Diploma Thesis

Gender differences in cardiovascular responses to orthostatic challenge in healthy older persons

submitted by

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Declaration

I hereby declare that I have authored this thesis independently, that I have not used other than the declared sources / resources, and that I have explicitly marked all material that have been quoted either literally or by content from the used sources.

Berlin, August 8, 2017 Catharina Sachse eh

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Abstract

Introduction: Premenopausal women show a higher incidence of orthostatic hypotension than age-matched men, but there is limited data available on gender differences in cardiovascular responses to orthostatic challenge in healthy older persons. Studying orthostatic hypotension is important because it is a major contributing factor of falls among older persons and it is associated with higher morbidity and mortality at an older age.

Aims: The overall aim of this study was to investigate gender differences in hemodynamic and autonomic responses to orthostatic challenge in healthy older males and females. Due to the effects of menopause on sex-steroid hormones, it was hypothesized that an orthostatic challenge in older persons will not lead to differences in cardiovascular and autonomic responses across gender.

Methodology: Fourteen older healthy women and ten age-matched men performed a sit to stand test (five minutes of sitting followed by six minutes of standing). Task Force® Monitor was used to continuously measure the following beat-to-beat hemodynamic parameters: heart rate, systolic blood pressure, diastolic blood pressure, mean blood pressure, stroke index, cardiac index and total peripheral resistance index. Cardiac autonomic activity was calculated using power spectral analysis of heart rate variability. Measurement of low-frequency (LF: 0.04 to 0.15 Hz) and high-frequency (HF: 0.15 to 0.4 Hz) components was made in absolute values and normalized units.

Results: More women than men fulfilled the criteria for orthostatic hypotension (35.7% vs. 11.1%). However, across all hemodynamic parameters, there were no significant differences between the sexes at baseline level and during standing. LFnuRRI (median: 70.2 vs. 52.3, $p<0.05$) and LF/HF ratio (median: 2.4 vs. 1.1, $p<0.05$) were significantly higher, while HFnuRRI (median 29.8 vs. 47.7, $p<0.05$) was lower among women during the baseline. All other heart rate variability measures did not differ between both sexes.

Conclusion: The data indicate that orthostatic hypotension occurs more frequently in older women than in men. Further study is required to determine the underlying mechanisms contributing to higher incidence of orthostatic hypotension in older females. Additionally, older women showed higher sympathetic and lower parasympathetic activity at rest compared to age-matched men. These results are

contradictory to the observations from previous studies, which showed a reduced sympathetic and enhanced parasympathetic activity in women in all ages.

Zusammenfassung

Einleitung: Prämenopausale Frauen sind häufiger von orthostatischer Hypotonie betroffen als Männer. Es existieren jedoch nur wenige Studien, die Geschlechtsunterschiede in den kardiovaskulären Reaktionen gesunder älterer Personen auf eine orthostatische Belastung untersuchen. Da orthostatische Hypotonie insbesondere im Alter einen Risikofaktor für Stürze darstellt und mit einer höheren Morbidität und Mortalität verbunden ist, ist es wichtig, die Auswirkungen orthostatischer Belastung in dieser Altersgruppe genauer zu untersuchen.

Zielsetzung: Ziel dieser Studie war es, geschlechtsspezifische Unterschiede bei gesunden älteren Personen in den hämodynamischen und autonomen Reaktionen auf eine orthostatische Belastung zu untersuchen. Angesichts des abnehmenden Einflusses weiblicher Sexualhormone auf die kardiovaskuläre Regulation nach der Menopause, gehen wir davon aus, dass sich in der von uns untersuchten Altersgruppe keine Geschlechtsunterschiede mehr finden.

Methoden: Vierzehn ältere gesunde Frauen und zehn gleichaltrige gesunde Männer unterzogen sich einem Sit-to-Stand Test mit einer fünfminütigen Sitz- und einer sechsminütigen Standphase. Mittels Task Force® Monitor erfolgte die kontinuierliche Messung der hämodynamischen Parameter (Herzfrequenz, Blutdruck, Schlagindex, Herzindex, totaler peripherer Gefäßwiderstandsindex). Die Beurteilung der autonomen Funktion erfolgte mittels Spektralanalyse der Herzfrequenzvariabilität. Die Power in den hochfrequenten (HF: 0.15-0.4 Hz) und niederfrequenten (LF: 0.04-0.15 Hz) Bändern wurde in absoluten Werten und normalisierten Einheiten angegeben.

Ergebnisse: Es erfüllten mehr Frauen als Männer die Kriterien für orthostatische Hypotonie (35.7% vs. 11.1%). Es fand sich jedoch kein signifikanter Geschlechtsunterschied in den gemessenen hämodynamischen Parametern während der Sitz- und der Standphase. Frauen zeigten während der Baseline signifikant höhere LFnuRRI (Median: 70.2 vs. 52.3, $p<0.05$) und LF/HF Ratio (Median: 2.4 vs. 1.1, $p<0.05$), sowie signifikant niedrigere HFnuRRI (median 29.8 vs. 47.7, $p<0.05$) im Vergleich zu Männern.

Schlussfolgerung: Die Daten belegen, dass gesunde ältere Frauen häufiger von orthostatischer Hypotonie betroffen sind als gleichaltrige gesunde Männer. Um

den unseren Ergebnissen zugrunde liegenden Mechanismen nachzugehen, sind weitere Studien erforderlich. Darüber hinaus zeigten die untersuchten Frauen eine höhere sympathische und eine niedrigere parasympathische Aktivität in Ruhe als die gleichaltrigen Männer. Diese Ergebnisse widersprechen den Beobachtungen früherer Studien, welche eine erniedrigte sympathische und eine erhöhte parasympathische Aktivität bei Frauen aller Altersklassen zeigten.

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List of Abbreviations

ACE	angiotensin converting enzyme
ADH	antidiuretic hormone
ANP	atrial natriuretic peptide
bpm	beats per minute
CI	cardiac index
CNS	central nervous system
CO	cardiac output
dBP	diastolic blood pressure
ECG	electrocardiography
FFT	fast Fourier transform
HF	high-frequency
HFnu	normalized high-frequency
HFRRI	low-frequency R-R interval
HR	heart rate
HRT	hormone replacement therapy
HRV	heart rate variability
HUT	head-up tilt
ICG	impedance cardiography
LBNP	lower body negative pressure
LF	low-frequency
LFnu	normalized low-frequency
LFRRI	low-frequency R-R interval
MAP	mean arterial pressure
mBP	mean blood pressure
mmHg	millimeter of mercury
MSNA	muscle sympathetic nerve activity
OH	orthostatic hypotension
$PaCO_2$	arterial partial pressure of carbon dioxide
PaO_2	arterial partial pressure of oxygen
RAAS	renin-angiotensin-aldosterone-system
SAN	sinoatrial node
sBP	systolic blood pressure

SD	standard deviation
SI	stroke index
SV	stroke volume
TFM	Task Force Monitor
TPR	total peripheral resistance
TPRI	total peripheral resistance index
VLF	very low-frequency
WMA	World Medical Association

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1 Introduction

1.1 The cardiovascular system: elements and function

A functioning circulatory system is essential for the survival. Its main functions are the distribution of dissolved gases, molecules for nutrition and the removal of waste products. In addition, the circulatory system plays an important role in the intercellular communication by transporting hormones and neurotransmitters from one organ to another, in the temperature regulation by emitting heat and in the inflammatory response. Generally the cardiovascular system maintains conditions for ideal survival and function of all cells, tissues and organs.

The cardiovascular system comprises three functional parts: the heart, the blood vessels and the blood, which are explained in more detail in the following subchapters.

1.1.1 The heart

The primary role of the heart is the supply of blood to the organs. The heart, shown in Figure 1, is a hollow muscular organ consisting of two pulsatile two-chamber pumps controlling the blood flow in two circuits, the pulmonary circulation and the systemic circulation. The right-sided heart receives blood from the vena cavae and pumps it through the lungs, whereas the left-sided heart pumps the oxygenated blood coming from the lungs through the peripheral organs (1,2). The cardiac rhythmicity ensures the efficiency of the pumping of the different chambers from the venous side to the arterial side. The direction of the blood flow is determined by the configuration of the heart valves, which assure an unidirectional way (Figure 1) (3).

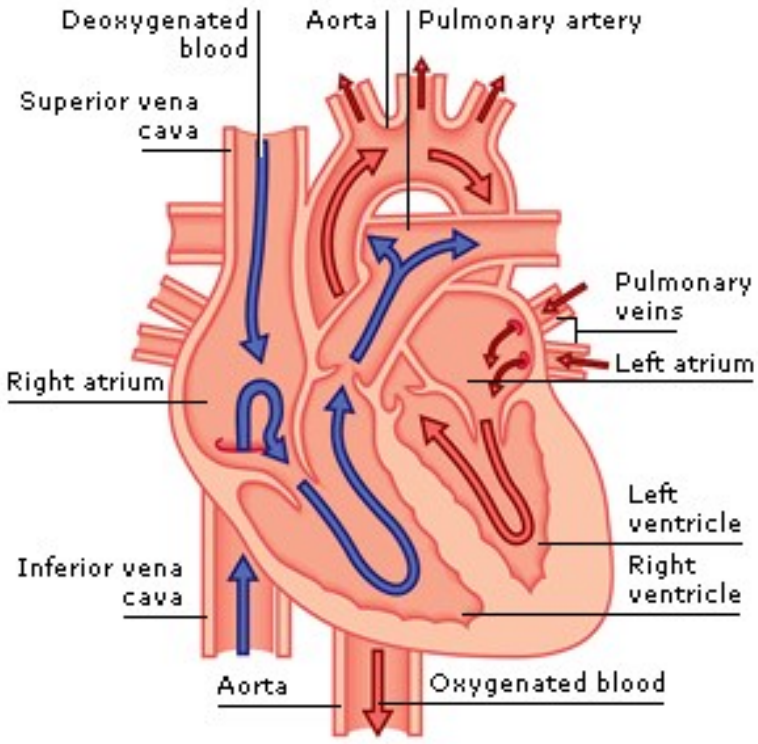


Figure 1: Blood flow through the heart.

Obtained from: https://www.aviva.co.uk/health-insurance/home-of-health/medical-centre/medical-encyclopedia/entry/function-blood-flow-through-the-heart (22.06.2017).

1.1.2 Vascular system

The vascular network consists of different types of vessels, which can be grouped by their function. The arteries primary function is the distribution of the oxygenated blood coming from the left-sided heart to specific organs or regions of the body. They are thick-walled and have an extensive elastic tissue and smooth muscle. Because of their vascular distensibility the aorta is able to dampen the pulsatile pressure out of the heart, whereas the large arteries do not contribute to regulation the blood flow and arterial pressure. The smallest branches of the arterial system are the arterioles, which have a diameter of <200μm (3). Arterioles, acting as the resistance vessels, are responsible for arterial blood pressure regulation and blood flow regulation. Their smooth muscle wall is innervated by autonomic nerve fibers and therefore the vessels are able to constrict or dilate regulated by the autonomic nerve system in response to the tissue´s needs. Furthermore the resistance vessels respond to circulating hormones and a variety of different chemical messengers, which are described in more detail in subchapter 1.2.2. Capillaries are the site of exchange of gases, water, nutrients and other substances and have the largest total cross-sectional and surface area. The capillaries merge into

venules, which also serve as exchange vessels. The veins, formed from merged venules, return the blood the right-sided heart. Veins, thin-walled and under low pressure, are easily distensible and represent the primary capacitance vessels and blood reservoir of the body. For instance, more than 50% of the entire blood volume is in the veins and venules (2,4). Constriction and dilation of the veins control the venous blood volume und venous blood pressure and thereby have an effect on the ventricular preload (3).

1.2 Physiology of the cardiovascular system

1.2.1 Physiology of the heart

1.2.1.1 Cardiac muscle and electrical conduction system

The cardiac muscle is a specialized striated muscle and is responsible for pumping blood throughout the body. It consists of two separated functional syncytiums, the ventricular syncytium and the atrial syncytium, which are conducted by the bundle of His. The syncytium allows fast coordinated contraction of muscles fibers along their length and the separation allows the atria to contract ahead of ventricular contraction (1).

The rhythmic pulsation of the heart originates from a specialized cardiac excitatory and conductive system and spreads by this system to all parts of the myocardium. This system consists of structures, which are capable of automatic rhythmical electrical discharge or conduction of action potentials. The action potential normally starts in the auto-rhythmic cells in the sinoatrial node (SAN). Therefore the sinoatrial node is the pacemaker region determining the rate of beat. The impulses generated in the SAN spread into the atrial muscle wall, pass through the atrial pathways to the atrioventricular node. From the atrioventricular node the signal is transmitted to the bundle of His, its branches and the Purkinje fibers, which are all part of the specialized conductive system. Finally, the Purkinje fibers carry the impulses to the ventricular myocardium (Figure 2). Thus, an impulse starting in the SAN leads to a complete contraction of the right and left atria and both ventricles (4–6).

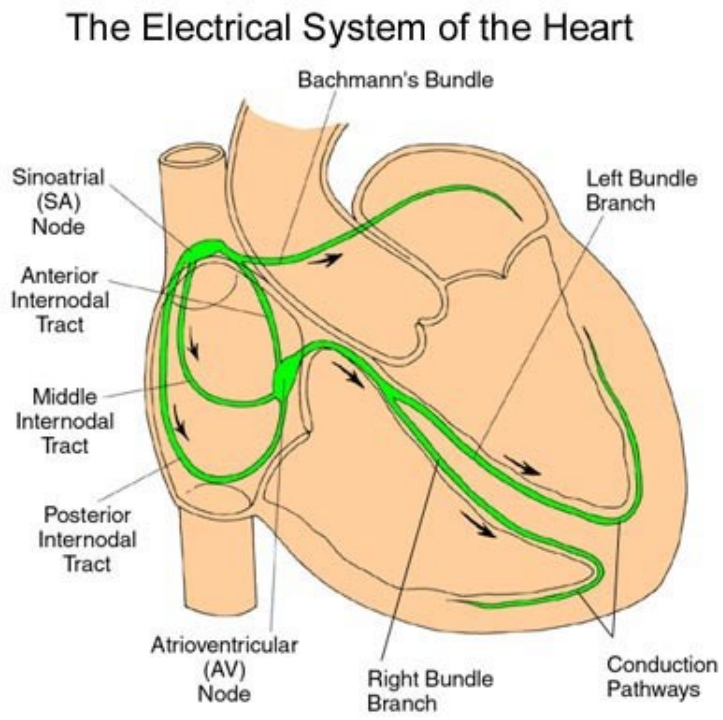


Figure 2: The electrical system of the heart.

Obtained from http://www.heart-consult.com/articles/electrical-conduction-system-heart (23.09.15).

1.2.1.2 Cardiac cycle

The spontaneous electrical activity in the sinoatrial node initiates the cardiac cycle, which is a repeating cycle of contraction and relaxation of the cardiac muscle. Figure 3 depicts the mechanical and electrical events of the cardiac cycle for the left ventricle. The opening and closing of the valves determine four distinct phases: isovolumetric contraction and ejection, both occurring in systole and isovolumetric relaxation and ventricular filling with atria contraction, both occurring in diastole.

During passive ventricular filling the atria and ventricles are relaxed and the A-V valve opens. At the end of the diastole the SAN fires off an electrical impulse, the atria depolarizes and cause a contraction of the atria, which raises the end-diastolic volume. Systole begins with isovolumetric contraction of the ventricle, leading to the closure of the atrioventricular valve, because of the rising pressure. The pressure increases and at the time as the left ventricular pressure exceeds that in the aorta (at 80mmHg) the aortic valve opens. During the ventricular ejection about 60% of the end-diastolic volume is ejected (=stroke volume). The closure of the semilunar valves, triggered by the ventricular pressure falling below that of the aorta, is the beginning of the isovolumetric relaxation phase of the

diastole. The ventricles repolarize and expand but do not fill. The ventricular pressure decreases, causing the atrioventricular valves to open. Figure 3 shows the described changes in the left ventricular pressure, left atrial pressure, ventricular volume as well as the electrocardiography (ECG) and the phonocardiogram.

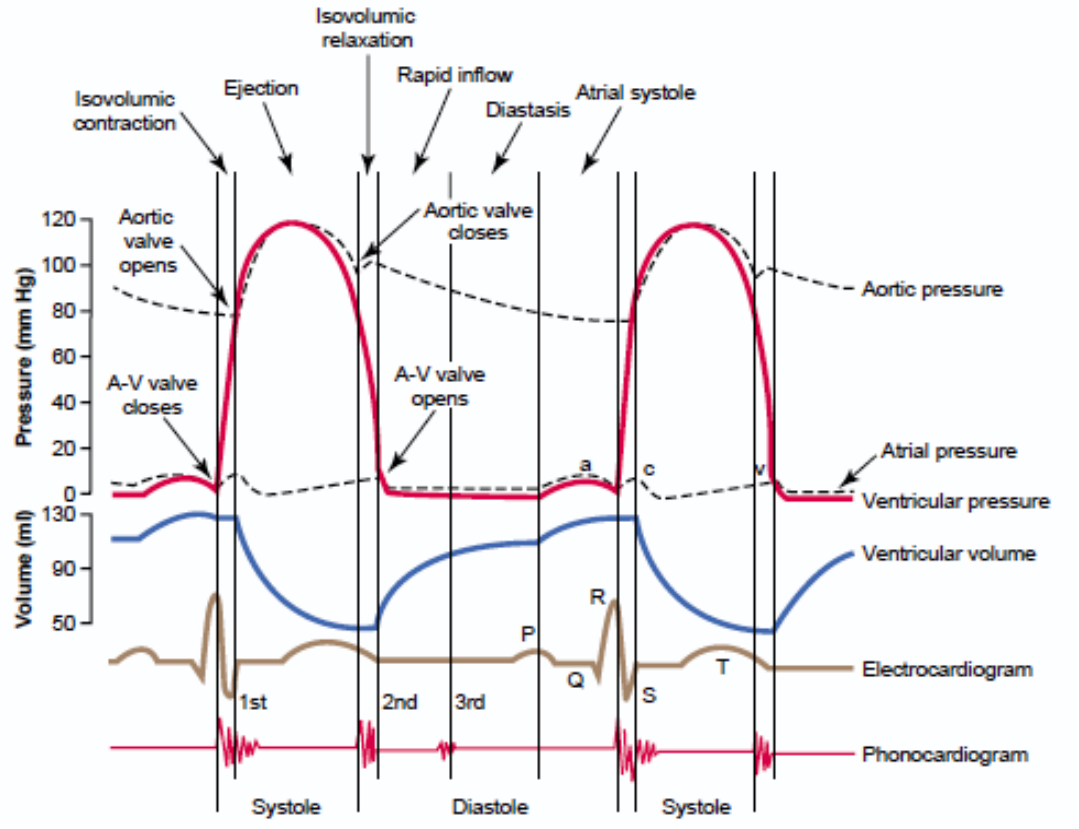


Figure 3: Cardiac cycle.
Obtained from (1).

1.2.1.3 Cardiac output and regulation of the heart

The amount of blood ejected by each ventricle is called stroke volume (SV), which is calculated according the following formula:

$$CO = HR \times SV$$

For a resting, average-sized young adult the cardiac output is about 5,0L/min (72 beats/min x 70ml/beat) (7). The heart rate and the stroke volume are regulated by two different mechanisms: the intrinsic and the extrinsic regulation.

1.2.1.3.1 Intrinsic regulation

The intrinsic regulation is expressed by the Frank-Starling law. It says that the stroke volume increases in reaction to an increase of preload i.e. the increased stretch of the ventricles during filling due to an increased venous return. The cardiac muscle has the ability to stretch up to an ideal length in order to contract with intensified force of contraction and that results in an increase amount of blood ejected in the aorta or the pulmonary arteries. The Frank-Starling mechanism enables the cardiac output to be synchronized with the venous return and maintains a balance between the output of both ventricles (1,3).

The force of contraction and thus the stroke volume also depend on the cardiac afterload. Afterload is the “load” that the contracting ventricular muscle must eject blood against.

An increase in afterload reduces the stroke volume because the greater the afterload, the less the velocity of fiber shortening during the contraction and the less the velocity by which the blood is ejected.

1.2.1.3.2 Extrinsic regulation

The extrinsic regulation is expressed by the activity of the sympathetic and parasympathetic nervous system and the effects of norepinephrine and epinephrine.

All parts of the heart receive sympathetic nerves, whereas vagal fibers distributed to the entire myocardium of the atria, but rarely innervate the ventricles. Sympathetic stimulation increases the heart rate (positive chronotropic), the force of the myocardial contraction (positive intropic) as well the conduction velocity (positive dromotropic). Conversely, parasympathetic stimulation decreases the heart rate, the contraction and the conduction velocity. Because of the distribution of the parasympathetic nerves, vagal stimulation mainly decreases the heart rate and only has a weak influence on ventricular contractility (3,6).

Besides neural control of the sympathetic nervous system, circulating catecholamines from the adrenal medulla enhance the activity of the heart mediated by beta-2 adrenergic receptors on the heart (6).

The cardiac function curve in Figure 4 overviews the effects of sympathetic and parasympathetic stimulation on the cardiac output. An increase in activity of the sympathetic stimulation can raise the heart rate in young, average-sized adults up

to 180-200 beats per minute (bpm) and the stroke volume to as much as double normal resulting in a maximum cardiac output as much as twofold to threefold. This alters the cardiac function curve by shifting it upwards (red curve). Conversely, the yellow curve of Figure 4 shows the alteration in cardiac output during increased parasympathetic stimulation. The vagal nerves can reduce the ventricular pumping function by 50% or more (1).

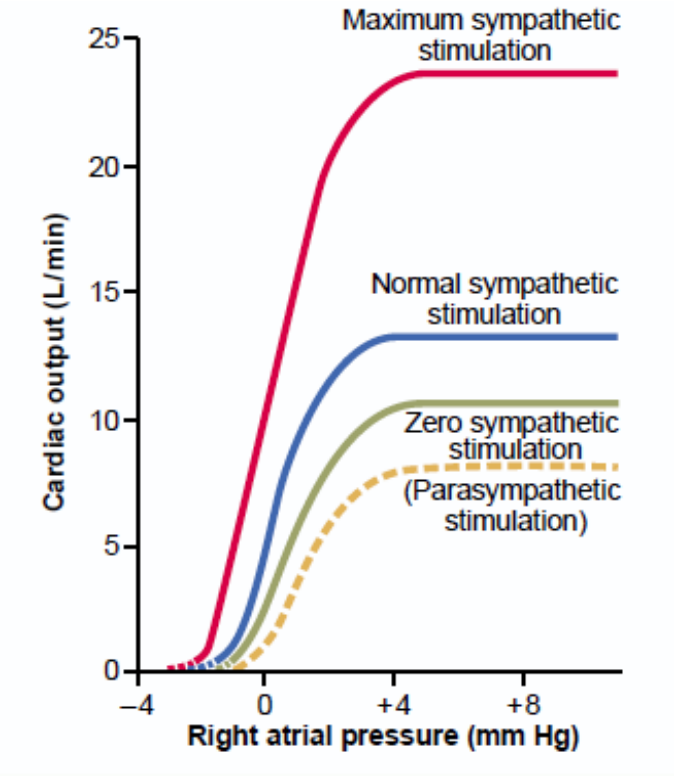


Figure 4: Regulation of the heart by the autonomic nervous system.
Obtained from (1).

1.2.2 Regulation of the cardiovascular function: arterial blood pressure regulation

The mean arterial pressure (MAP) can be determined from:

$$MAP = CO \times TPR$$

As a result, changes in the arterial pressure arise either from changes in cardiac output or TPR or both (2,7).

It is important to maintain the MAP within a normal range in order to ensure a sufficient perfusion pressure. There are multiple mechanisms that are part of the

homeostatic regulation of the arterial blood pressure, which are described in detail in the following subchapters.

1.2.2.1 Local control

The mean arterial pressure is equal throughout the body, therefore circulatory adjustments occurs through changing the diameter of the resistance vessels i.e. the arterioles. Thus, vasoconstriction increases the resistance to blood flow and thereby decreases the rate of blood flow through the organs and tissues. Conversely, relaxation of smooth muscle cells within the vessel walls (vasodilation) enhances the rate of blood flow.

Local control denotes the ability of tissues to control blood flow depending on their metabolic needs. It is an intrinsic, auto-regulating mechanism, independent of neurohumoral control. The regulation comprises paracrine and myogenic mechanisms. The release of vasoactive endothelial mediators (e.g. nitric oxide, prostacycline, endothelial derived hyperpolarizing factor and endothelin) and tissue factors (e.g. adenosine, hydrogen ions, potassiums ions, partial pressure of oxygen, carbon dioxide) affects the degree of vasoconstriction and vasodilation in response to the tissue´s requirements. In addition, myogenic mechanisms sustain a constant blood flow by modifying the vascular tone in response to changes in blood pressure (3,8).

1.2.2.2 Short term regulation and the autonomic nervous system: baroreceptor reflex, chemoreceptor reflex, CNS ischemic mechanism

The short-term regulation of arterial blood pressure is mainly mediated by the autonomic nervous system. The effects of sympathetic and parasympathetic stimulation of the heart were already discussed above. Sympathetic nerve fibers innervate blood vessels in all parts of the body, except capillaries and venules, to cause vasoconstriction. The neurons release norepinephrine, which binds alpha-1 adrenergic receptor. Through the sympathetic stimulation arterioles regulate the resistance to blood flow and veins regulate their blood volume. Parasympathetic innervation of the vasculature is far less important (4,5,7).

Sympathetic stimulation also results in a release of epinephrine and norepinephrine from the adrenal medullae. These circulating catecholamines

cause the same effects as direct sympathetic stimulation. They both act on alpha-1 adrenergic receptors on vessels causing vasoconstriction and on beta-1 adrenergic receptors causing excitation of cardiac muscle on the heart. Furthermore epinephrine interacts with beta-2 adrenergic receptors on vessels in skeletal muscle and the liver causing vasodilatation (1,6).

The homeostatic regulation of the arterial blood pressure requires the presence of receptors and negative feedback systems and needs the initiation of cardiovascular response by sympathetic and parasympathetic nervous pathways.

1.2.2.2.1 Baroreceptor reflex

High-pressure baroreceptors in the walls of the aortic arch and the carotid sinus sense changes in the arterial blood pressure. Figure 5 shows the baroreceptor arterial pressure control process. A sharp rise in mean arterial pressure causes direct stretching of the receptors and results in an increased firing of baroreceptor´s sensory nerve (4,6).

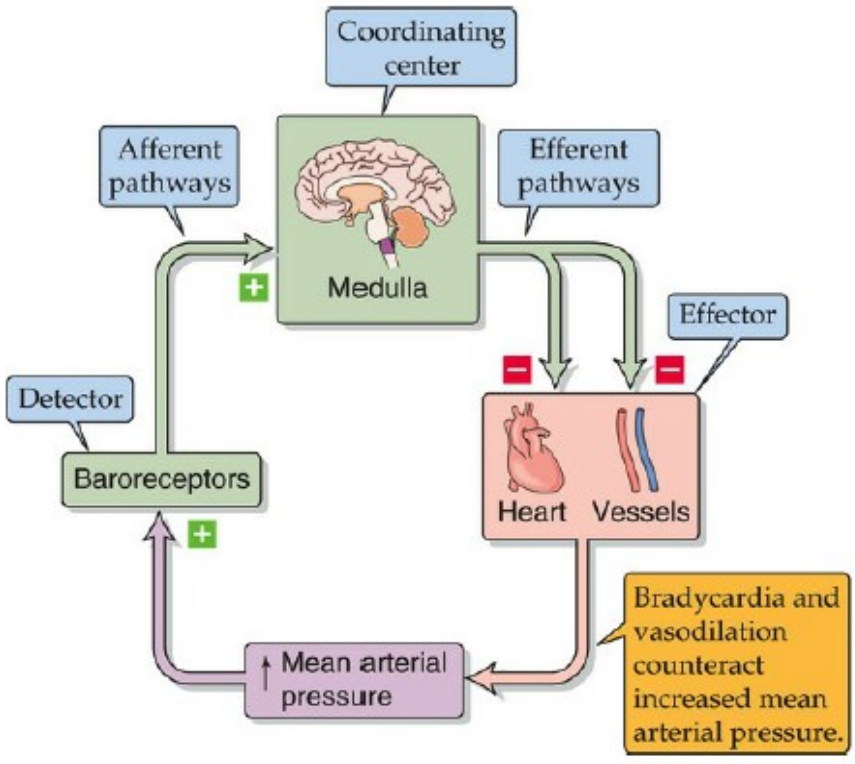


Figure 5: Baroreceptor arterial pressure control.

Obtained from (4).

The signals are transmitted to the brain stem, where they elicit a parasympathetic stimulation and a sympathetic inhibition. That results in bradycardia and vasodilation, which brings the arterial pressure back to the normal level. Contrary, a fall in arterial pressure causes tachycardia and vasoconstriction (4,6).

The baroreceptors respond to pressures in a definite range. They are active above a mean arterial pressure around 50 mmHg and have their maximum firing rate at 180-200 mmHg. Even though their sensitivity to changes in pressure around 100 mmHg is highest (2).

In addition, baroreceptors are much more sensitive to rapid changes in arterial pressure than to changes being continuous. In fact, baroreceptors readjust itself within a short period to different resting blood pressure (1).

Due to the high sensitivity to rapid changes in MAP, the baroreceptors play an important role in stabilizing the arterial pressure during changes in posture. The decrease in arterial pressure in the upper part of the body during changes in posture cause decreased firing of the baroreceptor, strong sympathetic discharge and hence restoration of normal MAP (6).

In addition to high-pressure baroreceptors, there are low-pressure baroreceptors in the walls of the atria and the pulmonary arteries. They are able to detect a rise in pressure caused by increased venous return. They induce reflexes, which are parallel to those of the baroreceptor reflex (1).

1.2.2.2.2 Chemoreceptor reflex

A second neural mechanism of short-term regulation of blood pressure is based on chemoreceptor reflex. Peripheral chemoreceptors in the carotid and aortic bodies are activated by a fall in arterial partial pressure of oxygen (PaO2) and hydrogen ions and a rise in arterial partial pressure of carbon dioxide (PaCO2). A fall in arterial pressure with diminished blood flow causes decreased PaO2 and pH and increased PaCO2, which elicit an increase in firing frequency of the afferent nerves to the vasomotor center in the brain stem. Even though the primary role of chemoreceptors is the regulation of the ventilation, they can cause, via the same pathway as baroreceptors, vasoconstriction and tachycardia. The chemoreceptor reflex is gaining importance when the MAP falls below 80 mmHg (1,4).

1.2.2.2.3 CNS ischemic response

Central nervous system (CNS) ischemic response denotes the elevation of MAP in response to cerebral ischemia. Cerebral hypoxia stimulates the vasomotor center, which initiate a strong increase in sympathetic discharge. The result is a powerful rise of arterial pressure, sometime up to 250 mmHg during severe cerebral ischemia (1,3).

1.2.2.3 Intermediate-term regulation and long-term regulation:

Whereas the short-term arterial blood pressure regulation is primarily mediated by the nervous system by altering the peripheral resistance, the heart rate and the stroke volume, the intermediate-term and long-term regulation underlies mainly hormonal controls and local control systems within the kidneys. The long-term processes for the regulation of blood pressure are based on the maintenance of a constant extracellular fluid volume and composition, because blood volume influences blood pressure and vice versa.

1.2.2.3.1 Renin-angiotensin-aldosterone-system

The renin-angiotensin-aldosterone-system (RAAS) is a hormone system, which plays an important role in the regulation of arterial blood pressure and fluid balance. The juxtaglomerular cells of the kidneys secrete renin, a non-vasoactive proteolytic enzyme, when blood pressure is reduced. Renin catalyzes the cleavage of angiotensinogen into the decapeptide angiotensin I. This in turn is converted into the octapeptide angiotensin II by angiotensin-converting enzyme (ACE). Angiotension II causes primarily vasoconstriction, which results in an increase of arterial blood pressure. Secondly, angiotensin II decreases the excretion of sodium and fluid by the kidneys. In the adrenal cortex, angiotensin II stimulates the release of aldosterone. Aldosterone causes an increased retention of salt and water in the distal tubule of the kidney. As a result, the blood volume increases and the blood pressure increases accordingly within hours and days (1,3). Figure 6 illustrates the effects of RAAS activity on blood pressure.

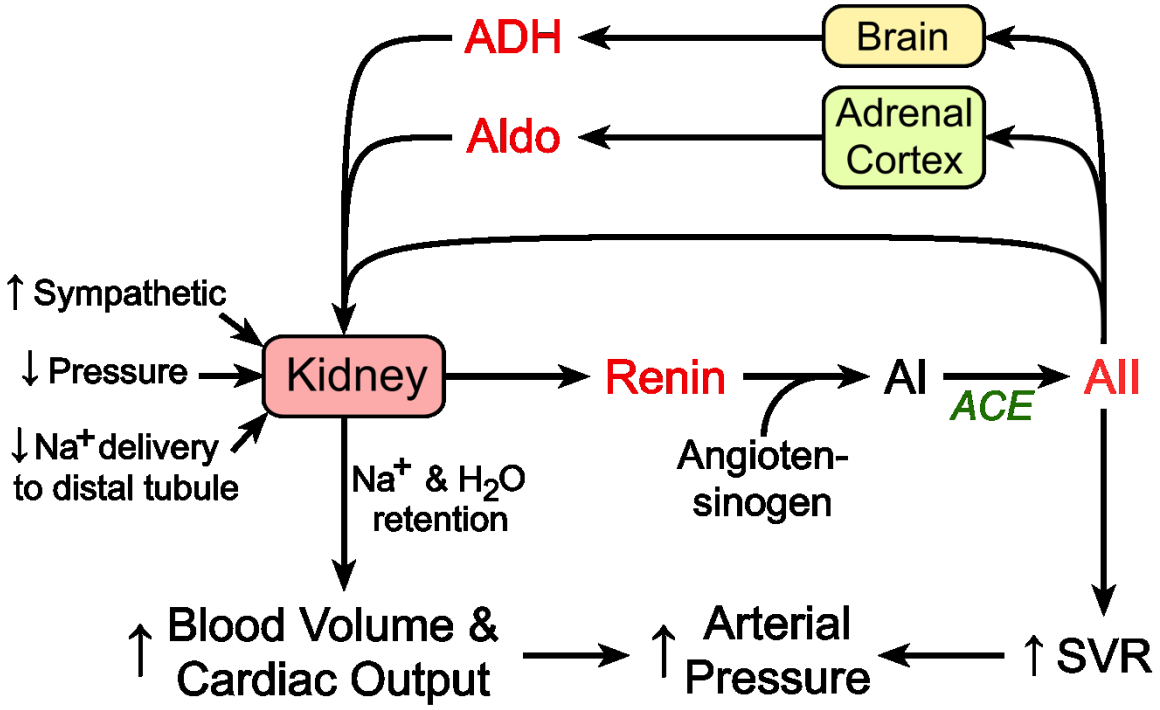


Figure 6: Renin-angiotensin-aldosterone-system.

Obtained from http://cvphysiology.com/Blood%20Pressure/BP015.htm (09.01.2016).

1.2.2.3.2 Vasopressin

Vasopressin (antidiuretic hormone, ADH) is a neurohypophysial hormone with two principal means. It increases the retention of water by an increase of the collecting duct water permeability and it is a strong vascular constrictor agent, which helps to maintain arterial blood pressure. Principally, Vasopressin release is stimulated by reduced plasma volume and increased extracellular osmolarity (3,5).

1.2.2.3.3 Atrial natriuretic peptide (ANP)

The heart muscle cells secrete ANP in response to increased atrial filling pressure. It is the inhibitor of the renin-angiotensin-aldosterone-system and produces diuresis and natriuresis, thereby reducing blood volume. Furthermore ANP is a strong vasodilator substance (3).

1.2.2.3.4 Stress relaxation and capillary fluid shift

Stress-relaxation and capillary fluid shift are both part of intermediate-term regulation mechanisms, which act within a few minutes to hours following arterial pressure changes. Capillary fluid shift means the slow shift of fluid between the blood plasma and the interstitial fluid volume. A rise in pressure increases the

capillary hydrostatic pressure, resulting in a transfer of fluid from the plasma into the interstitial fluid, thus decreasing the blood volume and blood pressure. Conversely, when capillary pressure falls, the osmotic pressure drives fluid back into the vessel. Thus the blood volume is increased which helps to return the blood pressure back to normal (1,2).

Stress relaxation refers to vascular tone adjustment due to stress on the vascular smooth muscle cells. A sustained raise in pressure causes the vascular smooth muscle to stretch for minutes or hours, resulting in a relaxation of the blood vessels. The vasodilatation leads to a fall of arterial blood pressure, because of the enlarged capacity of the arterial system (1,2).

1.2.2.3.5 Renal-body fluid pressure control

Kidneys have the ability for controlling arterial blood pressure through appropriate changes in extracellular fluid volume. Besides the renin-angiotensin system and the aldosterone-system, the kidneys possess direct mechanisms to control arterial blood pressure over a longer period: the renal-body fluid pressure control system. This system controls the body fluids and simultaneously corrects the arterial pressure through pressure diuresis and pressure naturiesis. When the arteriolar blood pressure rises because of an elevated blood volume, the flow through the kidneys also increases, resulting in an increased renal urinary output and sodium output. Conversely, a fall in blood pressure leads to a decrease of water and sodium excretion in order to reestablish a normal blood pressure. For maintenance of a constant blood pressure over a long time, the renal output of water and sodium must equal the intake.

This feedback mechanism acts very slowly, but it is the most potent control of the long-term regulation. It exhibits an infinite feedback gain capability (1).

Figure 7 outlines the several mechanisms, which maintain the mean arterial pressure within its normal range as described above.

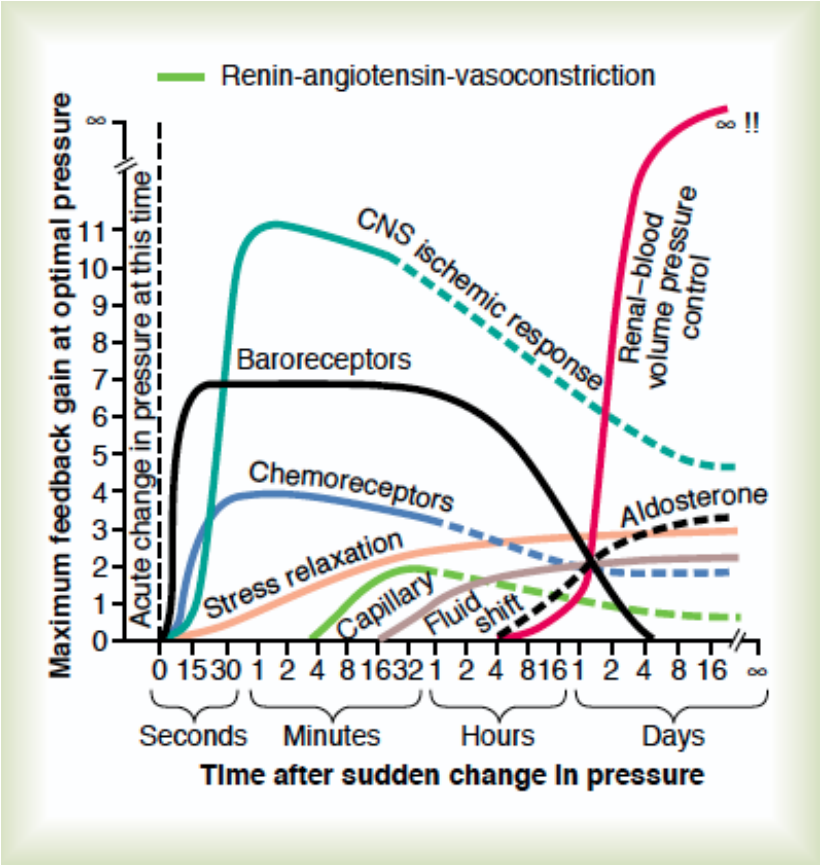


Figure 7: Arterial blood pressure regulation mechanisms.

Obtained from (1).

1.3 Orthostatis and orthostatic hypotension

The change from a supine to an upright position or the application of lower body negative pressure (LBNP) induces orthostatic stress. It leads to a redistribution of the blood volume and a subsequent activation of the cardiovascular regulatory mechanisms mentioned in chapter 1.2.2.2 (9).

In an upright position the capacitance vessels in the legs are exposed to an increased hydrostatic pressure, which leads to a blood volume shift to the lower extremities of 400-600 ml (10). The accumulation of blood in the veins of the legs results in a fall of central blood volume and preload. Consequently, this leads to a decrease in stroke volume and cardiac output and therefore reduced MAP. The decrease in arterial blood pressure causes a baroreflex mediated compensatory increase in peripheral resistance and heart rate, which contributes to the stabilization of arterial blood pressure and maintenance of cerebral blood flow (9,11,12).

The ability of the brain to maintain a stable blood flow during changes in blood pressure is called cerebral autoregulation. The regulation is achieved by alterations of cerebrovascular resistance. Autoregulation of cerebral blood flow operates in the range of approximately 60 to 140 mmHg MAP. Below and above, cerebral blood flow is dependent on MAP in a linear way (Figure 8) (13,14).

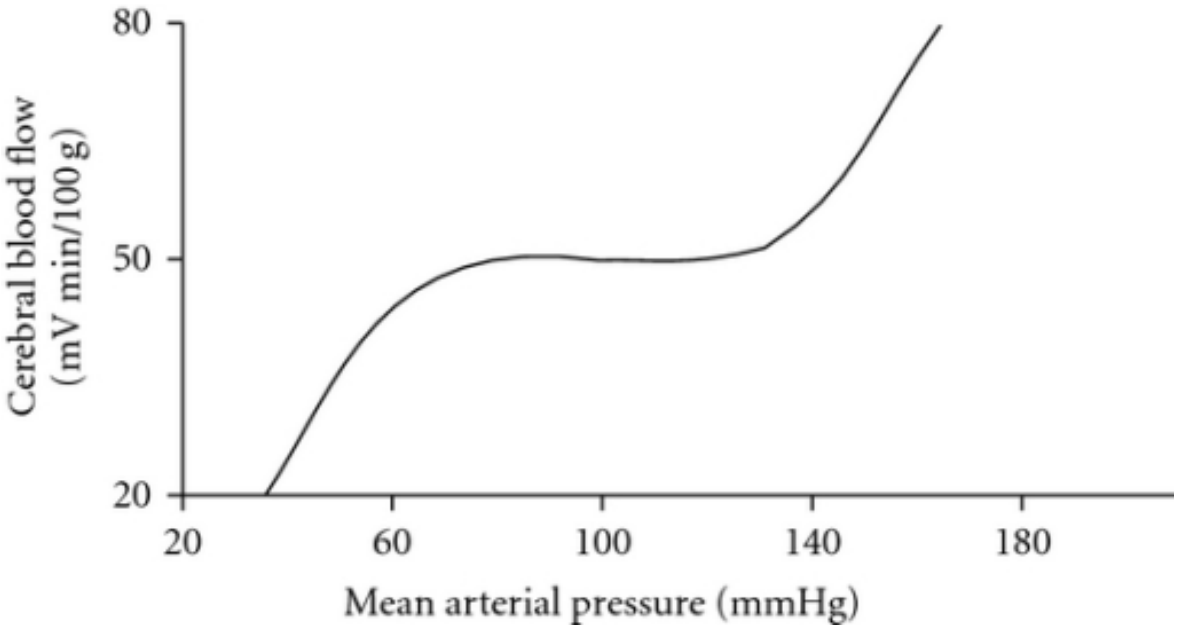


Figure 8: Cerebral autoregulation.
Obtained from (15).

Figure 9 shows the changes in heart rate, relative stroke volume, relative cardiac output, blood pressure and total peripheral resistance during orthostatic stress in young healthy adults (16).

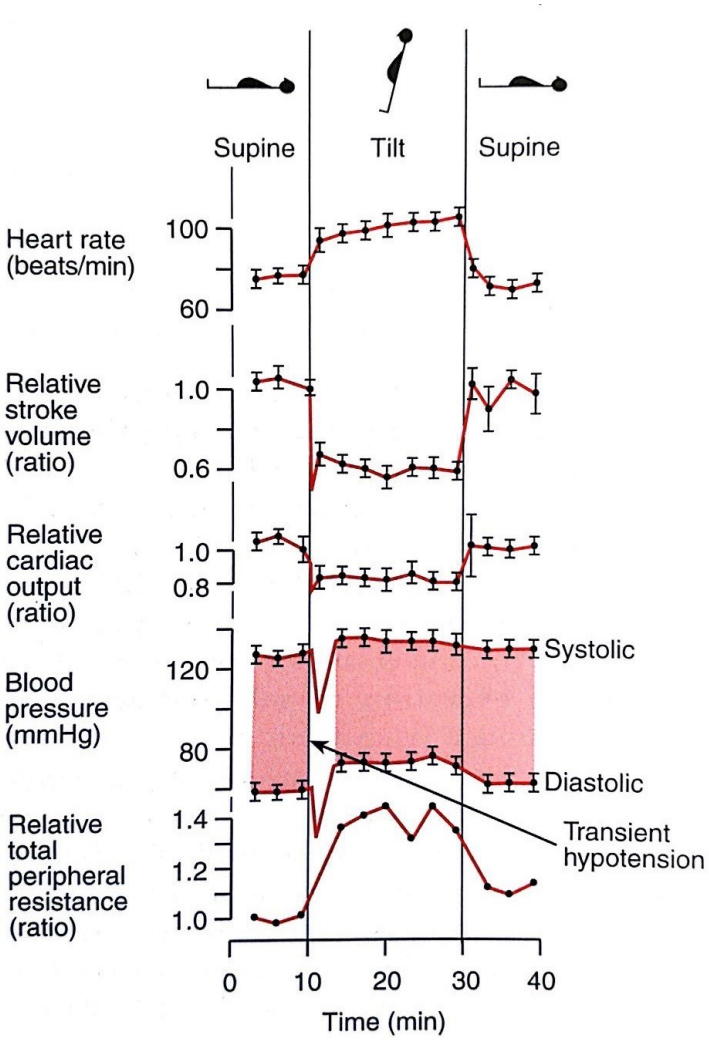


Figure 9: Changes in HR, SV, CO, blood pressure and TPR during a 20 minutes head-up tilt. Obtained from (16).

Furthermore the fall in central blood volume is more distinct during standing than during walking because of the skeletal-muscle pump in the legs. The distended veins are compressed and emptied due to the muscle contractions, which leads to an increased venous return to the heart.

Orthostatic hypotension (OH) is defined as a decrease of systolic blood pressure of ≥20 mmHg or diastolic blood pressure of ≥10 mmHg within three minutes of standing (17).

In some individuals or in certain circumstances, the cardiovascular adjustments are not sufficient to maintain an adequate circulatory function during orthostatic stress. Inadequate neurohumoral responses, impairment of the baroreceptor reflex or simply insufficient blood volume are three of the reasons for the development of orthostatic intolerance.

Symptoms like dizziness, lightheadedness or even loss of consciousness may occur as a result of cerebral hypoperfusion. Usually, all symptoms are relieved by recumbency (17,18).

1.4 Gender differences in cardiovascular regulation

Several studies investigated gender-specific differences in cardiovascular regulation and responses to postural stress and indicated differences in cardiovascular anatomy, hemodynamics and the autonomic nervous system.

Two main gender-related differences in the regulation of blood pressure can be seen clinically. Typically, blood pressure is lower in young women compared to age-matched men. Orthostatic hypotension and syncope as well as hypotensive disorders are more prevalent in premenopausal women than men (19,20).

In comparison to men, women show considerable differences in anatomical properties such as smaller hearts, reduced distensibility of the heart muscle and a greater blood pooling in the pelvic region with subsequent reduced cardiac preload compared to men. These factors result in a smaller stroke volume, probably because of a decreased cardiac filling during orthostatic loading and explain part of the gender differences in the susceptibility to orthostatic intolerance (20–22).

Furthermore men and women exhibit hormonal differences, which may have a strong impact on the neurohumoral regulation of the cardiovascular system (21,22). Women und men differ in that men respond primarily to cardiovascular stress with an increase in peripheral resistance, whereas women show greater heart rate increases (23,24).

Because of the greater susceptibility to orthostatic intolerance in premenopausal women and the rising incidence of hypertension around the onset of menopause, the influence of female sex hormones appears to play a key role in cardiovascular regulation (25,26). Indeed, several studies have shown the strong influence of female sex hormones on the arterial blood pressure regulation. There is strong evidence that female sex hormones show a protective effect against cardiovascular diseases such as hypertension (27,28). First, estrogens have a beneficial effect on lipoprotein profiles, making it an important atheroprotective factor (28). Furthermore, estradiol directly influences the vascular and endothelial function of the blood vessels. It increases the availability of endothelium-derived nitric oxide, thus augments vasodilation (23,24,26) and estrogen exposure leads to

attenuation of alpha adrenergic vasoconstriction (26). As a consequence, women have an inferior vasoconstrictor response to gravitational stress.

While in young men high sympathetic nerve activity is directly related to higher TPR, young women do not exhibit this relationship. Contrary to men, cardiac output does not seem to be balanced with sympathetic nerve activity in premenopausal women. This means that there are additional factors, which are modulating the vasoconstrictor tone in young women.

There is some evidence suggesting that beta adrenergic receptor-mediated vasodilatation offsets the vasoconstrictor effects of alpha adrenergic receptors in young females (25,27). The increased sensitivity of beta adrenergic receptors might probably be related to female sex hormones, but the exact mechanisms are not yet completely clarified (25,27).

The depicted vasodilatory effects reduce vascular resistance as well as venous return and may be reflected in lower orthostatic tolerance in premenopausal females.

Overall, one can say that greater sensitivity to estrogen may be reflected in lower orthostatic tolerance. Hence, sex hormones may cause differences between the sexes as well as among women.

Furthermore there is a difference in the sympathetic und parasympathetic autonomic regulation between men and women. Several studies determined that women have a reduced sympathetic and enhanced parasympathetic activity relative to men (29,30). Young healthy women demonstrate a high vagal cardiac tone during rest due to parasympathetic predominance, but a lower sympathetic vascular stimulation to peripheral vasculature, whereas men show high sympathetic input to vascular regulation (30–32). Therefore, women respond to orthostatic challenge primarily with vagal withdrawal while men respond with sympathetic activation and an increase in peripheral resistance (31,33,34).

In addition, hormonal differences may influence cardiovascular responses to postural stress. Women appear to have lower total circulating norepinephrine and epinephrine levels during orthostatic stress compared with men. Hence, lower concentrations of catecholamines in females may limit the compensatory mechanisms for preventing the onset of orthostatic hypotension (11,31–33).

Sex-specific conditions like the menstrual cycle, oral contraceptive administration, hormone replacement therapy or menopause may influence the determinants of

blood pressure regulation. Several studies have examined whether the different phases of the menstrual cycle or contraceptives affect the cardiovascular regulatory control, but the results are ambiguous. There are studies that showed that sympathetic baroreflex sensitivity and resting levels of circulating catecholamines are altered during different phases of the menstrual cycle (35,36). However, other studies demonstrated no significant changes (26,37,38).

Overall, it has not been definitively determined whether or not there are consistent changes in blood pressure or orthostatic tolerance during the phases of the menstrual cycle (35,36).

The impact of hormone replacement therapy (HRT) in postmenopausal women is as follows: Experiments have shown that estrogen treatment decreases blood pressure in hypertensive rats. Moreover, postmenopausal women taking hormone replacement therapy seem to have a reduced sympathetic activity compared with non-treated age-matched subjects (29) and HRT reduces the incidence of atherosclerosis diseases (28).

The effects of aging and further consequences of menopause are covered in Chapter 1.5

In summary, it can be stated that gender differences are multifactorial and not yet understood and interpreted in detail, but they appear to be due to different mechanisms and the similarities and differences include more than the sex hormones per se.

1.5 Effect of aging on the cardiovascular regulation

Aging is associated with significant cardiovascular modifications concerning structure and function. Gender differences vary depending on age. The normal aging process in humans is associated with the development of an endothelial dysfunction (39), an increase of arterial stiffness (40,41) and a blunted myocardial contractility (42). The venous compliance in aging humans is also reduced (43). The process of aging is further associated with a sizable impairment of the baroreceptor efficiency, with a greater involvement of the control of the heart than the peripheral vascular resistance (42,44). The sympathetic control of the heart is in decline, leading to a reduced heart rate, ejection fraction, cardiac output and blood pressure in response to beta adrenergic stimulation (33,45). Besides that, parasympathetic regulation of the heart is decreased with advanced aging as well,

resulting in less cardio acceleration during vagal withdrawal. Table 1 summarizes the age-related changes in blood pressure regulation.

Table 1: Age-related changes in blood pressure regulation.
Modified from (46).

Reduced baroreflex sensitivity
Decreased adrenergic response to sympathetic stimuli
Reduced parasympathetic regulation of the heart
Augmented vascular stiffness
Decreased left ventricular diastolic filling
Limited heart rate and cardiac output responses
Reduced venous compliance
Decreased renal water and salt conservation

Regarding orthostatic tolerance, aging has an adverse influence. Orthostatic problems and orthostatic hypotension occur relatively frequently in older people (46,47) Factors that may predispose to orthostatic intolerance are mentioned above (Table 1). Orthostatic hypotension is further known to be associated with syncope, falls and subsequent fractures, stroke, myocardial infarction and higher mortality (44,46).

As set out in chapter 1.4, young women show a predominance of parasympathetic control in cardiovascular regulation. Interestingly though, in postmenopausal women the sympathetic tone dominates (29,30). In addition, older women have a rise in sympathetic activity and the relationship between sympathetic nerve activity and total peripheral resistance as well as blood pressure becomes positive. Older males also show a positive relationship of sympathetic nerve activity to blood pressure, but muscle sympathetic nerve activity (MSNA) increases at a higher rate in older females (48). Furthermore, the ability to offset alpha adrenergic vasoconstriction by beta adrenergic receptor-mediated vasodilation, that is seen in younger women, is lost in older females and hence sympathetic control of blood pressure and the sensitivity of beta adrenergic receptors are altered (19,25,27). It seems that the lack of influence of female sex hormones in postmenopausal women is one cause of these changes.

The predomination of the sympathetic tone may contribute to the rising incidence of hypertension and cardiovascular diseases with aging (30). With growing age it often comes to a rise in blood pressure and a higher risk of hypertension, other cardiovascular diseases and morbidity in both sexes. However, these effects are more striking in postmenopausal women, which is reflected in the incidence of hypertension even exceeding that in age-matched men (19,27).

In conclusion, aging strongly influences the control of the cardiovascular system and leads to changes in the responses in the cardiovascular regulation.

2 Aims and objectives

Studies addressing gender differences in cardiovascular responses to orthostatic stress have primarily focused on young premenopausal women and age-matched men.

Furthermore, there are various studies documenting differences in cardiovascular responses according to age, but principally focused on differences between young and old subjects independent from gender.

The aim of this study is to examine how treatment (orthostatic loading provided by a sit to stand test) and gender contribute to changes in cardiovascular responses in healthy older people (subjects aged ≥55 years).

This diploma thesis provides an integrated insight into gender differences in blood pressure regulation and orthostatic tolerance in older people. It will attempt to answer the question if there are sex-related differences in cardiovascular aging or if the observed gender differences in young adults are diminished with aging.

Chapter 1.4 shows differences between men and women in terms of blood pressure regulatory mechanisms and illustrates the impact of the female sex hormones, which may lead in large part to disparities between the genders. Nonetheless, anatomical properties are also a contributor to lower orthostatic tolerance in women and still exist with advancing age.

In the light of the vanishing influence of sex hormones in postmenopausal women, we hypothesize that older men and women would respond similarly to orthostatic stress and that gender-related differences in hemodynamic and cardiac autonomic responses are no longer detectable.

As aging and gender have a major impact on cardiovascular risk, it is relevant to highlight the effects of aging and gender on the control of blood pressure and their impact on orthostatic tolerance.

By identifying gender differences in cardiovascular aging, it might be possible to develop sex-specific interventions to improve cardiovascular deconditioning and disease management and to prevent orthostatic hypotension-related hospitalization.

3 Methods

As part of the pilot study “Vascular Stimulus Responses in Health and Disease: A longitudinal Study”, a cooperation between the Institute of Physiology and the Department of Neurology at the Medical University of Graz, this diploma thesis examined gender differences in cardiovascular responses to orthostatic challenge in healthy older persons.

3.1 Subjects

Ten men and fourteen women were recruited by the Department of Neurology at the Medical University of Graz. All subjects fulfilled the following inclusion and exclusion criteria.

Inclusion criteria:

- Age ≥ 55

Exclusion criteria:

- Neurological disorder (stroke, epilepsy, dementia, Parkinson´s disease)

All study volunteers were instructed to refrain from stressful activity before testing and to abstain from caffeine or other stimulants 24 hours before testing.
The physical characteristics of the subjects in both groups (men and women) are listed in Table 2.
Each subject underwent clinical evaluation that included a review of medical history, clinical neurological examination and received medical clearance to participate in this study. All investigational procedures were performed at the Department of Neurology Graz from 12.11.2014-22.05.2015.
The participants were familiarized with the protocol and procedures and were explained to have the right to terminate the test at any time. Verbal and written informed consent was obtained from each subject and research was performed in accordance with the Declaration of Helsinki (1989) of the World Medical Association (WMA). The study protocol was approved by the Ethics Committee of the Medical University of Graz. All subjects received an expense allowance of 20 Euro.

3.2 Experimental protocol

The participants were requested to come to the neurological outpatient clinic at University Hospital Graz at 7:00 am The actual measurements were performed between 07:00 and 10:00 am. Studies were conducted in a sensory-minimized environment, i.e. a quiet, partially darkened room and maintained at a comfortable temperature. While the subject was sitting, hemodynamic and autonomic monitoring was applied, including measurement of intermittent (upper arm oscillometry) and continuous blood pressure (finger plethysmography), heart rate (3-lead ECG), and thoracic impedance measurements using a Task Force® Monitor (CNSystems, Graz, Austria). Additionally, a nasal alar sensor measured the oxygen saturation.

We decided to use a sit to stand test to induce orthostatic stress. After preparation and establishment of baseline measurements, data were collected during a five-minute period of sitting, followed by a six-minute period of standing. The subjects were then requested to sit down for additional five minutes (recovery period). Additionally, blood was sampled for testing hormones at two points in time: first blood draw at the end of the preparation period and second blood draw during the last minute of the standing period. A diagram of the experimental protocol is provided in Figure 10.

The subjects were told to sit quietly with arm relaxed by their sides during the five-minute period of rest, after which they are assisted into the standing position for six minutes. During this period, they were instructed to keep their eyes open, to fix a point at eye-level and not to alter foot placement to avoid the effect of the skeletal-muscle pump. All individuals were asked to breathe normally and not to speak except in case of discomfort. Precautions (assistance in standing, available medical personnel) were taken to ensure safety in case a subject becomes syncopal during standing.

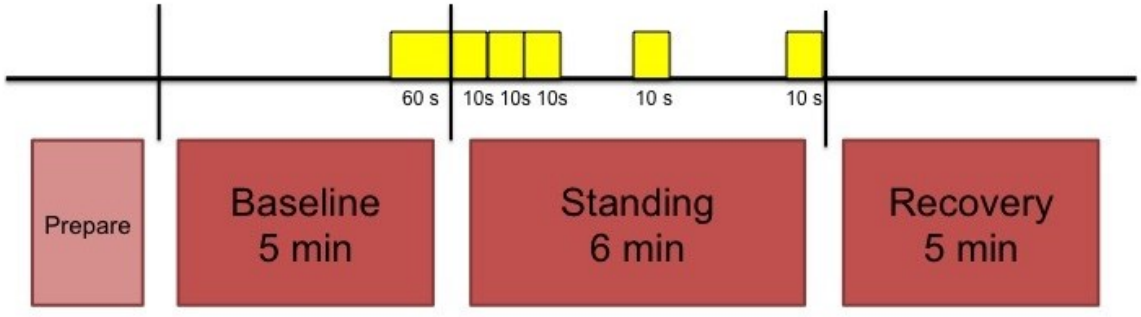


Figure 10: Experimental protocol.

3.3 Data collection

The hemodynamic and autonomic monitoring recorded during the experimental protocol included 3-lead electrocardiogram (FD-13, Fukuda Denshi Co. Ltd. Tokyo, Japan), upper arm oscillometry, finger plethysmography (Finometer, Finapres Medical Systems B.V., Amsterdam, Netherlands) and thoracic electrical impedance measurements.

ECG electrodes were attached for continuous recordings of heart rate. Beat-to-beat measurement of blood pressure was monitored using a finger plethysmography. In addition, intermittent blood pressure was measured from the left upper arm in order to calibrate continuous blood pressure values to the oscillometric measurement and providing absolute blood pressure values.

The double finger sensor for measurement of continuous non-invasive blood pressure was placed on the right hand, which was fixed by a sling at heart level throughout the testing periods. The device for continuous blood pressure measurement was attached to right forearm.

Four electrodes, one on the neck, two at the lateral side of the thorax at the xiphoid level and one neutral electrode on the lower leg, were attached for transthoracic bioimpedance cardiography (ICG) from which stroke volume, cardiac output and other cardiodynamic parameters were continuously processed.

ECG, continuous non-invasive arterial blood pressure measurement and thoracic impedance is synchronized and integrated in the Task Force® Monitor (TFM). The TFM is a hard- and software device for non-invasive monitoring and assessment of hemodynamic and autonomic parameters. The recorded measurements were displayed in real-time on a computer screen. The following Figure 11 shows a

screenshot of the hemodynamic trend recording of a healthy study participant during the experimental protocol.

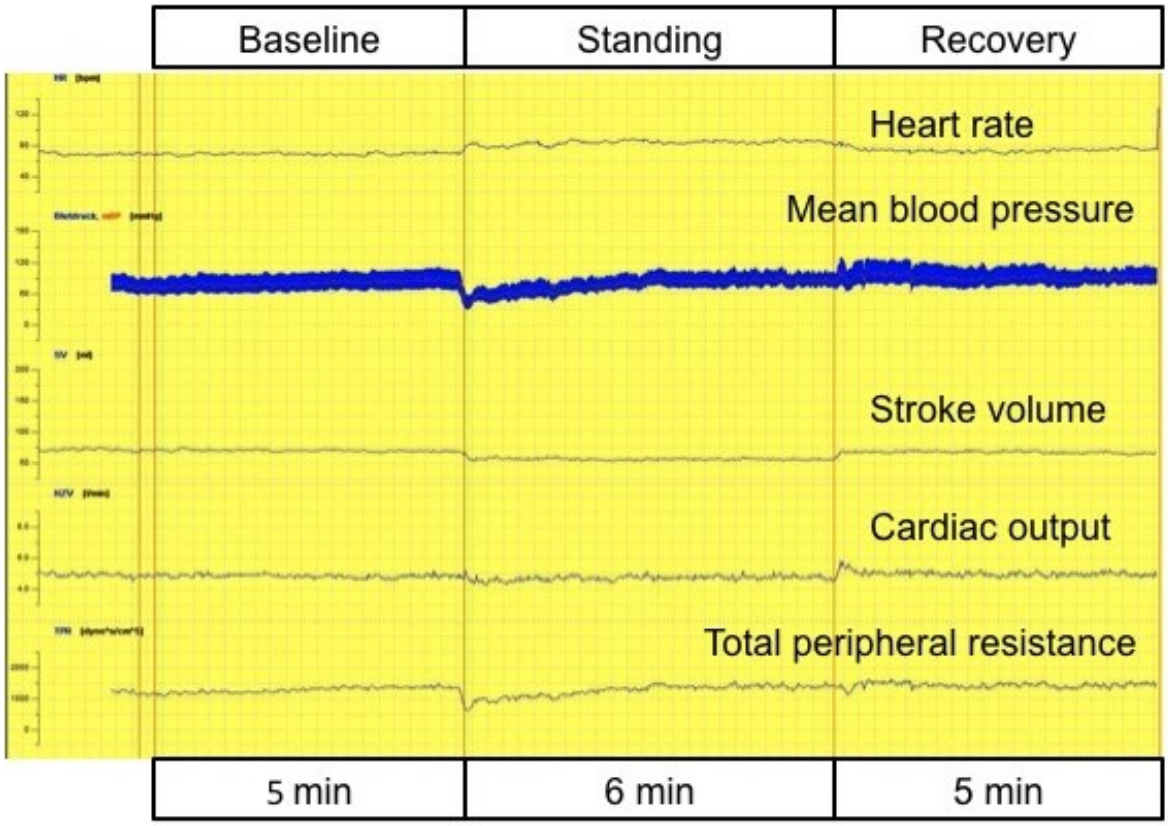


Figure 11: Trend view of a study participant during sit to stand test.

As can be seen in the figure, there is a clear rise in HR, a slight decrease in SV, whereas CO remains almost constant. After an immediate drop in TPR in the first seconds of standing, there is a subsequent rise in TPR. As is result, MAP stabilizes after an initial drop and a narrowing of pulse pressure is visible.

Mean arterial pressure was calculated from systolic and diastolic blood pressures. Stroke volume was computed from the signal of the transthoracic bioimpedance cardiography. ICG measurement was performed based on the original Kubicek design but using an improved estimate of thoracic volume (49). Cardiac output was calculated by dividing heart rate by stroke volume. Cardiac index and stroke index were obtained by dividing cardiac output and stroke volume by body surface area, respectively. For calculation of body surface area, formula of DuBois was used: $\mathrm{BSA}(m^2) = 0.007184 \times Height(cm)^{0.725} 0.725 \times Weight(kg)^{0.425}$. Total peripheral resistance index (TPRI) was calculated by dividing mean arterial pressure by cardiac index.

Besides the full hemodynamic assessment, the assessment of autonomic function and regulation using heart rate variability (HRV) was provided by the TFM.
Power spectral analysis of HRV using fast Fourier transform (FFT) assesses the balance between sympathetic und vagal activity. High frequency (HF: 0.15-0.4 Hz) and low frequency (LF: 0.04-0.15 Hz) power components were expressed in absolute values (ms^2) and normalized units. HRV normalization is performed as follows:

$$((LF\ or\ HF)\ /\ (Total\ Power - VLF)) * 100$$

In addition, the ratio between low und high frequency power (LF/HF) was presented, which is widely used as an index of sympathovagal balance (50). In the present study, high frequency oscillations were used to assess parasympathetic activity. Changes in the LF power are more complex, but increases in LFnuRRI are often assumed to represent increases in sympathetic activity, although there are indications that LF is probably affected by sympathetic and parasympathetic modulations (51–53).
Blood samples were taken from an antecubital vein for hormonal analysis, but the effect of orthostatic challenge on hormonal responses is not the focus of the diploma thesis and thus is not discussed further here.
All hemodynamic and autonomic function parameters were measured and assessed throughout the entire term of the study.

3.4 Data analysis

The acquired data were exported to Microsoft Excel®. Heart rate (HR), systolic blood pressure (sBP), diastolic blood pressure (dBP), mean blood pressure (mBP), cardiac index (CI), stroke index (SI) and total peripheral resistance index (TPRI) were averaged for each subject during different epochs of the sit to stand test: representative 60 seconds at the end of baseline, first 10 seconds, second 10 seconds, third 10 seconds of standing, 10 seconds in the third minute of standing and during the last 10 seconds of standing (before recovery). Values >± 2SD were detected and manually removed from the data set.
For the heart rate variability parameters periods of 4 minutes length in the sitting state und 4 minutes length in the standing position were selected from each test person. Thereafter, the median from the set of values was calculated. It was

chosen to use median because it is relatively unaffected by outliers and is more appropriate when data set is skewed (54).

Due to errors in the measurement und recording of heat rate and consequently CI, TPRI and HRV data sets of two study participants (one women and one man) were excluded from further analysis. Other hemodynamic parameters remained unaffected and were included in the statistical analysis. Additionally, blood pressure values and consequently TPRI values of one male participant were not recorded or stored and therefore excluded from further calculation. In case of missing values in an epoch of a test subject, the method of mean substitution was applied.

3.5 Statistical analysis

Data plots were transported to SPSS for statistical analysis. Shapiro-Wilk test was used on female and male samples at each epoch for each parameter to determine if data sets are well modeled by normal distribution.

As the hemodynamic variables show approximately normal distribution, mixed design ANOVAs with Bonferroni post hoc tests were applied; with the within-subjects factor (6 different epochs) and the between-subjects factor (male and female). In case of violation of sphericity, the degrees of freedom for the F-distribution were corrected using Greenhouse-Geisser Correction ($\varepsilon < 0.75$).

HRV-parameters did not follow normal distribution. Mann-Whitney U Test was used for gender comparisons, while Wilcoxon signed rank rest was used for the paired sample comparison of the two periods (sitting versus standing).

After it became apparent that some participants met the definition of orthostatic hypotension, statistical analysis via independent-sample-t-test was carried out to determine differences in hemodynamic parameters between the both groups (study participants with OH vs. study participants without OH).

All statistical analyses were performed using IBM SPSS Statistics 23. Statistical significance was assumed if $p < 0.05$.

4 Results

4.1 Subjects:

Twenty-four persons, 14 women and 10 men, were included in the study. Mean age for women and men was 62.8 ± 7.3 years and 64.0 ± 7.2 years, respectively. Women´s height, weight and body surface area were significantly lower than men´s. All subject characteristics are presented in Table 2.

Table 2: Subject characteristics.

	Women	Men
n	14	10
Age, yr	62.8 ± 7.3	64.0 ± 7.2
Height, cm	164.7 ± 6.6	177.3 ± 5.8 **
Weight, kg	69.6 ± 13.3	88.2 ± 11.7 *
Body surface area, m^2	1,76 ± 0.14	2.06 ± 0.15 **

Values are expressed as means ± SD.

* $p < 0.05$ compared with women

** $p < 0.001$ compared with women

Blood pressure data and TPRI data of one male participant and HR values along with CI, TPRI and HRV data of one male and one female participant were excluded from analysis due to errors in measurement and recording.

4.2 Hemodynamic parameters

The results of the mixed ANOVAs showed that here was a significant main effect of phase of the experimental protocol on heart rate, systolic blood pressure, diastolic blood pressure, mean blood pressure, stroke index, cardiac index and total peripheral resistance index (Table 3).

In the first ten seconds of standing, there was a transient decrease in mean arterial pressure (females: 108.2 ± 12.8 to 97.7 ± 20.1 mmHg; males: 101.2 ± 7.7 to 93.8 ± 11.8, $p = 0.004$). The same was observed for systolic and diastolic blood pressure parameters ($p = 0.02$, $p = 0.002$, respectively).

Thereafter blood pressure stabilized, reached baseline values after minute 3 of standing and remained almost unchanged in the following minutes. In response to

orthostatic challenge stoke volume decreased slightly, but the accompanying increase in heart rate was able to maintain cardiac output or even rather exceed baseline level. Similar to blood pressure values, TPRI temporarily decreased in the first ten seconds of standing (females: 4306.3 ± 1318.9 to 3497.9 ± 1380.1; males: 3846.6 ± 651.9 to 2985.2 ± 662.3, $p < 0.001$) followed by a subsequent stabilization within a few seconds. During orthostatic challenge, heart rate increased immediately (females: 73.1 ± 13.6 to 81.0 ± 14.4; males: 68.2 ± 6.8 to 77.0 ± 8.4, $p < 0.001$) and remained elevated throughout the standing period. Figure 12 illustrates the changes in hemodynamic parameters over time. There was no main effect gender on hemodynamic responses, with women and men performing similarly overall. All hemodynamic parameters are presented as mean ± standard deviation in Table 3. Heart rate data is presented in the first row of Table 3. Resting heart rate appears to be greater in females versus men (73. 1 ± 13.6 vs. 68.2 ± 6.8 bpm), however not statistically significant. Although greater heart rate values were recorded in females throughout the entire term of the experimental protocol, no significance was observed across gender. Blood pressure values at baseline were greater among women in comparison with men (sBP: 133.8 ± 18.7 vs. 122.7mmHg ± 10.2; dBP: 88.0 ± 11.0 vs. 85.5 ± 6.1 mmHg; mBP: 108.2 ± 12.8 vs. 101.2 ± 7.7 mmHg), but still not significant however. Also during the five epochs during standing, higher blood pressure values were recorded in women, but values did not differ significantly between genders.

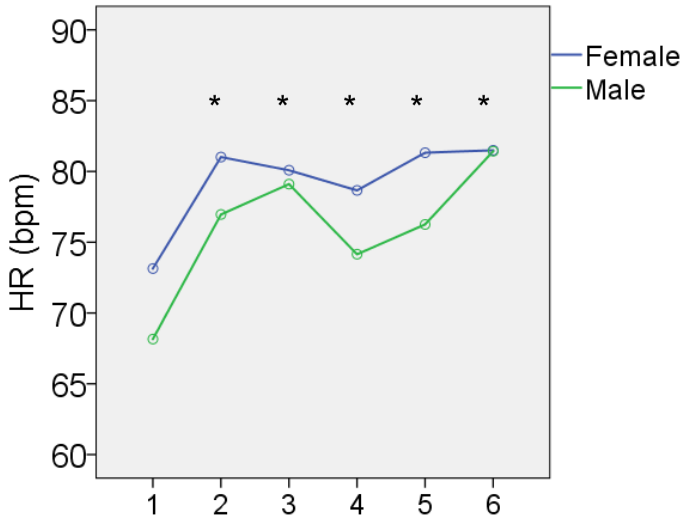
There was a tendency to greater decrease in blood pressure immediately after assumption of the upright position in females versus males (sBP: -10.8 bpm vs. -8.3 bpm; dBP: -9.2 bpm vs. 6.5 bpm; mBP: -10.5 bpm vs. -7.4 bpm). However, differences were not statistically significant.

Greater values of TPRI at rest were observed in women compared to men (4306.3 ± 1318.9 vs. 3846.6 ± 651.9 dyne*s*m^2/cm^5), but the differences did not reach statistical significance. Also, there was a tendency to higher TPRI values during standing in females compared to males.

In addition, no significant gender differences were seen for stroke index and cardiac index with similar changes observed throughout the sit to stand test. Furthermore, there was no significant interaction between epoch and gender.

Five female (35.7%) but only one male (11.1%) participant fulfilled the criteria for orthostatic hypotension, i.e. fall in systolic blood pressure of ≥20mmHg and/or diastolic blood pressure of ≥10mmHg within 3 minutes of standing (17). Despite that, presyncopal symptoms were not expressed. The decrease in systolic and diastolic blood pressure occurred immediately after standing up (in the first epoch of standing).

The independent-sample-t-test showed a significant difference in TPRI between subjects with and without initial orthostatic hypotension in the first ten seconds of standing. Subjects with rapid drop in blood pressure had significantly lower TPRI values in the first ten seconds of standing compared to subjects without indication of orthostatic hypotension (2342.91 ± 669.33 vs. 3686.47 ± 1110.13 dyne*s*m^2/cm^5), t(19) = 2.746, p = 0.013. There was no difference in baseline TPRI values between the two groups (3503.93 ± 937.53 vs. 4382.09 ± 1108.22 dyne*s*m^2/cm^5), t(19) = 1.705, p = 0.104. The decrease in TPRI in response to orthostatic challenge in subjects with OH amounted to 33.13%, while in subjects without OH the decrease in TPRI amounted to 15.87%. The independent-sample-t-tests showed no significant differences between the two groups for heart rate, stroke index and cardiac index.


HR (bpm)
90
85
80
75
70
65
60
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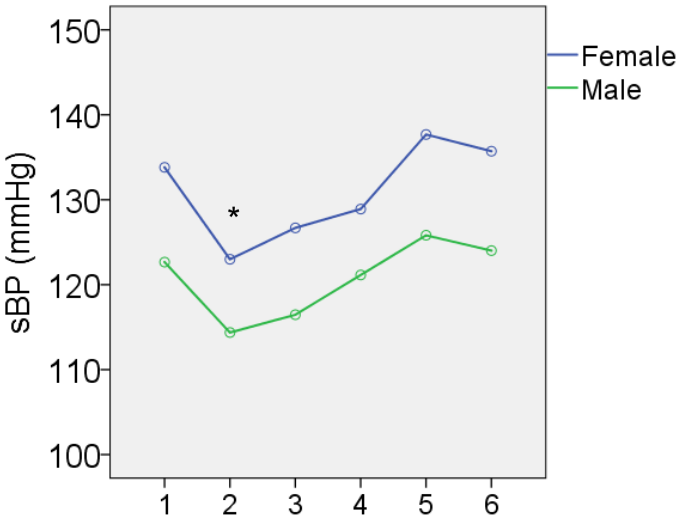
2
3
4
5
6
*
*
*
*
*
Female
Male


sBP (mmHg)
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130
120
110
100
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3
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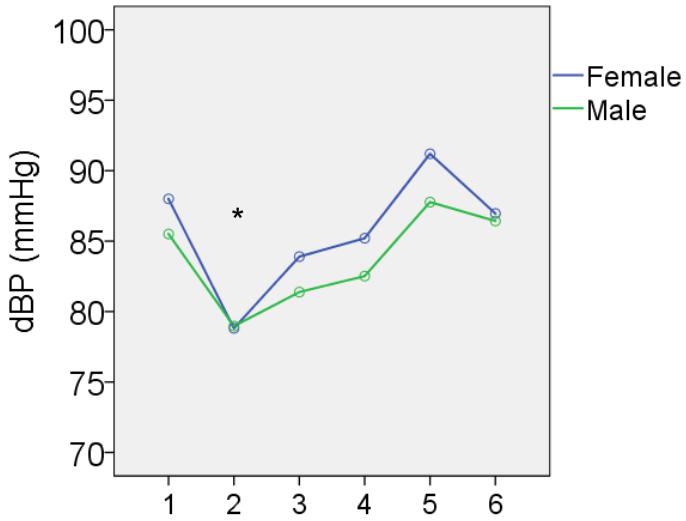
*
Female
Male


dBP (mmHg)
100
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75
70
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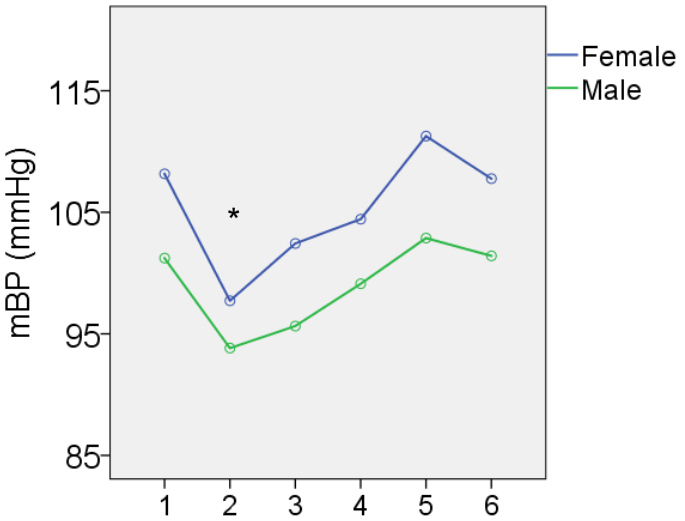
*
Female
Male


mBP (mmHg)
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105
95
85
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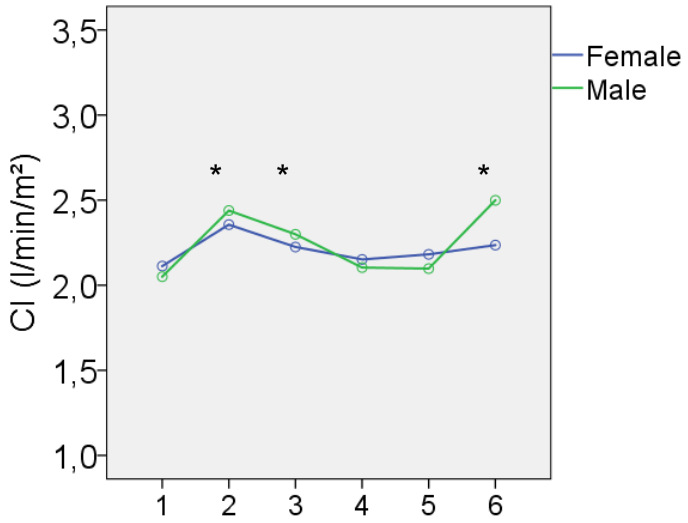
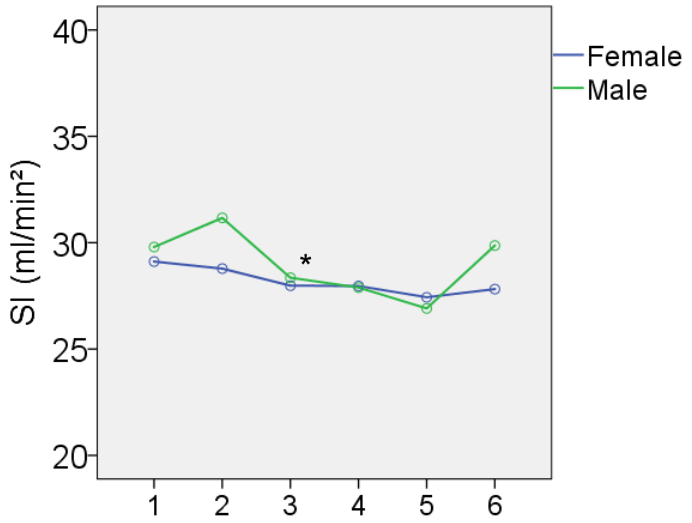
Female
Male





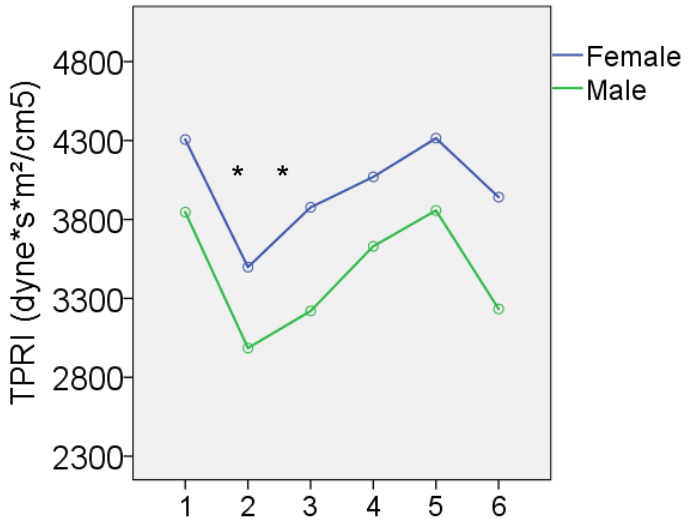


Figure 12: Hemodynamic responses during sit to stand test in females (blue line) and males (green line).
Epochs of the sit to stand test are plotted on the x-axis:
1: Baseline
2: First 10 seconds of standing
3: Second 10 seconds
4: Third 10 seconds of standing
5: 10 seconds in the third minute of standing
6: Last 10 seconds of standing
* $p < 0.05$ compared with baseline

Table 3: Hemodynamic parameters during sit to stand test by gender

		Baseline	Standing					Statistics	
		1. epoch	2. epoch	3. epoch	4. epoch	5. epoch	6. epoch	Effects	P
HR (bpm)	Female	73. 1 ± 13.6	81.0 ± 14.4	80.1 ± 16.9	78.7 ± 17.3	81.3 ±15.6	81.5 ± 14.6	Epoch	<0.001
	Male	68.2 ± 6.8	77.0 ± 8,4	79.1 ± 5.0	74.1 ± 7.5	76.3 ± 5.6	81.4 ±7.4	Epoch ∗ Gender	0.209
								Gender	0.541
sBP (mmHg)	Female	133.8 ±18.7	123.0 ± 27.0	126.7 ± 25.4	128.9 ± 27.4	137.7 ± 21.9	135.7 ± 24.3	Epoch	0.002
	Male	122.7 ±10.2	114.4 ± 13.3	116.5 ± 14.9	121.1 ± 9.5	125.8 ± 9.4	124.0 ± 20.9	Epoch ∗ Gender	0.893
								Gender	0.209
dBP (mmHg)	Female	88.0 ± 11.0	78.8 ± 17.5	83.9 ±16.0	85.2 ±17.6	91.2 ± 12.0	87.0 ± 14.7	Epoch	<0.001
	Male	85.5 ± 6.1	79.0 ± 9.7	81.4 ± 8.4	82.6 ±6.6	87.8 ± 3.7	86.4 ± 5.5	Epoch ∗ Gender	0.838
								Gender	0.695
mBP (mmHg)	Female	108.2 ± 12.8	97.7 ± 20.1	102.4 ± 18.2	104.4 ± 20.3	111.3 ± 14.7	107.8 ± 16,0	Epoch	<0.001
	Male	101.2 ± 7.7	93.8 ± 11.8	95.6 ± 11.1	99.1 ± 8.8	102.9 ± 5.7	101.4 ± 8.0	Epoch ∗ Gender	0.845
								Gender	0.281
SI (ml/m^2)	Female	29.1 ± 5.7	28.8 ± 4.1	28.0 ± 4.2	28.0 ± 4.6	27.4 ± 4.3	27.8 ± 4.5	Epoch	0.013
	Male	29.8 ± 5.5	31.2 ± 6.8	28.4 ± 5.2	27.9 ± 5.1	26.9 ± 5.2	29.9 ±5.3	Epoch ∗ Gender	0.299
								Gender	0.669
CI (l/min/m^2)	Female	2.1 ± 0.5	2.4 ± 0.5	2.2 ± 0.5	2.2 ± 0.5	2.2 ± 0.5	2.2 ± 0.4	Epoch	<0.001
	Male	2.1 ± 0.3	2.4 ± 0.4	2.3 ± 0.3	2.1 ± 0.3	2.1 ± 0.3	2.5 ± 0.5	Epoch ∗ Gender	0.67
								Gender	0.827
TPRI (dyne*s*m^2/cm$^{5)}$	Female	4306.3 ± 1318.9	3497.9 ± 1380.1	3876.9 ± 1336.6	4069.7 ± 1340.8	4314.7 ±1077.3	3941.5 ± 982.3	Epoch	<0.001
	Male	3846.6 ± 651.9	2985.2 ± 662.3	3220.1 ± 333.4	3630.1 ± 582.3	3857.3 ± 697.5	3233.1 ± 947.6	Epoch ∗ Gender	0.876
								Gender	0.240

Values are means ± SD.

The table shows changes of hemodynamic variables during orthostatic challenge (sit to stand test) along with significance of main effects and interactions for epoch and gender.

4.3 Autonomic parameters

Wilcoxon signed-rank tests with combined female and male data sets showed that the change from a sitting to a standing posture elicits statistically significant changes in heart rate variability parameters (Table 4).

A significant reduction occurred in spectral power in the high-frequency band (normalized units) (37.3 vs. 27.3, Z = -2.127, p = 0.033). In addition, there was n increase in LFnuRRI (62.7 vs. 72.4, Z = -2.127, p = 0.033) and LF/HF-ratio (1.7 vs. 2.7, Z = -2.289, p = 0.022) in response to orthostatic challenge. Furthermore, there were significant changes in HF-RRI and LF-RRI during orthostatic challenge (HF-RRI (ms^2): 53.1 vs. 26.3, Z = -2.873, p = 0.004; LF-RRI (ms^2): 101.9 vs. 71.8, Z = -2.613, p = 0.009).

Table 3: Heart rate variability during sit to stand test

	Baseline	**Standing**
LFnuRRI (%)	62.7 (49.4-75.6)	72.4* (59.3-81.3)
HFnuRRI (%)	37.3 (24.4-50.6)	27.3* (18.7-40.7)
LF-RRI (ms^2)	101.9 (39.2-143.7)	71.8* (27.3-138.2)
HF-RRI (ms^2)	53.1 (24.5-143.7)	26.3* (10.5-73.5)
LF/HF	1.7 (1.0-3.1)	2.7* (1.5-4.3)

Values are medians and 1st and 3rd quartiles.

* $p < 0.05$ compared with baseline

Finally, Mann-Whitney U tests were used to compare differences between females and males. Data is presented in Table 4. Regarding heart rate variability parameters, there were found lower HFnuRRI (marker of parasympathetic activity) (29.8 vs. 47.7, U = 28.000 p = 0.043), higher LFnuRRI (related to sympathetic modulation) (70.2 vs. 52.3, U = 28.000 p = 0.043) and higher LF/HF ratio (marker of sympathovagal balance) (2.4 vs. 1.1, U = 28.000 p = 0.043) in women compared with men at rest. No significant gender differences were found after standing up from the sitting position, albeit there was a similar tendency. No differences were found in absolute values of low frequency power components and high frequency power components of heart rate variability, neither at baseline nor in the standing position.

Table 4: Heart rate variability during sit to stand test by gender

	Baseline		Standing	
	Female	Male	Female	Male
LFnuRRI (%)	70.2 (60.1-77.1)	52.3* (39.4-61.0)	78.8 (65.9-82.8)	59.9 (55.2-80.2)
HFnuRRI (%)	29.8 (22.8-39.9)	47.7* (39.0-60.5)	21.2 (17.2-34.0)	40.1 (19.8-44.8)
LF-RRI (ms^2)	81.8 (39.2-211.2)	153.83 (38.6-247.7)	64.0 (35.3-118.3)	100.1 (24.5-195.1)
HF-RRI (ms^2)	31.1 (21.7-84.9)	123.1 (40.5-177.3)	23.5 (8.2-49.3)	42.6 (12.7-62.7)
LF/HF	2.4 (1.5-3.9)	1.1* (0.7-1.6)	3.7 (1.9-4.8)	1.5 (1.3-4.0)

Values are medians and 1st and 3rd quartile.

* $p \leq 0.05$ compared with women

5 Discussion

The study was designed to investigate how orthostatic challenge provided by a sit to stand test affects changes in hemodynamic and autonomic responses in healthy older males and females.

The major findings of the study are as the following:

1. Orthostatic hypotension occurs more frequently in older women than in men.
2. Women and men demonstrate no statistically significant differences in hemodynamic parameters during orthostatic challenge.
3. There are gender differences in the cardiac autonomic control, with women having higher LFnuRRI, lower HFnuRRI and higher LF/HF ratio at rest.

These unique data provide evidence that older women show greater occurrence of orthostatic hypotension. The above observations support the hypothesis that men and women have similar hemodynamic responses to an orthostatic challenge. However, the data do not support the hypothesis that cardiac autonomic control is similar in both sexes. The postmenopausal females showed a predominance of sympathetic control at rest when compared to men.

5.1 Gender differences in orthostatic hypotension

Standing up from a lying or sitting position creates a gravitational gradient with blood accumulation in the lower body parts, decrease in central blood volume and blood pressure. Compensatory effects including, among others, the baroreflex-mediated blood pressure regulation (parasympathetic withdrawal and sympathetic activation) maintain an adequate blood pressure. Orthostatic hypotension occurs when the change in blood pressure is ineffectively compensated.

In the light of the demographic change with growing proportion of the older population it is important to gain more detailed information of the cardiovascular regulation during changes in posture in older people and possible differences between men and women. Orthostatic hypotension is common in older persons with a prevalence that varies from 4% to 33% (47) and is associated with risk of falls, fractures, syncope and higher morbidity and mortality (44,46,55). Generally, the risk of falls increases with aging and women are more affected than men (56–58). According to the WHO global report on falls prevention in older age,

approximately 30% of people ≥65 years fall each year (55). Falls are the major cause for injury-related hospitalizations in people ≥65 years and therefore represent an important public health issue (57). Preventing falls is absolutely essential and when designing prevention strategies, one should not disregard gender-specific factors.

Numerous studies reported a higher incidence of orthostatic hypotension and greater orthostatic intolerance to various orthostatic challenges in females compared to males in younger age groups (20,21,29,31,59–61). However, most studies investigating gender differences focused on healthy young persons and the results are not fully transferable to the older population. In older subjects, gender differences in cardiovascular responses and orthostatic tolerance are not yet completely clear. This is probably due to the stronger interference with factors such as diseases and existing medication. Previous investigators mentioned that is uncertain whether the higher incidence of orthostatic hypotension is due to aging per se or due to age associated conditions (62). Because of the increasing frequency of orthostatic hypotension and circulatory lability with advancing age (44,46,47,62,63), it is important to detect sex-specific differences in older persons. Within this study, it was observed that more women met the criteria for OH than men. Orthostatic challenge led to rapid drop in blood pressure (systolic blood pressure ≥20 mmHg and/or diastolic blood pressure ≥10 mmHg) in 35.7% of women and 11.1% of men. These results indicate that postmenopausal women are more prone to orthostatic hypotension than age-matched men. These findings are surprisingly different with some studies in this area. Rutan et al. (1992) carried out a longitudinal study aimed at assessing the prevalence of OH and its association with several factors. 5201 men and women ≥65 years were enrolled in the study. Heart rate and blood pressure were measured in a supine position and after three minutes of standing. The prevalence of asymptomatic OH (sBP ≥20 mmHg and/or dBP ≥10 mmHg) was 16.2% and the overall prevalence increased to 18.2% when symptomatic OH was included. There were no gender differences in OH (47). Our unique data, which were obtained by selected epochs during different phases of standing, allowed us to examine the hemodynamic responses to orthostatic challenge comprehensively. This could explain the differences in the

data obtained by our study and that of Rutan et al. (1992). Furthermore, by measuring blood pressure solely three minutes after standing up, it might be possible that they underestimated the incidence of OH in the older population.

A study by Mellingsæter et al. (2003) on gender differences in orthostatic tolerance demonstrated that men ≥65 years have poorer orthostatic tolerance than age-matched women (59): more men terminated a head-up tilt (HUT) test because of presyncopal symptoms or syncope. However, it should be noted that we assessed the percentage of men and women who fulfilled the criteria of orthostatic hypotension, while Mellingsæter et al. focused on the development of presyncopal symptoms or syncope. Hence, it is possible that asymptomatic OH is more common in older females, whereas symptomatic OH with presyncopal symptoms such as lightheadedness, dizziness, blurred vision, pallor, nausea are more common in older men. Furthermore, their study protocol differed from ours in the mean age of the study participants (72 vs. 63 years, respectively) and method of inducing orthostatic challenge (30° and 70° HUT vs. sit to stand test, respectively). As we found no differences in hemodynamic responses between the sexes, the mechanisms responsible for the larger proportion of women in OH remain unclear and have to be ascertained in further studies. However, our data showed that the participants with a rapid fall in blood pressure meeting the definition of orthostatic hypotension had significantly lower TPRI values immediately after assuming the upright position. There was no difference in heart rate, stroke index and cardiac index, which indicates that vascular response, not cardiac response, is responsible for the transient hypotension. There was no evidence that systemic vascular resistance is generally attenuated in older women compared to men, which could explain the differences in the distribution of OH.

In the present investigation a fall in systolic blood pressure ≥20 mmHg and/or diastolic blood pressure ≥10 mmHg occurred immediately after standing up from a sitting position. Thereafter, blood pressure stabilized within a few seconds and no additional blood pressure drops could be detected after 30 seconds in the further course of the protocol. Rutan et al. (1992) stated that it is not known if an initial drastic but brief decrease in blood pressure or a less drastic but sustained reduction in blood pressure is pathophysiologically more important (47).

In our study, none of the affected persons expressed presyncopal symptoms during the orthostatic challenge. Orthostatic hypotension can be asymptomatic or symptomatic. However, even asymptomatic individuals have a higher risk for future falls as well as associated comorbidities and therefore asymptomatic OH should also be minimized (44,46,47).

5.2 Gender differences in hemodynamic parameters

Application of orthostatic challenge led to changes in the seven measured hemodynamic parameters (heart rate, systolic blood pressure, diastolic blood pressure, mean blood pressure, cardiac index, stroke index and total peripheral resistance index). There was a trend towards higher blood pressure, heart rate and TPRI values in the sitting and in the standing position in women compared to men. Additionally, there was a tendency to stronger decrease in systolic, diastolic and mean blood pressure immediately after standing up. All these differences were not statistically significant, which suggests that older men and women have comparable hemodynamic responses to postural change.

As Figure 12 illustrates, orthostatic challenge led to compensatory effects to maintain blood pressure and also cerebral blood flow. There was a minimal decrease in stroke index but the accompanying increase in heart rate was able to maintain cardiac output. TPRI returned to baseline level after an initial reduction. It appears that maintenance of blood pressure in older persons during postural changes was achieved primarily by increased heart rate and minimal changes in stroke volume, thus ensuring that the cardiac output remained stable. The results obtained in this study differ from the observations seen in other studies. For example, in comparison to supine position, assuming an upright position led to a greater drop of SV and lower CO as well as an increase over baseline level of TPR (62,64). Laitinen et al. (2003) postulated that older persons, in order to maintain their blood pressure, rely essentially on increase in peripheral resistance and less on increase in heart rate (62). The differences reported in these studies compared to ours could be due to the fact that the studies used HUT to provide orthostatic challenge, while in the present study a simple sit to stand test was used. It is conceivable that a sit to stand test constitutes a less strong stimulus than the formerly mentioned methods and therefore elicits less powerful cardiovascular responses. Additionally, the study protocol of Laitinen et al. differed

from ours in the duration of the stimulated condition (thirteen minutes 70° HUT vs. six minutes sit to stand test). Our data also showed that there was a tendency to stronger decrease of blood pressure immediately after assumption of the upright position in women versus men, even though the difference was not statistically significant. However, our results are in contradiction to the findings of other studies that found a tendency to larger drop in blood pressure among older men compared to age-matched women in response to orthostatic challenge (59,65). It is possible that the divergences arise from different protocols (HUT vs. sit to stand test) or from differences in the selected epochs. Mellingsæter et al. selected one epoch of four minutes length in the tilted position, while we analyzed five different epochs in the standing position. Moreover, Romero-Ortuno et al. (2010) reported that the observed difference between older men and women disappeared in the group, which was free from antihypertensives.

5.3 Gender differences in cardiac autonomic regulation

Heart rate variability is measured by the beat-to-beat variations of heart rate period (R-R interval). The autonomic nervous system has a major impact on HRV. Therefore, power spectral analysis of HRV (via fast Fourier transform) has become widely used for quantification of cardiac autonomic activity. Frequency components are divided in high frequency (0.15 to 0.40 Hz), low frequency (0.04 to 0.15 Hz) and very low frequency (<0.04 Hz).

High frequency oscillations of heart rate reflect the cardiac parasympathetic activity and are associated with respiratory sinus arrhythmia (66–68). Respiratory sinus arrhythmia is a vagal mediated variation in heart rate causing an increase in heart rate during inspiration and a decrease during expiration.

The role of low frequency oscillations of heart rate seems to be more complex. They are associated with the Traube-Hering-Mayer waves of blood pressure, which give information about the sympathetic activity. Previously, the LF peak was considered to solely represent sympathetic nerve activity, especially when expressed in normalized units (LFnuRRI). In recent works, the role of low frequency oscillations in heart rate is more controversial, because it depends on several factors (i.e. alterations in venous return, cardiac filling, the baroreceptor reflexes and the cardiac sympathetic activity) (49,52). Now it is widely believed

that LF activity is linked to the sympathetic and parasympathetic nervous system (51–53).

The ratio between LF and HF has been described in literature as a marker of autonomic balance. A higher LF/HF ratio reflects a shift to higher sympathetic activity, whereas a lower LF/HF ratio reflects a parasympathetic dominance. On the other hand, there are studies like Billmann et al. (2013) that demonstrate that LF/HF ratio do not accurately reflect sympathovagal balance, because the components are influenced by a complex mix of several factors (52).

It is assumed that female sex hormones are in a large part responsible for gender differences in autonomic regulation (19,25–27,69). Due to the declining influence of estrogen after menopause, a narrowing or vanishing of the gender differences seen in young men and women were anticipated. Contrary to this expectation, the data suggest that postmenopausal women have a dominant sympathetic influence at rest (higher LF power, lower HF power, lower LF/HF ratio) compared to age-matched men. Although not statistically significant, evidence of a transition in the balance of the autonomic nervous system towards higher sympathetic activity in the standing position was seen. The fact that significant gender differences were only evident during rest and not during orthostatis might be due to the small sample size or the susceptibility of HRV measures to small variations and disturbance. Another possible reason for disappearing sex differences in response to orthostatic challenge might be that older women show less pronounced increase in sympathetic tone and less vagal withdrawal, respectively.

Numerous clinical studies investigated how age and sex may affect heart rate variability (29,70,71). Several lines of evidence suggest that parasympathetic control is the major regulator of the cardiovascular system in young women, while in postmenopausal women the sympathetic tone is dominant (30). Convertino et al. (1998) hypothesized that young women respond to orthostatic stress with vagal withdrawal, while young men show greater sympathetic stimulation to the peripheral vasculature (31). The results of Agelink et al. (2001) support these observations. LF power and LF/HF ratio were significantly lower in young and middle-aged women compared to age-matched men (72), indicating that women of this age group show a predominance of parasympathetic control. Barnett et al. (1999) who investigated how age and gender influence autonomic control,

demonstrated that healthy women in all ages have elevated HF power compared to men (29).

Most of the studies in which HRV across age was investigated reported the frequency domain indexes of HRV to be significantly lower among older persons than among younger persons (62,71,73,74), which speaks for a decreased parasympathetic nervous system activity or increased sympathetic nervous system activity, respectively. Ryan et al. (1994) and Stein et al (1997) described a decrease in HF power and LF/HF ratio with physiological aging and Bigger et al. (1995) also found reduced VLF, LF and HF power with increasing age (71,73,74). Consistent with these reports, Lipsitz et al. (1990) found an overall reduction in HRV in supine position and during HUT 60° in older persons (mean age 76.5 years) and demonstrated that the reduction occurs in HF and LF power components (66). In a study by Laitinen et al. (2004) on age dependency of autonomic responses to HUT, it is shown that aging is associated with increased sympathetic and decreased parasympathetic activity during rest and attenuation of autonomic responses during stimulation in terms of HUT (62). Furthermore, they suggest that parasympathetic regulatory control in attenuated with advancing age. The majority of studies on gender differences in cardiovascular autonomic responses have found either no gender differences in healthy older persons or a still existing lower sympathetic and higher parasympathetic activity in women in comparison to men which is in contrast to our findings.

Huxley et al. (2007) stated that women at all ages show reduced sympathetic and enhanced parasympathetic activity than men accompanied by lower TPR and MAP (20). Other studies confirmed that parasympathetic activity is still elevated in women in all age groups (29,73). Accordingly, Bigger et al. (1995) reported that healthy women aged 40 to 69 years have significantly lower LF power and LF/HF ratio than their male counterparts (74). Their subject selection criteria differed from ours as they included younger subjects and covered a wide range of age. Therefore, their results are not representative for differences between postmenopausal women and older men.

In a study by Mellingsæter et al. (2013) with 65 healthy persons (mean age 72 years) a lower LF/HF ratio, higher HFnuRRI and lower LFnuRRI were found in women compared to men at rest and during 30° HUT (59). However, no differences were detected during 70° HUT.

Matsukawa et al. (1998) measured MSNA in 69 healthy women (20 to 79 years) and 76 men (16 to 80 years) at rest and investigated how gender affects the age-related increase in sympathetic nerve activity (69). They found out that MSNA is similar in females and males over the age of 50 years. MSNA values even had the tendency to be higher in older women rather than similar to those of men, though. These findings are in accordance with ours, which were obtained using HRV.

Narkiewicz et al. (2005) studied 120 men and 96 women (aged 20-72 years) to investigate the influence of gender on age-related increase in sympathetic traffic. MSNA, blood pressure and heart rate were measured during supine rest. Their results suggested that aging is associated with a stronger increase in sympathetic activity in women compared to men (75). This matches the results obtained from our study.

Several publications have highlighted that sympathetic activity markedly increased after menopause (69). Barnett et al. (1999) found out that women with hormone replacement therapy have reduced sympathetic activity than untreated postmenopausal women and suggested that estrogen increases baroreflex sensitivity and leads to a reduction of LF power (29). This would explain similar autonomic nervous system responses in older men and women, but not higher sympathetic and lower parasympathetic activity at rest in females, which was obtained in our study.

It is interesting that despite the differences in HRV parameters between women and men at rest, hemodynamic parameters were comparable in both groups. Higher sympathetic and lower parasympathetic activity does not seem to cause higher resting blood pressure or total peripheral resistance. The underlying mechanisms for higher LFnuRRI and LF/HF ratio as well as lower HFnuRRI in older women at rest remain unclear. Further investigations are needed to elucidate possible physiological mechanisms.

5.3.1 Aging, autonomic function and gender

The implications of altered HRV were investigated in some studies. Tsjui et al. (1994) demonstrated a link between reduced HRV and the risk of overall mortality in older persons (76). Higher sympathetic and/or lower parasympathetic tone are related to cardiovascular disease morbidity and mortality (29,32). As people age, the cardiovascular risk and in particular the risk of hypertension increases (75,77).

It has been well established that the incidence of hypertension in women around the menopause is similar or even higher than in age-matched men. This results in part from the diminishing protective effect of female sex hormones on the cardiovascular system in postmenopausal women (25,27). The positive autonomic profile seen in younger women (predominance of parasympathetic tone) is lost in older women, which explains the increasing risk of developing cardiovascular diseases.

5.4 Limitations

A limitation of this study was the small sample size. However this pilot study could be a good basis for future full-scale studies. With a larger sample size, it would be possible to uncover gender difference in older persons, which remained possibly undetected in the present investigation.

A sit to stand test was used to provide orthostatic challenge, whereas many other investigators used head-up tilt test or application of lower body negative pressure, which makes our results not fully applicable to theirs and could also contribute to the differences in our findings. However, standing up from a sitting position is an everyday challenge in the lives of older persons and therefore, a sit to stand test represents a more realistic life situation than the abovementioned methods.

A further potential limitation is that respiration was not controlled in this study. Respiration has significant effects on HRV parameters (66,70). Standardization of respiratory rate by metronomic breathing may reduce the impact of different respiratory frequencies on heart rate dynamics and could make clearer the differences in gender. However, Goldberger et al. (2001) showed that HRV does not differ whether the breathing rate is fixed to metronome or not (70). In our study, although we did not control breathing, the participants were asked to not carry out deep breaths or perform valsalva maneuver in order to prevent potentially interfering influence.

Furthermore, data on additional influencing factors potentially related to cardiovascular responses to orthostatic challenge and orthostatic hypotension, e.g. hormone replacement therapy, contraceptive history in the older women (influence of years of oral contraceptive use) were not available. There is some evidence that hormonal changes affect cardiovascular regulatory mechanisms by altering sympathetic activity, baroreflex sensitivity and circulating catecholamines

(30). For future studies it would be interesting to examine the effects of long term and short term ingestion of contraceptives on cardiovascular regulatory mechanism and how exactly hormone replacement therapy affects the cardiac autonomic responses.

5.5 Conclusions and perspectives

In this pilot study we investigated the influence of gender on cardiovascular responses to a change in posture in healthy older persons. Our results indicate that a sit to stand test induces orthostatic hypotension more frequently in older women than in men. The underlying reasons contributing to higher incidence of orthostatic hypotension in older females remain unclear. We could not find significant gender differences in responses of heart rate, blood pressure, cardiac index, stroke index and peripheral resistance index, which would have provided an explanation for the uneven distribution of orthostatic hypotension. It seems that cardiac autonomic regulation differs, in some respect, between the sexes, i.e. higher sympathetic and lower parasympathetic activity in females during rest. However, a difference in cardiac autonomic parameters in response to orthostatic challenge could not be observed. Moreover, the observed differences seem to have no significant impact on hemodynamic parameters. Further studies should be conducted to determine the underlying mechanisms. Nevertheless, the present investigation provides valuable insights into similarities and differences of blood pressure regulation and interaction of sympathetic and parasympathetic nervous system. Identifying and understanding mechanisms, which are responsible for gender differences in cardiovascular regulation, may contribute to implementation of gender-specific prevention and treatment of cardiovascular diseases and prevention strategies of falls in older persons.

As Huxley et al. (2006) pointed out, gender differences involve more than just sex hormones alone (20) and it is important to recognize that differences in cardiovascular regulation exist between older men and women. The present pilot study is valuable as it helps to determine the adequate sample size for large-scale research in order to receive more reliable results.

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Appendix

Probandeninformation und Einwilligungserklärung zur Teilnahme an der wissenschaftlichen Studie

„VASCULAR STIMULUS RESPONSE IN HEALTH AND DISEASE: A LONGITUDINAL STUDY“ (VESSELS)

„Gefäßstimulation bei Gesunden und Kranken: Eine Längsschnittstudie“

- Pilotstudie -

Sehr geehrte Teilnehmerin, sehr geehrter Teilnehmer!

Wir laden Sie ein an der oben genannten wissenschaftlichen Studie im Anschluss teilzunehmen. Die Aufklärung darüber erfolgt in einem ausführlichen Gespräch.

Die Teilnahme an dieser Studie ist freiwillig und kann jederzeit ohne Angabe von Gründen durch Sie beendet werden.

Wissenschaftliche Studien sind notwendig, um verlässliche neue Forschungsergebnisse zu gewinnen. Unverzichtbare Voraussetzung für die Durchführung einer wissenschaftlichen Studie ist jedoch, dass Sie Ihr Einverständnis zur Teilnahme an dieser Studie schriftlich erklären. Bitte lesen Sie den folgenden Text sorgfältig durch und zögern Sie nicht Fragen zu stellen.

Bitte unterschreiben Sie die Einwilligungserklärung nur

- wenn Sie Art und Ablauf der wissenschaftlichen Studie vollständig verstanden haben,

- wenn Sie bereit sind, der Teilnahme zuzustimmen und

- wenn Sie sich über Ihre Rechte als Teilnehmer an dieser wissenschaftlichen Studie im Klaren sind.

Zu dieser wissenschaftlichen Studie, sowie zur Probandeninformation und Einwilligungserklärung wurde von der zuständigen Ethikkommission eine befürwortende Stellungnahme abgegeben.

1. Was ist der Zweck der wissenschaftlichen Studie?

Das Projekt ist als Longitudinalstudie angelegt und untersucht Gefäßantworten auf orthostatische[1] Reizung bei gesunden jungen Probanden, Patienten mit Parkinson-Erkrankung (PD), Alzheimer-Krankheit (AD), Schlaganfall (S), sowie älteren gesunden Probanden.

Es wird ermittelt, wie orthostatische Belastung, Zeitfaktoren, Alter und Geschlecht das Gefäßverhalten beeinflussen und sich damit auf Blutdruckregulation und orthostatische Stabilität auswirken. Die Endothelfunktion[2] wird über reaktive Vasodilatation[3] der a. brachialis (Ultraschall-Dopppler), Fundus- und Blutuntersuchung abgeschätzt - d.h. es wird die Blutzirkulation in verschiedenen Körperregionen, z.B. Augen oder Unterarm, gemessen.

2. Wie läuft die wissenschaftliche Studie ab?

Diese wissenschaftliche Studie wird am Institut für Physiologie, MedUniGraz (Studie A) bzw. an der Univ.-Klinik für Neurologie, LKH Graz (Studie B und C) durchgeführt, und es werden **insgesamt** 180 Personen daran teilnehmen. Die Studie ist unterteilt in drei Probandengruppen (A, B, C). Ihre Teilnahme an dieser klinischen Studie wird voraussichtlich 1 Jahr dauern (4 Versuche in dreimonatigem Abstand) - siehe Table 1.

Studie A: 60 gesunde junge Personen werden (konsekutiv) passiver Kopf-hoch-Lagerung (head-up tilt, HUT) plus zunehmender Unterdruck am Unterkörper (lower body negative pressure, LBNP), intensitätsmäßig ansteigend bis zum Auftreten präsynlopaler Symptome, ausgesetzt.

Studie B: 60 ältere Patienten (20 PD, 20 AD, 20 S) durchlaufen einen orthostatischen Test (Sitzen > Stehen für 5 min) und ihre Gefäßreaktionen werden studiert. Im Anschluss an den ersten Studientag wird eine augenärztliche Untersuchung, bestehend aus einer Untersuchung an der Spaltlampe, einer Augeninnendruckmessung und eines Sehtests durchgeführt. Weiter werden die Gefäßdurchmesser mittels Retinal Vessel Analyzers (=eine spezielle Kamera zur Darstellung der Netzhautgefäße) für drei Minuten gemessen. In der zweiten Minute wird die Netzhaut mit flackerndem Licht beleuchtet und die Reaktion der Gefäße auf diesen Reiz bestimmt. Dabei müssen Sie ruhig auf eine Nadelspitze blicken. Die Messung der Gefäßweiten erfolgt berührungslos und absolut schmerzfrei. Sollten Sie oder ein Familienmitglied an einer Epilepsie leiden, können Sie an dieser Messung nicht teilnehmen. Zur Durchführung der augenärztlichen Untersuchung, sowie der Gefäßweitenmessung wird das Studienauge mit einem Mydriatikum (=eine pupillenerweiternde Substanz) eingetropft. Sie werden dadurch einige Stunden unscharf sehen, die Wirkung klingt nach 3-5 Stunden wieder ab. Sie dürfen daher nach Studientagende kein Fahrzeug lenken und keine Maschinen bedienen!
Sämtliche augenärztlichen Untersuchungen und Messungen werden an der Universtitäts-Augenklinik ausschließlich am ersten Studientag durchgeführt.

Erläuterungen: [1]orthostatisch = "in aufrechter Körperhaltung"
[2]Endothelfunktion = Steuerung des Kontraktionszustands von Blutgefäßen durch die innere Gefäßhaut
[3]Vasodilatation = Unter **Vasodilatation** versteht man die "Ausdehnung" bzw. „Erweiterung" (Dilatation) von Blutgefäßen

Studie C, Kontrolle: Wie B, aber mit gesunden Probanden der gleichen Altersgruppe (N=60). Wir erwarten uns wichtige neue Hinweise auf den Einfluss von Alter und Krankheit auf Endothelfunktion und orthostatische Stabilität, die für epidemiologische Studien und Risikoabschätzungen nutzbar sind.

Tabelle 1:
Zusammenfassung der drei Gruppen und detaillierte Auflistung der orthostatischen Herausforderung

Erklärung: HUT: Head up tilt = aufrechte Körperlage
LBNP: Lower body negative pressure = Unterdruck an Becken und Beinen
Präsynkope: Zeit vor Auftreten eines Kreislaufkollaps

Gruppe	Anzahl/ Geschlecht	Gesund	Alter	Orthostatische Herausforderung	Orthostat. Test / Jahr
		JÜNGERE PERSONEN			
A	**Anzahl = 60:** 30 Männer 30 Frauen	JA	18 -35	HUT + LBNP ➔Präsynkope	4-mal alle 3 Monate
		ÄLTERE PATIENTENGRUPPE			
B	**Anzahl = 60:** 10 Männer (mit Parkinson-Erkrankung) 10 Frauen (mit Parkinson-Erkrankung) 10 Männer (mit Alzheimer-Erkrankung) 10 Frauen (mit Alzheimer-Erkrankung) 10 Männer (Schlaganfall) 10 Frauen (Schlaganfall)	NEIN	60 -90	**Sitzen/Steh-Test** - sitzen (5 min) - stehen (5min) - wieder hinsetzen (5 min)	4-mal alle 3 Monate
C	**Anzahl = 60:** **(Kontrollgruppe)** 30 Männer 30 Frauen	JA	60 -90	**Sitzen/Steh-Test** - sitzen (5 min) - stehen (5min) - wieder hinsetzen (5 min)	4-mal alle 3 Monate

3. Worin liegt der Nutzen einer Teilnahme an der wissenschaftlichen Studie?

Es ist möglich, dass Sie durch Ihre Teilnahme an dieser wissenschaftlichen Studie keinen direkten Nutzen für Ihre Gesundheit ziehen. Bei dieser Studie (sind für den Probanden wertvolle kardioregulatorische (Werte die den Kreislauf betreffen: z.B.: Blutdruck, Herzfrequenz, Schlagvolumen der linken Herzkammer, Nervensystem …) Parameter (Werte)

mit Hilfe des TFM (Task Force Monitor – Gerät zur kontinuierlichen Prüfung des Blutdruckes, Herzfrequenz, des Nervensystems, Schlagvolumen ... - Hersteller ist eine Grazer Firma mit dem Namen CNSystems) bestimmbar und werden dem Probanden auch in Form eines Diagnosebogens (dieser wird vollautomatisch mit Hilfe der Auswertesoftware des Task Force Monitor erstellt) zugänglich gemacht.

4. Gibt es Risiken, Beschwerden und Begleiterscheinungen?

Nein.

5. Zusätzliche Einnahme von Arzneimitteln?

Nein.

6. Hat die Teilnahme an der wissenschaftlichen Studie sonstige Auswirkungen auf die Lebensführung und welche Verpflichtungen ergeben sich daraus?

Die Studie hat keine Auswirkungen auf Ihre Lebensführung. Sollten Sie verhindert sein und einen Termin nicht wahrnehmen können, bitten wir Sie umgehend den Koordinator dieser Studie zu informieren.

7. Was ist zu tun beim Auftreten von Symptomen, Begleiterscheinungen und/oder Verletzungen?

Sollten im Verlauf der wissenschaftlichen Studie irgendwelche Symptome, Begleiterscheinungen oder Verletzungen auftreten, müssen Sie diese dem Studienleiter mitteilen, bei schwerwiegenden Begleiterscheinungen umgehend.

8. Wann wird die wissenschaftliche Studie vorzeitig beendet?

Sie können **jederzeit** auch ohne Angabe von Gründen, Ihre Teilnahmebereitschaft widerrufen und aus der wissenschaftlichen Studie ausscheiden.

9. In welcher Weise werden die im Rahmen dieser wissenschaftlichen Studie gesammelten Daten verwendet?

Sofern gesetzlich nicht etwas anderes vorgesehen ist, haben nur die Prüfer und deren Mitarbeiter Zugang zu den vertraulichen Daten, in denen Sie namentlich genannt werden. Diese Personen unterliegen der Schweigepflicht.

Die Weitergabe der Daten erfolgt ausschließlich zu statistischen Zwecken und Sie werden ausnahmslos darin nicht namentlich genannt. Auch in etwaigen Veröffentlichungen der Daten dieser wissenschaftlichen Studie werden Sie nicht namentlich genannt.

10. Entstehen für die Teilnehmer Kosten? Gibt es einen Kostenersatz oder eine Vergütung?

Die Teilnehmer an der Studie erhalten eine finanzielle Vergütung in der Höhe von 20 Euro pro Versuch. Überdies bekommen Sie einen ausführlichen Bericht über Ihr Kreislaufverhalten in Form eines TFM–Auswertebogens, der Ihnen genau erklärt wird.

11. Möglichkeit zur Diskussion weiterer Fragen

Für weitere Fragen im Zusammenhang mit dieser wissenschaftlichen Studie stehen Ihnen Ihr Studienleiter und seine Mitarbeiter gern zur Verfügung. Auch Fragen, die Ihre Rechte als Proband und Teilnehmer an dieser wissenschaftlichen Studie betreffen, werden Ihnen gerne beantwortet.

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12. Sollten andere behandelnde Ärzte von der Teilnahme an der wissenschaftlichen Studie informiert werden?

Nein

13. Einwilligungserklärung

Name der Probandin/des Probanden in Druckbuchstaben:

.....

Geb.Datum: ..... Code: .....

Ich erkläre mich bereit, an der wissenschaftlichen Pilot-Studie

„Vascular Stimulus Response in Health and Disease: A Longitudinal Study" (VESSELS) -

„Gefäßstimulation bei Gesunden und Kranken: eine Längsschnittstudie"

teilzunehmen.

Ich bin von Herrn Priv.Doz. Dr. Nandu Goswami ausführlich und verständlich über mögliche Belastungen und Risiken, sowie über Wesen, Bedeutung und Tragweite der Studie und sich für mich daraus ergebenden Anforderungen aufgeklärt worden. Ich habe darüber hinaus den Text dieser Probandenaufklärung und Einwilligungserklärung, die insgesamt sieben Seiten umfasst, gelesen. Aufgetretene Fragen wurden mir vom Studienleiter oder von seinen Assistenten verständlich und genügend beantwortet. Ich hatte ausreichend Zeit, mich zu entscheiden. Ich habe zurzeit keine weiteren Fragen mehr.

Ich werde den wissenschaftlichen Anordnungen, die für die Durchführung der wissenschaftlichen Studie erforderlich sind, Folge leisten, behalte mir jedoch das Recht vor, meine freiwillige Mitwirkung jederzeit zu beenden, ohne dass mir daraus Nachteile für meine weitere Betreuung entstehen.

Ich bin zugleich damit einverstanden, dass meine im Rahmen dieser wissenschaftlichen Studie ermittelten Daten aufgezeichnet werden. Beim Umgang mit den Daten werden die Bestimmungen des Datenschutzgesetzes beachtet.

Eine Kopie dieser Probandeninformation und Einwilligungserklärung habe ich erhalten.
Das Original verbleibt beim Studienleiter.

.....
(Datum und Unterschrift der Probandin/des Probanden)

.....
(Datum, Name und Unterschrift des Studienleiters)

(Die Probandin/der Proband erhält eine unterschriebene Kopie der Probandeninformation und Einwilligungserklärung, das Original verbleibt im Studienordner des Studienleiters.)