;40:2368–2375.
25. **Fushimi K, Uchida S, Hara Y, Hirata Y, Marumo F, Sasaki S.** Cloning and expression of apical membrane water channel of rat kidney collecting tubule. *Nature*. 1993;361:549–552.
26. **Ecelbarger CA, Terris J, Frindt G, et al.** Aquaporin-3 water channel localization and regulation in rat kidney. *Am J Physiol*. 1995;269: F663–F672.
27. **Hasegawa H, Ma T, Skach W, Matthay MA, Verkman AS.** Molecular cloning of a mercurial-insensitive water channel expressed

in selected water-transporting tissues. *J Biol Chem*. 1994;269:5497–5500.
28. **Yasui M, Kwon TH, Knepper MA, Nielsen S, Agre P.** Aquaporin-6: an intracellular vesicle water channel protein in renal epithelia. *Proc Natl Acad Sci USA*. 1999;96:5808–5813.
29. **Katsura T, Gustafson CE, Ausiello DA, Brown D.** Protein kinase A phosphorylation is involved in regulated exocytosis of aquaporin-2 in transfected LLC-PK1 cells. *Am J Physiol*. 1997;272:F817–F822.
30. **Nielsen S, Terris J, Andersen D, et al.** Congestive heart failure in rats is associated with increased expression and targeting of aquaporin-2 water channel in collecting duct. *Proc Natl Acad Sci USA*. 1997;94:5450–5455.
31. **Hoffert JD, Pisitkun T, Wang G, Shen RF, Knepper MA.** Quantitative phosphoproteomics of vasopressin-sensitive renal cells: regulation of aquaporin-2 phosphorylation at two sites. *Proc Natl Acad Sci USA*. 2006;103:7159–7164.
32. **Moeller HB, Aroankins TS, Slengerik-Hansen J, Pisitkun T, Fenton RA.** Phosphorylation and ubiquitylation are opposing players in regulating endocytosis of the water channel Aquaporin-2. *J Cell Sci*. 2014;127(Pt 14):3174–3183.
33. **Hoffert JD, Fenton RA, Moeller HB, et al.** Vasopressin-stimulated increase in phosphorylation at Ser269 potentiates plasma membrane retention of aquaporin-2. *J Biol Chem*. 2008;283:24617–24627.
34. **Hasler U, Leroy V, Jeon US, et al.** NF-κB modulates aquaporin-2 transcription in renal collecting duct principal cells. *J Biol Chem*. 2008;283:28095–28105.
35. **Tamma G, Lasorsa D, Trimpert C, et al.** A protein kinase A-independent pathway controlling aquaporin 2 trafficking as a possible cause for the syndrome of inappropriate antidiuresis associated with polycystic kidney disease 1 haploinsufficiency. *J Am Soc Nephrol*. 2014;25(10):2241–2253.
36. **Tamma G, Robben JH, Trimpert C, Boone M, Deen PM.** Regulation of AQP2 localization by S256 and S261 phosphorylation and ubiquitination. *Am J Physiol Cell Physiol*. 2011;300:C636–C646.
37. **Schlanger LE, Bailey JL, Sands JM.** Electrolytes in the aging. *Adv Chronic Kidney Dis*. 2010;17:308–319.
38. **Combet S, Teillet L, Geelen G, et al.** Food restriction prevents age-related polyuria by vasopressin-dependent recruitment of aquaporin-2. *Am J Physiol Renal Physiol*. 2001;281:F1123–F1131.
39. **Beck LH, Lavizzo-Mourey R.** Geriatric hypernatremia (corrected). *Ann Intern Med*. 1987;107:768–769.
40. **Medina-Escobedo M, Romero-Campos S, Sansores-Espana D, Viveros-Cortes A, Villanueva-Jorge S.** [Changes in body composition according to kidney damage in patients with type 2 diabetes mellitus]. *Rev Med Inst Mex Seguro Soc*. 2013;51:26–33.
41. **Palin K, Moreau ML, Orcel H, et al.** Age-impaired fluid homeostasis depends on the balance of IL-6/IGF-I in the rat supraoptic nuclei. *Neurobiol Aging*. 2009;30:1677–1692.
42. **De Guchtenaere A, Vande Walle C, Van Sintjan P, et al.** Nocturnal polyuria is related to absent circadian rhythm of glomerular filtration rate. *J Urol*. 2007;178:2626–2629.
43. **Schwartz WJ, Reppert SM.** Neural regulation of the circadian vasopressin rhythm in cerebrospinal fluid: a pre-eminent role for the suprachiasmatic nuclei. *J Neurosci*. 1985;5:2771–2778.
44. **Forsling ML, Montgomery H, Halpin D, Windle RJ, Treacher DF.** Daily patterns of secretion of neurohypophysial hormones in man: effect of age. *Exp Physiol*. 1998;83:409–418.
45. **Hofman MA, Swaab DF.** Alterations in circadian rhythmicity of the vasopressin-producing neurons of the human suprachiasmatic nucleus (SCN) with aging. *Brain Res*. 1994;651:134–142.
46. **Asplund R.** Pharmacotherapy for nocturia in the elderly patient. *Drugs Aging*. 2007;24:325–343.
47. **Bosch JL, Weiss JP.** The prevalence and causes of nocturia. *J Urol*. 2010;184:440–446.
48. **Laureanno P, Ellsworth P.** Demystifying nocturia: identifying the cause and tailoring the treatment. *Urol Nurs*. 2010;30:276–286; quiz 287.
49. **Varilla V, Samala RV, Galindo D, Ciocon J.** Nocturia in the elderly: a wake-up call. *Cleve Clin J Med*. 2011;78:757–764.
50. **Madeira MD, Sousa N, Santer RM, Paula-Barbosa MM, Gundersen HJ.** Age and sex do not affect the volume, cell numbers, or cell size of the suprachiasmatic nucleus of the rat: an unbiased stereological study. *J Comp Neurol*. 1995;361:585–601.
51. **Froy O.** Circadian rhythms, aging, and life span in mammals. *Physiology (Bethesda)*. 2011;26:225–235.
52. **Roozendaal B, van Gool WA, Swaab DF, Hoogendijk JE, Mirmiran M.** Changes in vasopressin cells of the rat suprachiasmatic nucleus with aging. *Brain Res*. 1987;409:259–264.
53. **Fliers E, Swaab DF, Pool CW, Verwer RW.** The vasopressin and oxytocin neurons in the human supraoptic and paraventricular nucleus; changes with aging and in senile dementia. *Brain Res*. 1985;342:45–53.
54. **Goudsmit E, Hofman MA, Fliers E, Swaab DF.** The supraoptic and paraventricular nuclei of the human hypothalamus in relation to sex, age and Alzheimer's disease. *Neurobiol Aging*. 1990;11:529–536.
55. **Ishunina TA, Swaab DF.** Vasopressin and oxytocin neurons of the human supraoptic and paraventricular nucleus: size changes in relation to age and sex. *J Clin Endocrinol Metab*. 1999;84:4637–4644.
56. **Zuber AM, Centeno G, Pradervand S, et al.** Molecular clock is involved in predictive circadian adjustment of renal function. *Proc Nat Acad Sci USA*. 2009;106:16523–16528.
57. **Rezakhaniha B, Arianpour N, Siroosbakhat S.** Efficacy of desmopressin in treatment of nocturia in elderly men. *J Res Med Sci*. 2011;16:516–523.
58. **Avidan AY.** Sleep in the geriatric patient population. *Semin Neurol*. 2005;25:52–63.
59. **Mahowald MW, Bornemann MAC.** Sleep complaints in the geriatric patient. *Minn Med*. 2007;90:45–47.
60. **Subramanian S, Surani S.** Sleep disorders in the elderly. *Geriatrics*. 2007;62:10–32.
61. **Mack GW, Weseman CA, Langhans GW, Scherzer H, Gillen CM, Nadel ER.** Body fluid balance in dehydrated healthy older men: thirst and renal osmoregulation. *J Appl Physiol*. 1994;76:1615–1623.
62. **Phillips PA, Bretherton M, Johnston CI, Gray L.** Reduced osmotic thirst in healthy elderly men. *Am J Physiol*. 1991;261:R166–R171.
63. **Saez F, Reverte V, Paliege A, et al.** Sex-Dependent Hypertension and Renal Changes in Aged Rats with an Altered Renal Development. *Am J Physiol Renal Physiol*. 2014;307(4):F461–F470.
64. **Jain AK, Kalbaugh CA, Farber MA, Marston WA, Vallabhaneni R.** Race and gender affect early outcomes of lower extremity bypass. *J Vasc Surg*. 2014;60(5):1275–1281.
65. **Baylis C.** Sexual dimorphism: the aging kidney, involvement of nitric oxide deficiency, and angiotensin II overactivity. *J Gerontol Series A Biol Sci Med Sci*. 2012;67:1365–1372.
66. **Maric C, Sandberg K, Hinojosa-Laborde C.** Glomerulosclerosis and tubulointerstitial fibrosis are attenuated with 17β-estradiol in the aging Dahl salt sensitive rat. *J Am Soc Nephrol*. 2004;15:1546–1556.
67. **Reckelhoff JF, Zhang H, Granger JP.** Decline in renal hemodynamic function in aging SHR: role of androgens. *Hypertension*. 1997;30:677–681.
68. **Stachenfeld NS, Splenser AE, Calzone WL, Taylor MP, Keefe DL.** Sex differences in osmotic regulation of AVP and renal sodium handling. *J Appl Physiol*. 2001;91:1893–1901.
69. **Wang YX, Crofton JT, Bealer SL, Share L.** Sexual dimorphism in

regional blood flow responses to vasopressin in conscious rats. *Am J Physiol*. 1997;273:R1126–R1131.
70. **Graugaard-Jensen C, Hvistendahl GM, Frokiaer J, Bie P, Djurhuus JC.** Urinary concentration does not exclusively rely on plasma vasopressin. A study between genders. Gender and diurnal urine regulation. *Acta Physiol (Oxf)*. 2014;212(1):97–105.
71. **Hill AA, Hunter CP, Tsung BT, Tucker-Kellogg G, Brown EL.** Genomic analysis of gene expression in *C. elegans*. *Science*. 2000; 290:809–812.
72. **Kwekel JC, Desai VG, Moland CL, Vijay V, Fuscoe JC.** Life cycle analysis of kidney gene expression in male F344 rats. *PloS One*. 2013;8:e75305.
73. **Zou S, Meadows S, Sharp L, Jan LY, Jan YN.** Genome-wide study of aging and oxidative stress response in Drosophila melanogaster. *Proc Natl Acad Sci USA*. 2000;97:13726–13731.
74. **Lin TH, Chiu HC, Wang CL, et al.** The Gly460Trp polymorphism of alpha-adducin gene as a predictor of renal function decline over 4 years of follow-up in an apparently healthy Chinese population. *Transl Res*. 2012;160:162–163.
75. **Salminen A, Kaarniranta K.** Regulation of the aging process by autophagy. *Trends Mol Med*. 2009;15:217–224.
76. **Hackl M, Brunner S, Fortschegger K, et al.** miR-17, miR-19b, miR-20a, and miR-106a are down-regulated in human aging. *Aging Cell*. 2010;9:291–296.
77. **Ho J, Ng KH, Rosen S, Dostal A, Gregory RI, Kreidberg JA.** Podocyte-specific loss of functional microRNAs leads to rapid glomerular and tubular injury. *J Am Soc Nephrol*. 2008;19:2069–2075.
78. **Bolignano D, Mattace-Raso F, Sijbrands EJ, Zoccali C.** The aging kidney revisited: a systematic review. *Ageing Res Rev*. 2014;14: 65–80.
79. **Pawlikowska L, Hu D, Huntsman S, et al.** Association of common genetic variation in the insulin/IGF1 signaling pathway with human longevity. *Aging Cell*. 2009;8:460–472.
80. **Emran S, Yang M, He X, Zandveld J, Piper MD.** Target of rapamycin signalling mediates the lifespan-extending effects of dietary restriction by essential amino acid alteration. *Aging*. 2014;6:390–398.
81. **Tatar M, Post S, Yu K.** Nutrient control of Drosophila longevity. *Trends Endocrinol Metab*. 2014;25(10):509–517.
82. **Zhuo L, Cai G, Liu F, et al.** Expression and mechanism of mammalian target of rapamycin in age-related renal cell senescence and organ aging. *Mech Ageing Dev*. 2009;130:700–708.
83. **Hao CM, Haase VH.** Sirtuins and their relevance to the kidney. *J Am Soc Nephrol*. 2010;21:1620–1627.
84. **Kume S, Kitada M, Kanasaki K, Maegawa H, Koya D.** Anti-aging molecule, Sirt1: a novel therapeutic target for diabetic nephropathy. *Arch Pharm Res*. 2013;36:230–236.
85. **Kuro-o M, Matsumura Y, Aizawa H, et al.** Mutation of the mouse klotho gene leads to a syndrome resembling ageing. *Nature*. 1997; 390:45–51.
86. **Hu MC, Kuro-o M, Moe OW.** Renal and extrarenal actions of Klotho. *Semin Nephrol*. 2013;33:118–129.
87. **Hu MC, Kuro-o M, Moe OW.** Secreted klotho and chronic kidney disease. *Adv Exp Med Biol*. 2012;728:126–157.
88. **Hu MC, Moe OW.** Klotho as a potential biomarker and therapy for acute kidney injury. *Nat Rev Nephrol*. 2012;8:423–429.
89. **Fischer SS, Kempe DS, Leibrock CB, et al.** Hyperaldosteronism in Klotho-deficient mice. *Am J Physiol Renal Physiol*. 2010;299: F1171–F1177.
90. **Tang C, Pathare G, Michael D, Fajol A, Eichenmuller M, Lang F.** Downregulation of Klotho expression by dehydration. *Am J Physiol Renal Physiol*. 2011;301:F745–F750.
91. **Moreno JA, Izquierdo MC, Sanchez-Nino MD, et al.** The inflammatory cytokines TWEAK and TNFα reduce renal klotho expression through NFκB. *J Am Soc Nephrol*. 2011;22:1315–1325.
92. **Tilstra JS, Clauson CL, Niedernhofer LJ, Robbins PD.** NF-κB in aging and disease. *Aging Dis*. 2011;2:449–465.
93. **Hocherl K, Schmidt C, Kurt B, Bucher M.** Inhibition of NF-κB ameliorates sepsis-induced downregulation of aquaporin-2/V2 receptor expression and acute renal failure in vivo. *Am J Physiol Renal Physiol*. 2010;298:F196–F204.
94. **Mendoza-Nunez VM, Ruiz-Ramos M, Sanchez-Rodriguez MA, Retana-Ugalde R, Munoz-Sanchez JL.** Aging-related oxidative stress in healthy humans. *Tohoku J Exp Med*. 2007;213:261–268.
95. **Bucala R.** Diabetes, aging, and their tissue complications. *J Clin Invest*. 2014;124:1887–1888.
96. **Reckelhoff JF, Kanji V, Racusen LC, et al.** Vitamin E ameliorates enhanced renal lipid peroxidation and accumulation of F2-isoprostanes in aging kidneys. *Am J Physiol*. 1998;274:R767–R774.
97. **Palmieri D, Cafueri G, Mongelli F, Pezzolo A, Pistoia V, Palombo D.** Telomere shortening and increased oxidative stress are restricted to venous tissue in patients with varicose veins: a merely local disease? *Vasc Med*. 2014;19:125–130.
98. **Albertoni Borghese MF, Majowicz MP, Ortiz MC, Delgado MF, Sterin Speziale NB, Vidal NA.** Renal sodium-glucose cotransporter activity and aquaporin-2 expression in rat kidney during chronic nitric oxide synthase inhibition. *Nephron Physiol*. 2007;107:77–86.
99. **Arreche N, Fellet A, Lopez M, Lopez-Costa J, Arranz C, Balaszczuk AM.** Hypovolemic state: involvement of nitric oxide in the aged related alterations of aquaporins-2 abundance in rat kidney. *Vasc Pharmacol*. 2008;49:19–25.
100. **Tamma G, Ranieri M, Di Mise A, Centrone M, Svelto M, Valenti G.** Glutathionylation of the aquaporin-2 water channel: a novel post-translational modification modulated by the oxidative stress. *J Biol Chem*. 2014;289(40):27807–27813.
101. **Bueters RR, van de Kar NC, Schreuder MF.** Adult renal size is not a suitable marker for nephron numbers: an individual patient data meta-analysis. *Kidney Blood Press Res*. 2013;37:540–546.
102. **Kaysen GA, Myers BD.** The aging kidney. *Clin Geriatr Med*. 1985; 1:207–222.
103. **Nyengaard JR, Bendtsen TF.** Glomerular number and size in relation to age, kidney weight, and body surface in normal man. *Anat Rec*. 1992;232:194–201.
104. **Lindeman RD.** Assessment of renal function in the old. Special considerations. *Clin Lab Med*. 1993;13:269–277.
105. **Lindeman RD.** Renal physiology and pathophysiology of aging. *Contrib Nephrol*. 1993;105:1–12.
106. **Fenton RA, Knepper MA.** Mouse models and the urinary concentrating mechanism in the new millennium. *Physiol Rev*. 2007;87: 1083–1112.
107. **Lorenz JN, Schultheis PJ, Traynor T, Shull GE, Schnermann J.** Micropuncture analysis of single-nephron function in NHE3-deficient mice. *Am J Physiol*. 1999;277:F447–F453.
108. **Faull CM, Holmes C, Baylis PH.** Water balance in elderly people: is there a deficiency of vasopressin? *Age Ageing*. 1993;22:114–120.
109. **Sands JM.** Urine-concentrating ability in the aging kidney. *Sci Aging Knowl Environ*. 2003;2003:PE15.
110. **Al Zahrani A, Sinnert R, Gernsheimer J.** Acute kidney injury, sodium disorders, and hypercalcemia in the aging kidney: diagnostic and therapeutic management strategies in emergency medicine. *Clin Geriatr Med*. 2013;29:275–319.
111. **Miller M.** Role of arginine vasopressin receptor antagonists in hyponatremia in the elderly. *Geriatrics*. 2007;62:20–26.
112. **Moore K, Thompson C, Trainer P.** Disorders of water balance. *Clin Med*. 2003;3:28–33.
113. **Fraser CL, Arieff AI.** Epidemiology, pathophysiology, and management of hyponatremic encephalopathy. *Am J Med*. 1997;102: 67–77.
114. **Fenske W, Stork S, Blechschmidt A, Maier SG, Morgenthaler NG, Allolio B.** Copeptin in the differential diagnosis of hyponatremia. *J Clin Endocrinol Metab*. 2009;94:123–129.
115. **Chan WY, Wo NC, Stoev S, Cheng LL, Manning M.** Discovery of

novel selective hypotensive vasopressin peptides that exhibit little or no functional interactions with known oxytocin/vasopressin receptors. *Br J Pharmacol*. 1998;125:803–811.
116. **Schrier RW.** The sea within us: disorders of body water homeostasis. *Curr Opin Investig Drugs*. 2007;8:304–311.
117. **Jovanovich AJ, Berl T.** Where vaptans do and do not fit in the treatment of hyponatremia. *Kidney Int*. 2013;83:563–567.
118. **Peri A.** Clinical review: the use of vaptans in clinical endocrinology. *J Clin Endocrinol Metab*. 2013;98:1321–1332.
119. **Verbalis JG, Adler S, Schrier RW, et al.** Efficacy and safety of oral tolvaptan therapy in patients with the syndrome of inappropriate antidiuretic hormone secretion. *Eur J Endocrinol*. 2011;164:725–732.
120. **Gheorghiade M, Gattis WA, O'Connor CM, et al.** Effects of tolvaptan, a vasopressin antagonist, in patients hospitalized with worsening heart failure: a randomized controlled trial. *JAMA*. 2004;291:1963–1971.
121. **Konstam MA, Gheorghiade M, Burnett JC Jr, et al.** Effects of oral tolvaptan in patients hospitalized for worsening heart failure: the EVEREST Outcome Trial. *JAMA*. 2007;297:1319–1331.
122. **Lehrich RW, Greenberg A.** When is it appropriate to use vasopressin receptor antagonists? *J Am Soc Nephrol*. 2008;19:1054–1058.
123. **Miranda CA, Lee JW, Chou CL, Knepper MA.** Tolvaptan as a tool in renal physiology. *Am J Physiol Renal Physiol*. 2014;306:F359–F366.
124. **Tamma R, Sun L, Cuscito C, et al.** Regulation of bone remodeling by vasopressin explains the bone loss in hyponatremia. *Proc Natl Acad Sci USA*. 2013;110:18644–18649.
125. **Tamma R, Colaianni G, Zhu LL, et al.** Oxytocin is an anabolic bone hormone. *Proc Natl Acad Sci USA*. 2009;106:7149–7154.
126. **Long CA, Marin P, Bayer AJ, Shetty HG, Pathy MS.** Hypernatraemia in an adult in-patient population. *Postgrad Med J*. 1991;67:643–645.
127. **Adrogue HJ, Madias NE.** Hypernatremia. *N Engl J Med*. 2000;342:1493–1499.
128. **Seo W, OH H.** Alterations in serum osmolality, sodium, and potassium levels after repeated mannitol administration. *J Neurosci Nurs*. 2010;42:201–207.
129. **Borra SI, Beredo R, Kleinfeld M.** Hypernatremia in the aging: causes, manifestations, and outcome. *J Natl Med Assoc*. 1995;87:220–224.
130. **Chapagain A, Ashman N.** Hyperkalaemia in the age of aldosterone antagonism. *QJM*. 2012;105:1049–1057.
131. **Marples D, Frokiaer J, Dorup J, Knepper MA, Nielsen S.** Hypokalemia-induced downregulation of aquaporin-2 water channel expression in rat kidney medulla and cortex. *J Clin Invest*. 1996;97:1960–1968.
132. **Goldsmith SR, Francis GS, Levine TB, Cowley AW Jr, Cohn JN.** Impaired response of plasma vasopressin to orthostatic stress in patients with congestive heart failure. *J Am Coll Cardiol*. 1983;2:1080–1083.
133. **Lee RW, Standaert S, Lancaster LD, Buckley D, Goldman S.** Cardiac and peripheral circulatory responses to angiotension and vasopressin in dogs. *J Clin Invest*. 1988;82:413–419.
134. **Filippatos TD, Elisaf MS.** Hyponatremia in patients with heart failure. *World J Cardiol*. 2013;5:317–328.
135. **Japundzic-Zigon N.** Vasopressin and oxytocin in control of the cardiovascular system. *Curr Neuropharmacol*. 2013;11:218–230.
136. **Nakamura Y, Haneda T, Osaki J, Miyata S, Kikuchi K.** Hypertrophic growth of cultured neonatal rat heart cells mediated by vasopressin V(1A) receptor. *Eur J Pharmacol*. 2000;391:39–48.
137. **Farmakis D, Filippatos G, Parissis J, Kremastinos DT, Gheorghiade M.** Hyponatremia in heart failure. *Heart Fail Rev*. 2009;14:59–63.
138. **Romanovsky A, Bagshaw S, Rosner MH.** Hyponatremia and congestive heart failure: a marker of increased mortality and a target for therapy. *Int J Nephrol*. 2011;2011:732746.
139. **Gheorghiade M, Konstam MA, Burnett JC Jr, et al.** Efficacy of Vasopressin Antagonism in Heart Failure Outcome Study With Tolvaptan I. Short-term clinical effects of tolvaptan, an oral vasopressin antagonist, in patients hospitalized for heart failure: the EVEREST Clinical Status Trials. *JAMA*. 2007;297:1332–1343.
140. **Kaufmann JE, Vischer UM.** Cellular mechanisms of the hemostatic effects of desmopressin (DDAVP). *J Thromb Haemost*. 2003;1:682–689.
141. **Doepker BA, Lucarelli MR, Lehman A, Shirk MB.** Thromboembolic events during continuous vasopressin infusions: a retrospective evaluation. *Ann Pharmacother*. 2007;41:1383–1389.
142. **Dunser MW, Fries DR, Schobersberger W, et al.** Does arginine vasopressin influence the coagulation system in advanced vasodilatory shock with severe multiorgan dysfunction syndrome? *Anesth Analg*. 2004;99:201–206.

Bibliography

[1] R. H. Goetz, J. V. Warren, O. H. Gauer, J. L. Patterson, J. T. Doyle, E. N. Keen, M. McGREGOR, L. M. Tiller, M. Smith, and E. Mance, “Circulation of the Giraffe,” *Circ. Res.*, vol. 8, no. 5, pp. 1049–1058, Sep. 1960.

[2] R. F. Schmidt, F. Lang, and M. Heckmann, *Physiologie des Menschen: mit Pathophysiologie*, 31. Aufl. 2011. Springer, 2010.

[3] J. J. Smith, *Circulatory Response to the Upright Posture*, 1st edition. Boca Raton, Fla: CRC Press, 1990.

[4] D. S. Dock, W. L. Kraus, L. B. McGuire, J. W. Hyland, F. W. Haynes, and L. Dexter, “The Pulmonary Blood Volume in Man*,” *J. Clin. Invest.*, vol. 40, no. 2, pp. 317–328, Feb. 1961.

[5] R. J. Levick, *An Introduction to Cardiovascular Physiology 5E*, 5th edition. London: CRC Press, 2009.

[6] J. E. Hall and A. C. Guyton, *Guyton and Hall textbook of medical physiology*. 2011.

[7] D. Robertson, I. Biaggioni, G. Burnstock, P. A. Low, and J. F. R. Paton, Eds., *Primer on the Autonomic Nervous System, Third Edition*, 3rd edition. Amsterdam ; Boston: Academic Press, 2011.

[8] W. Zhang and Z. Wang, “Resetting baroreceptors to a lower arterial pressure level by enalapril avoids baroreflex mediated activation of sympathetic nervous system by nifedipine,” *Life Sci.*, vol. 68, no. 25, pp. 2769–2779, May 2001.

[9] P. B. Raven, P. J. Fadel, and S. Ogoh, “Arterial baroreflex resetting during exercise: a current perspective,” *Exp. Physiol.*, vol. 91, no. 1, pp. 37–49, Jan. 2006.

[10] L. B. Rowell, *Human Cardiovascular Control*. New York: Amer Academy of Religion, 1993.

[11] F. Horn, I. Moc, N. Schneider, C. Grillhösl, S. Berghold, G. Lindenmeier, S. Helferich, I. Hopf, and A. Hunsicker, *Biochemie des Menschen: Das Lehrbuch für das Medizinstudium*, 3., vollst. überarb. u. erw. Aufl. Thieme, 2005.

[12] R. Lüllmann-Rauch, *Taschenlehrbuch Histologie*, 3rd ed. Thieme, 2009.

[13] B. D. Rose, *Clinical Physiology of Acid-Base and Electrolyte Disorders*, 5. A. New York: Mcgraw-Hill Education Ltd, 2000.

[14] T. Koshimizu, K. Nakamura, N. Egashira, M. Hiroyama, H. Nonoguchi, and A. Tanoue, “Vasopressin V1a and V1b receptors: from molecules to physiological systems,” *Physiol. Rev.*, vol. 92, no. 4, pp. 1813–1864, Oct. 2012.

[15] S. Qureshi, S. Galiveeti, D. G. Bichet, and J. Roth, “Diabetes insipidus: celebrating a century of vasopressin therapy,” *Endocrinology*, vol. 155, no. 12, pp. 4605–4621, Dec. 2014.

[16] J. F. Laycock, *Perspectives on Vasopressin*. London : Hackensack, NJ: Imperial College Press, 2010.

[17] C. H. Nickel, R. Bingisser, and N. G. Morgenthaler, “The role of copeptin as a diagnostic and prognostic biomarker for risk stratification in the emergency department,” *BMC Med.*, vol. 10, no. 1, p. 7, 2012.

[18] R. Roussel, L. Fezeu, M. Marre, G. Velho, F. Fumeron, P. Jungers, O. Lantieri, B. Balkau, N. Bouby, L. Bankir, and D. G. Bichet, “Comparison between copeptin and vasopressin in a population from the community and in people with chronic kidney disease,” *J. Clin. Endocrinol. Metab.*, vol. 99, no. 12, pp. 4656–4663, Dec. 2014.

[19] N. G. Morgenthaler, J. Struck, C. Alonso, and A. Bergmann, “Assay for the Measurement of Copeptin, a Stable Peptide Derived from the Precursor of Vasopressin,” *Clin. Chem.*, vol. 52, no. 1, pp. 112–119, Jan. 2006.

[20] G. Szinnai, N. G. Morgenthaler, K. Berneis, J. Struck, B. Müller, U. Keller, and M. Christ-Crain, “Changes in plasma copeptin, the c-terminal portion of arginine vasopressin during water

deprivation and excess in healthy subjects," *J. Clin. Endocrinol. Metab.*, vol. 92, no. 10, pp. 3973–3978, Oct. 2007.
[21] S. Jochberger, N. G. Morgenthaler, V. D. Mayr, G. Luckner, V. Wenzel, H. Ulmer, S. Schwarz, W. R. Hasibeder, B. E. Friesenecker, and M. W. Dünser, "Copeptin and Arginine Vasopressin Concentrations in Critically Ill Patients," *J. Clin. Endocrinol. Metab.*, vol. 91, no. 11, pp. 4381–4386, Nov. 2006.
[22] L. Dobsa and K. C. Edozien, "Copeptin and its potential role in diagnosis and prognosis of various diseases," *Biochem. Medica*, vol. 23, no. 2, pp. 172–190, 2013.
[23] A. Rauber, F. Kopsch, H. Leonhardt, G. Töndury, and K. Zilles, *Anatomie des Menschen, in 4 Bdn., Bd.3, Nervensystem, Sinnesorgane*. Thieme, Stuttgart, 1987.
[24] P. Norsk, P. Ellegaard, R. Videbaek, C. Stadeager, F. Jessen, L. B. Johansen, M. S. Kristensen, M. Kamegai, J. Warberg, and N. J. Christensen, "Arterial pulse pressure and vasopressin release in humans during lower body negative pressure," *Am. J. Physiol.*, vol. 264, no. 5 Pt 2, pp. R1024–1030, May 1993.
[25] B. Egan, R. Grekin, H. Ibsen, K. Osterziel, and S. Julius, "Role of cardiopulmonary mechanoreceptors in ADH release in normal humans," *Hypertension*, vol. 6, no. 6 Pt 1, pp. 832–836, Dec. 1984.
[26] M. L. Forsling, H. Montgomery, D. Halpin, R. J. Windle, and D. F. Treacher, "Daily patterns of secretion of neurohypophysial hormones in man: effect of age," *Exp. Physiol.*, vol. 83, no. 3, pp. 409–418, May 1998.
[27] M. A. Hofman and D. F. Swaab, "Influence of aging on the seasonal rhythm of the vasopressin-expressing neurons in the human suprachiasmatic nucleus," *Neurobiol. Aging*, vol. 16, no. 6, pp. 965–971, Dec. 1995.
[28] M. A. Hofman and D. F. Swaab, "Alterations in circadian rhythmicity of the vasopressin-producing neurons of the human suprachiasmatic nucleus (SCN) with aging," *Brain Res.*, vol. 651, no. 1–2, pp. 134–142, Jul. 1994.
[29] R. Asplund, "Pharmacotherapy for nocturia in the elderly patient," *Drugs Aging*, vol. 24, no. 4, pp. 325–343, 2007.
[30] J. L. H. R. Bosch and J. P. Weiss, "The prevalence and causes of nocturia," *J. Urol.*, vol. 184, no. 2, pp. 440–446, Aug. 2010.
[31] P. Laureanno and P. Ellsworth, "Demystifying nocturia: identifying the cause and tailoring the treatment," *Urol. Nurs.*, vol. 30, no. 5, pp. 276–286; quiz 287, Oct. 2010.
[32] E. Beitz, *Aquaporins*, 2009 Berlin: Springer, 2008.
[33] J. E. Kaufmann and U. M. Vischer, "Cellular mechanisms of the hemostatic effects of desmopressin (DDAVP)," *J. Thromb. Haemost. JTH*, vol. 1, no. 4, pp. 682–689, Apr. 2003.
[34] M. A. Blount, A. C. Mistry, O. Fröhlich, S. R. Price, G. Chen, J. M. Sands, and J. D. Klein, "Phosphorylation of UT-A1 urea transporter at serines 486 and 499 is important for vasopressin-regulated activity and membrane accumulation," *Am. J. Physiol. Renal Physiol.*, vol. 295, no. 1, pp. F295–F299, Jul. 2008.
[35] L. Bankir, D. G. Bichet, and N. Bouby, "Vasopressin V2 receptors, ENaC, and sodium reabsorption: a risk factor for hypertension?," *Am. J. Physiol. Renal Physiol.*, vol. 299, no. 5, pp. F917–928, Nov. 2010.
[36] S. Nielsen, J. Frøkiaer, D. Marples, T.-H. Kwon, P. Agre, and M. A. Knepper, "Aquaporins in the kidney: from molecules to medicine," *Physiol. Rev.*, vol. 82, no. 1, pp. 205–244, Jan. 2002.
[37] K. Eto, Y. Noda, S. Horikawa, S. Uchida, and S. Sasaki, "Phosphorylation of aquaporin-2 regulates its water permeability," *J. Biol. Chem.*, vol. 285, no. 52, pp. 40777–40784, Dec. 2010.
[38] H. Wen, J. Frokiaer, T. H. Kwon, and S. Nielsen, "Urinary excretion of aquaporin-2 in rat is mediated by a vasopressin-dependent apical pathway," *J. Am. Soc. Nephrol. JASN*, vol. 10, no. 7, pp. 1416–1429, Jul. 1999.

[39] T. Pisitkun, R.-F. Shen, and M. A. Knepper, “Identification and proteomic profiling of exosomes in human urine,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 101, no. 36, pp. 13368–13373, Sep. 2004.
[40] A. Al-Dameh, J. J. Bedford, J. P. Leader, and R. J. Walker, “Urinary aquaporin-2 levels in healthy volunteers,” *Nephrol. Carlton Vic*, vol. 8, no. 3, pp. 139–141, Jun. 2003.
[41] S. Iacobelli, J. B. Gouyon, F. Bonsante, L. Mastrofrancesco, M. Svelto, and G. Valenti, “Aquaporin-2 urinary excretion in preterm infants: relationship to diuresis and vasopressin,” *Acta Physiol. Oxf. Engl.*, vol. 200, no. 4, pp. 339–345, Dec. 2010.
[42] J. Starklint, J. N. Bech, and E. B. Pedersen, “Down-regulation of urinary AQP2 and unaffected response to hypertonic saline after 24 hours of fasting in humans,” *Kidney Int.*, vol. 67, no. 3, pp. 1010–1018, Mar. 2005.
[43] Y. Higashijima, H. Sonoda, S. Takahashi, H. Kondo, K. Shigemura, and M. Ikeda, “Excretion of urinary exosomal AQP2 in rats is regulated by vasopressin and urinary pH,” *Am. J. Physiol. Renal Physiol.*, vol. 305, no. 10, pp. F1412–1421, Nov. 2013.
[44] J.-D. Li, K. J. Burton, C. Zhang, S.-B. Hu, and Q.-Y. Zhou, “Vasopressin receptor V1a regulates circadian rhythms of locomotor activity and expression of clock-controlled genes in the suprachiasmatic nuclei,” *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, vol. 296, no. 3, pp. R824–830, Mar. 2009.
[45] C. Li, W. Wang, C. J. Rivard, M. A. Lanaspa, S. Summer, and R. W. Schrier, “Molecular mechanisms of angiotensin II stimulation on aquaporin-2 expression and trafficking,” *Am. J. Physiol. Renal Physiol.*, vol. 300, no. 5, pp. F1255–1261, May 2011.
[46] M. J. Radin, M.-J. Yu, L. Stoedkilde, R. L. Miller, J. D. Hoffert, J. Frokiaer, T. Pisitkun, and M. A. Knepper, “Aquaporin-2 regulation in health and disease,” *Vet. Clin. Pathol. Am. Soc. Vet. Clin. Pathol.*, vol. 41, no. 4, pp. 455–470, Dec. 2012.
[47] E. M. Hasser, V. S. Bishop, and M. Hay, “Interactions between vasopressin and baroreflex control of the sympathetic nervous system,” *Clin. Exp. Pharmacol. Physiol.*, vol. 24, no. 1, pp. 102–108, Jan. 1997.
[48] A. W. Cowley, E. Monos, and A. C. Guyton, “Interaction of vasopressin and the baroreceptor reflex system in the regulation of arterial blood pressure in the dog,” *Circ. Res.*, vol. 34, no. 4, pp. 505–514, Apr. 1974.
[49] X. Zhang, H. W. Hense, G. A. Riegger, and H. Schunkert, “Association of arginine vasopressin and arterial blood pressure in a population-based sample,” *J. Hypertens.*, vol. 17, no. 3, pp. 319–324, Mar. 1999.
[50] M. J. Cipolla, *The Cerebral Circulation*. San Rafael (CA): Morgan & Claypool Life Sciences, 2009.
[51] S. C. Woodhouse, *English-Greek Dictionary*. B&R Samizdat Express, 2007.
[52] J. Park, C. Yun, and S. Kang, “Physical Condition Does Not Affect Gravity-Induced Loss of Consciousness during Human Centrifuge Training in Well-Experienced Young Aviators,” *PloS One*, vol. 11, no. 1, p. e0147921, 2016.
[53] D. A. Self, C. D. White, R. M. Shaffstall, B. L. Mtinangi, J. S. Croft, and R. Hainsworth, “Differences between syncope resulting from rapid onset acceleration and orthostatic stress,” *Aviat. Space Environ. Med.*, vol. 67, no. 6, pp. 547–554, Jun. 1996.
[54] J. E. Naschitz and I. Rosner, “Orthostatic hypotension: framework of the syndrome,” *Postgrad. Med. J.*, vol. 83, no. 983, pp. 568–574, Sep. 2007.
[55] G. Clément, *Fundamentals of Space Medicine*, 2004 edition. Dordrecht, NE: Springer, 2005.
[56] J. Hanousek, P. Dosel, J. Cmiral, and J. Petricek, “Physiological response of pilots to the load of lower body negative pressure,” *J. Gravitational Physiol. J. Int. Soc. Gravitational Physiol.*, vol. 4, no. 2, pp. P33–34, Jul. 1997.
[57] C. Hinojosa-Laborde, K. L. Ryan, C. A. Rickards, and V. A. Convertino, “Resting sympathetic baroreflex sensitivity in subjects with low and high tolerance to central

hypovolemia induced by lower body negative pressure," *Front. Physiol.*, vol. 5, p. 241, 2014.
[58] H. Hinghofer-Szalkay, I. Loder, A. Rossler, and D. Jezova, "'Neutral point titration': cardiovascular regulation during combined (LBNP/HDT vs. LBPP/HUT) stimulation," *J. Gravitational Physiol. J. Int. Soc. Gravitational Physiol.*, vol. 9, no. 1, pp. P93–94, Jul. 2002.
[59] N. Goswami, E. Grasser, A. Roessler, D. Schneditz, and H. Hinghofer-Szalkay, "The cardiovascular response to lower body negative pressure in humans depends on seal location," *Physiol. Res. Acad. Sci. Bohemoslov.*, vol. 58, no. 3, pp. 311–318, 2009.
[60] R. Peinado Peinado, "[Is the prognostic significance of presyncope the same as for syncope?]," *Rev. Esp. Cardiol.*, vol. 57, no. 7, pp. 613–616, Jul. 2004.
[61] A. Natale, M. Akhtar, M. Jazayeri, A. Dhala, Z. Blanck, S. Deshpande, A. Krebs, and J. S. Sra, "Provocation of Hypotension During Head-Up Tilt Testing in Subjects With No History of Syncope or Presyncope," *Circulation*, vol. 92, no. 1, pp. 54–58, Jul. 1995.
[62] V. A. Convertino, D. A. Ludwig, B. D. Gray, and J. Vernikos, "Effects of exposure to simulated microgravity on neuronal catecholamine release and blood pressure responses to norepinephrine and angiotensin," *Clin. Auton. Res. Off. J. Clin. Auton. Res. Soc.*, vol. 8, no. 2, pp. 101–110, Apr. 1998.
[63] P. Alboni and R. Furlan, Eds., *Vasovagal Syncope*, 2015 edition. New York, NY: Springer, 2014.
[64] B. P. G. MD and B. Olshansky, *Syncope: Mechanisms and Management*, 2nd edition. Malden, Mass: Wiley-Blackwell, 2005.
[65] H. Hinghofer-Szalkay, H. K. Lackner, A. Rössler, B. Narath, A. Jantscher, and N. Goswami, "Hormonal and plasma volume changes after presyncope," *Eur. J. Clin. Invest.*, vol. 41, no. 11, pp. 1180–1185, Nov. 2011.
[66] H. Edgell, A. Grinberg, N. Gagné, K. R. Beavers, and R. L. Hughson, "Cardiovascular responses to lower body negative pressure before and after 4 h of head-down bed rest and seated control in men and women," *J. Appl. Physiol. Bethesda Md 1985*, vol. 113, no. 10, pp. 1604–1612, Nov. 2012.
[67] A. Roessler, N. Goswami, B. Haditsch, J. A. Loeppky, F. C. Luft, and H. Hinghofer-Szalkay, "Volume regulating hormone responses to repeated head-up tilt and lower body negative pressure," *Eur. J. Clin. Invest.*, vol. 41, no. 8, pp. 863–869, Aug. 2011.
[68] J. E. Greenleaf, T. W. Petersen, A. Gabrielsen, B. Pump, P. Bie, N. J. Christensen, J. Warberg, R. Videbaek, S. R. Simonson, and P. Norsk, "Low LBNP tolerance in men is associated with attenuated activation of the renin-angiotensin system," *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, vol. 279, no. 3, pp. R822–829, Sep. 2000.
[69] S. R. Goldsmith, G. S. Francis, A. W. Cowley, and J. N. Cohn, "Response of vasopressin and norepinephrine to lower body negative pressure in humans," *Am. J. Physiol. - Heart Circ. Physiol.*, vol. 243, no. 6, pp. H970–H973, Dec. 1982.
[70] K. Sander-Jensen, N. H. Secher, A. Astrup, N. J. Christensen, M. Damkjaer-Nielsen, J. Giese, J. Warberg, and P. Bie, "Angiotensin II attenuates reflex decrease in heart rate and sympathetic activity in man," *Clin. Physiol. Oxf. Engl.*, vol. 8, no. 1, pp. 31–40, Feb. 1988.
[71] M. S. Hammerum, P. Bie, B. Pump, L. B. Johansen, N. J. Christensen, and P. Norsk, "Vasopressin, angiotensin II and renal responses during water immersion in hydrated humans," *J. Physiol.*, vol. 511, Pt 1, pp. 323–330, Aug. 1998.
[72] Z. László, A. Rössler, and H. G. Hinghofer-Szalkay, "Cardiovascular and hormonal changes with different angles of head-up tilt in men," *Physiol. Res. Acad. Sci. Bohemoslov.*, vol. 50, no. 1, pp. 71–82, 2001.
[73] A. Kitano, J. K. Shoemaker, M. Ichinose, H. Wada, and T. Nishiyasu, "Comparison of cardiovascular responses between lower body negative pressure and head-up tilt," *J. Appl. Physiol. Bethesda Md 1985*, vol. 98, no. 6, pp. 2081–2086, Jun. 2005.
[74] B. D. Levine, L. D. Lane, J. C. Buckey, D. B. Friedman, and C. G. Blomqvist, "Left

ventricular pressure-volume and Frank-Starling relations in endurance athletes. Implications for orthostatic tolerance and exercise performance," *Circulation*, vol. 84, no. 3, pp. 1016–1023, Sep. 1991.
[75] V. A. Convertino, L. D. Tripp, D. A. Ludwig, J. Duff, and T. L. Chelette, "Female exposure to high G: chronic adaptations of cardiovascular functions," *Aviat. Space Environ. Med.*, vol. 69, no. 9, pp. 875–882, Sep. 1998.
[76] J. R. Meendering, B. N. Torgrimson, B. L. Houghton, J. R. Halliwill, and C. T. Minson, "Menstrual cycle and sex affect hemodynamic responses to combined orthostatic and heat stress," *Am. J. Physiol. Heart Circ. Physiol.*, vol. 289, no. 2, pp. H631–642, Aug. 2005.
[77] Q. Fu, A. Arbab-Zadeh, M. A. Perhonen, R. Zhang, J. H. Zuckerman, and B. D. Levine, "Hemodynamics of orthostatic intolerance: implications for gender differences," *Am. J. Physiol. Heart Circ. Physiol.*, vol. 286, no. 1, pp. H449–457, Jan. 2004.
[78] W. D. Franke, C. P. Johnson, J. A. Steinkamp, R. Wang, and J. R. Halliwill, "Cardiovascular and autonomic responses to lower body negative pressure: do not explain gender differences in orthostatic tolerance," *Clin. Auton. Res. Off. J. Clin. Auton. Res. Soc.*, vol. 13, no. 1, pp. 36–44, Feb. 2003.
[79] W. W. Waters, M. G. Ziegler, and J. V. Meck, "Postspaceflight orthostatic hypotension occurs mostly in women and is predicted by low vascular resistance," *J. Appl. Physiol. Bethesda Md 1985*, vol. 92, no. 2, pp. 586–594, Feb. 2002.
[80] D. D. White, R. W. Gotshall, and A. Tucker, "Women have lower tolerance to lower body negative pressure than men," *J. Appl. Physiol. Bethesda Md 1985*, vol. 80, no. 4, pp. 1138–1143, Apr. 1996.
[81] S. S. Jarvis, J. P. Florian, M. J. Curren, and J. A. Pawelczyk, "Sex differences in vasoconstrictor reserve during 70 deg head-up tilt," *Exp. Physiol.*, vol. 95, no. 1, pp. 184–193, Jan. 2010.
[82] T. Russomano, F. May, G. Dalmarco, and R. R. Baptista, "A Gender comparison of Cardiovascular Responses to Lower Body Negative Pressure Exposure," *Am. J. Med. Biol. Res.*, vol. 3, no. 4, pp. 95–101, 2015.
[83] H. Tagawa, H. Shimokawa, T. Tagawa, M. Kuroiwa-Matsumoto, Y. Hirooka, and A. Takeshita, "Short-term estrogen augments both nitric oxide-mediated and non-nitric oxide-mediated endothelium-dependent forearm vasodilation in postmenopausal women," *J. Cardiovasc. Pharmacol.*, vol. 30, no. 4, pp. 481–488, Oct. 1997.
[84] M. Tanaka, M. Sato, S. Umehara, and T. Nishikawa, "Influence of menstrual cycle on baroreflex control of heart rate: comparison with male volunteers," *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, vol. 285, no. 5, pp. R1091–1097, Nov. 2003.
[85] C. T. Minson, J. R. Halliwill, T. M. Young, and M. J. Joyner, "Influence of the menstrual cycle on sympathetic activity, baroreflex sensitivity, and vascular transduction in young women," *Circulation*, vol. 101, no. 8, pp. 862–868, Feb. 2000.
[86] D. Hartwich, S. Aldred, and J. P. Fisher, "Influence of menstrual cycle phase on muscle metaboreflex control of cardiac baroreflex sensitivity, heart rate and blood pressure in humans," *Exp. Physiol.*, vol. 98, no. 1, pp. 220–232, Jan. 2013.
[87] T. Findley, "Role of the neurohypophysis in the pathogenesis of hypertension and some allied disorders associated with aging," *Am. J. Med.*, vol. 7, no. 1, pp. 70–84, Jul. 1949.
[88] L. E. Cowen, S. P. Hodak, and J. G. Verbalis, "Age-Associated Abnormalities of Water Homeostasis," *Endocrinol. Metab. Clin. North Am.*, vol. 42, no. 2, pp. 349–370, Jun. 2013.
[89] P. A. O'Neill and K. A. McLean, "Water homeostasis and ageing," *Med. Lab. Sci.*, vol. 49, no. 4, pp. 291–298, Dec. 1992.
[90] J. W. Rowe, N. W. Shock, and R. A. DeFronzo, "The influence of age on the renal response to water deprivation in man," *Nephron*, vol. 17, no. 4, pp. 270–278, 1976.
[91] J. M. Sands, "Urine concentrating and diluting ability during aging," *J. Gerontol. A. Biol.*

Sci. Med. Sci., vol. 67, no. 12, pp. 1352–1357, Dec. 2012.
[92] J. J. Figueroa, J. R. Basford, and P. A. Low, “Preventing and treating orthostatic hypotension: As easy as A, B, C,” *Cleve. Clin. J. Med.*, vol. 77, no. 5, pp. 298–306, May 2010.
[93] S. Combet, L. Teillet, G. Geelen, B. Pitrat, R. Gobin, S. Nielsen, M. M. Trinh-Trang-Tan, B. Corman, and J. M. Verbavatz, “Food restriction prevents age-related polyuria by vasopressin-dependent recruitment of aquaporin-2,” *Am. J. Physiol. Renal Physiol.*, vol. 281, no. 6, pp. F1123–1131, Dec. 2001.
[94] S. Combet, S. Gouraud, R. Gobin, V. Berthonaud, G. Geelen, B. Corman, and J.-M. Verbavatz, “Aquaporin-2 downregulation in kidney medulla of aging rats is posttranscriptional and is abolished by water deprivation,” *Am. J. Physiol. Renal Physiol.*, vol. 294, no. 6, pp. F1408–1414, Jun. 2008.
[95] C. A. Miranda, J. W. Lee, C.-L. Chou, and M. A. Knepper, “Tolvaptan as a tool in renal physiology,” *Am. J. Physiol. Renal Physiol.*, vol. 306, no. 3, pp. F359–366, Feb. 2014.
[96] R. W. Schrier, “The sea within us: disorders of body water homeostasis,” *Curr. Opin. Investig. Drugs Lond. Engl. 2000*, vol. 8, no. 4, pp. 304–311, Apr. 2007.
[97] A. J. Jovanovich and T. Berl, “Where vaptans do and do not fit in the treatment of hyponatremia,” *Kidney Int.*, vol. 83, no. 4, pp. 563–567, Apr. 2013.
[98] N. A. Bijan Rezakhaniha, “Efficacy of desmopressin in treatment of nocturia in elderly men.,” *J. Res. Med. Sci. Off. J. Isfahan Univ. Med. Sci.*, vol. 16, no. 4, pp. 516–23, 2011.
[99] S. R. Goldsmith, “The role of vasopressin in congestive heart failure,” *Cleve. Clin. J. Med.*, vol. 73 Suppl 3, pp. S19–23, Sep. 2006.
[100] Konstam MA, Gheorghiade M, Burnett JC, and et al, “Effects of oral tolvaptan in patients hospitalized for worsening heart failure: The everest outcome trial,” *JAMA*, vol. 297, no. 12, pp. 1319–1331, März 2007.
[101] L. Share, J. T. Crofton, and Y. Ouchi, “Vasopressin: sexual dimorphism in secretion, cardiovascular actions and hypertension,” *Am. J. Med. Sci.*, vol. 295, no. 4, pp. 314–319, Apr. 1988.
[102] J. T. Crofton, H. Dustan, L. Share, and D. P. Brooks, “Vasopressin secretion in normotensive black and white men and women on normal and low sodium diets,” *J. Endocrinol.*, vol. 108, no. 2, pp. 191–199, Feb. 1986.
[103] J. Perucca, N. Bouby, P. Valeix, and L. Bankir, “Sex difference in urine concentration across differing ages, sodium intake, and level of kidney disease,” *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, vol. 292, no. 2, pp. R700–705, Feb. 2007.
[104] M. Perinpam, E. B. Ware, J. A. Smith, S. T. Turner, S. L. R. Kardia, and J. C. Lieske, “Key influence of sex on urine volume and osmolality,” *Biol. Sex Differ.*, vol. 7, p. 12, 2016.
[105] J. T. Crofton, P. G. Baer, L. Share, and D. P. Brooks, “Vasopressin release in male and female rats: effects of gonadectomy and treatment with gonadal steroid hormones,” *Endocrinology*, vol. 117, no. 3, pp. 1195–1200, Sep. 1985.
[106] R. Punnonen, O. Viinamäki, R. Lammintausta, and L. Rauramo, “Effect of estrogens on plasma vasopressin and renin activity in postmenopausal women,” *Asia-Ocean. J. Obstet. Gynaecol. AOFOG*, vol. 8, no. 4, pp. 381–384, Dec. 1982.
[107] M. L. Forsling, P. Strömberg, and M. Åkerlund, “Effect of ovarian steroids on vasopressin secretion,” *J. Endocrinol.*, vol. 95, no. 1, pp. 147–151, Oct. 1982.
[108] N. S. Stachenfeld, L. DiPietro, S. F. Palter, and E. R. Nadel, “Estrogen influences osmotic secretion of AVP and body water balance in postmenopausal women,” *Am. J. Physiol.*, vol. 274, no. 1 Pt 2, pp. R187–195, Jan. 1998.
[109] E. Schillinger, P. Ramwell, and G. Rubanyi, *Sex Steroids and the Cardiovascular System*. Berlin ; New York: Springer-Verlag, 1992.
[110] M. L. Forsling, M. Akerlund, and P. Strömberg, “Variations in plasma concentrations of vasopressin during the menstrual cycle,” *J. Endocrinol.*, vol. 89, no. 2, pp. 263–266, May 1981.

[111] T. J. Vokes, N. M. Weiss, J. Schreiber, M. B. Gaskill, and G. L. Robertson, “Osmoregulation of thirst and vasopressin during normal menstrual cycle,” *Am. J. Physiol.*, vol. 254, no. 4 Pt 2, pp. R641–647, Apr. 1988.
[112] R. Punnonen, O. Viinamäki, and S. Multamäki, “Plasma vasopressin during normal menstrual cycle,” *Horm. Res.*, vol. 17, no. 2, pp. 90–92, 1983.
[113] B. A. Spruce, P. H. Baylis, J. Burd, and M. J. Watson, “Variation in osmoregulation of arginine vasopressin during the human menstrual cycle,” *Clin. Endocrinol. (Oxf.)*, vol. 22, no. 1, pp. 37–42, Jan. 1985.
[114] M. Stauber and T. Weyerstahl, *Duale Reihe Gynäkologie und Geburtshilfe*, 3., aktualisierte Auflage. Thieme, 2007.
[115] D. A. Holwerda, “A glycopeptide from the posterior lobe of pig pituitaries. I. Isolation and characterization,” *Eur. J. Biochem. FEBS*, vol. 28, no. 3, pp. 334–339, Jul. 1972.
[116] S. Mohanty and G. Asha, “A novel stress neurohormone copeptin: its potential role in diagnosis and prognosis of various diseases,” 2015. [Online]. Available: http://www.wjpr.net/dashboard/abstract_id/2075. [Accessed: 06-Apr-2016].
[117] F. M. de Bree and J. P. Burbach, “Structure-function relationships of the vasopressin prohormone domains,” *Cell. Mol. Neurobiol.*, vol. 18, no. 2, pp. 173–191, Apr. 1998.
[118] C. Barat, L. Simpson, and E. Breslow, “Properties of human vasopressin precursor constructs: inefficient monomer folding in the absence of copeptin as a potential contributor to diabetes insipidus,” *Biochemistry (Mosc.)*, vol. 43, no. 25, pp. 8191–8203, Jun. 2004.
[119] T. Hew-Butler, K. J. Stuempfle, and M. D. Hoffman, “Bone: an acute buffer of plasma sodium during exhaustive exercise?,” *Horm. Metab. Res. Horm. Stoffwechselforschung Horm. Métabolisme*, vol. 45, no. 10, pp. 697–700, Sep. 2013.
[120] G. Koch and I. Schnyder, “Model for characterizing copeptin kinetics and response in healthy subjects,” 2015. [Online]. Available: http://www.page-meeting.org/?abstract=3501. [Accessed: 25-Aug-2015].
[121] I. Schnyder, K. Strausz, G. Koch, C. Walti, M. Pfister, B. Allolio, W. K. Fenske, and M. Christ-Crain, “Physiological area of normality of copeptin in normal-to-hyperosmolar states,” *Endocr. Abstr.*, May 2015.
[122] S. S. Bhandari, I. Loke, J. E. Davies, I. B. Squire, J. Struck, and L. L. Ng, “Gender and renal function influence plasma levels of copeptin in healthy individuals,” *Clin. Sci. Lond. Engl. 1979*, vol. 116, no. 3, pp. 257–263, Feb. 2009.
[123] G. L. Robertson, E. A. Mahr, S. Athar, and T. Sinha, “Development and Clinical Application of a New Method for the Radioimmunoassay of Arginine Vasopressin in Human Plasma,” *J. Clin. Invest.*, vol. 52, no. 9, pp. 2340–2352, Sep. 1973.
[124] M. Kluge, S. Riedl, B. Erhart-Hofmann, J. Hartmann, and F. Waldhauser, “Improved extraction procedure and RIA for determination of arginine8-vasopressin in plasma: role of premeasurement sample treatment and reference values in children,” *Clin. Chem.*, vol. 45, no. 1, pp. 98–103, Jan. 1999.
[125] K. Narkiewicz, R. L. Cooley, and V. K. Somers, “Alcohol Potentiates Orthostatic Hypotension Implications for Alcohol-Related Syncope,” *Circulation*, vol. 101, no. 4, pp. 398–402, Feb. 2000.
[126] A. C. Arnold, C. E. Ramirez, L. Choi, L. E. Okamoto, A. Gamboa, A. Diedrich, S. R. Raj, D. Robertson, I. Biaggioni, and C. A. Shibao, “Combination ergotamine and caffeine improves seated blood pressure and presyncopal symptoms in autonomic failure,” *Front. Physiol.*, vol. 5, p. 270, 2014.
[127] A. W. Quail, F. M. Traugott, W. L. Porges, and S. W. White, “Thoracic resistivity for stroke volume calculation in impedance cardiography,” *J. Appl. Physiol.*, vol. 50, no. 1, pp. 191–195, Jan. 1981.
[128] G. L. Robertson and S. Athar, “The interaction of blood osmolality and blood volume in

regulating plasma vasopressin in man," *J. Clin. Endocrinol. Metab.*, vol. 42, no. 4, pp. 613–620, Apr. 1976.
[129] W. N. Leimbach, P. G. Schmid, and A. L. Mark, "Baroreflex control of plasma arginine vasopressin in humans," *Am. J. Physiol.*, vol. 247, no. 4 Pt 2, pp. H638–644, Oct. 1984.
[130] K. Sato, T. Kimura, K. Ota, M. Shoji, M. Ohta, T. Yamamoto, T. Funyu, and K. Abe, "Changes in plasma vasopressin levels and cardiovascular function due to postural changes in diabetic neuropathy," *Tohoku J. Exp. Med.*, vol. 177, no. 1, pp. 49–60, Sep. 1995.
[131] C. I. Saad, A. B. Ribeiro, M. T. Zanella, R. A. Mulinari, I. Gavras, and H. Gavras, "The role of vasopressin in blood pressure maintenance in diabetic orthostatic hypotension," *Hypertension*, vol. 11, no. 2 Pt 2, pp. I217–221, Feb. 1988.
[132] S. R. Goldsmith, G. S. Francis, A. W. Cowley, T. B. Levine, and J. N. Cohn, "Increased plasma arginine vasopressin levels in patients with congestive heart failure," *J. Am. Coll. Cardiol.*, vol. 1, no. 6, pp. 1385–1390, Jun. 1983.
[133] B. Pump, A. Gabrielsen, N. J. Christensen, P. Bie, M. Bestle, and P. Norsk, "Mechanisms of inhibition of vasopressin release during moderate antiorthostatic posture change in humans," *Am. J. Physiol.*, vol. 277, no. 1 Pt 2, pp. R229–235, Jul. 1999.
[134] T. Tsuchihashi, M. Ueno, H. Muratani, Y. Tomita, S. Takishita, and M. Fujishima, "Effects of Sodium Depletion and Orthostasis on Plasma and Urinary Vasopressin in Normal Subjects," *Endocrinol. Jpn.*, vol. 36, no. 2, pp. 237–243, 1989.
[135] J. R. Claybaugh, Y.-C. Lin, H. G. Schafstall, and P. B. Bennett, "Cardiovascular and endocrine responses to 90 degree tilt during a 35-day saturation dive to 46 and 37 ATA," *Aviat. Space Environ. Med.*, vol. 78, no. 11, pp. 1042–1049, Nov. 2007.
[136] M. Cignarelli, G. De Pergola, A. Paternostro, M. Corso, M. R. Cospite, G. M. Centaro, and R. Giorgino, "Arginine-vasopressin response to supine-erect posture change: an index for evaluation of the integrity of the afferent component of baroregulatory system in diabetic neuropathy," *Diabète Métabolisme*, vol. 12, no. 1, pp. 28–33, Feb. 1986.
[137] J. P. Wolf, N. U. Nguyen, A. Baulay, G. Dumoulin, and S. Berthelay, "The role of posture on the changes in plasma atrial natriuretic factor and arginine vasopressin levels during immersion," *Eur. J. Appl. Physiol.*, vol. 61, no. 3–4, pp. 284–288, 1990.
[138] A. Viti, C. Lupo, L. Lodi, M. Bonifazi, and G. Martelli, "Hormonal changes after supine posture, immersion, and swimming," *Int. J. Sports Med.*, vol. 10, no. 6, pp. 402–405, Dec. 1989.
[139] A. Gabrielsen, J. Warberg, N. J. Christensen, P. Bie, C. Stadeager, B. Pump, and P. Norsk, "Arterial pulse pressure and vasopressin release during graded water immersion in humans," *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, vol. 278, no. 6, pp. R1583–1588, Jun. 2000.
[140] M. Shiraishi, M. Schou, M. Gybel, N. J. Christensen, and P. Norsk, "Comparison of acute cardiovascular responses to water immersion and head-down tilt in humans," *J. Appl. Physiol. Bethesda Md 1985*, vol. 92, no. 1, pp. 264–268, Jan. 2002.
[141] S. W. Lord, S. Brady, P. H. Baylis, J. H. Dark, R. A. Kenny, and J. M. McComb, "Vasopressin release during orthostatic hypotension after cardiac transplantation," *Clin. Auton. Res. Off. J. Clin. Auton. Res. Soc.*, vol. 6, no. 6, pp. 351–357, Dec. 1996.
[142] B. D. Johnson, N. van Helmond, T. B. Curry, C. M. van Buskirk, V. A. Convertino, and M. J. Joyner, "Reductions in central venous pressure by lower body negative pressure or blood loss elicit similar hemodynamic responses," *J. Appl. Physiol. Bethesda Md 1985*, vol. 117, no. 2, pp. 131–141, Jul. 2014.
[143] C. Giannattasio, A. Del Bo, B. M. Cattaneo, C. Cuspidi, E. Gronda, M. Frigerio, M. Mangiavacchi, M. Marabini, C. De Vita, and G. Grassi, "Reflex vasopressin and renin modulation by cardiac receptors in humans," *Hypertension*, vol. 21, no. 4, pp. 461–469, Apr. 1993.
[144] P. Norsk, F. Bonde-Petersen, and J. Warberg, "Influence of central venous pressure change

on plasma vasopressin in humans," *J. Appl. Physiol.*, vol. 61, no. 4, pp. 1352–1357, Oct. 1986.
[145] B. Trimarco, N. De Luca, B. Ricciardelli, A. Cuocolo, A. De Simone, M. Volpe, A. F. Mele, and M. Condorelli, "Impaired responsiveness of the ventricular sensory receptor in hypertensive patients with left ventricular hypertrophy," *Circulation*, vol. 74, no. 5, pp. 980–990, Nov. 1986.
[146] B. Trimarco, G. Lembo, N. De Luca, M. Volpe, B. Ricciardelli, G. Condorelli, G. Rosiello, and M. Condorelli, "Blunted sympathetic response to cardiopulmonary receptor unloading in hypertensive patients with left ventricular hypertrophy. A possible compensatory role of atrial natriuretic factor," *Circulation*, vol. 80, no. 4, pp. 883–892, Oct. 1989.
[147] R. van Hoeyweghen, J. Hanson, M. J. Stewart, L. Dethune, I. Davies, R. A. Little, M. A. Horan, and E. Kirkman, "Cardiovascular response to graded lower body negative pressure in young and elderly man," *Exp. Physiol.*, vol. 86, no. 3, pp. 427–435, May 2001.
[148] P. I. Nedvetsky, G. Tamma, S. Beulshausen, G. Valenti, W. Rosenthal, and E. Klussmann, "Regulation of aquaporin-2 trafficking," *Handb. Exp. Pharmacol.*, no. 190, pp. 133–157, 2009.
[149] M. Buemi, F. Corica, G. Di Pasquale, C. Aloisi, M. Sofi, T. Casuscelli, F. Floccari, M. Senatore, A. Corsonello, and N. Frisina, "Water immersion increases urinary excretion of aquaporin-2 in healthy humans," *Nephron*, vol. 85, no. 1, pp. 20–26, May 2000.
[150] T. Rai, K. Sekine, K. Kanno, K. Hata, M. Miura, A. Mizushima, F. Marumo, and S. Sasaki, "Urinary excretion of aquaporin-2 water channel protein in human and rat," *J. Am. Soc. Nephrol. JASN*, vol. 8, no. 9, pp. 1357–1362, Sep. 1997.
[151] P. H. Baylis and D. A. Heath, "Influence of presyncope and postural change upon plasma arginine vasopressing concentration in hydrated and dehydrated man," *Clin. Endocrinol. (Oxf.)*, vol. 7, no. 1, pp. 79–83, Jul. 1977.
[152] K. Hu, F. A. J. L. Scheer, M. Laker, C. Smales, and S. A. Shea, "Endogenous circadian rhythm in vasovagal response to head-up tilt," *Circulation*, vol. 123, no. 9, pp. 961–970, Mar. 2011.