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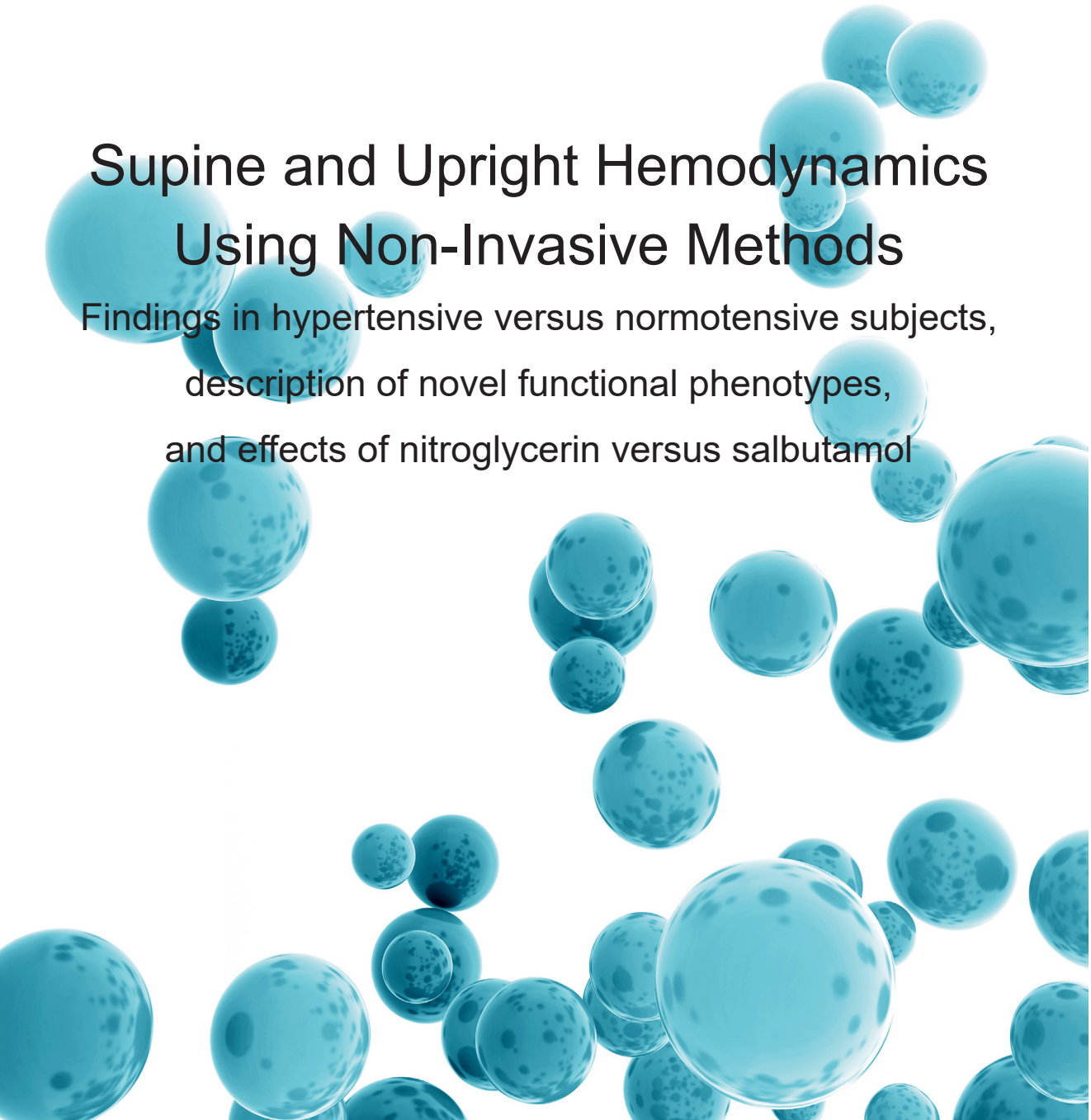
## Findings in hypertensive versus normotensive subjects, description of novel functional phenotypes, and effects of nitroglycerin versus salbutamol



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ACADEMIC DISSERTATION
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UNIVERSITY OF TAMPERE

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# List of original communications

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II. Tahvanainen A*, Tikkakoski A*, Koskela J, Nordhausen K, Viitala J, Leskinen M, Kähönen M, Kööbi T, Uitto M, Viik J, Mustonen J, Pörsti I. The type of the functional cardiovascular response to upright posture is associated with arterial stiffness: a cross-sectional study in 470 volunteers. *BMC Cardiovascular Disorders* 2016;16:101. *Contributed equally

III. Tahvanainen A, Tikkakoski A, Leskinen M, Nordhausen K, Kähönen M, Kööbi T, Mustonen J, Pörsti I. Supine and upright haemodynamic effects of sublingual nitroglycerin and inhaled salbutamol: a double-blind, placebo-controlled, randomized study. *Journal of Hypertension*, 2012;30:297-306.

IV. Tikkakoski AJ, Tahvanainen AM, Kangas P, Koskela JK, Viitala J, Kähönen MA, Kööbi T, Niemelä O, Mustonen JT, Pörsti IH. Salbutamol-induced decrease in augmentation index is related to the parallel increase in heart rate. *Basic and Clinical Pharmacology and Toxicology*. 2018;123(2):161-173.

# Abbreviations

AASI = Ambulatory arterial stiffness index

AP = Augmentation pressure

AIx = Augmentation index

AIx@75 = Augmentation index corrected to heart rate 75

BP = Blood pressure

PP = Pulse pressure.

PWV = Pulse wave velocity

NTS = Nucleus tractus solitarius

SVR = Systemic vascular resistance

SVRI = Systemic vascular resistance index

LCW = Left cardiac work

LCWI = Left cardiac work index

LF/HF-ratio = low frequency to high frequency ratio.

# Abstract

Essential hypertension is a major global health burden, but the primary reason why blood pressure changes in the course of the disease is still in many ways a mystery. Genome-wide studies have not found alleles that would account for more than 1 mmHg change in systolic or diastolic blood pressure in primary hypertension. Hypertension studies typically measure only blood pressure and heart rate, but blood pressure is the product of cardiac output and systemic vascular resistance. Additionally, there are more than 80 000 heart cycles in a single day, while hemodynamic variability is extensive during different physical provocations. Normally the measurements of blood pressure are performed in a seated position, which doesn't reveal anything about the variability or adaptation to normal daily activities. Better characterization of cardiovascular phenotypes would help in both genome research and characterization of primary hypertension.

Hypertension causes a decline of endothelial vasodilator function. Previously, inhalation of the $\beta_2$-adrenoceptor agonist salbutamol has been reported to induce an endothelium dependent vasodilatation, while sublingual administration of the nitric oxide donor nitroglycerin induces an endothelium independent vasodilatation in the vasculature. Global endothelial function has been suggested to be quantifiable by comparing the effects of salbutamol and nitroglycerin on the augmentation index, a measure of wave reflection in the arterial tree. However, salbutamol has a definite positive chronotropic effect which causes a rise in heart rate, while a rise in heart rate influences the magnitude of the augmentation index. So, an increase in heart rate could at least partially explain the change in augmentation index after salbutamol inhalation. Therefore, changes in augmentation index may not unequivocally represent alterations in endothelium mediated control of arterial tone.

Our aims were to examine whether subjects with primary hypertension have functional changes in cardiovascular regulation when compared with normotensive subjects. The response of the cardiovascular system to upright position was also examined to discover possible functional phenotypes which could be connected to known indicators of cardiovascular risk. Inhaled salbutamol was compared with sublingual nitroglycerin to examine their effects on hemodynamics during head-up tilt. In particular, we examined whether the effect of salbutamol on augmentation index was correlated with the chronotropic effect of $\beta_2$-adrenoceptor stimulation.

In study I, the hemodynamics of non-medicated normotensive and hypertensive subjects were studied. Hypertensive subjects had higher systemic vascular resistance and increased arterial stiffness, which correspond with the results from previous studies. Additionally, supine cardiac index was higher in hypertensives, so there was a measurable hyperdynamic component involved. Hypertensive subjects also had several rather small but measurable changes in cardiovascular adaptation to upright position.

In study II, we discovered three new phenotypes, which could only be found by inspecting the changes in hemodynamic variables during passive head-up tilt. The subjects with the constrictor phenotype had the largest increase in systemic vascular resistance and largest decrease in cardiac output. The constrictors had a significantly lower pulse wave velocity, an established marker of arterial stiffness, when compared with subjects in the intermediate and sustainer phenotype.

In studies III and IV, inhaled salbutamol was found to have a clearly smaller effect on cardiovascular regulation in the upright than in the supine position. In contrast, the hemodynamic effects of nitroglycerin were accentuated in the upright position. Additionally, the results in studies III and IV established that the change in augmentation index induced by salbutamol inhalation was predominantly correlated with the parallel changes in heart rate and ejection duration. This indicates that the observed simultaneous change in augmentation index does not reflect alterations in the endothelium mediated control of arterial tone.

Our results indicate that the phenotype of primary hypertension is not quantifiable by the mere measurements of supine blood pressure and heart rate. Additionally, not only the level of blood pressure and arterial stiffness, but also the functional phenotype of the cardiovascular system may be associated with cardiovascular risk. A small dose of nitroglycerin has a profound vasodilatory effect that is aggravated in the upright position. In contrast, the moderate effect of salbutamol on hemodynamics is diminished in the upright position, and is tightly connected to the positive chronotropic effect of the compound.

# Tiivistelmä

Primaari hypertensio on merkittävä verenkiertosairauksien päätetapahtumien riskitekijä. Hypoteeseja verenpaineen kohoamisen syiksi on useita, mutta perimmäinen syy edelleen on varmistamatta ja genetiikan tutkimuksissakin yksittäisten alleelien vaikutus verenpainetasoon on ollut minimaalinen. Verenkiertoelimistön tilaa arvioidaan pääsääntöisesti mittaamalla verenpaine ja syketaso istuma- tai makuuasennossa. Tämä antaa suppean kuvan verenkiertoelimistöstä. Kattavampaan verenpaineen osatekijöiden arviointiin tarvitaan sykkeen ja verenpaineen mittauksen lisäksi sydämen minuuttitilavuuden ja ääreisverenkierron vastuksen mittaukset. Verenpaineen säätelyn mekanismit aktivoituvat ja kohonneen verenpaineen hoidon ongelmat korostuvat pystyasennossa. On hyvin todennäköistä, että mittaukset myös pystyasennossa antavat pelkkiä lepomittauksia paremman arvion poikkeamista verenkierron säätelyssä.

Hypoteesimme oli, että kohonneen verenpaineen taustatekijöiden arviointia ja tutkimusmenetelmiä on mahdollista kehittää arvioimalla verenkierron säätelyä aikaisempaa kattavammin. Lisätavoitteena oli uusien kardiovaskulaaristen fenotyyppien löytäminen. Tutkimuksessa mitattiin verenkiertoelimistön toimintaa käyttäen hyväksi jatkuvaa verenpaineen mittausta, pulssiaallon analyysijärjestelmää ja impedanssikardiografiaa sekä levossa että kallistuskokeen aikana. Menetelmä mahdollistaa laaja-alaisen ja kajoamattoman hemodynaamisten muuttujien rekisteröinnin.

Lääkitsemättömässä aineistossa (n=387) normotensiivisten ja hypertensiivisten koehenkilöiden lepohemodynaamikan tulokset vahvistivat vallitsevan käsityksen. Kohonnut ääreisverenkierron vastus ja lisääntynyt suurten verisuonten jäykkyys ovat merkittävimmät tekijät hypertension taustalla. Hypertensioon vaikutti kuitenkin myös hyperdynaaminen komponentti, koska sydämen minuuttitilavuus oli merkitsevästi korkeampi hypertensiivillä kuin normotensiivisillä koehenkilöillä. Lisäksi hypertensiivisten koehenkilöiden reagointi pystyasentoon erosi normotensiivisistä siten, että useissa muuttujissa voitiin todeta pieniä mutta merkitseviä toiminnallisia eroja kardiovaskulaarisessa säätelyssä.

Koehenkilöt olivat pystyasennon hemodynamiikan säätelyn perusteella jaettavissa kolmeen eri fenotyyppiin. Yhteensä 470 koehenkilön otoksessa minuuttitilavuuden ja ääreisverenkierron vastuksen muutokset pystyasennossa olivat vähäisiä ”sustainer”-fenotyypissä. ”Constrictor” fenotyypissä havaittiin pystyasennossa huomattava ääreisverenkierron vastuksen nousu ja minuuttitilavuuden lasku. Näiden ryhmien lisäksi

havaitsimme "intermediate"-fenotyypin eli toiminnaltaan edellä mainittujen välimaastoon jääneen ryhmän. Havaitsimme muista riskitekijöistä riippumattoman merkitsevän yhteyden ”sustainer”-fenotyypin ja kardiovaskulaarista riskiä ennustavan lisääntyneen suurten suonten jäykkyyden välillä.

Osatyössä III osoitettiin, että salbutamolin ja nitroglyseriinin aiheuttamat vaikutukset hemodynamiikkaan olivat mitattavissa useissa muuttujissa. Salbutamolin vaikutus oli selvempi makuuasennossa, kun taas nitroglyseriini aiheutti merkittävimmän muutoksen pystyasennon hemodynamiikassa.

Salbutamoli-inhalaation on aiemmin väitetty aiheuttavan endoteelista riippuvan vasodilataation verenkiertoelimistössä ja tämän on ajateltu olevan kvantitoitavissa keskeisen verenkierron paineheijasteiden muutoksen avulla. Työn IV perusteella salbutamolin aiheuttamat hemodynaamiset muutokset johtuvat kuitenkin pääosin syketason noususta. Tämä löydös kumoaa aikaisemman käsityksen siitä, että salbutamolin aiheuttamaa keskeisen verenkierron paineheijasteiden muutosta voitaisiin käyttää kokonaisvaltaiseen endoteelin toiminnan arviointiin.

# Contents

# 1 Introduction

High blood pressure (BP) is the main risk factor for stroke and a significant risk factor for cardiovascular disease (Lopez et al., 2006). Many risk factors for the development of elevated BP have been recognized, but the fundamental hemodynamic changes and phenotypes behind primary hypertension remain unidentified. Additionally, hypertension is mainly diagnosed by solitary measurements of BP in the resting position and no continuous measurements are applied. These measurements give only restricted information about the hemodynamic status of the patients. As the change from supine to upright position causes major changes in autonomic tone and cardiovascular regulation (Avolio and Parati, 2011), measurements in the upright position would give additional information about hemodynamic changes during the active live.

BP is defined by the product of cardiac output and systemic vascular resistance (SVR). According to the current view, essential hypertension is mainly related to high SVR (Lund-Johansen, 1989). Aging and hypertension can cause changes in the vascular structure and cardiovascular regulation. For example, aging is closely associated with increased large arterial stiffness. An old paradigm is that aging can cause the phenotype of hypertension to change from one caused by high cardiac output to one caused by high vascular resistance (Lund-Johansen, 1986). These different phenotypes cannot be investigated by mere measurements of heart rate and BP (Connors et al., 1990), but cardiac output and SVR should also be registered.

Endothelial function is typically deteriorated in hypertension (Wilkinson et al., 2002). Endothelial function can be measured by various methods, but most of them are invasive or laborious. As such, they are not suited for clinical work. Pulse wave analysis has risen as a promising method to evaluate endothelial function non-invasively (Wilkinson et al., 2002). However, human hemodynamics comprise a complex system with numerous variables. Many hemodynamic variables change at the same time due to compensatory mechanisms. Often these effects are not adjusted for, and many of the studies have focused on a single variable of interest. The above-mentioned pulse wave analysis has not been validated in the evaluation of endothelium-mediated control of arterial tone in large populations, which would enable adjusting for these compensatory effects. For example, salbutamol has been used to evaluate endothelial function, but it has a positive chronotropic effect which raises heart rate (Hayward et al., 2002; Kallergis et al., 2005; Wong et al., 1990). A rise in heart rate causes compensatory changes in other

hemodynamic variables (Stefanadis et al., 1998), and this should be taken into account in the interpretation of the results.

Our aim was to measure possible deviations in hemodynamic regulation in subjects with primary hypertension and search for novel cardiovascular phenotypes. Additionally, we registered the hemodynamic influences of two vasodilating agents, inhaled salbutamol and sublingual nitroglycerin. We applied measurements in supine and upright positions to gain insight about functional cardiovascular regulation.

# 2 Review of the literature

## 2.1 Regulation and measurement of blood pressure

### 2.1.1 Determinants of blood pressure

BP is the product of cardiac output and SVR (Guyton et al., 1972). Cardiac output is the product of heart rate and stroke volume (Warner and Toronto, 1960). Stroke volume is affected by the amount of blood flowing into the left ventricle and the fraction of blood that is ejected forward to the aorta. The factors that affect stroke volume are preload, afterload, and size and contractility of the heart (Bugge-Asperheim and Kiil, 1973; Higginbotham et al., 1986; Jacob et al., 1992).

The arterial system is historically modelled as a 'Windkessel', which means that arteries work as an elastic and capacitive reservoir against pumping of the heart (Patel et al., 1963). The refined theory takes into account the difference between peripheral arteries and aorta in their capacitive and resistive components (Westerhof et al., 1969; Westerhof and Stergiopulos, 2000). This model also accounts for the wave reflection, which is an important factor in the pulse wave analysis and modelling of the arterial system (Taylor, 1957). In a specific artery, the vascular resistance is determined by the length and radius of the arteries, viscosity of blood and elasticity of vessels (Nichols et al., 2011). Of these, the arterial radius is the key factor. Resistance is inversely proportional to the fourth power of the radius, and smaller vessels contribute the largest increase to the resistance.

The aorta is an elastic artery, and its diameter is sensitive to pressure changes (Asmar et al., 2001; Hirata et al., 2005). Femoral, brachial and radial artery are muscular arteries which also distend if pressure rises, but less than the aorta, and are sensitive to vasodilatory drugs (Armentano et al., 1995; Safar et al., 1983). Most of the peripheral arterial resistance is attributed to small arteries that are less than 400 μm in diameter, and they are referred to as the resistance vessels (Mulvany, 1993; Schiffrin, 1992; Schiffrin and Hayoz, 1997; van Varik et al., 2012). The aggregate resistance of certain size vessels is inversely proportional to the number of their branches (Christensen and Mulvany, 2001; Mulvany, 1993). Capillaries are the smallest vessels, but they do not participate in the regulation of vascular resistance.

### 2.1.2 Regulation of blood pressure at rest

BP regulation is classically divided into short and long-term regulation. Short-term regulation of BP is mostly through the sympathetic nervous system, but it additionally participates in the long-term BP regulation (Izzo, 1989, 1984). Parasympathetic nervous system contributes to the short term regulation (Hall and Guyton, 2015). Baroreceptors in the wall of the carotid sinus and aortic arch primarily regulate short-term changes in BP and are connected to nucleus tractus solitarius (NTS) (Guyenet, 2006). Heart rate and to a smaller degree myocardial contractility are regulated by the sinoatrial node that is under the control of parasympathetic tone via the vagus nerve, which is connected to the NTS. Sympathetic signals to heart are transmitted directly through nerve-endings which secrete noradrenaline locally and indirectly via secretion of adrenaline to the blood from the adrenal gland (Saper, 2002). The sympathetic nervous system is directly connected to renal tubular segments and juxtaglomerular granular cells and can cause direct secretion of renin and influence renal tubular transport function (DiBona, 2005). Sympathetic regulation of vascular resistance is direct through innervation of small arteries and arterioles, and through secretion of adrenaline from the adrenal gland and secretion of renin as well (Hall and Guyton, 2015).

Kidneys are the main players in the long-term regulation of BP, but sympathetic nervous system regulates the kidneys and also has a direct role in the long-term regulation (Guyton, 1991; Morimoto et al., 2001). If blood volume increases for example through ingestion of either water or sodium, BP increases if the vascular capacitance is not altered. Increased BP activates pressure diuresis and natriuresis. As a result, water and salt are expelled to the urine until the previous BP level has been reached again (Hall and Guyton, 2015). BP has a certain 24-hour set point, which can be altered in the short term by provocations such as eating or exercise, but it's under question what system is the main long-term regulator of that set point and why that set point rises in the hypertensive individuals (Guyton, 1991; Osborn, 2005; Zanutto et al., 2010). The most popular hypothesis is that the kidney's pressure natriuresis states the BP set point, however recently it has been proposed that sympathetic nervous system can also be the main determinant (Guyenet, 2006; Guyton, 1991; Hall and Guyton, 2015; Osborn, 2005).

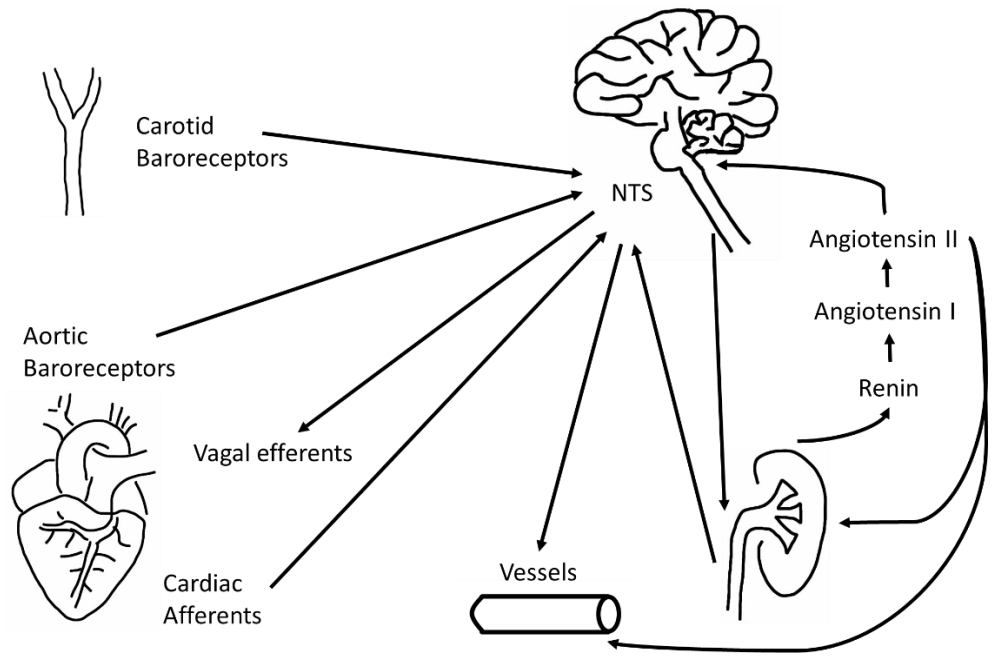


Figure 1. A highly simplified illustration of the main pathways in BP regulation. The main vessels that participate in the regulation of BP are small arteries and arterioles. Short-term regulation is mainly through carotid and aortic baroreceptors, which sense changes in BP and are connected to nucleus tractus solitarius. Kidneys have a major role in the long-term regulation of BP through pressure diuresis and natriuresis, the mechanisms of which are regulated through hormonal and neural mechanisms. BP has a certain set point, which can change in response to provocations such as physical stress. NTS = nucleus tractus solitarius (Modified from Martin and Victor, 2011).

In hormonal regulation, angiotensin II is a potent vasoconstrictor and the most vasoactive part of the renin-angiotensin II-aldosterone axis. When perfusion pressure in the kidney lowers, renin is secreted which breaks angiotensinogen into angiotensin I and this is converted to angiotensin II by the angiotensin converting enzyme (Riet et al., 2015). Aldosterone and angiotensin II increase the reabsorption of salt and water by kidneys (Hall and Guyton, 2015). Vasopressin is a hormone secreted by the central nervous system; it is secreted by the posterior pituitary. It has a major function in water reabsorption, but it is additionally a powerful vasoconstrictor (Hall and Guyton, 2015). Atrial myocytes lower the blood volume via the release of atrial natriuretic peptide which increases vascular permeability, increases renal excretion of salt and water and vasodilates resistance vessels (Curry, 2005). The heart secretes the atrial natriuretic peptide if the atriums are stretched due to a rise of central volume and pressure.

Local tissue blood flow regulation is mainly responsible for providing enough blood flow to tissues so that the metabolic needs are met (Hall and Guyton, 2015). Shear and circumferential stress on the vessel walls ensuing from blood flow and pressure, correspondingly cause biological responses, which control the smooth muscle tone and structural remodeling. In addition to the vasodilator transmitter nitric oxide, the endothelium releases prostacyclin, hydrogen sulphide, potassium, and hyperpolarizing factors that regulate the tone of the underlying smooth muscle (Daiber et al., 2017a; Galley and Webster, 2004). Prostaglandins and nitric oxide are potent vasodilators, and arterial tone is strongly locally regulated by the endothelial cell monolayer. The discovery on endothelium-dependent relaxation led gradually to the widely accepted concept that the arterial system is in a state of active vasodilatation mediated by the endothelium-derived factors (Galley and Webster, 2004; Vallance et al., 1989). The endothelium also releases vasoconstrictor substances like endothelin-1 and superoxide anion (Daiber et al., 2017a; Galley and Webster, 2004).

### 2.1.3 Hemodynamic changes in response to upright posture

When body position is changed from supine to upright, either actively or passively, this causes major changes in blood distribution, autonomic tone and pressure dynamics (Avolio and Parati, 2011; Hall and Guyton, 2015; Olufsen et al., 2005). At the start of the upright position, right after posture change, gravity hinders the flow back from veins, venous pooling increases, and stroke volume is reduced (Hall and Guyton, 2015; Jardine, 2013). This drop-in stroke volume lowers the cardiac output and BP. However, this is rapidly sensed by the baroreceptors in the carotid sinus, which stimulate the efferent output of the sympathetic nervous system. This restores the BP homeostasis by increasing heart rate and constricting resistance arteries, and this increases SVR (Smith et al., 1994; Tahvanainen et al., 2009a). Prolonged orthostasis activates the renin-angiotensin-aldosterone system and the release of vasopressin (Egan et al., 1983; Jacob et al., 1998). These hormonal mechanisms are activated in a positive correlation with the extent of standing (Jacob et al., 1998).

### 2.1.4 Non-invasive measurement of blood pressure and its limitations

Hypertension is defined as mean systolic BP of 140 mmHg or higher or diastolic BP of 90 mmHg or higher in office measurements. The corresponding limits for home measurements are 135 mmHg and 85 mmHg(Mancia et al., 2013; O'Brien et al., 2013). Additionally, the new American College of Cardiology and American Heart Association

recommendations define hypertension as BP over 130/80 mmHg (Whelton et al., 2017). In clinical studies and clinical work, these pressure measurements are most often performed with the subjects in the seated position after a period of at least 5 min of rest.

Usually, the mean of two consecutive BP measurements are recorded. This gives a rather limited window into true BP level because there are over 80 000 heart cycles during a day if the heart rate is near normal. Not surprisingly, multiple home BP measurements do not replicate the variability observed during ambulatory BP monitoring (Juhanoja et al., 2016). Even in a 24-hour ambulatory BP measurement, the recorded cycles represent less than 0.1% of the daily total count of systolic and diastolic BPs.

In the doctor's office, patients are susceptible to a white-coat reaction, which can cause inappropriately high values (Pickering, 1996). Masked hypertension is also recognized, where the situation is reversed and hypertension is evident in home measurements, but not in the clinical environment (O'Brien et al., 2013; Schwartz et al., 2016). There are also additional subject-related factors, which can cause inappropriate values such as anxiety, acute smoking, caffeine ingestion, hangover, distended bladder, physical exercise and recent meal (Dickinson et al., 2014; Ogedegbe et al., 2008; Schultz et al., 2013; Smith et al., 2003).

Ambulatory BP measurement is a better predictor of mortality than office BP (Dolan et al., 2005; Hansen et al., 2007). Some guidelines regard ambulatory BP monitoring as the gold standard in BP monitoring (O'Brien et al., 2013). There is however some doubt regarding what is the correct threshold for ambulatory BP, because ambulatory measurement analysis methods are not meticulously standardized and office BP has been studied more extensively (Whelton et al., 2017).

In the real life situation and even after training the office BP measurements by a health care professional have been shown to be inaccurate (Sebo et al., 2014). The exclusion of the effects mentioned above is encouraged in recommendations, but it is questionable how well these measurements then replicate normal daily BP level (Mancia et al., 2013; Whelton et al., 2017). Short, mid and long term BP variability have all been associated with cardiovascular outcomes (Stevens et al., 2016). Additionally, visit-to-visit BP variability has been associated with cardiovascular events (Mehlum et al., 2018). Since BP variability, recorded by the present BP means from infrequent intervals, is an inferior predictor of cardiovascular events when compared with the actual BP level, at least the measurement of the exact BP level should be the goal (Asayama et al., 2014; O'Brien et al., 2013; Schutte et al., 2012).

### 2.1.5 Non-invasive measurement of blood pressure components using bioimpedance

Normal office or home measurements give only limited information about the control of cardiovascular homeostasis, as only BP and heart rate are measured. The evaluation of SVR and cardiac output would give us more insight about the cardiovascular status and also yield information about various cardiovascular phenotypes (Avolio and Parati, 2011; Korner, 2010). Impedance cardiography can be used to measure stroke volume non-invasively (Kööbi et al., 1997a). Measured stroke volume, BP and heart rate are used to calculate cardiac output and SVR. Impedance cardiography is operator-independent and easily performed. It requires mathematical calculations and some methods use correction coefficients for sex, weight and hematocrit (Kööbi et al., 1997a). Use of mathematical calculations have raised criticism against impedance methods (Geerts et al., 2011). However, preliminary reports have suggested that adding impedance cardiography to standard BP treatment could aid with BP control (Krzesiński et al., 2016; Sharman et al., n.d.; Smith et al., 2006).

## 2.2 Passive head-up tilt

Head-up tilt causes significant changes in autonomic tone and induces numerous changes in hemodynamics (Avolio and Parati, 2011; Lu et al., 2014; Tahvanainen et al., 2009a; van den Bogaard et al., 2011). Thus, head-up tilt can be regarded as a simple way to activate the cardiovascular system, much like in a stress test. Additionally, we spend a significant amount of our daily life in the upright position. Hence, head-up tilt can also be regarded as a test which simulates the conditions in our daily life.

When used as a diagnostic tool, the head-up tilt is often performed by raising the individual from supine to a 60-70-degree angle. This position is kept for 30-40 min. Head-up tilt has been traditionally applied as a safe and non-invasive method to evaluate the underlying pathology after a syncope (Grubb and Kosinski, 1997). The tilt table test can diagnose vasovagal syncope with rather good certainty, and especially differentiate between cardioinhibitory, vasodepressor or mixed types of syncope (Brignole et al., 2000). As a drawback the head-up tilt test can give false positive results, which are unwarranted because diagnoses of primary diseases that can be life-threatening should be avoided (Natale et al., 1995).

The simple head-up tilt can reveal differences in BP regulation even in subjects with no history of syncope (Brignole et al., 2000). It has been hypothesized that the head-up tilt could be used in the research of previously unidentified cardiovascular phenotypes (Avolio and Parati, 2011; Plomin et al., 2009).

## 2.3 Hypertension

Primary hypertension is the most prevalent form of hypertension. Increased vascular resistance and decreased vascular distensibility are the main pathophysiological changes in primary hypertension (Lund-Johansen, 1989; Simon, 2004). High SVR in primary hypertension has been demonstrated by invasive and non-invasive studies (Lund-Johansen, 1989; Messerli et al., 1987). Usually cardiac output remains normal in primary hypertension (Izzard and Heagerty, 1995).

Age greatly influences the hemodynamic profile of a hypertensive subject. Young and tall male subjects can be labeled as hypertensive from brachial artery measurements, but central BP can be normal because backward pressure wave augments the brachial pressure (amplification) (Agabiti-Rosei et al., 2007; McEniery et al., 2005a). Aging causes an increase in media-to-lumen ratio and vascular remodeling in arterioles (Kanbay et al., 2011; Lakatta et al., 2009; Safar et al., 2011). Studies have suggested that aging decreases the total cross-section of arterioles, and causes capillary rarefaction (Folkow and Svanborg, 1993; Hajdu et al., 1990; Kanbay et al., 2011; Safar et al., 2011). The aforementioned changes lead to an increase in SVR which causes an increase in BP. In aged normotensive individuals the SVR seems to be unaffected (Lakatta et al., 2009; Tahvanainen et al., 2009c). The aorta and central large arteries become stiffer and their diameter increases with aging. The above changes rise the systolic BP and lower the diastolic, so the mean pressure is often not affected (Lakatta and Levy, 2003).

Many risk factors for increased vascular resistance and decreased arterial distensibility have been recognized. If hypertension is persistent, arterial degeneration is accelerated and pathophysiological changes characteristic of aging occur prematurely. Central arteries dilate and stiffen, while peripheral muscular arteries are characterized by impaired endothelial function (Lind, 2006; Nichols et al., 1992; Taddei et al., 1997). High BP in the kidney vasculature causes arteriolosclerosis, glomerulosclerosis and secondary changes which reduce renal blood flow (Kaplan, 1992), with subsequent activation of the renin-angiotensin-aldosterone system, with a further rise of BP. However, the underlying reason for primary hypertension remains unknown (Hall and Guyton, 2015; Oparil et al., 2003; Riet et al., 2015). Reasons such as renal microvascular disease, hyperuricemia, problems in baroreflex regulation and high salt intake have been proposed as the main reason (Carretero and Oparil, 2000; Korner, 2010; Oparil et al., 2003). Because the primary reason for hypertension has been elusive, it has been proposed that further phenotypic patterns behind hypertension must be discovered in order to promote research of the underlying reasons and genetics (Korner, 2010).

## 2.4 Cardiovascular phenotypes

Recent advances in genome wide association research have revealed that multiple genes with small effect sizes are accountable for multifactorial diseases such as hypertension (Plomin et al., 2009). Thus, the phenotype of diseases such as hypertension is the result of a large combination of different genes modified by environmental factors. It has been proposed that more detailed detection of phenotypes could give more power to the genome wide analyses and discovery of phenotypes could improve the interpretation of the results of the large-scale genomic studies. Additionally, new vascular phenotypes could become instruments for more precise cardiovascular risk evaluation (Olsen et al., 2016).

### 2.4.1 Known cardiovascular phenotypes

#### 2.4.1.1 Hyperdynamic circulation

For a long time primary hypertension has been hypothesized to be partly caused by hyperdynamic circulation (Frohlich, 1972). Some studies have found that this can indeed be true. Primary hypertension is usually defined by high SVR (Lund-Johansen, 1989; Messerli et al., 1987). However, in young individuals hypertension can be caused by high cardiac output and it has been suggested that with aging the phenotype of primary hypertension changes from one with high cardiac output to one with high SVR (Lund-Johansen, 1989).

#### 2.4.1.2 Different forms of hypertension: isolated systolic, isolated diastolic, and systolic and diastolic

Hypertension is defined as either high systolic or diastolic BP, and thus both of the pressures can be high or only one of them (Chobanian et al., 2003; Hay, 1931; Whelton et al., 2017). In isolated systolic hypertension the systolic BP is over the chosen cut-off and diastolic BP is under the cut-off. In isolated diastolic hypertension the situation is reverse. Large studies have suggested that all these forms carry additional risk (Lewington et al., 2002; Rapsomaniki et al., 2014). However, different cardiovascular end-points are connected to different forms of hypertension (Rapsomaniki et al., 2014). Recent reports suggest that isolated systolic hypertension, when compared with isolated diastolic hypertension, is a stronger predictor of future cardiovascular events (Strandberg et al., 2002; Sundström et al., 2011). Isolated systolic hypertension is most

prevalent in elderly subjects, but it is sometimes identified also in younger individuals (Franklin et al., 2001; McEniery et al., 2005b). There are differences in hemodynamics between the different forms of hypertension. For example in younger individuals, isolated systolic hypertension is connected to increased stroke volume and aortic stiffness (McEniery et al., 2005b). In a recent large study, isolated diastolic hypertension was related to female sex and high peripheral vascular resistance (McEniery et al., 2017). It has been suggested that the finding of new patterns in hemodynamics could lead to new insights in intervention and risk stratification (McEniery et al., 2017).

#### 2.4.1.3 Orthostatic hypotension and neurally mediate syncope

Significant hypotension during standing is most often neurally mediated. Orthostatic hypotension is defined as a decrease of systolic BP exceeding 20 mmHg or diastolic BP exceeding 10 mmHg during the first 3 min of standing or head up tilt (Freeman et al., 2011). The underlying pathophysiology is not well identified (Jardine, 2013; Tahvanainen et al., 2011). The decrease in BP is caused either by a fall in cardiac output, insufficient vascular resistance, or a combination of these factors.

Neurally mediated syncope is a heterogenous group, where different mechanisms can account for the upright loss of consciousness (Brignole et al., 2000; Kurbaan et al., 2003). Different mechanisms are linked to putative explanations, such as vasovagal, cough or micturition initiated or carotid sinus derived syncope. These conditions are characterized by a reflex which affects afferent, central and efferent pathways, and result in a sudden change in autonomic nervous system activity, leading to a fall in BP, heart rate and cerebral perfusion (Freeman et al., 2011; Rogers and O'Flynn, 2011). These conditions are caused by different cardiovascular regulatory problems and their research can reveal phenotypic differences in regulation. For example in a recent study, higher supine wave reflections where associated with orthostatic hypotension in the upright position (Sung et al., 2014). In patients with neurodegenerative diseases that affect the autonomic nerves, inadequate release of norepinephrine from sympathetic vasomotor neurons leads to a depressed vasoconstrictor stimulus, impairment of the cardiac sympathetic innervations and to orthostatic hypotension (Freeman et al., 2011; Hanyu et al., 2006; Metzler et al., 2013).

## 2.5 Large arterial compliance and arterial stiffness

In a classic windkessel model of arterial tree, smaller arteries work mainly as a 'cushioning' system in the arterial tree and larger arteries as a 'conduit' system (Nichols et al., 2011). If smaller arteries contract and rise the peripheral resistance, this causes the systolic and diastolic BP to rise. If compliance of large arteries decreases, this causes the pulse pressure (PP) and pulse wave velocity (PWV) to rise. However, this division is somewhat oversimplified. There is marked heterogeneity between arteries of different sizes. It has been proposed that most accurate model of the arterial tree is a visco-elastic tube with branch points (Laurent et al., 2006).

### 2.5.1 Measurement methods

#### 2.5.1.1 Pulse wave velocity

PWV is the speed of the pressure wave travelling from the heart to the periphery. Usually PWV is calculated by measuring the pressure change in two arterial locations and using the time difference to calculate the velocity. Carotid-to-femoral PWV is the most commonly used and it is often referred as aortic PWV, because aorta mostly accounts for the calculated velocity (Laurent et al., 2006; Weber et al., 2015). In a study done in 1999, it was first shown in end stage renal disease patients that PWV is an independent predictor for cardiovascular outcomes (Blacher et al., 1999). After that, multiple studies have proven this to be true in other patient groups as well, including elderly subjects, and those suffering from hypertension or acute coronary syndrome (Boutouyrie et al., 2002; Meaume et al., 2001; Tomiyama et al., 2005). In a recent meta-analysis, adding PWV to standard risk factors reclassified the risk for cardiovascular events (Ben-Shlomo et al., 2014). Additionally, PWV increases in conjunction with aging (Vaitkevicius et al., 1993). PWV has been proposed to be an indicator of aortic degeneration and stiffening (Hirata et al., 2006).

#### 2.5.1.2 Pulse wave analysis

The pressure wave that starts from the heart is reflected back from arterial branches, while most of the reflection occurs in the high-resistance arterioles. The reflected waves can cause a difference between the systolic BP in the aorta and in the more distal arteries. High PWV (arterial stiffness) in large arteries causes the pressure difference between

central and peripheral sites to decline because the reflected wave returns earlier and augments the central systolic pressure (Figure 2). The reflected wave is most often measured from radial artery and mathematically transformed to depict the central wave. The rise in central BP due to increased wave reflection is called the augmentation pressure (AP). It is used to calculate the augmentation index (AIx), which is the pressure rise in proportion to PP. AIx is the most commonly used variable to express the wave reflection (Nichols et al., 2011).

A change in heart rate also affects the AP and AIx (Wilkinson et al., 2000) (Figure 3). If heart rate is lower and R–R-interval longer, the time of the reflected pressure wave stays the same and the backward pressure wave overlaps more with systolic pressure. Due to this, AIx is often corrected to heart rate 75/min (Wilkinson, 2002). However, this method does not consider variances in the correlations between heart rate and AIx in different study populations (Stoner et al., 2014).

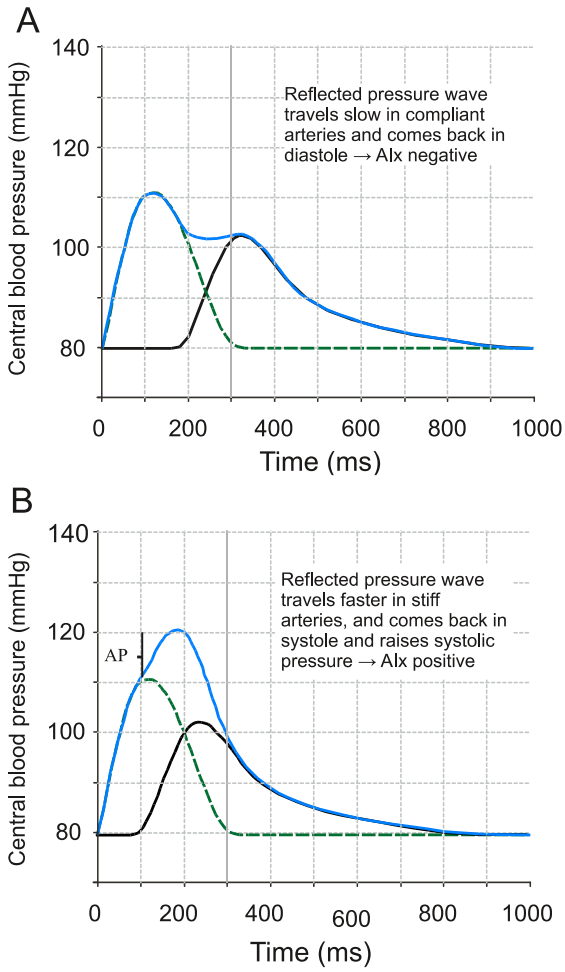


Figure 2. In compliant arteries (A) the reflected pressure wave travels back slowly and comes back mostly in diastole. However, in stiff arteries (B) the pressure wave travels faster and returns mostly during systole rising the systolic pressure. The rise in systolic pressure is called augmentation pressure (AP). AIx = Augmentation index, AP = Augmentation pressure. (Modified from Hirata et al., 2006).

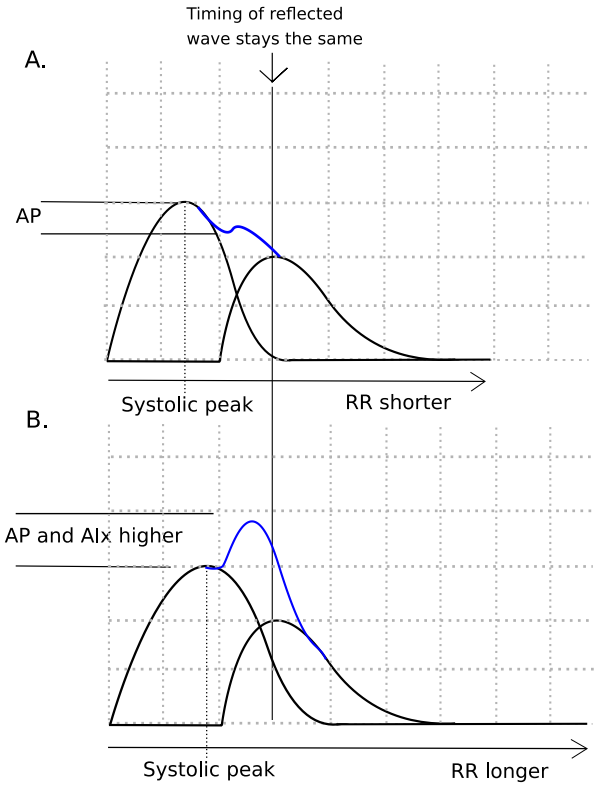


Figure 3. During higher heart rate, ejection duration and RR-interval are shorter, and reflected wave comes back mostly during diastole, and AP and AIx are low (A). If heart rate is lower, the backward pressure wave overlaps more with central systolic pressure and rises AP and AIx (B). RR = R-R-interval, AP = Augmentation pressure, AIx = Augmentation index.

#### 2.5.1.3 Other stiffness evaluation methods: pulse pressure, ambulatory arterial stiffness index, forward wave amplitude and stroke volume to central pulse pressure ratio

Because the above mentioned methods are known to have problems (Laurent et al., 2006; Gary F. Mitchell et al., 2010), other means for arterial stiffness measurement have also been devised. PP is simple method which has been proposed to measure large arterial stiffness. However, in addition to arterial stiffness, PP is correlated to amplification due to backward pressure wave, mean BP, and pattern and duration of heart contractions (Laurent et al., 2006; O'Rourke et al., 2004). Thus, PP alone should probably be used with caution. Pressure wave can be separated into forward and backward travelling components (Nichols, 2005). Backward component is the more

commonly used component in arterial stiffness research. However, forward traveling pressure wave amplitude is generated by left ventricular contraction and has been suggested to be a main determinant of pressure augmentation and AIx (Torjesen et al., 2014). Ambulatory arterial stiffness index (AASI) has been proposed to be a measure of global arterial stiffness (Li et al., 2006). In the calculation of AASI, the symmetric regression coefficient of diastolic on systolic pressure is obtained and it is subtracted from one. Typically, in stiff arteries diastolic pressure doesn't rise with systolic pressure and AASI is higher. AASI has been shown to correlate with cardiovascular event (Aznaouridis et al., 2012; Kollias et al., 2012). However, there are conflicting reports about how well AASI correlates with established measures of arterial stiffness (Matsui et al., 2012; Schillaci et al., 2007). It has been hypothesized that forward pressure wave and stroke index measurement can be applied in the calculation of total arterial compliance (Chemla et al., 2008). This hypothesis relies on the notion that aortic stiffness increases impedance to the left ventricular pulsatile flow and increases forward pressure wave amplitude. The variable calculated as the inverse of total arterial compliance has been proposed to be a more sensitive method in measurement of total arterial stiffness than PWV (Chemla et al., 2008).

## 2.6 Evaluation of autonomic tone

### 2.6.1 Heart rate variability analysis

The autonomic nervous system balances the sympathetic and parasympathetic signals to maintain a symmetry of vital hemodynamic functions. Heart rate is largely controlled by the nervous system. The balancing of the autonomic nervous system is a dynamic process and variations in the autonomic tone can be recorded from intervals of succeeding heart beats. When the intervals between heart beats are analyzed to evaluate the sympathetic and parasympathetic tone, the method is called the analysis of heart rate variability (HRV). There are, however, different methods to measure heart rate variability (Electrophysiology, 1996; Thayer et al., 2010).

#### 2.6.1.1 Time domain parameters

The simplest heart rate variability analysis methods are time the domain methods. The time domain parameters are derived by determining the intervals between two normally conducted sinus heart beats. The most popular methods are standard deviation of

normal to normal intervals and the square root of the mean squared differences of normal to normal intervals. These values are estimates of overall HRV and short-term components, respectively (Electrophysiology, 1996).

#### 2.6.1.2 Frequency domain parameters

Heart rate variability can be divided into its frequency components. The power of these frequencies can be calculated with various mathematical methods. This gives the power spectral density analysis. Four different frequency areas are computed for the analysis: ultra-low frequency (≤ 0.003 Hz), very low frequency (> 0.003 to ≤ 0.04 Hz), low frequency (> 0.04 to ≤ 0.15 Hz), and high frequency (> 0.15 to ≤ 0.4 Hz) (Electrophysiology, 1996). The frequency domain analysis gives an estimate of how much either sympathetic and parasympathetic pathways affect the heart rate (Pomeranz et al., 1985). Vagotomy and electrical vagal stimulation have been confirmed to eliminate the high frequency variability (Chess et al., 1975; Malliani et al., 1991). These results demonstrate that parasympathetic control is associated with high frequency variability. On the other hand, low frequency variability (Electrophysiology, 1996) has been mainly connected with the activity of the sympathetic nervous system, although it also contains a minor parasympathetic component (Akselrod et al., 1985; Malliani et al., 1991).

#### 2.6.1.3 Muscle sympathetic nerve activity

The direct recording of nerve activity from postganglionic sympathetic efferent nerve fibers regulating skeletal muscle vasculature is the gold standard in the measurement of regional sympathetic activity (Delius et al., 1972). Initially, the nerve pathway is mapped subcutaneously. Then a microelectrode is positioned near the nerve fascicle and its activity can be recorded. This method has a good time resolution and thus it can detect fast changes which are typical for the sympathetic nervous system. This method is also highly reproducible (Fagius and Wallin, 1993). However, it can only measure nerve activity regionally from muscle or skin, and so it can't directly measure global adrenergic activity (Lambert et al., 1997).

#### 2.6.1.4 Noradrenaline spillover technique

The release of noradrenaline from sympathetic nerves can be measured from plasma with the so called "noradrenaline spillover"-technique (Esler et al., 1988, 1979). Noradrenaline spillover can be measured with radioenzymatic or radioisotope methods.

It is an invasive method and can be applied to measure sympathetic nerve activity either specifically in an organ or in the whole body (Esler et al., 1984). Organ specific methods have been applied to kidneys, heart, and the upper limbs (Esler et al., 1984, 1979; Sudhir et al., 1989). However, noradrenaline spillover has a low sensitivity in detecting increased sympathetic activity during various disease states (G. Grassi et al., 1995; Guido Grassi et al., 1995; Mancia and Grassi, 2014). Additionally, noradrenaline spillover technique has a suboptimal repeatability compared to muscle sympathetic nerve activity measurement (Grassi et al., 1997).

## 2.7 Endothelium-dependent and endothelium-independent control of vascular tone

Endothelial cells cover the inner lining of vasculature called intima from the luminal side in the entire vascular system. The endocrine function of the endothelial cells is influenced by neurotransmitters, different hormones and vasoactive factors, the level of BP, pulsatile flow, and the shear stress of streaming blood flow (Galley and Webster, 2004). During disease states, several mechanisms can lead to endothelial dysfunction, including endothelial nitric oxide synthase uncoupling, activation of the endothelin-1 system, direct inactivation of nitric oxide by superoxide, nitration and inactivation of prostacyclin synthase, and desensitization of soluble guanylate cyclase in smooth muscle (Bachschmid et al., 2005; Daiber et al., 2017a; Kähler et al., 2001). So, in brief, damaged endothelium leads to a distorted balance in the production of vasoactive factors which influence vasomotion, thrombosis, aggregation of platelets, and inflammation. These effects cause endothelial dysfunction, which has also been shown to be an early indicator of atherosclerosis (Lerman, 2005) and to correlate with prognosis (Schächinger et al., 2000). It has additionally been linked to the classical cardiovascular risk factors such as hypertension and diabetes (Calver et al., 1992; Panza et al., 1990).

### 2.7.1 Endothelium-derived regulators of arterial tone

Several different vasoactive substances are released by endothelium. These are divided into vasodilatory factors and vasoconstrictive factors (Galley and Webster, 2004). The main vasodilatory substances are nitric oxide, prostacyclin and the endothelium derived hyperpolarizing factor. The vasoconstrictive factors include thromboxane-$A_2$ and endothelin-1. The basal vasodilatory tone of blood vessels has been mainly attributed to the release of nitric oxide (Vallance et al., 1989). The endothelial nitric oxides synthase

converts amino acid L-arginine to L-citrulline in the process of nitric oxide synthesis in the vasculature (Palmer et al., 1988).

### 2.7.2 Drug-induced vasodilatation

The measurement of blood vessel dilatation in response to a stimulus is the basis of most methods to assess the function of the endothelium. Vasodilatation can be either endothelium-dependent or independent. Endothelium-independent dilatation is mediated by changes in the contractile function of arterial smooth muscle. Endothelium-dependent dilatation is by definition mediated by the release of vasoactive substances from the endothelium. It is vital to distinguish between these two when assessing the function of the endothelium (Sandoo et al., 2010).

#### 2.7.2.1 Endothelium-dependent vasodilatation via activation of ß$_2$-adrenoceptors

It has been shown that the ß$_2$-adrenoceptor agonist salbutamol can induce endothelium-dependent vasodilatation of arteries (Chowienczyk et al., 1999; Dawes et al., 1997; Graves and Poston, 1993; Wang et al., 1993; Xu et al., 2000). This response can be blunted by removing the endothelium or by treating with N$^G$-nitro-L-arginine methyl ester, which blocks the nitric oxide L-arginine pathway in the endothelium (Dawes et al., 1997; Graves and Poston, 1993). These results show that salbutamol causes endothelium dependent vasodilatation. In addition, the $\beta_2$-sympatomimetic influence of salbutamol induces a direct relaxing action in vascular smooth muscle via activation of the adenylate cyclase (Dawes et al., 1997; Xu et al., 2000).

#### 2.7.2.2 Endothelium-independent vasodilatation induced by nitroglycerin

Exogenous nitric oxide donor such as sublingual nitroglycerin tablet is usually used to determine the magnitude of endothelium-independent vasodilatation. The endothelium-independent vasodilatation reflects the vasodilatory function of vascular smooth muscle and the peak effect of sublingual nitroglycerin is measurable 3 to 4 min after its administration (Ducharme et al., 1999). It has been customary to compare the endothelium-independent vasodilatation to the endothelium-dependent vasodilatation when evaluating endothelial function (Duffy et al., 2011; Lind, 2005; Lind et al., 2011; McEniery et al., 2006; Rambaran et al., 2008; Wilkinson, 2002). New review by Maruhashi et al., has highlighted the evidence that endothelium-independent

vasodilatation alone is impaired in atherosclerosis and could be a risk factor for cardiovascular events (Adams et al., 1998; Maruhashi et al., 2018, 2013)

### 2.7.3 Evaluation of endothelial dysfunction

#### 2.7.3.1 Venous occlusion plethysmography

Widely regarded as the gold standard for measurement of endothelial function is the venous occlusion plethysmography (Daiber et al., 2017b; Schnabel et al., 2011; Wilkinson and Webb, 2001). In this technique the venous blood flow from the forearm is occluded while arterial blood flow is measured by means of the swelling of the forearm. After venous occlusion the swelling of the forearm is directly related to the magnitude of blood flow. The brachial artery is cannulated, and used to apply different test different drugs into the forearm vasculature (van de Ree et al., 2001; Wilkinson and Webb, 2001). For example, intra-arterial infusion of nitroprusside and acetylcholine can be used to evaluate endothelial function and dysfunction. These drugs cause endothelium-independent and dependent vasodilatation, respectively. The drawbacks of this method are that it is invasive and only examines the forearm vasculature.

#### 2.7.3.2 Flow-mediated dilatation

If blood flow is occluded into the upper arm by means of a cuff and the occlusion is subsequently relieved, the acceleration of flow increases shear stress on arteries. The increase in shear-stress causes local vasodilatation, which can be measured using ultrasound (Corretti et al., 2002). This phenomenon is applied in the recording of flow-mediated vasodilatation (Corretti et al., 2002). The vasodilating effect of shear stress is usually compared to the effect of sublingual nitroglycerin, so that endothelial function can be quantified. This method is non-invasive, but can be painful due to ischemia and is highly operator-dependent (Corretti et al., 2002). However, decreased flow-mediated dilatation has been shown to correlate with numerous cardiovascular risk factors (Lind et al., 2011; Mitchell et al., 2004; Shechter et al., 2014). Nevertheless, the shear stress stimulus to the endothelium can also be reduced because of various risk factors and vascular pathologies, so the true magnitude of impaired endothelium-dependent vasodilatation can be hard to determine (Mitchell et al., 2004).

#### 2.7.3.3 Global endothelial function using pulse wave analysis

Flow-mediated dilatation and venous occlusion plethysmography both evaluate endothelial function locally. Global endothelial function has been proposed to be measurable by the use of pulse wave analysis (Hayward et al., 2002; Wilkinson, 2002). As the heart contracts, it releases a PP wave, which travels distally and reflects partially back from the periphery. The increase in systolic BP due to the reflected wave is characterized by the magnitude of the AIx, and it changes during vasodilatation because active relaxation reduces the resistance to flow and magnitude of reflection in the small arteries (Chowienczyk, 2011) (Figure 2).

Both nitroglycerin and salbutamol have been used to induce endothelium-independent and endothelium-dependent vasodilatation, respectively, in measurements that have applied the pulse wave analysis approach. Both drugs have been shown to induce measurable changes in the AIx, and the changes have been linked to the level of vasodilatation. Moreover, the difference in change between these variables has been proposed to be a marker of global endothelial function *in vivo* (Chowienczyk et al., 1999; Hayward et al., 2002; McEniery et al., 2006). Pulse wave analysis is safe, non-invasive and easy to perform. Although it has not been thoroughly compared with more robust methods, it has gained popularity in research due to the ease of use.

#### 2.7.3.4 Finger pulse amplitude tonometry

New emerging method is the assessment of endothelial function from the output of finger pulse tonometers (Kuvin et al., 2003). This method has been shown to be reproducible and is not as operator-dependent as other methods (Aizer et al., 2009). Finger pulse amplitude tonometry is performed by simultaneously measuring the pulse amplitude from fingers of both hands with cuff tonometers. First the initial amplitude is measured and then blood flow is occluded in one arm by rising upper arm cuff pressure to a suprasystolic level. After 5 min occlusion, blood flow is restored and the post-occlusion amplitude changes are measured. The normal reaction is a significant post-ishaemic increase in the amplitude of the signal from the finger tonometer (Kuvin et al., 2003). Finger pulse amplitude tonometry has been shown to correlate with cardiovascular risk factors (Hamburg et al., 2008; Kuvin et al., 2003).

#### 2.7.3.5 Other methods

The diameter of coronary arteries can be measured using angiography. Both acetylcholine and nitroglycerin have been infused locally into the coronary artery. The drug-induced effect on vessel diameter is quantified and the comparison between the dilatory responses induced by the above compounds is used as a measure of endothelial function (Ludmer et al., 1986). Acetylcholine, usually considered as an endothelium-dependent vasodilator, can cause a paradoxical vasoconstriction in patients with coronary artery disease, probably due to the release of constrictor substances from the endothelium and a direct effect on smooth muscle (Ludmer et al., 1986).

Laser-doppler flowmetry evaluates the blood-flow and function of skin microvasculature. Post-occlusive hyperemia and acetylcholine administered through iontophoresis have been used to induce vasodilatation in the skin microvasculature. However, this method is under criticism, as it is highly sensitive to the skin temperature and the results have a poor reproducibility (Cracowski et al., 2006).

# 3 Aims of the study

Our aims were to characterize differences in hemodynamic regulation between normotensive and hypertensive subjects, search for novel cardiovascular phenotypes, and compare the hemodynamics influences of two vasodilating agents, inhaled salbutamol and sublingual nitroglycerin. The recordings were performed in supine and upright positions to gain insight about functional cardiovascular regulation.

The detailed aims were:

1. To investigate if primary hypertension is associated with functional changes in cardiovascular regulation during orthostatic challenge.

2. To examine whether the hemodynamic response to upright posture can be divided into different functional phenotypes, and whether the observed phenotypes are associated with known determinants of cardiovascular risk.

3. To examine the effects of nitroglycerin and salbutamol on hemodynamics in supine and upright positions.

4. To study whether the positive chronotropic properties of salbutamol inhalation can explain the observed subsequent changes in AIx.

# 4 Subjects and methods

## 4.1 Study Subjects

The subjects to this study were selected from individuals enrolled to the DYNAMIC-study (clinical trials registration NCT01742702). The primary aim of the DYNAMIC-study is to examine hemodynamic changes during supine and upright position in primary and secondary hypertension by comparing them to normotensive subjects. The subjects were enrolled by announcements among the local occupational health care providers, at the start of long-distance running program for beginners at Varala Sports Institute in Tampere, Tampere University Hospital and University of Tampere. Additionally, two announcements were published in a local newspaper. The volunteers were recruited in the order they contacted the research nurse (recruitment years from 2006 to 2011).

All subjects underwent physical examination by a medical doctor. Medication use was verified by the doctor, so that it could be precisely documented. Additionally, during the doctor's examination lifestyle habits, weekly alcohol intake, family history of cardiovascular disease, smoking in pack-years, number of cigarettes per day and medical history were documented. Office BP measurements were recorded during the doctor's visit. Patients with a history of coronary artery disease, chronic renal disease, diabetes, stroke, heart valve disease, chronic arrythmia or secondary hypertension were excluded in all of the present the studies. All subjects were over 18 years of age. In all the studies, patients with antihypertensive medication or any other medication directly influencing cardiovascular status were excluded.

In the Study I, the hemodynamics of 387 (173 males and 214 females) subjects with either never-medicated primary hypertension or normotensive BP values were compared. Hypertension was defined as laboratory BP ≥135/85 mmHg in supine position. Laboratory BP was measured using Colin radial artery sensor (Colin Medical Instruments Corp., San Antonia, Texas, USA) from the left hand, which was calibrated periodically from oscillometric measurements in the right upper arm. The measured BP was most stable during the last three minutes of supine measurement and it was chosen as the cut-off BP. According to our analyses, this corresponds to a BP of 139/95 mmHg in our office measurements. Office measurements were done by measuring blood pressure twice in a seated position during the doctor examination. Of the 387 subjects, 155 were classified as hypertensive, and they presented with male predominance, higher age and higher body mass index than normotensive subjects.

Altogether 470 subjects (230 males, 240 females) comprised the population in Study II, which examined the hemodynamic response to upright position (Figure 4). The study II population was divided into three clusters according to their hemodynamic responses to head-up tilt using hierarchical clustering. Ward's method for squared Euclidean distances was chosen. Each subject served as a cluster, and at each step two clusters closest to each other were combined, until only one cluster remained. To consider the scaling effects, all differences were standardized. The three clusters were termed: the constrictors, the sustainers and the intermediate group.

In the Study III, the population consisted of 35 (19 females and 16 males) healthy normotensive individuals. In these 35 subjects, the effects of sublingual nitroglycerin and inhaled salbutamol were examined in a placebo-controlled double-blinded randomized study setting.

In the Study IV, a total of 335 subjects (161 males and 174 females) were included to compare the haemodynamic effects of salbutamol inhalation and sublingual nitroglycerin especially on the level of the AIx.

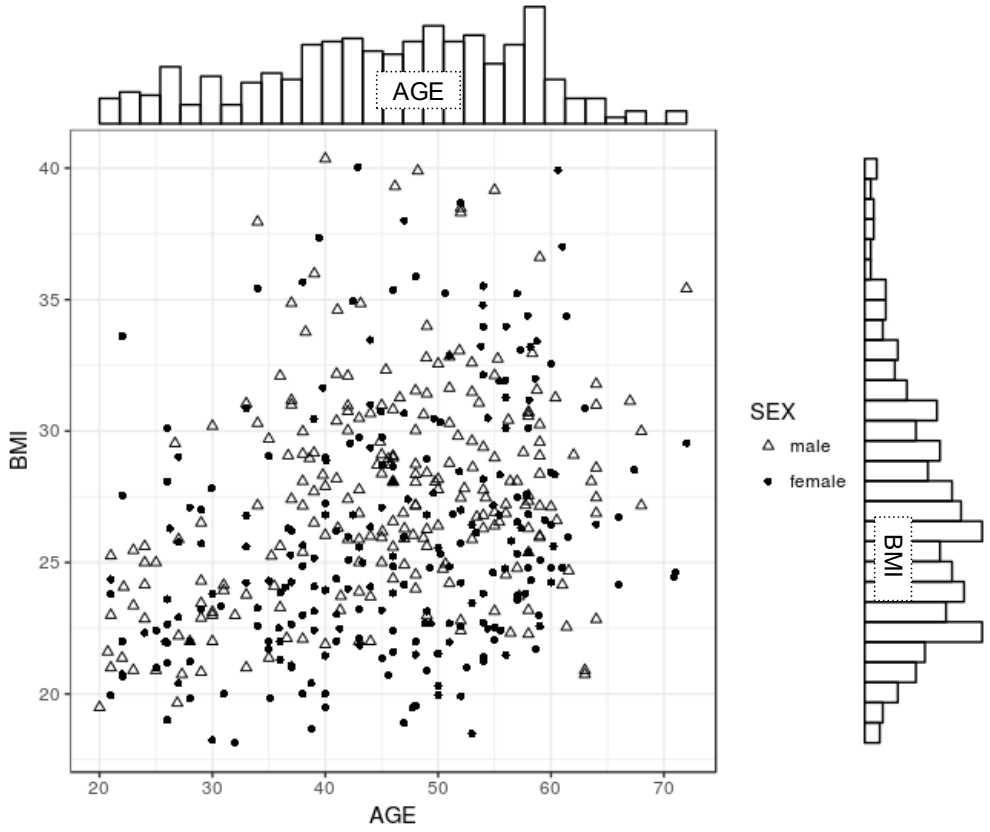


Figure 4. Age and body mass index (BMI) distribution of the largest study (study II, n = 470). The distributions in studies I and IV were quite similar.

Table 1. Demographics of the study subjects. Data represented either as medians and quartiles in parenthesis or means ± standard deviation.

| | Study I | | | Study II | | Study III | Study IV |
|---|---|---|---|---|---|---|---|
| | Hypertensive | Normotensive | Sustainer | Intermediate | Constrictor | All subjects | All subjects |
| **Number of patients** | 155 | 232 | 222 | 139 | 109 | 35 | 335 |
| **Age (years)** | 49 ± 11 | 42 ± 12 | 47 (38–55) | 46 (38–57) | 46 (37–53) | 33 (26–42) | 46 ± 12 |
| **BMI (kg/m$^2$)** | 28 ± 4 | 25 ± 3 | 27 (25-31) | 27 (24–29) | 23 (22–26) | 22 (22–25) | 26 ± 4 |
| **Sex (male)** | 55 % | 38 % | 68 % | 42 % | 20 % | 46 % | 48 % |
| **Smoking (present/ previous)** | 17 % /25 % | 8 % /33 % | 10 % / 35 % | 10 % / 25 % | 15 % / 25 % | 11 % / 14 % | 13 % / 30 % |
| **HDL cholesterol (mmol/l)** | 1.5 ± 0.4 | 1.7 ± 0.4 | 1.4 (1.2–1.7) | 1.5 (1.2–1.8) | 1.8 (1.5–2.1) | 1.7 (1.5–1.9) | 1.6 ± 0.4 |
| **LDL cholesterol (mmol/l)** | 3.1 ± 0.9 | 2.6 ± 0.9 | 3.1 (2.3–3.7) | 3.0 (2.4–3.6) | 2.5 (2.1–3.2) | 2.2 (1.8–2.6) | 2.9 ± 0.9 |
| **Triglycerides (mmol/l)** | 1.3 ± 1.4 | 1.0 ± 0.6 | 1.1 (0.8–1.7) | 1.1 (0.8–1.4) | 0.9 (0.6–1.2) | 0.8 (0.7–1.1) | 1.1 ± 0.7 |
| **Fasting glucose (mmol/l)** | 5.5 ± 0.6 | 5.2 ± 0.4 | 5.5 (5.1–5.8) | 5.4 (5.1–5.7) | 5.2 (4.9–5.4) | 5.1 (4.7–5.4) | 5.4 ± 0.5 |
| **Creatinine (µmol/l)** | 74 ± 13 | 73 ± 13 | 76 (69–84) | 70 (64–80) | 66 (59–74) | 75 (69–87) | 73 ± 14 |
| **eGFR (ml/min per 1.73 m$^2$)** | 110 ± 14 | 112 ± 14 | 89 (78–99) | 88 (79–97) | 89 (80–97) | | 111 ± 14 |

## 4.2 Laboratory values

A rather large number of laboratory analyses was included in the examination to exclude pathological conditions such as secondary hypertension and to collect vital confounders for multivariate analyses. The included laboratory values were basic blood count, sodium, potassium, glucose, creatinine, triglyceride, and total and high-density lipoprotein cholesterol concentrations. Low density lipoprotein was in most subjects directly measured, while in less than 10% of the participants it was calculated using the Friedewald formula. Estimated creatinine-based glomerulus filtration rate was calculated using the RULE formula (Rule et al., 2004) or by the Modification of Diet in Renal Disease (MDRD) formula (Levey et al., 1999). Laboratory analyses were collected after a fast of ~12 hours.

## 4.3 Measurement methods

### 4.3.1 Whole body impedance cardiography

Changes in body electrical impedance during cardiac cycles were recorded using whole-body impedance cardiography (CircMon®, JR Medical Ltd., Tallinn, Estonia) to determine heart rate, stroke volume, and cardiac output, as previously described (Kööbi et al., 2003, 1997a, 1997b). SVR and left cardiac work (LCW) were calculated using the BP signal from the radial tonometric sensor and the cardiac index (CI) measured by the CircMon device (Figure 5). SVR was calculated by subtracting central venous pressure from mean arterial pressure and dividing it by cardiac output. LCW was calculated by subtracting pulmonary arterial occlusion pressure from mean arterial pressure and multiplying it by cardiac output. Mean normal values of central venous (3 mmHg) and pulmonary arterial occlusion (6 mmHg) pressures were used in calculations. Cardiac output, SVR and LCW were related to body surface area and presented as indexes. A detailed description of the method and electrode configuration has been previously reported (Kööbi et al., 2003, 1997a). The cardiac output values measured with CircMon whole-body impedance cardiography are in good agreement with the values measured by the thermodilution method, in both supine position and during head-up tilt (Kööbi et al., 1997a, 1997b). PWV was not assessed during the head-up tilt due to less accurate timing of left ventricular ejection during reduced stroke volume (Kööbi et al., 2003).

### 4.3.2 Pulse wave analysis

Radial BP and pulse waveform were determined from the radial pulsation by an automatic tonometric sensor (Colin BP-508T, Colin Medical Instruments Corp., San Antonia, Texas, USA), calibrated approximately every 2.5 min by contralateral brachial systolic and diastolic BP measurements. Before the actual haemodynamic recordings, BP was measured manually 2 times using an ordinary sphygmomanometer to verify that the automated BP readings were correct. Continuous aortic BP and wave reflections were derived from the radial tonometric signal with the SphygmoCor pulse wave monitoring system (SphygmoCor PWMx, AtCor Medical, Australia) using the previously validated generalized transfer function (Chen et al., 1997) (Figure 5). Heart rate, aortic PP (aortic systolic pressure – aortic diastolic pressure), ejection duration, aortic AP and AIx (aortic AP/aortic PP x 100%) were determined.

### 4.3.3 Heart rate variability

HRV analysis from electrocardiograms was used to assess cardiac autonomic tone. The electrocardiograms were recorded by the CircMon device at 200 Hz sampling rate, and data analyzed using Matlab software (MathWorks Inc., Natick, Massachusetts, USA). Normal R-R intervals were recognized, and a beat was considered ectopic if the interval differed over 20% from the previous values. The artifacts were processed using the cubic spline interpolation method. Since the data were collected from short-term recordings, the frequency domain method was applied (Peltola, 2012). The following variables were calculated from the recordings in supine (0-5 min) and upright positions (5-10 min) using the Fast Fourier Transformation method: i) total power, ii) power in low frequency (LF) range (0.04-0.15 Hz), iii) power in high frequency (HF) range (0.15-0.40 Hz), and iv) LF/HF ratio.

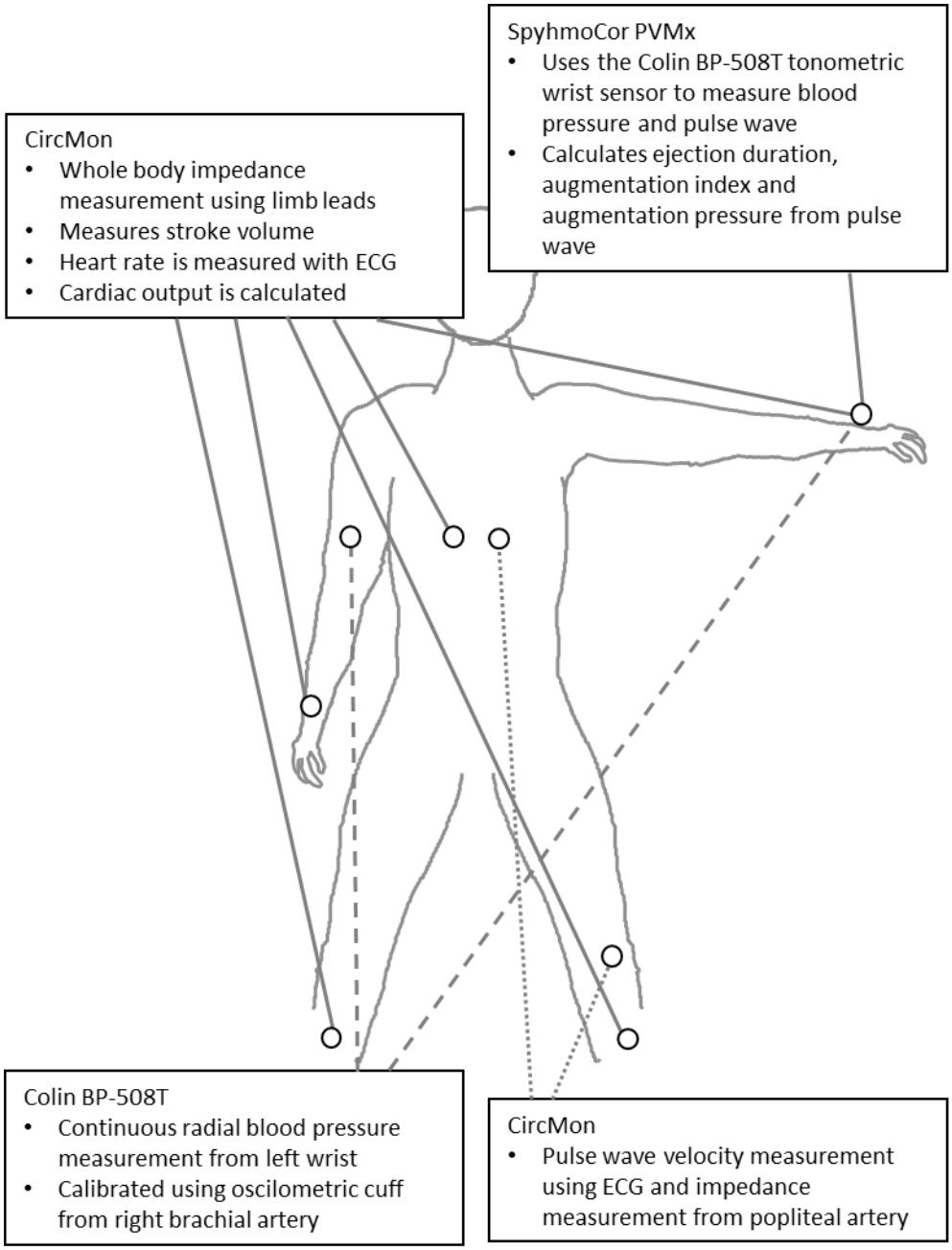


Figure 5. Main measurement methods and measurement points. The left hand is extended to the side throughout the measurement, so that wrist is at the level of the heart in supine and upright positions. ECG = electrocardiography.

## 4.4 Hemodynamic measurement protocol

### 4.4.1 Head-up tilt test and research drugs

Hemodynamic measurements were performed in a quiet, temperature-controlled laboratory by a trained research nurse (Koskela et al., 2013; Tahvanainen et al., 2009b, 2009a, 2012). Subjects were instructed to avoid caffeine containing products, smoking and heavy meal for at least 4 hours and alcohol for at least 24 hours prior to the measurements. The subjects were resting supine on the tilt-table and the left arm with the tonometric wrist sensor was abducted to 90 degrees in an arm support, which held the extended arm at the level of the heart in supine and upright positions (Koskela et al., 2013; Tahvanainen et al., 2009b, 2009a, 2012).

To accommodate the subject, an introductory head-up tilt was performed. The measurement contained three 5 min periods with continuous capture of data. First 5 min supine, then 5 min of head-up tilt at 60 degrees, and lastly 5 min supine (Figure 6). In study IV, nitroglycerin 0.25 mg (Nitro resoriblet; Orion Pharma, Espoo, Finland) was given sublingually and the 15 min protocol was repeated. After nitroglycerin recordings, the measurements were paused for at least an hour. Then the whole protocol was repeated using salbutamol 400 μg inhalation as the test drug (2 times 200 μg at 1-min intervals, Ventoline$^{R}$ with Volumatic$^{R}$ spacer device, GlaxoSmithKline, Uxbridge, Middlesex, UK). The repeatability and reproducibility of these haemodynamic measurements is good, both supine and upright (Tahvanainen et al., 2009c). In study III, after the unmedicated 15 min period patients were given either the above-mentioned nitroglycerin or salbutamol, or placebo resoriblet (Pharmacy of University, Helsinki, Finland) or placebo inhalation (GlaxoSmithKline). The effect of nitroglycerin returns to baseline 20 min after drug administration, while plasma salbutamol concentration is stable 5 to 20 min after administration (Wilkinson, 2002). Both drugs retain their effect throughout the 15-min tilt-protocol.

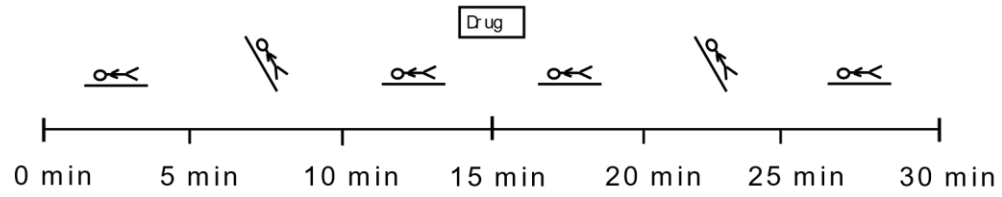


Figure 6. Whole 30-minute hemodynamic measurement protocol. Studies I-II used data from the beginning of the measurement protocol (5 min supine, 5 min in upright position and 5 min supine). Studies III-IV used also the data after the drug administration when the 15-minute protocol was repeated. Salbutamol and nitroglycerin were given in separate 30-minute tests and a drug free 15-minute reference trial was performed before either drug.

## 4.5 Statistical analyses

In Study I data were analysed using SPSS version 17.0 (SPSS Inc., Chicago, Illinois, USA) and software package R version 2.15.1. Differences between the groups in either supine or upright position were examined using analysis of variance for repeated measures. One-minute means of minutes 1 to 5 in each phase were used in analysis of variance for repeated measures. The analyses were adjusted for age, BMI and sex. Backward linear regression was used to examine the changes in the hemodynamic variables in response to head up-tilt. The values in the analyses were adjusted for the supine value of each variable, sex, BMI, and age. The results were reported as constants and coefficients. Example of interpretation: mean change (constant) in heart rate during head-up tilt in normotensives was 13.4/min, and in hypertensives (13.4 - 3.4) = 10/min (mean - coefficient). The variables were centered on a chosen value in the multiple variables model to ease the interpretation of results. Variables with P values exceeding 0.1 were excluded when deciding which to keep in the linear regression model (so-called backward method of elimination).

In study II, due to skewed distribution, PWV and HRV parameters were logarithmically transformed before statistical analyses. To evaluate the impact of the phenotype on PWV, a L1-regression was fitted using sex, categorized BMI, categorized age, categorized hypertension, phenotype, interaction between sex and phenotype (Model 1); and fasting plasma lipids and glucose, and haematocrit (Model 2, with variables of Model 1 included) as variables. In addition, Model 1 was used to evaluate the impact of phenotype on HRV parameters. BMI, age, and presence of hypertension were categorized due to lack of linearity in the data. We performed additional analyses

using continuous variables without categorization to examine the association of changes in SVR index (SVRI) and cardiac index with PWV. The same approach was applied previously, with testing of different cut-off levels. As summary statistics, median and 25th to 75th percentile, or mean and standard deviation (SD) or 95 % confidence intervals (CI) were reported. Kruskal-Wallis rank sum test, Fisher's exact test and Wilcoxon rank sum test were used to compare haemodynamics and demographics between the phenotypes. In regression analysis, average of 3 minutes of each phase was for calculated, because during that the period the values were most stable. All analyses were performed using R software version 2.14.1 (R Development Core Team, 2011).

In study IV, the data were analyzed using R software version 2.14.1 (R Development Core Team, 2011). Differences between non-medicated and medicated phases were examined using t-test for independent samples and linear regression. Linear regression was also employed to examine the changes in the hemodynamic variables in response to salbutamol or nitroglycerin. Variables were included in multivariate regression analysis if there was a statistically significant change after either salbutamol or nitroglycerin administration in the variable in question. The variables included in the analyses were heart rate, stroke index, PWV, ejection duration, and SVR. In regression analysis the mean values of the last 3 supine min preceding the head-up tilt, the last 3 min during the head-up tilt and the last 3 supine min after the head-up tilt were calculated, i.e. during the period when the signal was most stable. The values recorded during the last two min at rest before test-drug administrations served as the reference values for the medication effects. P-values $<0.05$ were considered statistically significant. In some studies, AIx has been adjusted to heart rate 75 beats/min using previously measured gender- and age-specific reference values (Wilkinson et al., 2002). However, this method does not take into account differences in interactions between heart rate and AIx in different populations (Stoner et al., 2014). Therefore, we used heart rate as an independent predictor in the statistical analyses rather than using the AIx values that were adjusted to heart rate 75 beats/min with the SphygmoCor software.

In all the studies, P values less than 0.05 were considered statistically significant. There is no accepted cut-off level to define elevated BP (hypertension) during tilt-table measurements, while office BP is usually higher than home BP. As the supine BP during haemodynamic measurements was on average 8/12 mmHg (systolic/diastolic) lower than the seated office BP ($n = 470$), we applied the accepted cut-off level for home and ambulatory daytime measurements, i.e. values $\geq 135/85$ mmHg, to define hypertension during the recordings. Also other cut-off values were tested, and they yielded corresponding results.

## 4.6 Ethical aspects

All participants gave written informed consent and the study was approved by the ethics committee of Tampere University Hospital District (study number R06086M) and the Finnish Medicines Agency (Eudra-CT registration number 2006–002065–39). The investigation conforms to the principles outlined in the Declaration of Helsinki. The study was registered to an international database (clinicaltrials.gov NCT01742702).

# 5 Results

## 5.1 Hemodynamic profile in primary hypertension versus normotension in supine and upright positions

In study I, subjects were classified either as hypertensive or normotensive using the chosen cut-off values. The three 5-minute phases (supine, head-up tilt and supine) and the response to head-up tilt were compared between the groups. Before the hemodynamic measurements seated office BP was measured manually using a brachial cuff. During the hemodynamic measurements supine radial systolic BP was 4 mmHg lower and diastolic BP was 10 mmHg lower than that measured in office. To take this difference into account, we used the cut-off 135 mmHg for systolic pressure and 85 mmHg for diastolic pressure for hypertension. This boundary was chosen, because it has been applied clinically in ambulatory measurements. The main analyses were additionally performed with the cut-off 140/90 mmHg from office BP and 136/80 mmHg from supine laboratory BP, and the results did not practically change.

When the above-mentioned 135/85 mmHg boundary was used in the classification of hypertension, the average supine radial BP was 148/88 mmHg and supine aortic BP was 136/89 mmHg in the hypertensive subjects, and the corresponding values were 118/68 mmHg and 107/69 mmHg in the normotensive subjects. Mean systolic office BP was 149 ± 15 mmHg in hypertensive subjects and 125 ± 13 mmHg in normotensive subjects. Mean diastolic office BP was 93 ± 9 mmHg in hypertensive subjects and 82 ± 10 mmHg in normotensive subjects. Additionally, heart rate was significantly lower during laboratory measurements than in the office (64 beats/min vs. 67 beats/min, $p<0.05$).

Radial and aortic BP and PP values were higher both supine and upright in hypertensive subjects in multivariate analyses adjusted with age, sex and BMI ($p < 0.001$ for all). Aortic AP, cardiac index, SVRI and LCW index (LCWI) were also higher in presence of hypertension ($p \leq 0.020$ for all, SVRI shown in Figure 7A, Table 2). AIx adjusted to heart rate 75 was higher in hypertensive subjects in both positions ($p < 0.05$), but there was no significant difference in the unadjusted AIx values ($p = 0.129$-$0.162$, Table 2). Stroke index was lower and heart rate was higher in hypertensive subjects in the supine position ($p \leq 0.001$ for both), however there were no statistical differences in the upright position ($p = 0.069$-$0.236$). In supine position, hypertensive subjects had a 1.3 m/s higher PWV than normotensive subjects (Figure 7B) ($p < 0.001$ after

logarithmic correction in both unadjusted analysis and in analysis adjusted with age, BMI and sex).

Table 2. Supine differences between variables in the presence of hypertension (backward regression analysis with gender, age and body mass index as covariates) reported as coefficients for hypertension and as their percentage of normotensive means. SVRI = systemic vascular resistance index, LCWI = left cardiac work index, AP = augmentation pressure, AIx@75 = augmentation index adjusted for heart rate 75/min.

| | Coefficient for hypertension | Percentage of normotensive supine mean | P-value |
|---|---|---|---|
| SVRI (dyn*s/cm$^5$*m$^2$) | 439.2 | +19.1 % | <0.001 |
| Cardiac index (l/min/m$^2$) | 0.141 | +4.9 % | 0.011 |
| Heart rate (1/min) | 3.652 | +5.9 % | <0.001 |
| LCWI (kg*min/m$^2$) | 1.034 | +31 % | <0.001 |
| Aortic AP (mmHg) | 2.907 | +35.3 % | <0.001 |
| AIx@75 (%) | 3.237 | +21.2 % | <0.001 |

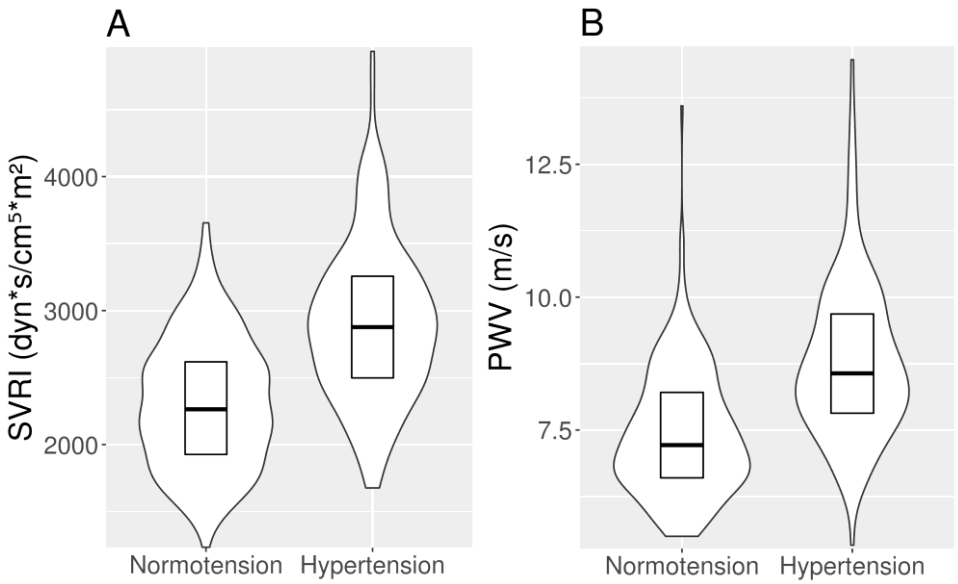


Figure 7. Supine systemic vascular resistance index (SVRI) (A) and pulse wave velocity (PWV) (B) in normotensive subjects and hypertensive subjects. Graph outlines illustrate the distribution. The horizontal line inside the box depicts median and the box outlines the quartiles from median. $P < 0.001$ for the comparisons between normotension and hypertension.

During the head-up tilt aortic systolic BP, LCWI, aortic AP and AIx adjusted to heart rate 75 decreased and this decrease was larger in normotensive subjects in unadjusted analysis ($P < 0.05$). Additionally, aortic diastolic BP and heart rate increased in response to head-up tilt and this increase was larger in normotensive subjects in unadjusted analysis ($P < 0.05$). The decrease in radial systolic BP, radial PP, AIx and cardiac index, and increase in radial diastolic BP and SVR were not different between normotensive and hypertensive subjects. If the supine value of the variable in question, age, sex and body mass index were used as covariates, the decrease in normotensive subjects was still larger during the head-up tilt in aortic systolic BP, stroke index, LCWI and aortic PP than in hypertensive subjects ($P < 0.05$). Additionally, in analyses adjusted with the above-mentioned variables during head-up tilt heart rate increased less, while SVRI and aortic diastolic BP increased more in hypertensive than in normotensive subjects ($P < 0.05$).

Table 3. Difference for change during head-up tilt between variables in the presence of hypertension (backward regression analysis with gender, age and body mass index as covariates) reported as coefficients for hypertension and as percentage of change in normotensive subjects. SVRI = systemic vascular resistance index, LCWI = left cardiac work index, PP = pulse pressure, AIx@75 = augmentation index adjusted for heart rate 75/min.

| | Coefficient for hypertension | Percentage of normotensive upright change | P-value |
|---|---|---|---|
| SVRI (dyn*s/cm$^5$*m$^2$) | 177.4 | +30.1 % | <0.001 |
| Heart rate (1/min) | -2.660 | -22.3 % | <0.001 |
| LCWI (kg*min/m$^2$) | 0.144 | -26.5 % | 0.018 |
| Aortic PP (mmHg) | 2.404 | -30.8 % | 0.002 |
| AIx@75 (%) | -0.996 | +29.2 % | 0.099 |

## 5.2 Hemodynamic phenotypes uncovered by the use of head-up tilt

In study II, the subjects were classified into three different clusters by their hemodynamic response to head-up tilt. The responses in SVR and cardiac index were used in cluster classification. Because supine values of both variables differed between clusters, the cluster analysis was additionally performed using supine values and these clusters did not correlate with the clusters that were classified using response to head-up tilt.

The clusters were named as 'constrictor', 'intermediate' and 'sustainer'. In the constrictor group, SVRI increase in response to head-up tilt was greatest, both relatively (+45 %) and absolutely (mean ± SD increase +944 ± 361 dyn*s/cm$^5$*m$^2$). Additionally, the cardiac index decrease was largest in the 'constrictor' group (-27 %, -0.89 ± 51 l /min/m$^2$) (Figure 8). The group with the smallest changes was named as 'sustainer. In the 'sustainer'-group the increase in SVRI (+2 %, 60 ± 254 dyn*s/cm$^5$*m$^2$) and the decrease in cardiac index (-2 %, -0.06 ± 46 l /min/m$^2$) were both almost non-existent when compared with the 'constrictor'-group (Figure 8). The cluster analysis additionally exposed a third group, which was named as 'intermediate'. In the 'intermediate'-group, the increase in SVRI (+22 %, 554 ± 197 dyn*s/cm$^5$*m$^2$) and the decrease cardiac index (-13 %, -0.37 ± 29 l /min/m$^2$) were between the 'sustainers' and 'constrictors' in

magnitude (Figure 8). Systolic BP was highest in sustainers in supine position, but systolic BP in the head-up position did not differ between the groups (Figure 9). Diastolic BP was lowest in constrictors, both upright and supine (Figure 9). SVRI was lowest in the constrictors in the supine position and lowest in the sustainers in the head-up position (Figure 8). The constrictors had the highest supine and lowest upright cardiac index and stroke index (Figure 8). Heart rate was different in sustainers when compared with constrictors and intermediates, both supine and upright.

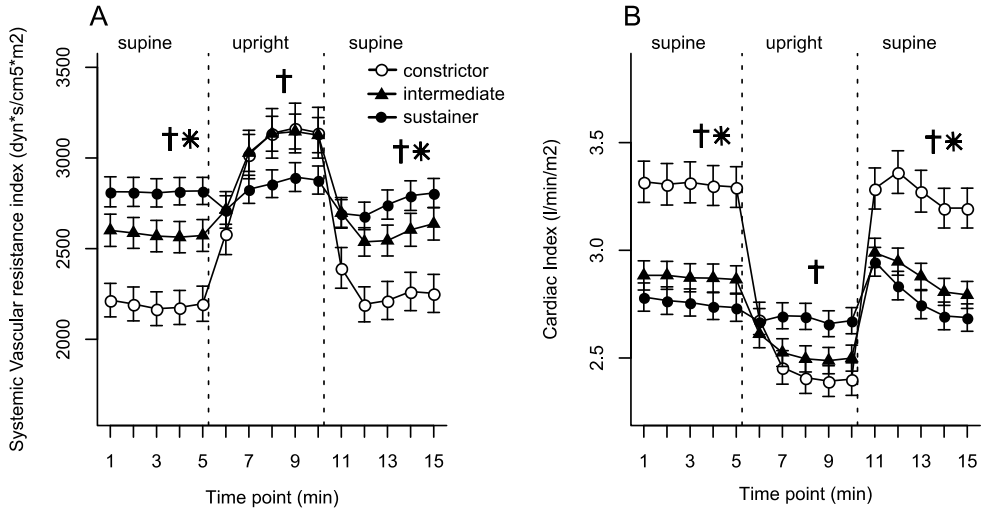


Figure 8. Hemodynamics in the three phenotypes in response to upright posture. Systemic vascular resistance index (A) and cardiac index (B) during the measurement protocol, respectively, in the constrictor (○), intermediate (▲), and sustainer phenotypes (●). Data represents one-minute means and confidence intervals. † if p-value < 0.05 for difference of last three-minute means in selected phase between constrictor and sustainer group, and * if p-value < 0.05 between constrictor and intermediate group.

Of the subjects classified as sustainers a larger percentage had BP values over 135/85 mmHg in the laboratory (both $p < 0.010$), and they were predominantly male ($p < 0.001$) and had higher median BMI ($p < 0.001$). However, age was not significantly different between groups ($p = 0.216$). Creatinine, hematocrit, LDL cholesterol and fasting glucose were lowest, and HDL cholesterol highest in the constrictors. After multivariate analysis, with the covariates gender, BMI and hypertension prevalence, only fasting glucose and creatinine remained significantly different in constrictors. Creatinine was used to estimate eGFR with the MDRD-formula and this was not different between groups. Less than half of the subjects used some medication (42 %) and no subjects used cardiovascular medication. The most common medication was female low-dose hormones (estrogen, progestin or their combination as contraception or as hormone

replacement therapy), which were used by 75 subjects (31% of females). The other medications were antidepressants (22 subjects), intranasal or inhaled corticosteroids (21), thyroxin (16), statins (12), proton pump inhibitors (11), antihistamines (11), non-steroidal anti-inflammatory drugs (5) and glucosamine (5). However, inhaled or intranasal corticosteroids was the only medication, the use of which differed between the groups (2.3 % in sustainers versus 6.4-6.5 % in the two other groups, $p = 0.038$).

In the sustainer subject group, supine median PWV (8.65 m/s) was markedly higher than in the subject groups classified as intermediate (8.00 m/s) or constrictor (7.35 m/s) (Figure 10). These analyses were adjusted with the confounding factors BMI (tertiles), age (tertiles), gender and hypertension. In adjusted analysis, classification into sustainer or intermediate phenotype remained as a statistically significant predictor of PWV. In this model, classification into the sustainer cluster predicted a rise of 0.62 m/s in median supine PWV when compared to the constrictor cluster with female sex predominance, and lowest age and BMI tertiles. Additionally, when the key laboratory values (plasma lipids, glucose and haematocrit) were included in the model, the intermediate and sustainer clusters remained as significant predictors of the supine level of PWV. In the multivariate model, the factors that explained supine PWV were intermediate and sustainer phenotypes, interaction between female sex and intermediate phenotype, BMI tertiles, age tertiles, hypertension and fasting triglycerides.

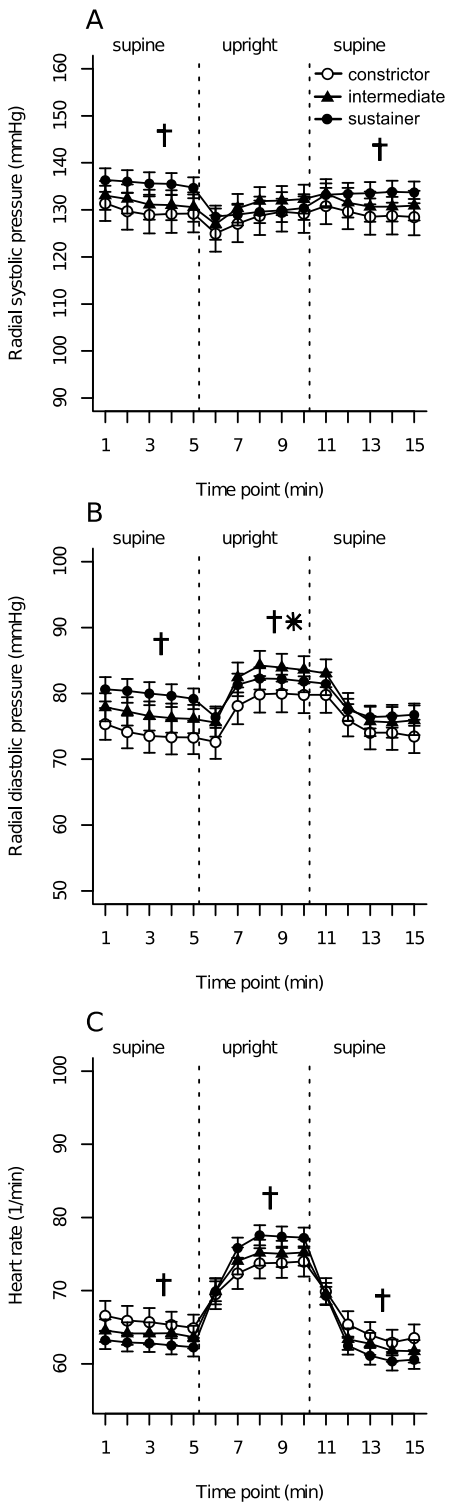


Figure 9. Hemodynamics in the three phenotypes in response to upright posture. Radial systolic pressure (A), radial diastolic pressure (B) and heart rate (C) during the measurement protocol, respectively, in the constrictor (○), intermediate (▲), and sustainer phenotypes (●). The data represents one-minute means and confidence intervals. † if p-value < 0.05 for difference of last three-minute means in selected phase between constrictor and sustainer group, and * if p-value < 0.05 between constrictor and intermediate group.

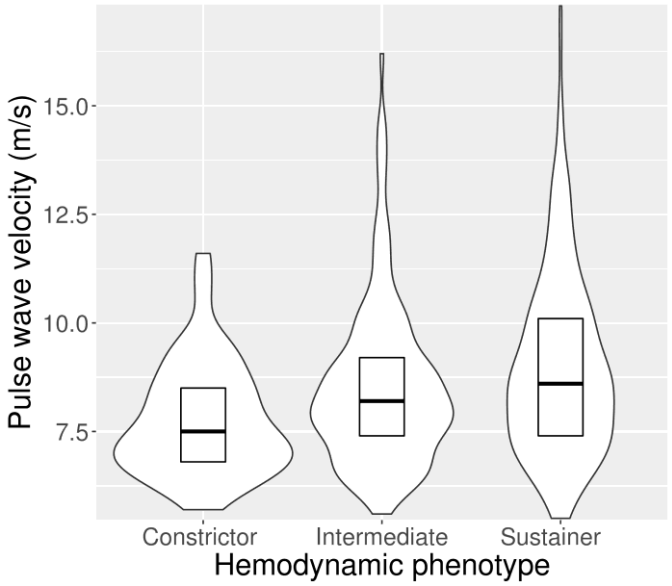


Figure 10. Pulse wave velocity in the three phenotypic clusters. Graph outlines illustrates the distribution. The horizontal line inside the box depicts median and the box outlines the quartiles from median. $P < 0.05$ between intermediate and sustainer, $P < 0.01$ between constrictor and intermediate and $P < 0.001$ between constrictor and sustainer,

In the supine position low frequency to high frequency ratio (LF/HFratio) of HRV was higher in sustainers than in constrictors, while in the upright position sustainers had a higher LF/HF-ratio than constrictors and intermediates. There were no significant differences in the LF power in supine and upright positions, and in the HF power in supine position. Upright HF power was lowest in sustainers and highest in constrictors. However, in adjusted analyses there were no significant differences in HRV between the groups (adjusted with gender, BMI and presence of hypertension).

An important finding was that the clusters could not be found when using BP and heart rate values alone in the classification (Figure 9). Therefore, the measurement of cardiac output and SVR were absolute requirements for the classification.

## 5.3 Salbutamol and nitroglycerin effects on hemodynamics

In study III, the effects of nitroglycerin resoriblet and salbutamol inhalation were compared in a placebo-controlled study. The three 5-minute medicated phases (supine, head-up tilt and supine) were compared to the unmedicated phases with both drugs separately. This effect of medication was compared to the effect of placebo in both drugs.

After the placebo effect was accounted for, nitroglycerin caused a statistically significant decrease in finger, radial and aortic BPs. Additionally, SVRI, AIx and PWV decreased. Heart rate, cardiac index, stroke index and aortic reflection time increased after nitroglycerin administration. The effect of nitroglycerin on hemodynamic variables was different between supine and upright positions, and there was a significantly greater effect during the head-up tilt on finger BPs, radial and aortic diastolic BP, heart rate, SVRI, cardiac index and AIx.

Salbutamol and placebo inhalation were compared in an analogous fashion as nitroglycerin and placebo resoriblet. Salbutamol inhalation caused a statistically significant decrease in radial and aortic BP, aortic PP, finger diastolic BP, AIx and SVRI. Salbutamol also caused an increase in heart rate and cardiac index. The hemodynamic effects were generally smaller after salbutamol than after nitroglycerin.

In study IV, the salbutamol and nitroglycerin effects were examined in a much larger population, but without placebo. In this study, after salbutamol inhalation AIx decreased by 3 percentage points in supine position before and after head-up tilt when compared with the level before drug administration ($p < 0.001$) (Figure 11). Additionally, there was a statistically significant rise in heart rate (Figure 12) and cardiac index. SVRI decreased after the inhalation. The change in heart rate was strongly correlated with the change in AIx ($r^2 = 0.225\text{-}0.389$, $p<0.001$). Multivariate regression analysis was applied to correlate the change in AIx to the change in the other hemodynamic variables. In multivariate regression analysis in supine position the decrease in AIx after salbutamol inhalation was only correlated with the parallel increase in heart rate. During the head-up tilt the decrease in AIx was correlated with the increase heart rate and additionally with the decrease in ejection duration.

After nitroglycerine resoriblet the AIx decreased by 15 percentage points when compared with the level before drug administration ($p < 0.001$) (Figure 11). Additionally, nitroglycerin increased heart rate (Figure 12), stroke index and cardiac index, and decreased ejection duration and SVRI. The decrease in AIx was correlated with the increase in heart rate after AIx, but this correlation was significantly less marked than with salbutamol ($r^2 = 0.111\text{-}0.142$, $p<0.001$). In multivariate regression analysis in both supine and upright positions the decrease in augmentation after nitroglycerin was correlated with heart rate, SVR and ejection duration. The changes in hemodynamic

variables during the head up-tilt were substantial after nitroglycerine, while salbutamol did not affect the hemodynamic response during the head-up tilt to the same extent and the effects were modest.

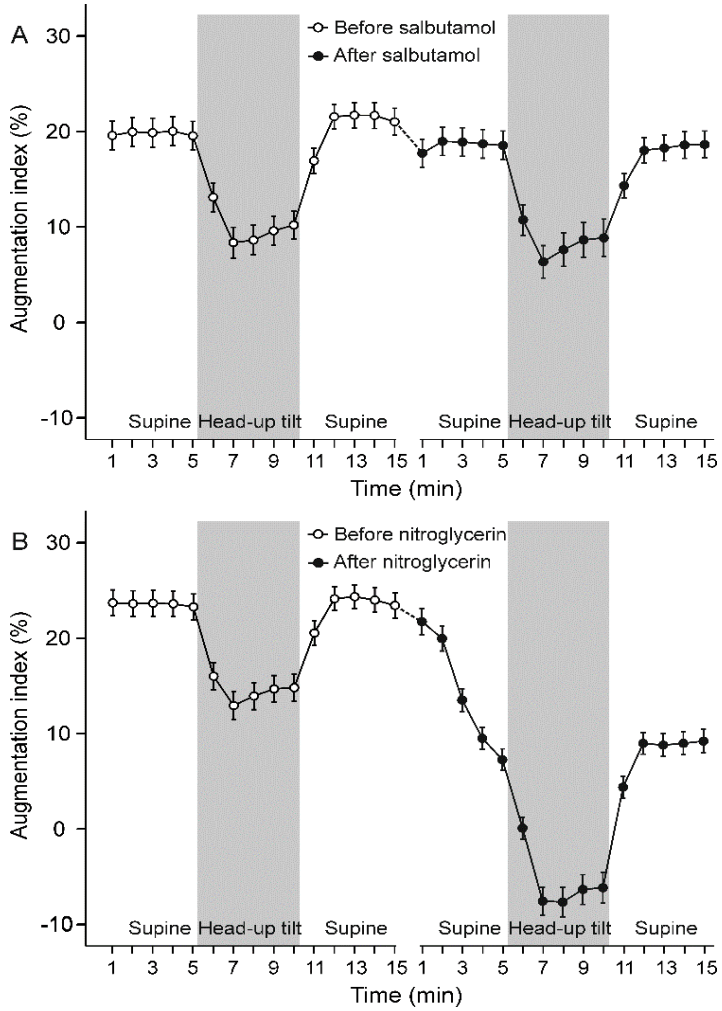


Figure 11. Augmentation index during the whole 30-minute period in salbutamol (A) and nitroglycerin (B) measurements. Data is represented as mean and confidence intervals. $p < 0.001$ in all phases after both drugs.

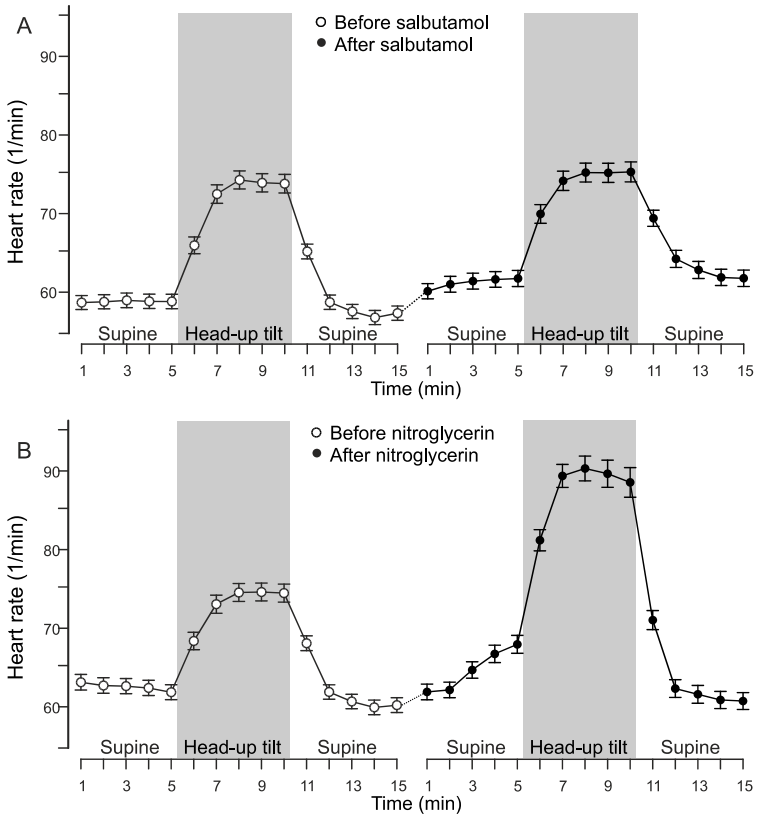


Figure 12. Heart rate during the whole 30-minute period in salbutamol (A) and nitroglycerin (B) measurements. Data is represented as mean and confidence intervals. $p < 0.001$ in all phases after salbutamol and $p < 0.01$ in first supine phase and in head-up tilt after nitroglycerin, and $p = 0.116$ in second supine phase after head-up tilt after nitroglycerin.

# 6 Discussion

## 6.1 Methods: strengths and weaknesses

Invasive measurement is the gold standard for the measurement of central BP. However, invasive methods carry a risk of complications and are not ethically suitable for a large group of healthy subjects (Tavakol et al., 2012). A generalized transfer function has been derived, which calculates the central BP from non-invasively measured radial BP waveform. This method has been shown to correlate well with invasive methods in key studies (Chen et al., 1997; Fetics et al., 1999; Karamanoglu et al., 1993), but at least three studies have also reported a substandard correlation and have suggested that diabetes or gender specific functions should be applied (Cloud et al., 2003; Hope et al., 2004, 2003). So, criticism has been raised against this mathematical derivation of central BP, but no other non-invasive methods have been validated.

Whole body impedance cardiography was used to evaluate stroke volume and cardiac output non-invasively. The invasively measured cardiac output is the gold standard, and all non-invasive measurements results should be observed critically. For ethical reasons, a good clinical justification is needed for invasive measurements. However, whole body impedance cardiography measured cardiac output has been shown to agree well with the invasive thermodilution method (Kööbi et al., 1997b, 1997a; Paredes et al., 2006). For additional verification, we have validated the stroke volume measurements against 3-dimensional ultrasound (Koskela et al., 2013).

SVR is derived mathematically from cardiac output and BP. As a limitation, the central venous pressure is added into the equation of SVR as a constant and it is not estimated individually (Kööbi et al., 1997b). Venous system contains a marked part of blood volume, and venous pressure affects the return of blood into the right atrium (Gelman, 2008). Measurement of venous characteristics would give additional information about the vascular system and would help in the analysis of hemodynamic interactions. Possible venous measurement methods are for example ultrasound measurement of venous diameters and near-infrared spectroscopy (Stone et al., 2016). We also used impedance cardiography to measure PWV and it has been validated against doppler ultrasound (Kööbi et al., 2003) and the gold standard i.e. the tonometric method (Wilenius et al., 2016). Our measurement method of PWV is thus different from the gold standard.

Generally, in studies using the transfer function to calculate AIx, radial BP waveform is measured with a tonometric pen-shaped sensor. This recording is performed chronologically within varied time limits after non-invasive recording of BP, and the wave form is captured during 10-20 heart beats (Hayward et al., 2002). In most of the previous AIx measurement studies, BP and heart rate have been measured with the non-continuous methods (Hayward et al., 2002; McEniery et al., 2006; Wilkinson, 2002). Additionally, most studies have only measured BP and heart rate in addition to AIx. This gives very limited information about the hemodynamical regulation.

In most studies, the intervals between BP and other measurements like AIx have not been reported. In our studies, we measured all variables simultaneously and continuously. In addition, BP was measured continuously with two distinct methods: from the wrist (tonometer) and from the index finger (plethysmography). Heart rate was measured with a single channel recording of ECG. Cardiac output was measured continuously with impedance cardiography (Kööbi et al., 1997b; Tahvanainen et al., 2009a). The continuous cardiac output and BP measurements enabled the continuous measurement of SVR. The time interval from the start of the measurement to the recordings was standardized in all subjects. Additionally, the laboratory conditions and time spent in the supine position did not vary between the subjects in our studies. These factors give additional strength to our study results. There are more than 80 000 heart cycles in a single day with a large amount of normal variation. Without continuous measurements and a standardized challenge that stimulates the hemodynamics, we can only get an incomplete assessment of normal physiology.

Humans change positions repeatedly and do various activities during a single day. However, studies often measure BP only in supine or seated position. This gives only restrained information of normal hemodynamics because a large part of a day is spent in the upright position. The head-up tilt can be thought as a simple provocation trial mimicking normal daily routines (Avolio and Parati, 2011). We measured hemodynamics in the upright position because we hypothesized that the regulation of head-up tilt would reveal additional information which is not found in the supine recordings alone (Tahvanainen et al., 2009a).

Upright position causes major changes in blood distribution and pressures, which affects venous and arterial tone, and stimulates the autonomic nervous system. As a limitation, head-up tilt causes fluctuations in hemodynamics and a longer supine measurement would give more stable measurements in same period length. However, we have previously shown that measurements in supine and upright position are reproducible and repeatable (Tahvanainen et al., 2009a). Additionally, a supine 5-minute period of continuous measurement alone is quite long when compared to several other studies (Hayward et al., 2002; McEniery et al., 2006; Waring et al., 2006; Wilkinson, 2002) and gives stable readings in all measured variables (Tahvanainen et al., 2009a).

## 6.2 Study subjects: strengths and weaknesses

Study subjects represented different age and body mass index groups. We had a rigorous screening protocol, and all our subjects went through a thorough medical examination by a licensed physician. This confirmed that medications, background information, and diseases were correctly reported. Our studies excluded subjects with medications with direct cardiovascular influences. The fact that subjects with cardiovascular medications were excluded should be regarded as a major strength because such exclusion has not been the norm in hemodynamic research (Abdelhammed et al., 2005; Kovács et al., 2010; Smith et al., 2006). It is well known that various medications have marked effects on the measurements of hemodynamics and the responses to physiological stimuli (Lund-Johansen, 1979; Roberts et al., 1987; Tapp et al., 2010; Zusman et al., 1992). During our study, the medical examination by a doctor also revealed several clinically significant diseases among the participants. This was beneficial to the individuals and the inclusion of such patients would have altered the results.

Our subjects were recruited through various channels, and thus the sample was not randomly selected from a population. The goal was especially to recruit unmedicated subjects and the enrollment would have been more laborious from a population sample. The non-random selection method can be regarded as a limitation. The studies I and II were cross-sectional studies and the compared groups were not age, sex and BMI matched. The power was calculated to be sufficient for multivariate analysis and statistics were adjusted with demographical variables. However, the statistical adjustment is always secondary to well-matched groups, and lack of matching can be regarded as a limitation (Stuart, 2010). Cut-off BP for hypertension in the study I was ≥ 135/85 from the laboratory BP. The gold standard is 24-hour ambulatory blood pressure monitoring. The lack of ambulatory monitoring can be regarded as a limitation. However, the main results of study I were additionally analyzed using cut-off levels ≥ 136/80 mmHg and ≥ 140/90 mmHg, and the results remained comparable.

## 6.3 Hemodynamics of hypertension

Under 50 % of hypertensive patients achieve the accepted BP target (Messerli et al., 2007). More detailed measurement of hemodynamics and hypertensive phenotypes have been shown to improve the treatment results of high BP (Smith et al., 2006; Taler et al., 2002). Large genome-wide studies have tried to map the genes that are responsible for primary hypertension and some risk alleles have been found, but the effect of different alleles have been small, largest single contribution around 1 mmHg (Levy et al., 2009). Phenotype characterization has been proven to help with treatment and could also be

valuable in genome analyses. In study I, unmedicated hypertensive subjects had a higher SVRI than normotensive subjects, in line with previous studies (Galarza et al., 1997; Lund-Johansen, 1989; Gary F. Mitchell et al., 2010; Omvik et al., 2000). Despite SVRI being higher in the supine position, it also increased more in the hypertensive subjects in the upright position. This result confirms the hypothesis that the regulation of vascular resistance is altered in primary hypertension (Lund-Johansen, 1989; Messerli et al., 1987). Furthermore, hypertensive patients had a higher heart rate, and even though stroke index was lower, cardiac output remained higher than in normotensive subjects. This cardiac component in hypertension has been verified in a study in 175 individuals (Kovács et al., 2010). As the small cardiac component contributed to the high BP, hypertension is not merely mediated by high vascular resistance. During head-up tilt, in addition to the greater increase SVRI, the increase in heart rate and a decrease in stroke index were smaller in hypertensives. Thus, the differences in adaptation to upright position show that there are functional alterations in hypertensive individuals in the autonomic regulation of the cardiovascular system.

Large arterial status measured using PWV has an independent prognostic impact on cardiovascular events in population samples and in specific groups such as elderly subjects, and patients with renal disease, diabetes or hypertension (Blacher et al., 1999; Boutouyrie et al., 2002; Cruickshank et al., 2002; G. F. Mitchell et al., 2010). Age and BMI correlate well with PWV (Bouthier et al., 1985; Wildman et al., 2003). In our study I, unmedicated hypertensive patients had a markedly higher PWV than normotensive patients, which is in line with previous studies (G. F. Mitchell et al., 2010; Wallace et al., 2007).

## 6.4 Cardiovascular phenotype of head-up tilt

The discovery of cardiovascular phenotypes gives tools for genetic research to find genes that influence our cardiovascular disease risk (Plomin et al., 2009). It is additionally important to find new ways to chart independent risk factors for cardiovascular disease so that we can reveal subjects who are at increased risk without established risk factors or whose risk is augmented when compared with traditional measurements (Ezzati et al., 2002). This has previously been proven possible: hemodynamic response to physiological stimuli such as cold pressor test or exercise test predicts cardiovascular risk (de Liefde et al., 2011; Leino et al., 2009).

In study II, we showed that the hemodynamic response profile to head-up tilt correlates independently with large arterial status evaluated using measurements of PWV. In multivariate statistics of study II, the coefficients for increased PWV in the intermediate and sustainer phenotypes were of the same magnitude as those of two

established the risk factors BMI and elevated BP. There was no statistical difference in age between phenotypes. These results indicate that a simple head-up tilt could be used as a tool in when evaluating an individual's cardiovascular risk. Our study was cross-sectional, so we did not have end-point data, and this can be considered as a limitation. However, due to its nature as the first report on this subject, our study should be regarded as "hypothesis generating" and as thus should be credited for the introduction of a novel idea.

Arterial stiffness has been associated with changes in markers of autonomic tone in type 1 diabetics and prehypertensive subjects (Jaiswal et al., 2013; Pal et al., 2013). However, in a recent study Mäki-Petäjä et al. showed that in normotensive healthy individuals the acute PWV changes are mediated by BP and there is no pressure independent effect on arterial stiffness by the autonomic nervous system (Mäki-Petäjä et al., 2016). The authors pointed out that the results may not apply to patients with increased sympathetic tone or hypertension.

In study II, the differences in HF power and LF/HF ratio were not different between the three clusters after adjustment with demographic differences. This suggests that the observed differences were indeed explained by the demographic deviations between the clusters. We, however, did not measure baroreflex sensitivity. The measurement of baroreflex sensitivity could give further insight for the underlying reasons for the differences in arterial stiffness that were characteristic for the different functional phenotype categories.

The autonomic regulation of BP could still be a key factor in the pathogenesis of primary hypertension (Guyenet, 2006; Hall and Guyton, 2015). Thus, an interesting subject for future studies would be to measure both autonomic nervous tone and baroreflex sensitivity when comparing the hemodynamics between normotensive and hypertensive subjects. These examinations could reveal further different phenotypes in the regulation of hemodynamics.

## 6.5 Hemodynamical changes after salbutamol or nitroglycerin administration

In study III we found that nitroglycerin's effect on hemodynamics was most evident during head-up-tilt. It is known that nitroglycerin causes an endothelium independent vasodilatation and lowers the SVR (Mason and Braunwald, 1965; Oliver et al., 2005; Tahvanainen et al., 2011). However, the main hemodynamic changes induced by small doses of nitroglycerin were suggested to be mediated through venodilatation, which causes the blood to pool into veins. This subsequently lowers the preload in the left ventricle (Mason and Braunwald, 1965; Raviele et al., 1994). In contrast, the arterial

vasodilatory effect was thought to be of lesser significance. However, our study showed that a marked vasodilatation was evident even with the small 0.25 mg nitroglycerin dose that induced a striking decrease in SVR. Simultaneously, cardiac output increased, which additionally suggests that vasodilatation was the major contributor to the nitroglycerin induced hemodynamic changes. In the future, measurement of leg blood volume with near infrared spectroscopy along with recording of SVR could help when comparing the venous and arterial changes in hemodynamics induced by nitroglycerin (Stone et al., 2016).

Study III was a methodological study to see if salbutamol and nitroglycerin cause quantifiable effects in hemodynamics. The effect of salbutamol on the hemodynamic variables was clearly smaller than that of nitroglycerin. The salbutamol effect on heart rate and cardiac index was smaller in the upright than in the supine position. In the case of nitroglycerin the differences were accentuated in the upright when compared with the supine position. The salbutamol effect was nevertheless quantifiable. As a result, we hypothesized that the comparison of nitroglycerin and salbutamol effects on AIx could be indeed used in quantification of global endothelial function, as suggested previously (McEniery et al., 2006).

However, the beta-2-adrenergic effect of salbutamol raises heart rate (Kallergis et al., 2005; Wong et al., 1990), and AIx is known to be markedly heart rate dependent (Stefanadis et al., 1998; Wilkinson et al., 2000). Correspondingly, in our study IV salbutamol caused a significant increase in heart rate and we even found a very good correlation between the salbutamol-induced decrease in AIx and the rise in heart rate. In multivariate analysis, heart rate and ejection duration were the only significant predictors of the salbutamol-elicited change in AIx. There was no independent effect on AIx with salbutamol. In comparison, nitroglycerin had a marked heart-rate independent effect on AIx.

Our study IV results differ from previous studies. It has been proven that salbutamol causes an endothelium dependent vasodilatation (Chowienczyk et al., 1999; Gray and Marshall, 1992; Xu et al., 2000). Removal of endothelium abolishes this vasodilatation in a vascular bed, which validates that the effect is endothelium dependent (Dawes et al., 1997; Graves and Poston, 1993). Salbutamol's effect on AIx has been predominantly associated with endothelium dependent vasodilatation and the correlation of heart rate to AIx has been non-existent (Hayward et al., 2002; McEniery et al., 2006; Wilkinson, 2002). Subsequently owing to these results, the comparison of salbutamol and nitroglycerin effects on AIx was recognized as a practical means to evaluate endothelial function *in vivo*.

In the primary study by Chowienczyk et al. salbutamol caused a 26 % decrease in wave reflection measured from finger and a simultaneous increase of 18 % in heart rate (Chowienczyk et al., 1999). If the NO-synthase inhibitor $N^G$-monomethyl-L-arginine

was administered before salbutamol, the mean changes in both AIx and heart rate were markedly smaller (5 % and 7 % respectively). Similarly, terbutaline has also been used to measure AIx indirectly by measuring radial reflectance index (Lind, 2005; Lind et al., 2011, 2003). In the first study, Lind et al. showed that terbutaline caused a decrease in radial reflectance index, but heart rate rose simultaneously. The correlation between the increase in heart rate and the reduction in reflectance index was actually quite good (r~0.5). As in the above study by Chowienczyk et al., the increase in heart rate and the decrease in AIx was reduced by concomitant NO-synthase inhibition with $N^G$-monomethyl-L-arginine. The NO-synthase inhibitor effect can, however, be due to the increase in BP, which causes a decrease in heart rate due to a reduction in sympathetic tone by baroreflex activation. Thus, the reduction in heart rate causes a parallel increase in AIx (Bernardi et al., 2011). As a strength of our study, we measured both heart rate and AIx continuously when compared to the sequential measurements in previous studies (Hayward et al., 2002; McEniery et al., 2006; Wilkinson, 2002).

The terbutaline induced change in radial reflectance index was not correlated with stroke, myocardial infarct or 5-year risk of composite death (Lind et al., 2011). On the other hand, acetylcholine-induced vasodilatation, an established method in the evaluation of endothelial function, was correlated with end-points mentioned above (Lind et al., 2011). This additionally suggests that the beta-2-agonist effect on AIx was not mainly endothelium dependent.

As a limitation, we did not evaluate endothelium mediated vascular tone with more established methods such as flow-mediated or acetylcholine-induced vasodilatation (Lind, 2005). Such a comparison could reveal if there is any residual endothelium dependent effect induced on AIx with salbutamol. However, our results suggest that this comparison should be performed with a large group of subjects to have sufficient power because the absolute change in AIx with salbutamol is small. In a recent study it was proposed that the effect of nitroglycerin on backward pressure wave was secondary to changes in the forward pressure wave, and that ventricular contraction plays a role in changes observed in AP (Fok et al., 2014). In the future, quantification of backward and forward pressure wave components could reveal whether salbutamol causes changes in the contractile properties of the heart that could additionally affect AP.

# 7 Summary and conclusions

I. Hypertensive and normotensive patients show clear differences in the hemodynamic reactions during passive head-up tilt. Supine SVR and arterial stiffness are higher in hypertensive patients, as suggested by previous studies. However, a distinct hyperdynamic component also contributed to hypertension. In addition, hypertensive subjects were characterized higher upright cardiac index, lower increase in heart rate, lower decrease in stroke index and LCW, and higher increase in SVRI. These results emphasize that the measurement of mere supine BP and heart rate gives a very limited view of the hemodynamics of primary hypertension.

II. The phenotype of the hemodynamic response to upright posture is independently related to arterial stiffness, a widely acknowledged indicator of cardiovascular risk. In particular, the functional phenotype with low upright vasoconstriction and sustained upright cardiac output is related to stiff large arteries, as evaluated from recordings of PWV. Importantly, such phenotypes cannot be discovered by mere measurements of BP and heart rate, or by the recordings of supine hemodynamics, but require assessments of cardiac output and SVR in the upright posture.

III. Salbutamol administration by inhalation elicits moderate but statistically significant changes in several hemodynamic variables. A small dose of nitroglycerin causes remarkable hemodynamic changes that are clearly larger than those following salbutamol inhalation. Both drugs reduce BP, AIx, and peripheral arterial resistance, and increase cardiac output. However, the salbutamol induced changes are more apparent in the supine position, whereas the nitroglycerin induced changes in hemodynamics are highlighted in the upright position.

IV. The measurement of global endothelial function has been proposed to be achievable by comparing the effects of inhaled salbutamol and sublingual nitroglycerin on AIx. However, salbutamol causes a significant parallel rise in heart rate and a decrease in AIx, indicating that the change is heart rate dependent. Indeed, the decrease in AIx after salbutamol administration is

strongly correlated with the simultaneous rise in heart rate with no observable independent effect. Therefore, the change in AIx mainly reflects the beta-2-agonist chronotropic effect of salbutamol on heart rate and does not reflect endothelium dependent vasodilatation in the vasculature.

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# 9 References


Abdelhammed, A., Smith, R., Levy, P., Smits, G., Ferrario, C., 2005. Noninvasive hemodynamic profiles in hypertensive subjects. Am. J. Hypertens. 18, 51–59.

Adams, M.R., Robinson, J., McCredie, R., Seale, J.P., Sorensen, K.E., Deanfield, J.E., Celermajer, D.S., 1998. Smooth muscle dysfunction occurs independently of impaired endothelium-dependent dilation in adults at risk of atherosclerosis. J. Am. Coll. Cardiol. 32, 123–127.

Agabiti-Rosei, E., Mancia, G., O'Rourke, M.F., Roman, M.J., Safar, M.E., Smulyan, H., Wang, J.-G., Wilkinson, I.B., Williams, B., Vlachopoulos, C., 2007. Central Blood Pressure Measurements and Antihypertensive Therapy: A Consensus Document. Hypertension 50, 154–160.

Aizer, J., Karlson, E.W., Chibnik, L.B., Costenbader, K.H., Post, D., Liang, M.H., Gall, V., Gerhard-Herman, M.D., 2009. A controlled comparison of brachial artery flow mediated dilation (FMD) and digital pulse amplitude tonometry (PAT) in the assessment of endothelial function in systemic lupus erythematosus. Lupus 18, 235–242.

Akselrod, S., Gordon, D., Madwed, J.B., Snidman, N.C., Shannon, D.C., Cohen, R.J., 1985. Hemodynamic regulation: investigation by spectral analysis. Am. J. Physiol. 249, H867-875.

Armentano, R., Megnien, J.L., Simon, A., Bellenfant, F., Barra, J., Levenson, J., 1995. Effects of hypertension on viscoelasticity of carotid and femoral arteries in humans. Hypertens. Dallas Tex 1979 26, 48–54.

Asayama, K., Schutte, R., Li, Y., Hansen, T.W., Staessen, J.A., 2014. Blood pressure variability in risk stratification: What does it add? Clin. Exp. Pharmacol. Physiol. 41, 1–8.

Asmar, R.G., London, G.M., O'Rourke, M.E., Safar, M.E., Investigators, for the R.P. coordinators and, 2001. Improvement in Blood Pressure, Arterial Stiffness and Wave Reflections With a Very-Low-Dose Perindopril/Indapamide Combination in Hypertensive Patient: A Comparison With Atenolol. Hypertension 38, 922–926.

Avolio, A., Parati, G., 2011. Reflecting on posture: J. Hypertens. 29, 655–657.

Aznaouridis, K., Vlachopoulos, C., Protogerou, A., Stefanadis, C., 2012. Ambulatory Systolic-Diastolic Pressure Regression Index as a Predictor of Clinical Events: A Meta-Analysis of Longitudinal Studies. Stroke 43, 733–739.

Bachschmid, M., Schildknecht, S., Ullrich, V., 2005. Redox regulation of vascular prostanoid synthesis by the nitric oxide-superoxide system. Biochem. Biophys. Res. Commun. 338, 536–542.

Ben-Shlomo, Y., Spears, M., Boustred, C., May, M., Anderson, S.G., Benjamin, E.J., Boutouyrie, P., Cameron, J., Chen, C.-H., Cruickshank, J.K., Hwang, S.-J., Lakatta, E.G., Laurent, S., Maldonado, J., Mitchell, G.F., Najjar, S.S., Newman, A.B., Ohishi, M., Pannier, B., Pereira, T., Vasan, R.S., Shokawa, T., Sutton-Tyrell, K., Verbeke, F., Wang, K.-L., Webb, D.J., Willum Hansen, T., Zoungas, S., McEniery, C.M., Cockcroft, J.R., Wilkinson, I.B., 2014. Aortic Pulse Wave Velocity Improves Cardiovascular Event Prediction. J. Am. Coll. Cardiol. 63, 636–646.

Bernardi, L., Spallone, V., Stevens, M., Hilsted, J., Frontoni, S., Pop-Busui, R., Ziegler, D., Kempler, P., Freeman, R., Low, P., Tesfaye, S., Valensi, P., Toronto Consensus Panel on Diabetic Neuropathy, 2011. Methods of investigation for cardiac autonomic dysfunction in human research studies. Diabetes Metab. Res. Rev. 27, 654–664.

Blacher, J., Guerin, A.P., Pannier, B., Marchais, S.J., Safar, M.E., London, G.M., 1999. Impact of Aortic Stiffness on Survival in End-Stage Renal Disease. Circulation 99, 2434–2439.

Bouthier, J.D., De Luca, N., Safar, M.E., Simon, A.C., 1985. Cardiac hypertrophy and arterial distensibility in essential hypertension. Am. Heart J. 109, 1345–1352.

Boutouyrie, P., Tropeano, A.I., Asmar, R., Gautier, I., Benetos, A., Lacolley, P., Laurent, S., 2002. Aortic stiffness is an independent predictor of primary coronary events in hypertensive patients: a longitudinal study. Hypertens. Dallas Tex 1979 39, 10–15.

Brignole, M., Menozzi, C., Del Rosso, A., Costa, S., Gaggioli, G., Bottoni, N., Bartoli, P., Sutton, R., 2000. New classification of haemodynamics of vasovagal syncope: beyond the VASIS classification. Analysis of the pre-syncopal phase of the tilt test without and with nitroglycerin challenge. Vasovagal Syncope International Study. Eur. Eur. Pacing Arrhythm. Card. Electrophysiol. J. Work. Groups Card. Pacing Arrhythm. Card. Cell. Electrophysiol. Eur. Soc. Cardiol. 2, 66–76.

Bugge-Asperheim, B., Kiil, F., 1973. Preload, contractility, and afterload as determinants of stroke volume during elevation of aortic blood pressure in dogs. Cardiovasc. Res. 7, 528–541.

Calver, A., Collier, J., Vallance, P., 1992. Inhibition and stimulation of nitric oxide synthesis in the human forearm arterial bed of patients with insulin-dependent diabetes. J. Clin. Invest. 90, 2548–2554.

Carretero, O.A., Oparil, S., 2000. Essential hypertension part I: definition and etiology. Circulation 101, 329–335.

Chemla, D., Plamann, K., Nitenberg, A., 2008. Towards New Indices of Arterial Stiffness Using Systolic Pulse Contour Analysis: A Theoretical Point of View: J. Cardiovasc. Pharmacol. 51, 111–117.

Chen, C.H., Nevo, E., Fetics, B., Pak, P.H., Yin, F.C., Maughan, W.L., Kass, D.A., 1997. Estimation of central aortic pressure waveform by mathematical transformation of radial tonometry pressure. Validation of generalized transfer function. Circulation 95, 1827–1836.

Chess, G.F., Tam, R.M., Calaresu, F.R., 1975. Influence of cardiac neural inputs on rhythmic variations of heart period in the cat. Am. J. Physiol. 228, 775–780.

Chobanian, A.V., Bakris, G.L., Black, H.R., Cushman, W.C., Green, L.A., Izzo, J.L., Jones, D.W., Materson, B.J., Oparil, S., Wright, J.T., Roccella, E.J., the National High Blood Pressure Education Program Coordinating Committee, 2003. Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. Hypertension 42, 1206–1252.

Chowienczyk, P., 2011. Pulse Wave Analysis: What Do the Numbers Mean? Hypertension 57, 1051–1052.

Chowienczyk, P.J., Kelly, R.P., MacCallum, H., Millasseau, S.C., Andersson, T.L., Gosling, R.G., Ritter, J.M., Anggård, E.E., 1999. Photoplethysmographic assessment of pulse wave reflection: blunted response to endothelium-dependent beta2-adrenergic vasodilation in type II diabetes mellitus. J. Am. Coll. Cardiol. 34, 2007–2014.

Christensen, K.L., Mulvany, M.J., 2001. Location of resistance arteries. J. Vasc. Res. 38, 1–12.

Cloud, G.C., Rajkumar, C., Kooner, J., Cooke, J., Bulpitt, C.J., 2003. Estimation of central aortic pressure by SphygmoCor requires intra-arterial peripheral pressures. Clin. Sci. Lond. Engl. 1979 105, 219–225.

Connors, A.F., Dawson, N.V., Shaw, P.K., Montenegro, H.D., Nara, A.R., Martin, L., 1990. Hemodynamic status in critically ill patients with and without acute heart disease. Chest 98, 1200–1206.

Corretti, M.C., Anderson, T.J., Benjamin, E.J., Celermajer, D., Charbonneau, F., Creager, M.A., Deanfield, J., Drexler, H., Gerhard-Herman, M., Herrington, D., Vallance, P., Vita, J., Vogel, R., International Brachial Artery Reactivity Task Force, 2002. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J. Am. Coll. Cardiol. 39, 257–265.

Cracowski, J.-L., Minson, C.T., Salvat-Melis, M., Halliwill, J.R., 2006. Methodological issues in the assessment of skin microvascular endothelial function in humans. Trends Pharmacol. Sci. 27, 503–508.

Cruickshank, K., Riste, L., Anderson, S.G., Wright, J.S., Dunn, G., Gosling, R.G., 2002. Aortic pulse-wave velocity and its relationship to mortality in diabetes and glucose intolerance: an integrated index of vascular function? Circulation 106, 2085–2090.

Curry, F.-R.E., 2005. Atrial natriuretic peptide: an essential physiological regulator of transvascular fluid, protein transport, and plasma volume. J. Clin. Invest. 115, 1458–1461.

Daiber, A., Di Lisa, F., Oelze, M., Kröller-Schön, S., Steven, S., Schulz, E., Münzel, T., 2017a. Crosstalk of mitochondria with NADPH oxidase via reactive oxygen and nitrogen species signalling and its role for vascular function. Br. J. Pharmacol. 174, 1670–1689.

Daiber, A., Steven, S., Weber, A., Shuvaev, V.V., Muzykantov, V.R., Laher, I., Li, H., Lamas, S., Münzel, T., 2017b. Targeting vascular (endothelial) dysfunction: Targeting vascular (endothelial) dysfunction. Br. J. Pharmacol. 174, 1591–1619.

Dawes, M., Chowienczyk, P.J., Ritter, J.M., 1997. Effects of Inhibition of the l-Arginine/Nitric Oxide Pathway on Vasodilation Caused by β-Adrenergic Agonists in Human Forearm. Circulation 95, 2293–2297.

de Liefde, I.I., Welten, G.M.J.M., Verhagen, H.J.M., van Domburg, R.T., Stolker, R.J., Poldermans, D., 2011. Exercise blood pressure response and perioperative complications after major vascular surgery. Coron. Artery Dis. 22, 228–232.

Delius, W., Hagbarth, K.E., Hongell, A., Wallin, B.G., 1972. General characteristics of sympathetic activity in human muscle nerves. Acta Physiol. Scand. 84, 65–81.

DiBona, G.F., 2005. Physiology in perspective: The Wisdom of the Body. Neural control of the kidney. Am. J. Physiol. Regul. Integr. Comp. Physiol. 289, R633-641.

Dickinson, K.M., Clifton, P.M., Burrell, L.M., Barrett, P.H.R., Keogh, J.B., 2014. Postprandial effects of a high salt meal on serum sodium, arterial stiffness, markers of nitric oxide production and markers of endothelial function. Atherosclerosis 232, 211–216.

Dolan, E., Stanton, A., Thijs, L., Hinedi, K., Atkins, N., McClory, S., Hond, E.D., McCormack, P., Staessen, J.A., O'Brien, E., 2005. Superiority of Ambulatory Over Clinic Blood Pressure Measurement in Predicting Mortality: The Dublin Outcome Study. Hypertension 46, 156–161.

Ducharme, A., Dupuis, J., McNicoll, S., Harel, F., Tardif, J.C., 1999. Comparison of nitroglycerin lingual spray and sublingual tablet on time of onset and duration of brachial artery vasodilation in normal subjects. Am. J. Cardiol. 84, 952–954, A8.

Duffy, M.J., Mullan, B.A., Craig, T.R., Shyamsundar, M., MacSweeney, R.E., Thompson, G., Stevenson, M., McAuley, D.F., 2011. Impaired endothelium-dependent vasodilatation is a novel predictor of mortality in intensive care. Crit. Care Med. 39, 629–635.

Egan, B.M., Julius, S., Cottier, C., Osterziel, K.J., Ibsen, H., 1983. Role of cardiovascular receptors on the neural regulation of renin release in normal men. Hypertension 5, 779–786.

Electrophysiology, T.F. of the E.S. of C. the N.A.S. of P., 1996. Heart Rate Variability Standards of Measurement, Physiological Interpretation, and Clinical Use. Circulation 93, 1043–1065.

Esler, M., Jackman, G., Bobik, A., Kelleher, D., Jennings, G., Leonard, P., Skews, H., Korner, P., 1979. Determination of norepinephrine apparent release rate and clearance in humans. Life Sci. 25, 1461–1470.

Esler, M., Jennings, G., Korner, P., Blombery, P., Sacharias, N., Leonard, P., 1984. Measurement of total and organ-specific norepinephrine kinetics in humans. Am. J. Physiol. 247, E21-28.

Esler, M., Jennings, G., Korner, P., Willett, I., Dudley, F., Hasking, G., Anderson, W., Lambert, G., 1988. Assessment of human sympathetic nervous system activity

from measurements of norepinephrine turnover. Hypertens. Dallas Tex 1979 11, 3–20.

Ezzati, M., Lopez, A.D., Rodgers, A., Hoorn, S.V., Murray, C.J., 2002. Selected major risk factors and global and regional burden of disease. The Lancet 360, 1347–1360.

Fagius, J., Wallin, B.G., 1993. Long-term variability and reproducibility of resting human muscle nerve sympathetic activity at rest, as reassessed after a decade. Clin. Auton. Res. Off. J. Clin. Auton. Res. Soc. 3, 201–205.

Fetics, B., Nevo, E., Chen, C.H., Kass, D.A., 1999. Parametric model derivation of transfer function for noninvasive estimation of aortic pressure by radial tonometry. IEEE Trans. Biomed. Eng. 46, 698–706.

Fok, H., Guilcher, A., Li, Y., Brett, S., Shah, A., Clapp, B., Chowienczyk, P., 2014. Augmentation Pressure Is Influenced by Ventricular Contractility/Relaxation Dynamics Novel Mechanism of Reduction of Pulse Pressure by Nitrates. Hypertension 63, 1050–1055.

Folkow, B., Svanborg, A., 1993. Physiology of cardiovascular aging. Physiol. Rev. 73, 725–764.

Franklin, S.S., Jacobs, M.J., Wong, N.D., L'Italien, G.J., Lapuerta, P., 2001. Predominance of isolated systolic hypertension among middle-aged and elderly US hypertensives: analysis based on National Health and Nutrition Examination Survey (NHANES) III. Hypertens. Dallas Tex 1979 37, 869–874.

Freeman, R., Wieling, W., Axelrod, F.B., Benditt, D.G., Benarroch, E., Biaggioni, I., Cheshire, W.P., Chelimsky, T., Cortelli, P., Gibbons, C.H., Goldstein, D.S., Hainsworth, R., Hilz, M.J., Jacob, G., Kaufmann, H., Jordan, J., Lipsitz, L.A., Levine, B.D., Low, P.A., Mathias, C., Raj, S.R., Robertson, D., Sandroni, P., Schatz, I., Schondorff, R., Stewart, J.M., van Dijk, J.G., 2011. Consensus statement on the definition of orthostatic hypotension, neurally mediated syncope and the postural tachycardia syndrome. Clin. Auton. Res. 21, 69–72.

Frohlich, E.D., 1972. Hyperdynamic Circulation and Hypertension. Postgrad. Med. 52, 68–73.

Galarza, C.R., Alfie, J., Waisman, G.D., Mayorga, L.M., Cámera, L.A., del Río, M., Vasvari, F., Limansky, R., Farías, J., Tessler, J., Cámera, M.I., 1997. Diastolic pressure underestimates age-related hemodynamic impairment. Hypertens. Dallas Tex 1979 30, 809–816.

Galley, H.F., Webster, N.R., 2004. Physiology of the endothelium. Br. J. Anaesth. 93, 105–113.

Geerts, B.F., Aarts, L.P., Jansen, J.R., 2011. Methods in pharmacology: measurement of cardiac output: Methods to measure cardiac output. Br. J. Clin. Pharmacol. 71, 316–330.

Gelman, S., 2008. Venous Function and Central Venous PressureA Physiologic Story. Anesthesiol. J. Am. Soc. Anesthesiol. 108, 735–748.

Grassi, G., Bolla, G., Seravalle, G., Turri, C., Lanfranchi, A., Mancia, G., 1997. Comparison between reproducibility and sensitivity of muscle sympathetic nerve traffic and plasma noradrenaline in man. Clin. Sci. Lond. Engl. 1979 92, 285–289.

Grassi, G., Seravalle, G., Cattaneo, B.M., Bolla, G.B., Lanfranchi, A., Colombo, M., Giannattasio, C., Brunani, A., Cavagnini, F., Mancia, G., 1995. Sympathetic activation in obese normotensive subjects. Hypertens. Dallas Tex 1979 25, 560–563.

Grassi, Guido, Seravalle, G., Cattaneo, B.M., Lanfranchi, A., Vailati, S., Giannattasio, C., Bo, A.D., Sala, C., Bolla, G.B., Pozzi, M., Mancia, G., 1995. Sympathetic Activation and Loss of Reflex Sympathetic Control in Mild Congestive Heart Failure. Circulation 92, 3206–3211.

Graves, J., Poston, L., 1993. β-Adrenoceptor agonist mediated relaxation of rat isolated resistance arteries: a role for the endothelium and nitric oxide. Br. J. Pharmacol. 108, 631–637.

Gray, D.W., Marshall, I., 1992. Novel signal transduction pathway mediating endothelium-dependent beta-adrenoceptor vasorelaxation in rat thoracic aorta. Br. J. Pharmacol. 107, 684–690.

Grubb, B.P., Kosinski, D., 1997. Tilt table testing: concepts and limitations. Pacing Clin. Electrophysiol. PACE 20, 781–787.

Guyenet, P.G., 2006. The sympathetic control of blood pressure. Nat. Rev. Neurosci. 7, 335–346.

Guyton, A.C., 1991. Blood pressure control--special role of the kidneys and body fluids. Science 252, 1813–1816.

Guyton, A.C., Coleman, T.G., Granger, H.J., 1972. Circulation: overall regulation. Annu. Rev. Physiol. 34, 13–46.

Hajdu, M.A., Heistad, D.D., Siems, J.E., Baumbach, G.L., 1990. Effects of aging on mechanics and composition of cerebral arterioles in rats. Circ. Res. 66, 1747–1754.

Hall, J., Guyton, A., 2015. Guyton and Hall Textbook of Medical Physiology, 13e ed. Elsevier.

Hamburg, N.M., Keyes, M.J., Larson, M.G., Vasan, R.S., Schnabel, R., Pryde, M.M., Mitchell, G.F., Sheffy, J., Vita, J.A., Benjamin, E.J., 2008. Cross-sectional relations of digital vascular function to cardiovascular risk factors in the Framingham Heart Study. Circulation 117, 2467–2474.

Hansen, T.W., Kikuya, M., Thijs, L., Björklund-Bodegård, K., Kuznetsova, T., Ohkubo, T., Richart, T., Torp-Pedersen, C., Lind, L., Jeppesen, J., Ibsen, H., Imai, Y., Staessen, J.A., IDACO Investigators, 2007. Prognostic superiority of daytime ambulatory over conventional blood pressure in four populations: a meta-analysis of 7,030 individuals. J. Hypertens. 25, 1554–1564.

Hanyu, H., Shimizu, S., Hirao, K., Kanetaka, H., Iwamoto, T., Chikamori, T., Usui, Y., Yamashina, A., Koizumi, K., Abe, K., 2006. Comparative value of brain perfusion SPECT and [(123)I]MIBG myocardial scintigraphy in distinguishing between dementia with Lewy bodies and Alzheimer's disease. Eur. J. Nucl. Med. Mol. Imaging 33, 248–253.

Hay, J., 1931. A British Medical Association Lecture on THE SIGNIFICANCE OF A RAISED BLOOD PRESSURE. Br. Med. J. 2, 43–47.

Hayward, C.S., Kraidly, M., Webb, C.M., Collins, P., 2002. Assessment of endothelial function using peripheral waveform analysis: a clinical application. J. Am. Coll. Cardiol. 40, 521–528.

Higginbotham, M.B., Morris, K.G., Williams, R.S., McHale, P.A., Coleman, R.E., Cobb, F.R., 1986. Regulation of stroke volume during submaximal and maximal upright exercise in normal man. Circ. Res. 58, 281–291.

Hirata, K., Kawakami, M., O'Rourke, M.F., 2006. Pulse wave analysis and pulse wave velocity a review of blood pressure interpretation 100 years after Korotkov. Circ. J. 70, 1231–1239.

Hirata, K., Vlachopoulos, C., Adji, A., O'Rourke, M.F., 2005. Benefits from angiotensin-converting enzyme inhibitor "beyond blood pressure lowering": beyond blood pressure or beyond the brachial artery? J. Hypertens. 23, 551–556.

Hope, S.A., Tay, D.B., Meredith, I.T., Cameron, J.D., 2003. Use of arterial transfer functions for the derivation of aortic waveform characteristics. J. Hypertens. 21, 1299–1305.

Hope, S.A., Tay, D.B., Meredith, I.T., Cameron, J.D., 2004. Use of Arterial Transfer Functions for the Derivation of Central Aortic Waveform Characteristics in Subjects With Type 2 Diabetes and Cardiovascular Disease. Diabetes Care 27, 746–751.

Izzard, A.S., Heagerty, A.M., 1995. Hypertension and the vasculature: arterioles and the myogenic response. J. Hypertens. 13, 1–4.

Izzo, J.L., 1984. The sympathoadrenal system in the maintenance of elevated arterial pressure. J. Cardiovasc. Pharmacol. 6 Suppl 3, S514-521.

Izzo, J.L., 1989. Sympathoadrenal activity, catecholamines, and the pathogenesis of vasculopathic hypertensive target-organ damage. Am. J. Hypertens. 2, 305S-312S.

Jacob, G., Ertl, A.C., Shannon, J.R., Furlan, R., Robertson, R.M., Robertson, D., 1998. Effect of standing on neurohumoral responses and plasma volume in healthy subjects. J. Appl. Physiol. 84, 914–921.

Jacob, R., Dierberger, B., Kissling, G., 1992. Functional significance of the Frank-Starling mechanism under physiological and pathophysiological conditions. Eur. Heart J. 13, 7–14.

Jaiswal, M., Urbina, E.M., Wadwa, R.P., Talton, J.W., D'Agostino, R.B., Hamman, R.F., Fingerlin, T.E., Daniels, S.R., Marcovina, S.M., Dolan, L.M., Dabelea, D., 2013. Reduced Heart Rate Variability Is Associated With Increased Arterial Stiffness in Youth With Type 1 Diabetes: The SEARCH CVD study. Diabetes Care 36, 2351–2358.

Jardine, D.L., 2013. Vasovagal Syncope. Cardiol. Clin. 31, 75–87.

Juhanoja, E.P., Niiranen, T.J., Johansson, J.K., Puukka, P.J., Jula, A.M., 2016. Agreement between ambulatory, home, and office blood pressure variability: J. Hypertens. 34, 61–67.

Kähler, J., Ewert, A., Weckmüller, J., Stobbe, S., Mittmann, C., Köster, R., Paul, M., Meinertz, T., Münzel, T., 2001. Oxidative stress increases endothelin-1 synthesis in human coronary artery smooth muscle cells. J. Cardiovasc. Pharmacol. 38, 49–57.

Kallergis, E.M., Manios, E.G., Kanoupakis, E.M., Schiza, S.E., Mavrakis, H.E., Klapsinos, N.K., Vardas, P.E., 2005. Acute electrophysiologic effects of inhaled salbutamol in humans. CHEST J. 127, 2057–2063.

Kanbay, M., Sánchez-Lozada, L.-G., Franco, M., Madero, M., Solak, Y., Rodriguez-Iturbe, B., Covic, A., Johnson, R.J., 2011. Microvascular disease and its role in the brain and cardiovascular system: a potential role for uric acid as a cardiorenal toxin. Nephrol. Dial. Transplant. Off. Publ. Eur. Dial. Transpl. Assoc. - Eur. Ren. Assoc. 26, 430–437.

Kaplan, N.M., 1992. Commentary on "Blood pressure and fitness for work" by Michael B. Murphy. Am. J. Hypertens. 5, 335.

Karamanoglu, M., O'rourke, M.F., Avolio, A.P., Kelly, R.P., 1993. An analysis of the relationship between central aortic and peripheral upper limb pressure waves in man. Eur. Heart J. 14, 160–167.

Kollias, A., Stergiou, G.S., Dolan, E., O'Brien, E., 2012. Ambulatory arterial stiffness index: A systematic review and meta-analysis. Atherosclerosis 224, 291–301.

Kööbi, T., Kähönen, M., Iivainen, T., Turjanmaa, V., 2003. Simultaneous non-invasive assessment of arterial stiffness and haemodynamics - a validation study. Clin. Physiol. Funct. Imaging 23, 31–36.

Kööbi, T., Kaukinen, S., Ahola, T., Turjanmaa, V.M.H., 1997a. Non-invasive measurement of cardiac output: whole-body impedance cardiography in simultaneous comparison with thermodilution and direct oxygen Fick methods. Intensive Care Med. 23, 1132–1137.

Kööbi, T., Kaukinen, S., Turjanmaa, V.M., Uusitalo, A.J., 1997b. Whole-body impedance cardiography in the measurement of cardiac output. Crit. Care Med. 25, 779–785.

Korner, P.I., 2010. The Phenotypic Patterns of Essential Hypertension Are the Key to Identifying" High Blood Pressure" Genes. Physiol. Res. 59, 841.

Koskela, J.K., Tahvanainen, A., Haring, A., Tikkakoski, A.J., Ilveskoski, E., Viitala, J., Leskinen, M.H., Lehtimäki, T., Kähönen, M.A., Kööbi, T., Niemelä, O., Mustonen, J.T., Pörsti, I.H., 2013. Association of resting heart rate with cardiovascular function: a cross-sectional study in 522 Finnish subjects. BMC Cardiovasc. Disord. 13, 102.

Kovács, K.R., Szekeres, C.C., Bajkó, Z., Csapó, K., Molnár, S., Oláh, L., Magyar, M.T., Bereczki, D., Kardos, L., Soltész, P., Bojtor, A.B., Csiba, L., 2010. Cerebro- and

cardiovascular reactivity and neuropsychological performance in hypertensive patients. J. Neurol. Sci. 299, 120–125.

Krzesiński, P., Gielerak, G., Stańczyk, A., Piotrowicz, K., Skrobowski, A., 2016. Who benefits more from hemodynamically guided hypotensive therapy? The experience from two randomized, prospective and controlled trials. Ther. Adv. Cardiovasc. Dis. 10, 21–29.

Kurbaan, A.S., Bowker, T.J., Wijesekera, N., Franzén, A.-C., Heaven, D., Itty, S., Sutton, R., 2003. Age and hemodynamic responses to tilt testing in those with syncope of unknown origin. J. Am. Coll. Cardiol. 41, 1004–1007.

Kuvin, J.T., Patel, A.R., Sliney, K.A., Pandian, N.G., Sheffy, J., Schnall, R.P., Karas, R.H., Udelson, J.E., 2003. Assessment of peripheral vascular endothelial function with finger arterial pulse wave amplitude. Am. Heart J. 146, 168–174.

Lakatta, E.G., Levy, D., 2003. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part I: aging arteries: a "set up" for vascular disease. Circulation 107, 139–146.

Lakatta, E.G., Wang, M., Najjar, S.S., 2009. Arterial Aging and Subclinical Arterial Disease are Fundamentally Intertwined at Macroscopic and Molecular Levels. Med. Clin. North Am. 93, 583–604.

Lambert, G.W., Thompson, J.M., Turner, A.G., Cox, H.S., Wilkinson, D., Vaz, M., Kalff, V., Kelly, M.J., Jennings, G.L., Esler, M.D., 1997. Cerebral noradrenaline spillover and its relation to muscle sympathetic nervous activity in healthy human subjects. J. Auton. Nerv. Syst. 64, 57–64.

Laurent, S., Cockcroft, J., Van Bortel, L., Boutouyrie, P., Giannattasio, C., Hayoz, D., Pannier, B., Vlachopoulos, C., Wilkinson, I., Struijker-Boudier, H., on behalf of the European Network for Non-invasive Investigation of Large Arteries, 2006. Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur. Heart J. 27, 2588–2605.

Leino, J., Minkkinen, M., Nieminen, T., Lehtimäki, T., Viik, J., Lehtinen, R., Nikus, K., Kööbi, T., Turjanmaa, V., Verrier, R.L., Kähönen, M., 2009. Combined assessment of heart rate recovery and T-wave alternans during routine exercise testing improves prediction of total and cardiovascular mortality: the Finnish Cardiovascular Study. Heart Rhythm 6, 1765–1771.

Lerman, A., 2005. Endothelial Function: Cardiac Events. Circulation 111, 363–368.

Levey, A.S., Bosch, J.P., Lewis, J.B., Greene, T., Rogers, N., Roth, D., 1999. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. Ann. Intern. Med. 130, 461–470.

Levy, D., Ehret, G.B., Rice, K., Verwoert, G.C., Launer, L.J., Dehghan, A., Glazer, N.L., Morrison, A.C., Johnson, A.D., Aspelund, T., Aulchenko, Y., Lumley, T., Köttgen, A., Vasan, R.S., Rivadeneira, F., Eiriksdottir, G., Guo, X., Arking, D.E., Mitchell, G.F., Mattace-Raso, F.U.S., Smith, A.V., Taylor, K., Scharpf, R.B., Hwang, S.-J., Sijbrands, E.J.G., Bis, J., Harris, T.B., Ganesh, S.K., O'Donnell, C.J., Hofman, A., Rotter, J.I., Coresh, J., Benjamin, E.J., Uitterlinden, A.G., Heiss, G., Fox, C.S., Witteman, J.C.M., Boerwinkle, E., Wang, T.J., Gudnason, V., Larson, M.G., Chakravarti, A., Psaty, B.M., van Duijn, C.M., 2009. Genome-wide association study of blood pressure and hypertension. Nat. Genet. 41, 677–687.

Lewington, S., Clarke, R., Qizilbash, N., Peto, R., Collins, R., Prospective Studies Collaboration, 2002. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. Lancet Lond. Engl. 360, 1903–1913.

Li, Y., Wang, J.-G., Dolan, E., Gao, P.-J., Guo, H.-F., Nawrot, T., Stanton, A.V., Zhu, D.-L., O'Brien, E., Staessen, J.A., 2006. Ambulatory Arterial Stiffness Index Derived From 24-Hour Ambulatory Blood Pressure Monitoring. Hypertension 47, 359–364.

Lind, L., 2005. A Comparison of Three Different Methods to Evaluate Endothelium-Dependent Vasodilation in the Elderly: The Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) Study. Arterioscler. Thromb. Vasc. Biol. 25, 2368–2375.

Lind, L., 2006. Systolic and diastolic hypertension impair endothelial vasodilatory function in different types of vessels in the elderly: the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study. J. Hypertens. 24, 1319–1327.

Lind, L., Berglund, L., Larsson, A., Sundström, J., 2011. Endothelial function in resistance and conduit arteries and 5-year risk of cardiovascular disease. Circulation 123, 1545–1551.

Lind, L., Pettersson, K., Johansson, K., 2003. Analysis of endothelium-dependent vasodilation by use of the radial artery pulse wave obtained by applanation tonometry. Clin. Physiol. Funct. Imaging 23, 50–57.

Lopez, A.D., Mathers, C.D., Ezzati, M., Jamison, D.T., Murray, C.J.L., 2006. Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data. Lancet 367, 1747–1757.

Lu, D.-Y., Sung, S.-H., Yu, W.-C., Cheng, H.-M., Chuang, S.-Y., Chen, C.-H., 2014. Wave reflections, arterial stiffness, heart rate variability and orthostatic hypotension. Hypertens. Res. 37, 1056–1061.

Ludmer, P.L., Selwyn, A.P., Shook, T.L., Wayne, R.R., Mudge, G.H., Alexander, R.W., Ganz, P., 1986. Paradoxical Vasoconstriction Induced by Acetylcholine in Atherosclerotic Coronary Arteries. N. Engl. J. Med. 315, 1046–1051.

Lund-Johansen, P., 1979. Hemodynamic consequences of long-term beta-blocker therapy: a 5-year follow-up study of atenolol. J. Cardiovasc. Pharmacol. 1, 487–495.

Lund-Johansen, P., 1986. Hemodynamic patterns in the natural history of borderline hypertension. J. Cardiovasc. Pharmacol. 8 Suppl 5, S8-14.

Lund-Johansen, P., 1989. Central haemodynamics in essential hypertension at rest and during exercise: a 20-year follow-up study. J. Hypertens. Suppl. Off. J. Int. Soc. Hypertens. 7, S52-55.

Mäki-Petäjä, K.M., Barrett, S.M.L., Evans, S.V., Cheriyan, J., McEniery, C.M., Wilkinson, I.B., 2016. The Role of the Autonomic Nervous System in the Regulation of Aortic Stiffness. Hypertens. Dallas Tex 1979 68, 1290–1297.

Malliani, A., Pagani, M., Lombardi, F., Cerutti, S., 1991. Cardiovascular neural regulation explored in the frequency domain. Circulation 84, 482–492.

Mancia, G., Fagard, R., Narkiewicz, K., Redón, J., Zanchetti, A., Böhm, M., Christiaens, T., Cifkova, R., De Backer, G., Dominiczak, A., Galderisi, M., Grobbee, D.E., Jaarsma, T., Kirchhof, P., Kjeldsen, S.E., Laurent, S., Manolis, A.J., Nilsson, P.M., Ruilope, L.M., Schmieder, R.E., Sirnes, P.A., Sleight, P., Viigimaa, M., Waeber, B., Zannad, F., Task Force Members, 2013. 2013 ESH/ESC Guidelines for the management of arterial hypertension: the Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). J. Hypertens. 31, 1281–1357.

Mancia, G., Grassi, G., 2014. The Autonomic Nervous System and Hypertension. Circ. Res. 114, 1804–1814.

Martin, E.A., Victor, R.G., 2011. Premise, promise, and potential limitations of invasive devices to treat hypertension. Curr. Cardiol. Rep. 13, 86–92.

Maruhashi, T., Kihara, Y., Higashi, Y., 2018. Assessment of endothelium-independent vasodilation: from methodology to clinical perspectives. J. Hypertens. 36, 1460–1467.

Maruhashi, T., Soga, J., Fujimura, N., Idei, N., Mikami, S., Iwamoto, Y., Kajikawa, M., Matsumoto, T., Hidaka, T., Kihara, Y., Chayama, K., Noma, K., Nakashima, A., Goto, C., Higashi, Y., 2013. Nitroglycerine-induced vasodilation for assessment of vascular function: a comparison with flow-mediated vasodilation. Arterioscler. Thromb. Vasc. Biol. 33, 1401–1408.

Mason, D.T., Braunwald, E., 1965. The effects of nitroglycerin and amyl nitrite on arteriolar and venous tone in the human forearm. Circulation 32, 755–766.

Matsui, Y., O'Rourke, M.F., Ishikawa, J., Shimada, K., Kario, K., 2012. Association of Changes in Ambulatory Arterial Stiffness Index and Pulse Wave Velocity During Antihypertensive Treatment: The J-CORE Study. Am. J. Hypertens. 25, 862–868.

McEniery, C., Yasmin, Y., McDonnell, B., Cockcroft, J., Wilkinson, I., 2017. Haemodynamic Mechanisms underlying Essential Hypertension Change Across the Adult Age-Span: The Anglo-Cardiff Collaborative Trial. FASEB J. 31, 1011.15-1011.15.

McEniery, C.M., Wallace, S., Mackenzie, I.S., McDonnell, B., Yasmin, Newby, D.E., Cockcroft, J.R., Wilkinson, I.B., 2006. Endothelial Function Is Associated With Pulse Pressure, Pulse Wave Velocity, and Augmentation Index in Healthy Humans. Hypertension 48, 602–608.

McEniery, C.M., Yasmin, Hall, I.R., Qasem, A., Wilkinson, I.B., Cockcroft, J.R., 2005a. Normal Vascular Aging: Differential Effects on Wave Reflection and Aortic Pulse Wave Velocity: The Anglo-Cardiff Collaborative Trial (ACCT). J. Am. Coll. Cardiol. 46, 1753–1760.

McEniery, C.M., Yasmin, Wallace, S., Maki-Petaja, K., McDonnell, B., Sharman, J.E., Retallick, C., Franklin, S.S., Brown, M.J., Lloyd, R.C., Cockcroft, J.R., Wilkinson, I.B., 2005b. Increased Stroke Volume and Aortic Stiffness Contribute to Isolated Systolic Hypertension in Young Adults. Hypertension 46, 221–226.

Meaume, S., Benetos, A., Henry, O.F., Rudnichi, A., Safar, M.E., 2001. Aortic pulse wave velocity predicts cardiovascular mortality in subjects >70 years of age. Arterioscler. Thromb. Vasc. Biol. 21, 2046–2050.

Mehlum, M.H., Liestøl, K., Kjeldsen, S.E., Julius, S., Hua, T.A., Rothwell, P.M., Mancia, G., Parati, G., Weber, M.A., Berge, E., 2018. Blood pressure variability and risk of cardiovascular events and death in patients with hypertension and different baseline risks. Eur. Heart J. 39, 2243–2251.

Messerli, F.H., Garavaglia, G.E., Schmieder, R.E., Sundgaard-Riise, K., Nunez, B.D., Amodeo, C., 1987. Disparate cardiovascular findings in men and women with essential hypertension. Ann. Intern. Med. 107, 158–161.

Messerli, F.H., Williams, B., Ritz, E., 2007. Essential hypertension. The Lancet 370, 591–603.

Metzler, M., Duerr, S., Granata, R., Krismer, F., Robertson, D., Wenning, G.K., 2013. Neurogenic orthostatic hypotension: pathophysiology, evaluation, and management. J. Neurol. 260, 2212–2219.

Mitchell, G. F., Hwang, S.-J., Vasan, R.S., Larson, M.G., Pencina, M.J., Hamburg, N.M., Vita, J.A., Levy, D., Benjamin, E.J., 2010. Arterial Stiffness and Cardiovascular Events: The Framingham Heart Study. Circulation 121, 505–511.

Mitchell, G.F., Parise, H., Vita, J.A., Larson, M.G., Warner, E., Keaney, J.F., Keyes, M.J., Levy, D., Vasan, R.S., Benjamin, E.J., 2004. Local Shear Stress and Brachial Artery Flow-Mediated Dilation The Framingham Heart Study. Hypertension 44, 134–139.

Mitchell, Gary F., Wang, N., Palmisano, J.N., Larson, M.G., Hamburg, N.M., Vita, J.A., Levy, D., Benjamin, E.J., Vasan, R.S., 2010. Hemodynamic correlates of blood pressure across the adult age spectrum: noninvasive evaluation in the Framingham Heart Study. Circulation 122, 1379–1386.

Morimoto, S., Cassell, M.D., Beltz, T.G., Johnson, A.K., Davisson, R.L., Sigmund, C.D., 2001. Elevated blood pressure in transgenic mice with brain-specific expression of human angiotensinogen driven by the glial fibrillary acidic protein promoter. Circ. Res. 89, 365–372.

Mulvany, M.J., 1993. Resistance vessel structure and the pathogenesis of hypertension. J. Hypertens. Suppl. Off. J. Int. Soc. Hypertens. 11, S7-12.

Natale, A., Akhtar, M., Jazayeri, M., Dhala, A., Blanck, Z., Deshpande, S., Krebs, A., Sra, J.S., 1995. Provocation of hypotension during head-up tilt testing in subjects with no history of syncope or presyncope. Circulation 92, 54–58.

Nichols, W., O'Rourke, M., Vlachopoulos, C., 2011. McDonald's Blood Flow in Arteries, Sixth Edition: Theoretical, Experimental and Clinical Principles

[WWW Document]. CRC Press. URL https://www.crcpress.com/McDonalds-Blood-Flow-in-Arteries-Sixth-Edition-Theoretical-Experimental/Nichols-ORourke-Vlachopoulos/p/book/9780340985014 (accessed 12.18.16).

Nichols, W.W., 2005. Clinical measurement of arterial stiffness obtained from noninvasive pressure waveforms. Am. J. Hypertens. 18, 3S-10S.

Nichols, W.W., Nicolini, F.A., Pepine, C.J., 1992. Determinants of isolated systolic hypertension in the elderly. J. Hypertens. Suppl. Off. J. Int. Soc. Hypertens. 10, S73-77.

O'Brien, E., Parati, G., Stergiou, G., Asmar, R., Beilin, L., Bilo, G., Clement, D., de la Sierra, A., de Leeuw, P., Dolan, E., Fagard, R., Graves, J., Head, G.A., Imai, Y., Kario, K., Lurbe, E., Mallion, J.-M., Mancia, G., Mengden, T., Myers, M., Ogedegbe, G., Ohkubo, T., Omboni, S., Palatini, P., Redon, J., Ruilope, L.M., Shennan, A., Staessen, J.A., vanMontfrans, G., Verdecchia, P., Waeber, B., Wang, J., Zanchetti, A., Zhang, Y., European Society of Hypertension Working Group on Blood Pressure Monitoring, 2013. European Society of Hypertension position paper on ambulatory blood pressure monitoring. J. Hypertens. 31, 1731–1768.

Ogedegbe, G., Pickering, T.G., Clemow, L., Chaplin, W., Spruill, T.M., Albanese, G.M., Eguchi, K., Burg, M., Gerin, W., 2008. The misdiagnosis of hypertension: the role of patient anxiety. Arch. Intern. Med. 168, 2459–2465.

Oliver, J.J., Bowler, A., Beudeker, Q., Cate, T. ten, Webb, D.J., 2005. Dose-response relationship of sublingual nitroglycerin with brachial artery dilatation and change in central and peripheral augmentation index. Clin. Pharmacol. Ther. 77, 337–338.

Olsen, M.H., Angell, S.Y., Asma, S., Boutouyrie, P., Burger, D., Chirinos, J.A., Damasceno, A., Delles, C., Gimenez-Roqueplo, A.-P., Hering, D., López-Jaramillo, P., Martinez, F., Perkovic, V., Rietzschel, E.R., Schillaci, G., Schutte, A.E., Scuteri, A., Sharman, J.E., Wachtell, K., Wang, J.G., 2016. A call to action and a lifecourse strategy to address the global burden of raised blood pressure on current and future generations: the Lancet Commission on hypertension. The Lancet 388, 2665–2712.

Olufsen, M.S., Ottesen, J.T., Tran, H.T., Ellwein, L.M., Lipsitz, L.A., Novak, V., 2005. Blood pressure and blood flow variation during postural change from sitting to standing: model development and validation. J. Appl. Physiol. Bethesda Md 1985 99, 1523–1537.

Omvik, P., Gerdts, E., Lund-Johansen, P., Myking, O.L., 2000. Long-term central hemodynamic effects at rest and during exercise of losartan in essential hypertension. Am. Heart J. 140, 624–630.

Oparil, S., Zaman, M.A., Calhoun, D.A., 2003. Pathogenesis of hypertension. Ann. Intern. Med. 139, 761–776.

O'Rourke, M.F., Nichols, W.W., Safar, M.E., 2004. Pulse waveform analysis and arterial stiffness: realism can replace evangelism and scepticism. J. Hypertens. 22, 1633–1634; author reply 1634.

Osborn, J.W., 2005. Hypothesis: set-points and long-term control of arterial pressure. A theoretical argument for a long-term arterial pressure control system in the brain rather than the kidney. Clin. Exp. Pharmacol. Physiol. 32, 384–393.

Pal, G.K., Adithan, C., Ananthanarayanan, P.H., Pal, P., Nanda, N., Thiyagarajan, D., Syamsunderkiran, A.N., Lalitha, V., Dutta, T.K., 2013. Association of sympathovagal imbalance with cardiovascular risks in young prehypertensives. Am. J. Cardiol. 112, 1757–1762.

Palmer, R.M., Ashton, D.S., Moncada, S., 1988. Vascular endothelial cells synthesize nitric oxide from L-arginine. Nature 333, 664–666.

Panza, J.A., Quyyumi, A.A., Brush, J.E., Epstein, S.E., 1990. Abnormal Endothelium-Dependent Vascular Relaxation in Patients with Essential Hypertension. N. Engl. J. Med. 323, 22–27.

Paredes, O.L., Shite, J., Shinke, T., Watanabe, S., Otake, H., Matsumoto, D., Imuro, Y., Ogasawara, D., Sawada, T., Yokoyama, M., 2006. Impedance cardiography for cardiac output estimation: reliability of wrist-to-ankle electrode configuration. Circ. J. Off. J. Jpn. Circ. Soc. 70, 1164–1168.

Patel, D.J., Defreitas, F.M., Fry, D.L., 1963. Hydraulic input impedance to aorta and pulmonary artery in dogs. J. Appl. Physiol. 18, 134–140.

Peltola, M., 2012. Role of editing of R-R intervals in the analysis of heart rate variability. Front. Physiol. 3.

Pickering, T.G., 1996. White coat hypertension. Curr. Opin. Nephrol. Hypertens. 5, 192–198.

Plomin, R., Haworth, C.M.A., Davis, O.S.P., 2009. Common disorders are quantitative traits. Nat. Rev. Genet. 10, 872–878.

Pomeranz, B., Macaulay, R.J., Caudill, M.A., Kutz, I., Adam, D., Gordon, D., Kilborn, K.M., Barger, A.C., Shannon, D.C., Cohen, R.J., 1985. Assessment of autonomic function in humans by heart rate spectral analysis. Am. J. Physiol. 248, H151-153.

R Development Core Team, 2011. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Rambaran, C., Jiang, B., Ritter, J.M., Shah, A., Kalra, L., Chowienczyk, P.J., 2008. Assessment of endothelial function: comparison of the pulse wave response to $\beta_2$ -adrenoceptor stimulation with flow mediated dilatation. Br. J. Clin. Pharmacol. 65, 238–243.

Rapsomaniki, E., Timmis, A., George, J., Pujades-Rodriguez, M., Shah, A.D., Denaxas, S., White, I.R., Caulfield, M.J., Deanfield, J.E., Smeeth, L., Williams, B., Hingorani, A., Hemingway, H., 2014. Blood pressure and incidence of twelve cardiovascular diseases: lifetime risks, healthy life-years lost, and age-specific associations in 1·25 million people. Lancet Lond. Engl. 383, 1899–1911.

Raviele, A., Gasparini, G., Di Pede, F., Menozzi, C., Brignole, M., Dinelli, M., Alboni, P., Piccolo, E., 1994. Nitroglycerin infusion during upright tilt: a new test for the diagnosis of vasovagal syncope. Am. Heart J. 127, 103–111.

Riet, L. te, Esch, J.H.M. van, Roks, A.J.M., Meiracker, A.H. van den, Danser, A.H.J., 2015. Hypertension: Renin–Angiotensin–Aldosterone System Alterations. Circ. Res. 116, 960–975.

Roberts, D.H., Tsao, Y., Grimmer, S.F., Winstanley, P.A., Orme, M.L., Breckenridge, A.M., 1987. Haemodynamic effects of atenolol, labetalol, pindolol and captopril: a comparison in hypertensive patients with special reference to changes in limb blood flow, heart rate and left ventricular function. Br. J. Clin. Pharmacol. 24, 163–172.

Rogers, G., O'Flynn, N., 2011. NICE guideline: transient loss of consciousness (blackouts) in adults and young people. Br. J. Gen. Pract. 61, 40–42.

Rule, A.D., Larson, T.S., Bergstralh, E.J., Slezak, J.M., Jacobsen, S.J., Cosio, F.G., 2004. Using serum creatinine to estimate glomerular filtration rate: accuracy in good health and in chronic kidney disease. Ann. Intern. Med. 141, 929–937.

Safar, M.E., Bouthier, J.A., Levenson, J.A., Simon, A.C., 1983. Peripheral large arteries and the response to antihypertensive treatment. Hypertens. Dallas Tex 1979 5, III63-68.

Safar, M.E., Henry, O., Blacher, J., 2011. Vascular Aging: Biology and Implications. Curr. Cardiovasc. Risk Rep. 5, 450.

Sandoo, A., van Zanten, J.J.C.. V., Metsios, G.S., Carroll, D., Kitas, G.D., 2010. The Endothelium and Its Role in Regulating Vascular Tone. Open Cardiovasc. Med. J. 4, 302–312.

Saper, C.B., 2002. The central autonomic nervous system: conscious visceral perception and autonomic pattern generation. Annu. Rev. Neurosci. 25, 433–469.

Schächinger, V., Britten, M.B., Zeiher, A.M., 2000. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. Circulation 101, 1899–1906.

Schiffrin, E.L., 1992. Reactivity of small blood vessels in hypertension: relation with structural changes. State of the art lecture. Hypertens. Dallas Tex 1979 19, II1-9.

Schiffrin, E.L., Hayoz, D., 1997. How to assess vascular remodelling in small and medium-sized muscular arteries in humans: J. Hypertens. 15, 571–584.

Schillaci, G., Parati, G., Pirro, M., Pucci, G., Mannarino, M.R., Sperandini, L., Mannarino, E., 2007. Ambulatory Arterial Stiffness Index Is Not a Specific Marker of Reduced Arterial Compliance. Hypertension 49, 986–991.

Schnabel, R.B., Schulz, A., Wild, P.S., Sinning, C.R., Wilde, S., Eleftheriadis, M., Herkenhoff, S., Zeller, T., Lubos, E., Lackner, K.J., Warnholtz, A., Gori, T., Blankenberg, S., Munzel, T., 2011. Noninvasive Vascular Function Measurement in the Community: Cross-Sectional Relations and Comparison of Methods. Circ. Cardiovasc. Imaging 4, 371–380.

Schultz, M.G., Otahal, P., Cleland, V.J., Blizzard, L., Marwick, T.H., Sharman, J.E., 2013. Exercise-induced hypertension, cardiovascular events, and mortality in patients undergoing exercise stress testing: a systematic review and meta-analysis. Am. J. Hypertens. 26, 357–366.

Schutte, R., Thijs, L., Liu, Y.-P., Asayama, K., Jin, Y., Odili, A., Gu, Y.-M., Kuznetsova, T., Jacobs, L., Staessen, J.A., 2012. Within-Subject Blood Pressure Level—Not Variability—Predicts Fatal and Nonfatal Outcomes in a General Population. Hypertension 60, 1138–1147.

Schwartz, J.E., Burg, M.M., Shimbo, D., Broderick, J.E., Stone, A.A., Ishikawa, J., Sloan, R., Yurgel, T., Grossman, S., Pickering, T.G., 2016. Clinic Blood Pressure Underestimates Ambulatory Blood Pressure in an Untreated Employer-Based

US Population: Results From the Masked Hypertension Study. Circulation 134, 1794–1807.

Sebo, P., Pechère-Bertschi, A., Herrmann, F.R., Haller, D.M., Bovier, P., 2014. Blood pressure measurements are unreliable to diagnose hypertension in primary care. J. Hypertens. 32, 509–517.

Sharman, D.L., Gomes, C.P., Rutherford, J.P., n.d. Improvement In Blood Pressure Control With Impedance Cardiography-Guided Pharmacologic Decision Making. Congest. Heart Fail. 10, 54–58.

Shechter, M., Shechter, A., Koren-Morag, N., Feinberg, M.S., Hiersch, L., 2014. Usefulness of brachial artery flow-mediated dilation to predict long-term cardiovascular events in subjects without heart disease. Am. J. Cardiol. 113, 162–167.

Simon, G., 2004. Pathogenesis of structural vascular changes in hypertension. J. Hypertens. 22, 3–10.

Smith, J.J., Porth, C.M., Erickson, M., 1994. Hemodynamic response to the upright posture. J. Clin. Pharmacol. 34, 375–386.

Smith, N.L., Psaty, B.M., Rutan, G.H., Lumley, T., Yanez, D., Chaves, P.H.M., Kronmal, R.A., 2003. The association between time since last meal and blood pressure in older adults: the cardiovascular health study. J. Am. Geriatr. Soc. 51, 824–828.

Smith, R.D., Levy, P., Ferrario, C.M., for the Consideration of Noninvasive Hemodynamic Monitoring to Target Reduction of Blood Pressure Levels Study Group, 2006. Value of Noninvasive Hemodynamics to Achieve Blood Pressure Control in Hypertensive Subjects. Hypertension 47, 771–777.

Stefanadis, C., Dernellis, J., Vavuranakis, M., Tsiamis, E., Vlachopoulos, C., Toutouzas, K., Diamandopoulos, L., Pitsavos, C., Toutouzas, P., 1998. Effects of ventricular pacing-induced tachycardia on aortic mechanics in man. Cardiovasc. Res. 39, 506–514.

Stevens, S.L., Wood, S., Koshiaris, C., Law, K., Glasziou, P., Stevens, R.J., McManus, R.J., 2016. Blood pressure variability and cardiovascular disease: systematic review and meta-analysis. BMJ 354, i4098.

Stone, K.J., Fryer, S.M., Ryan, T., Stoner, L., 2016. The validity and reliability of continuous-wave near-infrared spectroscopy for the assessment of leg blood volume during an orthostatic challenge. Atherosclerosis 251, 234–239.

Stoner, L., Faulkner, J., Lowe, A., M Lambrick, D., M Young, J., Love, R., S Rowlands, D., 2014. Should the augmentation index be normalized to heart rate? J. Atheroscler. Thromb. 21, 11–16.

Strandberg, T.E., Salomaa, V.V., Vanhanen, H.T., Pitkälä, K., Miettinen, T.A., 2002. Isolated diastolic hypertension, pulse pressure, and mean arterial pressure as predictors of mortality during a follow-up of up to 32 years. J. Hypertens. 20, 399–404.

Stuart, E.A., 2010. Matching methods for causal inference: A review and a look forward. Stat. Sci. Rev. J. Inst. Math. Stat. 25, 1–21.

Sudhir, K., Jennings, G.L., Esler, M.D., Korner, P.I., Blombery, P.A., Lambert, G.W., Scoggins, B., Whitworth, J.A., 1989. Hydrocortisone-induced hypertension in humans: pressor responsiveness and sympathetic function. Hypertens. Dallas Tex 1979 13, 416–421.

Sundström, J., Neovius, M., Tynelius, P., Rasmussen, F., 2011. Association of blood pressure in late adolescence with subsequent mortality: cohort study of Swedish male conscripts. BMJ 342, d643.

Sung, S.-H., Chen, Z.-Y., Tseng, T.-W., Lu, D.-Y., Yu, W.-C., Cheng, H.-M., Chen, C.-H., 2014. Wave Reflections, Arterial Stiffness, and Orthostatic Hypotension. Am. J. Hypertens. 27, 1446–1455.

Taddei, S., Virdis, A., Mattei, P., Ghiadoni, L., Fasolo, C.B., Sudano, I., Salvetti, A., 1997. Hypertension causes premature aging of endothelial function in humans. Hypertens. Dallas Tex 1979 29, 736–743.

Tahvanainen, A., Koskela, J., Leskinen, M., Ilveskoski, E., Nordhausen, K., Kähönen, M., Kööbi, T., Mustonen, J., Pörsti, I., 2011. Reduced systemic vascular resistance in healthy volunteers with presyncopal symptoms during a nitrate-stimulated tilt-table test. Br. J. Clin. Pharmacol. 71, 41–51.

Tahvanainen, A., Koskela, J., Tikkakoski, A., Lahtela, J., Leskinen, M., Kähönen, M., Nieminen, T., Kööbi, T., Mustonen, J., Pörsti, I., 2009a. Analysis of cardiovascular responses to passive head-up tilt using continuous pulse wave analysis and impedance cardiography. Scand. J. Clin. Lab. Invest. 69, 128–137.

Tahvanainen, A., Leskinen, M., Koskela, J., Ilveskoski, E., Alanko, J., Kähönen, M., Kööbi, T., Lehtimäki, L., Moilanen, E., Mustonen, J., Pörsti, I., 2009b. Non-invasive measurement of the haemodynamic effects of inhaled salbutamol, intravenous L-arginine and sublingual nitroglycerin. Br. J. Clin. Pharmacol. 68, 23–33.

Tahvanainen, A., Leskinen, M., Koskela, J., Ilveskoski, E., Nordhausen, K., Oja, H., Kähönen, M., Kööbi, T., Mustonen, J., Pörsti, I., 2009c. Ageing and cardiovascular responses to head-up tilt in healthy subjects. Atherosclerosis 207, 445–451.

Tahvanainen, A.M., Tikkakoski, A.J., Leskinen, M.H., Nordhausen, K., Kähönen, M., Kööbi, T., Mustonen, J.T., Pörsti, I.H., 2012. Supine and upright haemodynamic effects of sublingual nitroglycerin and inhaled salbutamol: a double-blind, placebo-controlled, randomized study. J. Hypertens. 30, 297–306.

Taler, S.J., Textor, S.C., Augustine, J.E., 2002. Resistant Hypertension: Comparing Hemodynamic Management to Specialist Care. Hypertension 39, 982–988.

Tapp, R.J., Sharp, A., Stanton, A.V., O'Brien, E., Chaturvedi, N., Poulter, N.R., Sever, P.S., Thom, S.A.M., Hughes, A.D., Mayet, J., ASCOT Investigators, 2010. Differential effects of antihypertensive treatment on left ventricular diastolic function: an ASCOT (Anglo-Scandinavian Cardiac Outcomes Trial) substudy. J. Am. Coll. Cardiol. 55, 1875–1881.

Tavakol, M., Ashraf, S., Brener, S.J., 2012. Risks and Complications of Coronary Angiography: A Comprehensive Review. Glob. J. Health Sci. 4, 65–93.

Taylor, M.G., 1957. An Approach to an Analysis of the Arterial Pulse Wave I. Oscillations in an Attenuating Line. Phys. Med. Biol. 1, 258.

Thayer, J.F., Yamamoto, S.S., Brosschot, J.F., 2010. The relationship of autonomic imbalance, heart rate variability and cardiovascular disease risk factors. Int. J. Cardiol. 141, 122–131.

Tomiyama, H., Koji, Y., Yambe, M., Shiina, K., Motobe, K., Yamada, J., Shido, N., Tanaka, N., Chikamori, T., Yamashina, A., 2005. Brachial -- ankle pulse wave velocity is a simple and independent predictor of prognosis in patients with acute coronary syndrome. Circ. J. Off. J. Jpn. Circ. Soc. 69, 815–822.

Torjesen, A., Wang, N., Larson, M.G., Hamburg, N.M., Vita, J.A., Levy, D., Benjamin, E.J., Vasan, R.S., Mitchell, G.F., 2014. Forward and backward wave morphology and central pressure augmentation in men and women in the Framingham Heart Study. Hypertension 64, 259–265.

Vaitkevicius, P.V., Fleg, J.L., Engel, J.H., O'Connor, F.C., Wright, J.G., Lakatta, L.E., Yin, F.C., Lakatta, E.G., 1993. Effects of age and aerobic capacity on arterial stiffness in healthy adults. Circulation 88, 1456–1462.

Vallance, P., Collier, J., Moncada, S., 1989. Effects of endothelium-derived nitric oxide on peripheral arteriolar tone in man. Lancet Lond. Engl. 2, 997–1000.

van de Ree, M.A., Huisman, M.V., de Man, F.H., van der Vijver, J.C., Meinders, A.E., Blauw, G.J., 2001. Impaired endothelium-dependent vasodilation in type 2 diabetes mellitus and the lack of effect of simvastatin. Cardiovasc. Res. 52, 299–305.

van den Bogaard, B., Westerhof, B.E., Best, H., Davis, S.C.A.T., Secher, N.H., van den Born, B.-J.H., van Lieshout, J.J., 2011. Arterial wave reflection decreases gradually from supine to upright. Blood Press. 20, 370–375.

van Varik, B.J., Rennenberg, R.J.M.W., Reutelingsperger, C.P., Kroon, A.A., de Leeuw, P.W., Schurgers, L.J., 2012. Mechanisms of arterial remodeling: lessons from genetic diseases. Front. Genet. 3.

Wallace, S.M.L., Yasmin, McEniery, C.M., Maki-Petaja, K.M., Booth, A.D., Cockcroft, J.R., Wilkinson, I.B., 2007. Isolated Systolic Hypertension Is Characterized by Increased Aortic Stiffness and Endothelial Dysfunction. Hypertension 50, 228–233.

Wang, Y.X., Poon, K.S., Randall, D.J., Pang, C.C., 1993. Endothelium-derived nitric oxide partially mediates salbutamol-induced vasodilatations. Eur. J. Pharmacol. 250, 335–340.

Waring, W.S., Sinclair, H.M., Webb, D.J., 2006. Effects of salbutamol and glyceryl trinitrate on large arterial stiffness are similar between patients with hypertension and adults with normal blood pressure. Br. J. Clin. Pharmacol. 62, 621–626.

Warner, H.R., Toronto, A.F., 1960. Regulation of Cardiac Output Through Stroke Volume. Circ. Res. 8, 549–552.

Weber, T., Wassertheurer, S., Hametner, B., Parragh, S., Eber, B., 2015. Noninvasive methods to assess pulse wave velocity: comparison with the invasive gold standard and relationship with organ damage. J. Hypertens. 33, 1023–1031.

Westerhof, N., Bosman, F., De Vries, C.J., Noordergraaf, A., 1969. Analog studies of the human systemic arterial tree. J. Biomech. 2, 121–143.

Westerhof, N., Stergiopulos, N., 2000. Models of the arterial tree. Stud. Health Technol. Inform. 71, 65–77.

Whelton, P.K., Carey, R.M., Aronow, W.S., Casey, D.E., Collins, K.J., Dennison Himmelfarb, C., DePalma, S.M., Gidding, S., Jamerson, K.A., Jones, D.W.,

MacLaughlin, E.J., Muntner, P., Ovbiagele, B., Smith, S.C., Spencer, C.C., Stafford, R.S., Taler, S.J., Thomas, R.J., Williams, K.A., Williamson, J.D., Wright, J.T., 2017. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults. J. Am. Coll. Cardiol.

Wildman, R.P., Mackey, R.H., Bostom, A., Thompson, T., Sutton-Tyrrell, K., 2003. Measures of obesity are associated with vascular stiffness in young and older adults. Hypertens. Dallas Tex 1979 42, 468–473.

Wilenius, M., Tikkakoski, A.J., Tahvanainen, A.M., Haring, A., Koskela, J., Huhtala, H., Kähönen, M., Kööbi, T., Mustonen, J.T., Pörsti, I.H., 2016. Central wave reflection is associated with peripheral arterial resistance in addition to arterial stiffness in subjects without antihypertensive medication. BMC Cardiovasc. Disord. 16, 131.

Wilkinson, I.B., 2002. Pulse-Wave Analysis: Clinical Evaluation of a Noninvasive, Widely Applicable Method for Assessing Endothelial Function. Arterioscler. Thromb. Vasc. Biol. 22, 147–152.

Wilkinson, I.B., Hall, I.R., MacCallum, H., Mackenzie, I.S., McEniery, C.M., van der Arend, B.J., Shu, Y.-E., MacKay, L.S., Webb, D.J., Cockcroft, J.R., 2002. Pulse-wave analysis clinical evaluation of a noninvasive, widely applicable method for assessing endothelial function. Arterioscler. Thromb. Vasc. Biol. 22, 147–152.

Wilkinson, I.B., MacCallum, H., Flint, L., Cockcroft, J.R., Newby, D.E., Webb, D.J., 2000. The influence of heart rate on augmentation index and central arterial pressure in humans. J. Physiol. 525, 263–270.

Wilkinson, I.B., Webb, D.J., 2001. Venous occlusion plethysmography in cardiovascular research: methodology and clinical applications. Br. J. Clin. Pharmacol. 52, 631–646.

Wong, C.S., Pavord, I.D., Williams, J., Britton, J.R., Tattersfield, A.E., 1990. Bronchodilator, cardiovascular, and hypokalaemic effects of fenoterol, salbutamol, and terbutaline in asthma. Lancet 336, 1396–1399.

Xu, B., Li, J., Gao, L., Ferro, A., 2000. Nitric oxide-dependent vasodilatation of rabbit femoral artery by beta(2)-adrenergic stimulation or cyclic AMP elevation in vivo. Br. J. Pharmacol. 129, 969–974.

Zanutto, B.S., Valentinuzzi, M.E., Segura, E.T., 2010. Neural set point for the control of arterial pressure: role of the nucleus tractus solitarius. Biomed. Eng. OnLine 9, 4.

Zusman, R.M., Christensen, D.M., Higgins, J., Boucher, C.A., 1992. Comparison of the cardiac and hemodynamic effects of lisinopril and atenolol in patients with hypertension: therapeutic implications. J. Cardiovasc. Pharmacol. 20, 216–222.

# 10 Original communications

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RESEARCH ARTICLE Open Access

# The type of the functional cardiovascular response to upright posture is associated with arterial stiffness: a cross-sectional study in 470 volunteers

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## Abstract

**Background:** In a cross-sectional study we examined whether the haemodynamic response to upright posture could be divided into different functional phenotypes, and whether the observed phenotypes were associated with known determinants of cardiovascular risk.

**Methods:** Volunteers ($n = 470$) without medication with cardiovascular effects were examined using radial pulse wave analysis, whole-body impedance cardiography, and heart rate variability analysis. Based on the passive head-up tilt induced changes in systemic vascular resistance and cardiac output, the principal determinants of blood pressure, a cluster analysis was performed.

**Results:** The haemodynamic response could be clustered into 3 categories: upright increase in vascular resistance and decrease in cardiac output were greatest in the first (+45 % and -27 %, respectively), smallest in the second (+2 % and -2 %, respectively), and intermediate (+22 % and -13 %, respectively) in the third group. These groups were named as 'constrictor' ($n = 109$), 'sustainer' ($n = 222$), and 'intermediate' ($n = 139$) phenotypes, respectively. The sustainers were characterized by male predominance, higher body mass index, blood pressure, and also by higher pulse wave velocity, an index of large arterial stiffness, than the other groups ($p < 0.01$ for all). Heart rate variability analysis showed higher supine and upright low frequency/high frequency (LF/HF) ratio in the sustainers than constrictors, indicating increased sympathovagal balance. Upright LF/HF ratio was also higher in the sustainer than intermediate group. In multivariate analysis, independent explanatory factors for higher pulse wave velocity were the sustainer ($p < 0.022$) and intermediate phenotypes ($p < 0.046$), age ($p < 0.001$), body mass index ($p < 0.001$), and hypertension ($p < 0.001$).

**Conclusions:** The response to upright posture could be clustered to 3 functional phenotypes. The sustainer phenotype, with smallest upright decrease in cardiac output and highest sympathovagal balance, was independently associated with increased large arterial stiffness. These results indicate an association of the functional haemodynamic phenotype with an acknowledged marker of cardiovascular risk.

**Trial registration:** ClinicalTrials.gov NCT01742702

**Keywords:** Arterial stiffness, Cardiac output, Heart rate, Head-up tilt, Systemic vascular resistance

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## Background

Elevated blood pressure (BP) and related cardiovascular (CV) complications are leading causes of morbidity and mortality, and early recognition of individuals with increased CV risk is of foremost importance [1]. All new cases of CV disease cannot be predicted using classical risk factors like family history, obesity, smoking, hypertension, diabetes, or dyslipidaemias. Therefore, studies aiming at the discovery of novel risk factors are still needed [2, 3]. Furthermore, also psychosocial factors, like hostility and anger, have been linked with worse cardiovascular outcome [4].

The CV phenotype in clinical practice has mainly been determined by measuring brachial BP and HR, even though the value of repeated single BP measurements in the diagnosis of hypertension has been questioned [5, 6]. The haemodynamic changes causing similar elevations of BP may differ between patients and disorders. For example, systemic vascular resistance is typically elevated in essential hypertension [7], while changes in fluid and electrolyte balance are characteristic causes of elevated BP during chronic kidney disease [8].

The age-related decrease in large arterial compliance is accelerated in various CV disorders [9, 10]. Increased large arterial stiffness is an acknowledged CV risk factor in both general populations and subjects with medical disorders [9, 11]. Increased arterial stiffness also predisposes to exaggerated upright decrease in central BP and orthostatic hypotension [12, 13]. The determination of pulse wave velocity (PWV) is the gold standard when evaluating large arterial stiffness [10].

The assessment of CV status is usually performed at rest, but several studies have shown that haemodynamic reactivity to physical stimuli provides further information about CV risk. Enhanced BP response to cold pressor test, or to 4-min 2-step exercise test, predicted the development of hypertension in Japanese populations [14, 15]. Reduced heart rate (HR) recovery after bicycle ergometer testing predicted mortality in a Finnish study [6]. As the change in body posture from supine to upright induces changes in autonomic tone and arterial resistance, orthostatic challenge can also be regarded as a stress test addressing CV reactivity [16, 17].

In the course of our studies on haemodynamics, we have observed that there are reproducible individual variations in the changes in cardiac output and systemic vascular resistance in response to upright posture [17–19]. The objective of the present study was to examine the hypothesis whether functional differences exist in CV responses to upright posture, and whether these differences are associated with known determinants of cardiovascular risk. The results show that not only age, body mass index (BMI), and the presence of hypertension, but also the phenotype of the haemodynamic response to upright posture is associated with arterial stiffness.

## Methods

### Study subjects

All subjects participated in an ongoing study on haemodynamics (clinical trials registration NCT01742702). An announcement for recruitment was distributed at the Tampere University Hospital, University of Tampere, local occupational health care providers, Varala Sports Institute, and two announcements were published in a newspaper. By the time of the present analysis, 694 subjects had been recruited. The participants were interviewed and examined by a physician. Subjects with diabetes, coronary artery disease, cardiac insufficiency, atherosclerotic vascular disease, cerebrovascular disease, renal disease, or medication influencing CV status were excluded.

### Ethics, consent and permissions

All participants gave written informed consent and the study was approved by the ethics committee of Tampere University Hospital District (study number R06086M). The investigation conforms to the principles outlined in the Declaration of Helsinki.

### Laboratory analyses

Blood and urine samples were obtained after about 12 h of fasting. Plasma sodium, potassium, calcium, glucose, creatinine, triglyceride, and total, high-density and low-density lipoprotein cholesterol concentrations were determined using Cobas Integra 700/800 or Cobas 6000, module c501 (F. Hoffmann-LaRoche Ltd, Basel, Switzerland), and blood cell count by ADVIA 120 or 2120 (Siemens Healthcare GmbH, Erlangen, Germany). Estimated glomerulus filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease (MDRD) formula [20].

### Pulse wave recording

An automatic tonometric sensor on the left wrist was used to continuously capture radial BP and pulse wave form (Colin BP-508 T, Colin Medical Instruments Corp., USA). The radial BP signal was calibrated every 2–4 min by contralateral brachial BP measurements [12, 17, 21, 22].

### Whole-body impedance cardiography

Changes in body electrical impedance during cardiac cycles were recorded using whole-body impedance cardiography (CircMon$^R$, JR Medical Ltd., Tallinn, Estonia) to determine HR, stroke volume, and cardiac output, as previously described [23–25]. Cardiac output and stroke volume were related to estimated body surface area to derive cardiac index (l/min/m$^2$) and stroke index (ml/

$m^2$). Systemic vascular resistance index (SVRI, dyn*s/$cm^{5}$*$m^2$) was calculated from tonometric BP signal and cardiac index. In supine position and during head-up tilt, the cardiac output values of CircMon$^R$ are in good agreement with values measured using thermodilution [23, 24].

PWV was determined using whole-body impedance cardiography [25, 26]. As the impedance-based method slightly overestimates PWV when compared with Doppler ultrasound method, a validated equation was applied to calculate values that correspond to the ultrasound method (PWV = (PWVimpedance *0.696) + 0.864) [25].

The method for PWV measurement using whole body ICG has been described previously [25, 26]. Briefly, the distal impedance is recorded from popliteal artery at knee joint level, and the active electrode is placed on the lateral side of the knee and the reference electrode on the calf below knee. When the pulse pressure wave enters the aortic arch and the diameter of the aorta changes, the whole-body impedance decreases, and this is measured by the voltage electrodes. To calculate the PWV value, the CircMon software measures the time difference between onset of the decrease in impedance in the whole-body impedance signal and the popliteal artery signal (aortic-popliteal PWV). The aortic-popliteal impedance cardiography measurement of PWV has been validated against aortic-popliteal PWV measurements using Doppler ultrasound [25]. The PWV results obtained using CircMon show good repeatability, and normal values for PWV in 799 individuals (age 25–76 years) have been published [26]. We have also shown that the determination of stroke volume using impedance cardiography versus 3-dimensional echocardiography show good correlation [19]. In addition, we have performed carotid-femoral PWV measurements using applanation tonometry and compared them with ICG measurements of aortic-popliteal PWV, and the correlation between these methods was excellent ($r = 0.82$).

### Frequency domain analysis of heart rate variability (HRV)

HRV analysis was used to assess cardiac autonomic tone. The electrocardiograms were recorded by the CircMon$^R$ device at 200 Hz sampling rate, and data analyzed using Matlab software (MathWorks Inc., Natick, Massachusetts, USA). Normal R-R intervals were recognized, and a beat was considered ectopic if the interval differed more than 20 % from the previous values. The artifacts were processed using the cubic spine interpolation method [27]. The following frequency domain variables were calculated using the Fast Fourier Transformation method: i) power in low frequency (LF) range (0.04–0.15 Hz), ii) power in high frequency (HF) range (0.15–0.40 Hz), and iii) LF/HF ratio [28].

### Experimental protocol

Caffeine containing products, smoking and a heavy meal for at least 4 h, and alcohol for at least 24 h prior to the investigation were to be avoided. Data was captured in a temperature-controlled laboratory by research nurses [12, 17, 22]. The left arm with the tonometric sensor was abducted 90 degrees in an arm support, which held it at the level of the heart in supine and upright positions. The recording comprised continuous capture of data during three consecutive 5-min periods (total 15 min): 5 min supine, 5 min of head-up tilt to 60 degrees, 5 min supine. The repeatability and reproducibility of the supine and upright measurements are good [22].

### Classification into phenotypes by means of clustering

SVRI and cardiac index were chosen for the phenotypic classification, since peripheral arterial resistance and cardiac output are the principal determinants of BP [17, 23, 29]. To characterize the reaction to upright posture, the differences in SVRI and cardiac index between supine values (5th minute average) and upright values (8th and 10th minute averages) were examined. Two values during the head-up tilt (8th and 10th minute) were chosen to take into account possible changes during upright posture. To avoid scaling effects, all differences were standardized to have a mean value of 0 and a variance of 1. Hierarchical clustering was performed using Ward's criterion for squared Euclidean distances: at first each subject served as a cluster, and at each step two clusters closest to each other were combined, until only one cluster remained (R software version 2.14.1 [30]). Based on the dendrogram, the 3 main clusters were analysed (Fig. 1; classification to clusters I, II and III). We also formed a practical rule for clustering, which was tested in a small validation population (Additional file 1).

### Statistical analyses

Due to skewed distribution, PWV and HRV parameters were logarithmically transformed before statistical analyses. To evaluate the impact of phenotype on PWV, a L1-regression was fitted using sex, categorized BMI, categorized age, categorized hypertension, phenotype, interaction between sex and phenotype (Model 1); and fasting plasma lipids and glucose, and haematocrit (Model 2, with variables of Model 1 included) as variables. In addition, Model 1 was used to evaluate the impact of phenotype on HRV parameters. BMI, age, and presence of hypertension were categorized due to lack of linearity in the data. We performed additional analyses using continuous variables without categorization to examine the association of changes in SVRI and cardiac index with PWV.

There is no accepted cut-off level to define elevated BP during tilt-table measurements, while office BP is

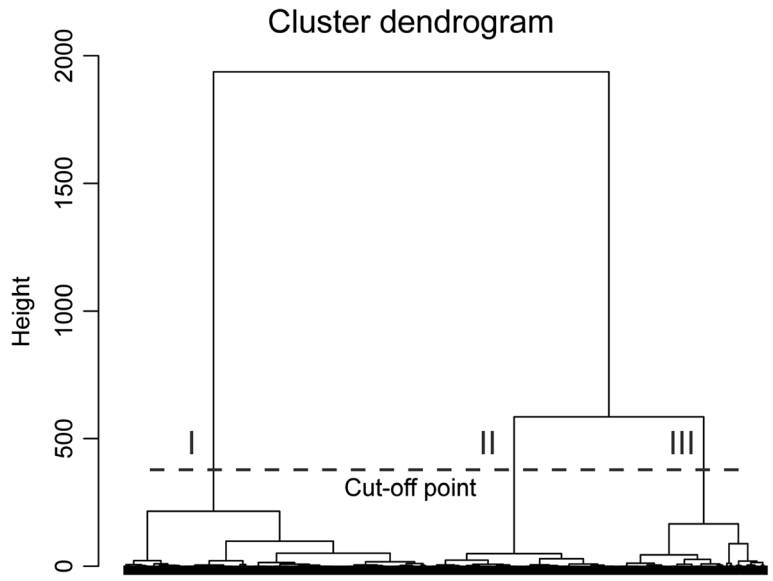


**Fig. 1** Clustering to phenotypes. Cluster dendrogram according to hierarchical clustering using Ward's criterion for the squared Euclidean distances. Dashed line indicates the cut-off point for the three phenotypic clusters (I, II and III). The classification was based on the changes in systemic vascular resistance index and cardiac index in response to passive head-up tilt ($n = 470$)

usually higher than home BP [5, 31]. As the supine BP during haemodynamic measurements was on average 8/12 mmHg (systolic/diastolic) lower than the seated office BP ($n = 470$), we applied the accepted cut-off level for home and ambulatory daytime measurements, i.e. values ≥135/85 mmHg [31], to define hypertension during the recordings. The same approach was applied previously, with testing of different cut-off levels [17].

As summary statistics, median and 25th to 75th percentile, or mean and standard deviation (SD) or 95 % confidence intervals (CI) were reported. Kruskal-Wallis rank sum test, Fisher's exact test and Wilcoxon rank sum test were used to compare haemodynamics and demographics between the phenotypes. All analyses were performed using R software version 2.14.1 [30].

## Results

### Study population and average haemodynamic responses to head-up tilt

The study population consisted of 470 (240 female and 230 male) individuals aged 20–72 years (average 46 years). Average systolic and diastolic BP, SVRI and cardiac index in all subjects during the 15-min measurement protocol are shown in Fig. 2. In response to upright posture, systolic BP slightly decreased and diastolic BP increased, while SVRI increased by about 15 %, and cardiac index decreased by about 15 %. Both SVRI and cardiac index were stabilized during the time-points chosen for clustering into phenotypes (at 5 min of recording while supine, and at 8 and 10 min of recording while upright) (Fig. 2).

### Classification into phenotypes

The classification to phenotypes was based on the changes in systemic vascular resistance and cardiac output in response to passive head-up tilt. The cluster that was characterised by the greatest increases in SVRI (+45 %) and the greatest decreases in cardiac index (−27 %) in response to upright posture was named 'constrictor' (Fig. 3a, d). The cluster characterised by the smallest changes in SVRI (+2 %) and cardiac index (−2 %), so that these variables were sustained on corresponding levels in supine and upright positions, was named 'sustainer' (Fig. 3c, f). The cluster with the intermediary changes (+22 %, -13 %, respectively) was named 'intermediate' (Fig. 3b, e). The mean (±SD) head-up tilt -induced changes in SVRI were +944 (±361), +554 (±197) and +60 (±254) dyn*s/cm$^{5}$*m$^{2}$ in the constrictor, intermediate and sustainer phenotypes, respectively (Additional file 2). The mean changes in cardiac index were -0.89 (±0.51), -0.37 (±0.29) and -0.06 (±0.46) l/min/m$^{2}$, respectively (Additional file 2).

Since supine SVRI and cardiac index differed between the clusters, we performed additional analyses in which the subjects were classified to three new clusters merely on the basis of supine levels of SVRI and cardiac index. These new clusters did not systematically or significantly correspond to the constrictor, intermediate and sustainer clusters (data not shown). This indicates that the above phenotypes were not discovered because of differences in the resting levels of SVRI and cardiac index between the groups.

The characteristics and laboratory values of the phenotypes are shown in Table 1. Age did not differ between

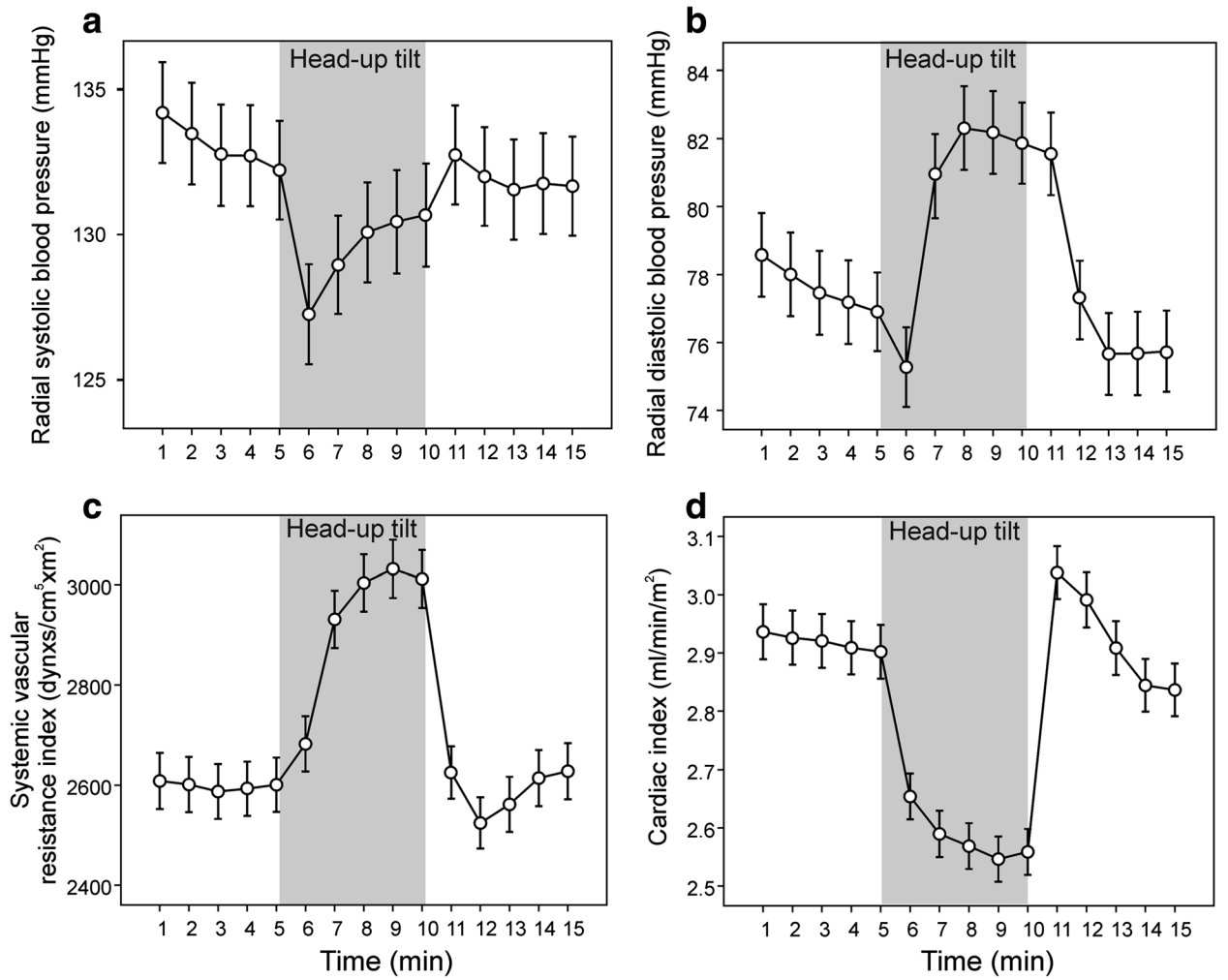


**Fig. 2** Average haemodynamics during the head-up tilt protocol. Average radial systolic (**a**) and diastolic (**b**) blood pressure, systemic vascular resistance index (**c**), and cardiac index (**d**) during the 15-min measurement protocol (5 min supine – 5 min upright – 5 min supine) in all subjects ($n = 470$). Mean ± 95 % confidence interval

the groups ($p = 0.216$). The gender distribution ($p < 0.001$), prevalence of laboratory BP ≥135 and/or ≥85 mmHg ($p = 0.009$), BMI ($p < 0.001$), and office BP values ($p < 0.008$) were different in the phenotypes, i.e. the sustainers were more likely to be males with elevated BP and higher BMI than in the other phenotypes (Table 1). In addition, total and LDL cholesterol and triglycerides were lowest, and HDL cholesterol highest in the constrictors (Table 1). Fasting plasma glucose values were lowest in the constrictors, but the mean values were within the normal range in all groups. Haematocrit and creatinine concentrations were lowest in the constrictors, probably due to the female predominance, but the eGFR (MDRD-formula) values did not differ between the phenotypes (Table 1). In analyses adjusted for differences in gender distribution, prevalence of laboratory BP ≥135/85, and BMI, the above differences in total, LDL, and HDL cholesterol, triglycerides, and haematocrit were no more significant between the phenotypes (p-values for all >0.12).

#### Detailed haemodynamics and arterial stiffness

The sustainers showed highest supine systolic BP, while upright systolic BP did not differ between the phenotypes (Additional files 3 and 4). Constrictors showed numerically lowest supine and upright diastolic BP. Supine heart rate was lowest and upright heart rate highest in sustainers, while supine stroke index was numerically highest and upright stroke index lowest in the constrictors (Additional file 3). Subsequently, supine cardiac index was highest but upright cardiac index was lowest in the constrictors. Supine SVRI was lowest in the constrictors, while upright SVRI was lowest in the sustainers (Additional file 3, Fig. 3).

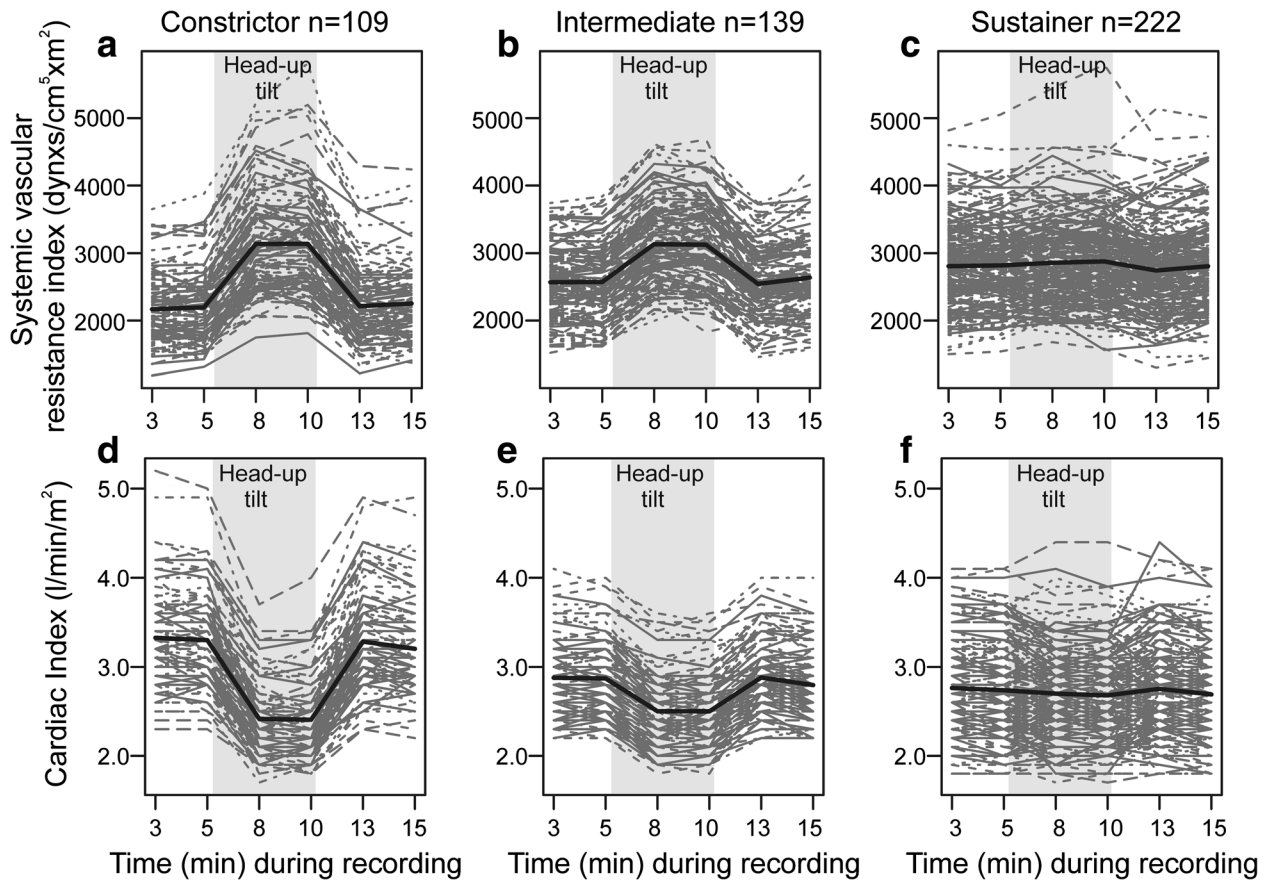


**Fig. 3** Haemodynamics in the 3 phenotypes in response to upright posture. Systemic vascular resistance index and cardiac index during the measurement protocol, respectively, in the constrictor (**a**, **d**), intermediate (**b**, **e**), and sustainer phenotypes (**c**, **f**). Mean (*bold line*) and individual curves (*grey lines*)

Median PWV was different between the phenotypes, sustainers showing the highest values (7.35, 8.00 and 8.65 m/s in the constrictor, intermediate and sustainer phenotypes, respectively) (Fig. 4). In multivariate analysis, sustainer and intermediate phenotypes, high and middle BMI tertiles, high and middle age tertiles, BP ≥135/85 mmHg, and interaction between female sex and intermediate phenotype were significant explanatory factors for supine PWV (Table 2, Model 1). If the laboratory variables that were different between the phenotypes (plasma lipids and glucose, haematocrit) were included in the multivariate model, the outcome was that sustainer and intermediate phenotype, high and middle BMI tertiles, high and middle age tertiles, BP ≥135/85 mmHg, interaction between female sex and intermediate phenotype, and plasma triglyceride level were significant explanatory factors for supine PWV (Table 2, Model 2). In additional analyses using continuous variables without categorization, the supine to upright change in cardiac index was still independently associated with PWV, while the association between the change in SVRI and PWV was not significant (Additional file 5).

#### Heart rate variability in the phenotypes

The HRV variables in supine and upright position are depicted in Fig. 5. No significant differences were found between the phenotypes in supine or upright power in LF range, a variable predominantly reflecting cardiac sympathetic tone [28, 32]. Supine power in HF range did not differ between the phenotypes, either. However, upright HF power was highest in the constrictors, followed by the intermediate phenotype, while the sustainer phenotype showed lowest level, suggesting reduced cardiac parasympathetic tone [28, 32]. The sympathovagal balance, as reflected by LF/HF ratio, was higher in supine position in the sustainers than in the constrictors, while upright LF/HF ratio was higher in the sustainers than in the two other phenotypes (Fig. 5). In all phenotypes, LF/HF ratio clearly increased in the upright position ($p < 0.001$). When the analyses were adjusted for differences in sex, BMI, and presence of hypertension, there were no statistically significant differences in the HRV variables between the phenotypes (Additional file 6).

**Table 1** Demographics and laboratory values in the three phenotypes

| Variable | Constrictor | Intermediate | Sustainer | *p*-value |
|---|---|---|---|---|
| Number of subjects | 109 | 139 | 222 | |
| Age (years) | 46 (37–53) | 46 (38–57) | 47 (38–55) | 0.216 |
| Female (%) | 80 | 58* | 32*† | <0.001 |
| BP ≥ 135/85 mmHg (%) in the laboratory | 34 | 42* | 51*† | 0.009 |
| Body mass index (kg/m$^2$) | 23.1 (21.6–25.6) | 26.5 (24.1–29.3)* | 27.4 (24.8–30.6)* | <0.001 |
| Waist circumference (cm) | | | | |
| Female | 79 (73–87) | 86 (79–97)* | 88 (82–99)* | <0.001 |
| Male | 85 (80–92) | 98 (91–99)* | 100 (94–107)* | <0.001 |
| Smoking (no/present/previous) | 66/16/27 | 90/14/35 | 121/23/78 | 0.090 |
| Office systolic BP (mmHg) | 134 (119–148) | 139 (123–152) | 141 (123–157)* | 0.007 |
| Office diastolic BP (mmHg) | 84 (75–91) | 90 (81–98)* | 91 (82–99)* | <0.001 |
| Haematocrit | 0.40 (0.39–0.42) | 0.42 (0.39–0.44)* | 0.43 (0.41–0.45)*† | <0.001 |
| Creatinine (μmol/l) | 66 (59–74) | 70 (64–80)* | 76 (69–84)*† | <0.001 |
| eGFR (ml/min/1.73 m$^2$) | 89 (80–97) | 88 (79–97) | 89 (78–99) | 0.631 |
| Total Cholesterol (mmol/l) | 4.80 (4.20–5.50) | 5.20 (4.60–5.80)* | 5.20 (4.4–5.8)* | 0.018 |
| LDL Cholesterol (mmol/l) | 2.45 (2.10–3.18) | 3.00 (2.40–3.60)* | 3.10 (2.30–3.70)* | <0.001 |
| HDL cholesterol (mmol/l | 1.78 (1.49–2.13) | 1.54 (1.23–1.84)* | 1.43 (1.17–1.71)* | <0.001 |
| Triglycerides (mmol/l) | 0.92 (0.61–1.16) | 1.08 (0.82–1.43)* | 1.11 (0.76–1.67)*† | <0.001 |
| Fasting plasma glucose (mmol/l) | 5.2 (4.9–5.4) | 5.4 (5.1–5.7)* | 5.5 (5.1–5.8)* | <0.001 |

Values are median (25th-75t$^h$ percentile); BP, blood pressure; eGFR, estimated creatinine-based glomerulus filtration rate using the MDRD formula;[23] *$p < 0.05$ when compared with constrictor phenotype; †$p < 0.05$ when compared with intermediate phenotype

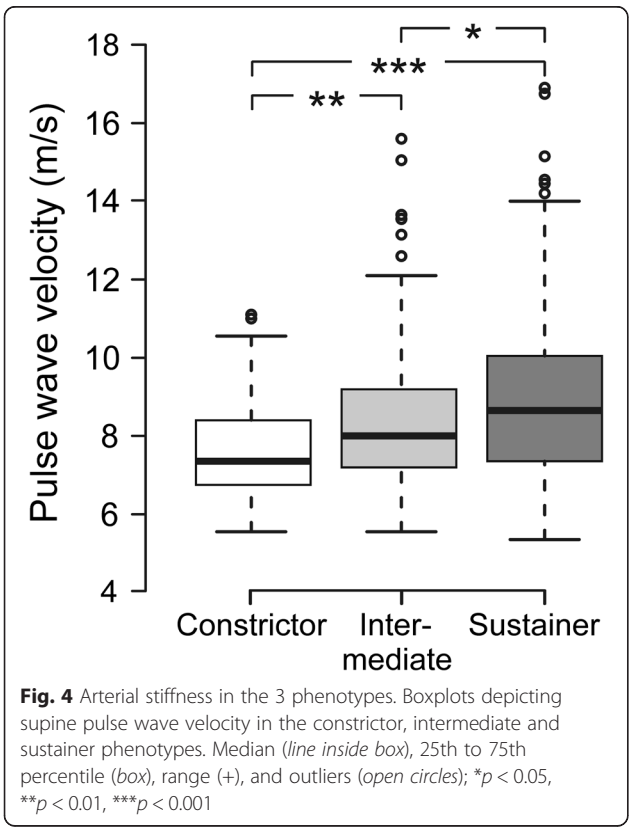


**Fig. 4** Arterial stiffness in the 3 phenotypes. Boxplots depicting supine pulse wave velocity in the constrictor, intermediate and sustainer phenotypes. Median (*line inside box*), 25th to 75th percentile (*box*), range (+), and outliers (*open circles*); *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

### Medications used by the subjects

The majority (58 %) of subjects were without any medications, and none had medication for cardiovascular disorders or diabetes. The established medications used by the participants were: 75 female subjects on low-dose hormones (oestrogen, progestin or combination) for contraception or hormone replacement therapy, 22 treated with antidepressants, 16 with thyroxin, 21 with intranasal or inhaled corticosteroids, 12 with statins, 11 with proton pump inhibitors, 11 with antihistamines, 5 with non-steroidal anti-inflammatory drugs, and 5 with glucosamine. Female hormone regimens were used by 31 % of the female subjects, but the proportions of low-dose hormone users did not differ between the phenotypes. Only the use of inhaled or intranasal corticosteroids ($p = 0.038$) differed between the groups so that these compounds were used by 6.4 %, 6.5 % and 2.3 % of the subjects in the constrictor, intermediate, and sustainer groups, respectively.

## Discussion

The evaluation of the CV status only in the supine or seated position gives rather limited information about haemodynamics [16, 17, 22]. Although head-up tilt for 5 min is a short period of observation, we found that haemodynamic adaptation differed between individuals of similar age who only showed small differences in BP

**Table 2** Analysis of determinants of arterial stiffness

| | Model 1: Constant 1.83258 | | Model 2: Constant 1.74717 | |
|---|---|---|---|---|
| Variable | Coefficient | *p*-value | Coefficient | *p*-value |
| Phenotype: intermediate | 0.09103 | 0.046 | 0.12729 | 0.005 |
| Phenotype: sustainer | 0.09103 | 0.022 | 0.11514 | 0.005 |
| BMI: middle tertile | 0.07742 | <0.001 | 0.07209 | 0.002 |
| BMI: high tertile | 0.12824 | <0.001 | 0.11146 | <0.001 |
| Age: middle tertile | 0.09143 | <0.001 | 0.08398 | <0.001 |
| Age: high tertile | 0.23804 | <0.001 | 0.23729 | <0.001 |
| Blood pressure ≥135/85 mmHg | 0.10273 | <0.001 | 0.08734 | <0.001 |
| Female sex | 0.03609 | 0.377 | 0.08017 | 0.065 |
| Interaction: female sex and intermediate phenotype | −0.11124 | 0.043 | −0.14789 | 0.007 |
| Interaction: female sex and sustainer phenotype | −0.07442 | 0.143 | −0.09407 | 0.071 |
| Fasting plasma triglycerides | - | - | 0.05707 | 0.004 |

Analysis of explanatory factors for supine pulse wave velocity (after logarithmic transformation) in multivariate analysis. The male sex, constrictor phenotype, lowest BMI tertile, lowest age tertile, and BP < 135/85 mmHg served as reference categories in both models, i.e. for these variables the numeric value of the coefficient is 0

Model 1 was based on phenotype, BMI-category, age-category, BP-category, and sex. *Model 1 formula for log(PWV)* ~ Constant + phenotype + BMI-category + AGE-category + BP-category + sex + phenotype*sex

Example of interpretation: median log(PWV) for a woman with the sustainer phenotype with BMI and age in the highest tertile and BP ≥ 135/85 mmHg: 1.83258 + 0.09103 + 0.12824 + 0.23804 + 0.10273+ 0.03609 + (-0.07442) = 2.35429; median PWV = exp(2.35429) = 10.53 m/s

Model 2 was based on Model 1 and fasting plasma total, LDL, and HDL cholesterol, triglycerides, glucose, and haematocrit

*Model 2 formula for log(PWV)* ~ Constant + phenotype + BMI-category + AGE-category + BP-category + sex + phenotype *sex + 0.05707*plasma triglyceride concentration

(Additional files 2 and 3). The type of haemodynamic reaction to upright posture was also related to PWV, an acknowledged measure of large arterial stiffness [9–11]. Previously, the majority of studies utilizing head-up tilt have been performed to examine the mechanisms of syncope [33, 34], and differences in upright haemodynamics have not been systematically investigated in non-syncopal subjects.

Subjects with increased CV risk should be identified before the manifestations of a clinical disease [1]. In addition to the classical risk factors, efforts have been made to identify novel risk factors to increase the sensitivity of risk evaluation. For example, haemodynamic responses to physical challenge (bicycle exercise test, step exercise test, cold pressor test) can predict CV outcome [6, 15, 35]. As change in posture from supine to upright activates sensory and neurogenic responses in the body, with subsequent changes in autonomic tone, cardiac function, and peripheral arterial resistance, passive head-up tilt can be regarded as a clinical haemodynamic stress test [12, 16, 17].

Here we used systemic vascular resistance and cardiac index, the principal determinants of BP [17, 23, 29], in the classification of haemodynamic response to upright posture into three phenotypes. The constrictor phenotype showed highest increase in SVRI, greatest decrease in cardiac output, and highest upright HF power as an indicator of parasympathetic cardiac autonomic tone [28, 32]. The sustainers showed lowest increase in vascular resistance and smallest decrease in cardiac output, and greatest upright LF/HF ratio indicating highest sympathovagal balance [28, 32]. The constrictors were also characterised by a more favourable CV risk profile than the sustainers, with lower total and LDL cholesterol, triglycerides and glucose (Table 1). However, in analyses adjusted for the differences in gender distribution, BMI and BP, the differences in the lipid and glucose values were no more significant.

In multivariate analysis including BMI, age, BP, gender, haematocrit, plasma glucose and lipid profile, the sustainer and intermediate phenotypes were associated with higher PWV, i.e. increased large arterial stiffness, than constrictors. Of note, the statistical coefficients relating the intermediate and sustainer phenotypes to higher PWV corresponded to those of increased BMI and elevated BP (Table 2). Higher PWV has been recognised as an independent CV risk factor in hypertensive subjects, elderly subjects, diabetics, and even in the general population [9, 36, 37]. The close relation between PWV and the well-known CV risk factors, increased BMI and BP, has been repeatedly shown [17, 38, 39]. As ageing is a strong determinant of PWV [37, 40], it is important to note that the average age did not differ between the three phenotypes in the present study.

In supine position, HRV analysis showed a higher LF/HF ratio (increased symphatovagal balance) in the sustainers than in the constrictors [28, 32]. Supine SVRI was also highest in the sustainers, who were characterised by male predominance, and highest BMI and BP, all factors associated with increased sympathetic tone

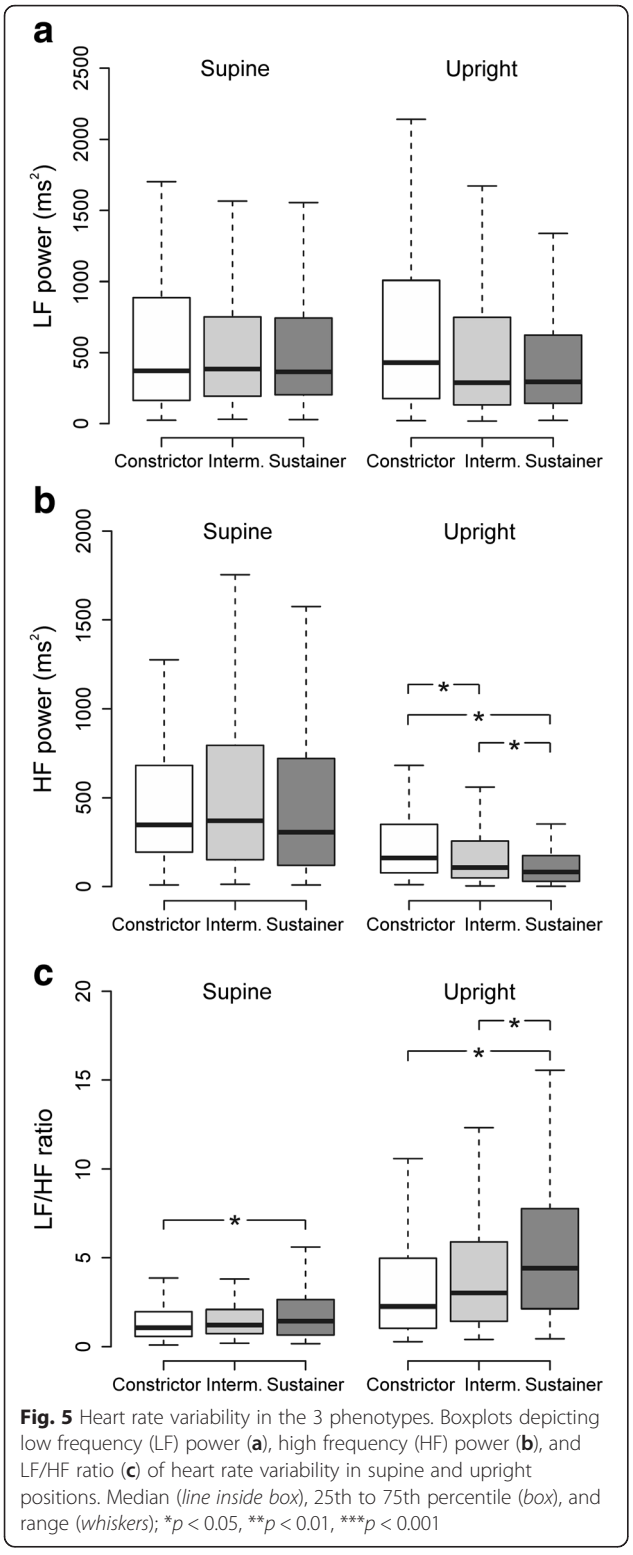


**Fig. 5** Heart rate variability in the 3 phenotypes. Boxplots depicting low frequency (LF) power (**a**), high frequency (HF) power (**b**), and LF/HF ratio (**c**) of heart rate variability in supine and upright positions. Median (*line inside box*), 25th to 75th percentile (*box*), and range (*whiskers*); *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

[41–43]. The head-up tilt uncovered differences in cardiac autonomic tone that largely resulted from the suppression of cardiac parasympathetic tone (decrease in HF power) in response to upright posture in the intermediate and sustainer phenotypes (Fig. 4b). Subsequently, the sustainers showed the highest upright LF/HF ratio, which corresponds to the smallest upright decrease in cardiac output in this phenotype. Previously, sympathovagal imbalance in young prehypertensive subjects has been attributed to increased sympathetic and decreased parasympathetic tone [44].

Increased arterial stiffness is known to influence the control of autonomic tone. Higher arterial stiffness has been associated with reduced heart rate variability (HRV) [45–47]. As baroreceptors are located in the arterial wall, arterial stiffening attenuates baroreceptor responses to changes in BP [48]. Increased large arterial stiffness and impaired cardiac baroreflex control are also typical features of hypertension, and haemodynamic changes in hypertensive subjects have been attributed to reduced vagal inhibitory influence and overdrive of the sympathetic nervous system (for a review, see [49]). Yet, reduced baroreflex sensitivity in the elderly can persist during methodological elimination of the influence of the stiffness of the vessel wall [50]. Therefore, increased arterial stiffness is not the sole explanation for baroreflex changes, and these may also result from changes in the control of neural baroreflex pathways [50].

In the present study, a putative explanation for the association between the sustainer phenotype and increased arterial stiffness would be alterations in baroreflexes, and this topic is a subject for further studies. However, it should be noted that the differences in HF power and LF/HF ratio were no more significant in adjusted analyses. This indicates that the demographic differences (sex distribution, BMI, and presence of hypertension) between the phenotypes largely explained the observed deviations in cardiac autonomic tone. Importantly, the differences in PWV between the phenotypes persisted after the adjustments, indicating that the deviations in autonomic tone did not explain the differences in arterial stiffness. The cross-sectional design of our study does not allow conclusions about causality, and an unanswered question is whether modifications in the associating risk profiles (like weight reduction) would result in changes of the functional cardiovascular phenotype.

Gender distribution was different between the phenotypes, with male predominance in the sustainers. Previously, haemodynamic differences between men and women have been observed in central wave reflections [51], but the functional CV differences have been less thoroughly characterized. In women, autonomic responses to orthostasis may be attenuated due to lower baroreceptor sensitivity [52]. Smaller body size and lower centre of gravity have been suggested to increase venous pooling of blood to lower extremities in women during orthostatic challenge [53]. In the present study, the decrease in cardiac index during upright posture was greatest in the

constrictors with female predominance, which could imply an increase in venous pooling of blood during head-up tilt. However, BP was well maintained in the constrictors, probably due to the pronounced increase in vascular resistance. Despite the differences in gender distribution between the groups, PWV was significantly associated with the functional phenotype, while the association of PWV with sex was only found as an interaction in the intermediate phenotype.

The present non-invasive methods have been validated against invasive methods [23, 29, 54], and the repeatability and reproducibility of the measurements are good [22]. The non-invasive nature of the recordings is a limitation, as the calculation of cardiac output from the bioimpedance signal requires mathematical equations and simplification of physiology [24]. Invasive haemodynamic measurements, however, are not justified in humans without a clinical reason. The tonometric measurement of carotid-femoral PWV is considered as the gold standard for the assessment of arterial stiffness [55], and the lack of this method in our study can be considered as a limitation. The impedance cardiography-derived PWV shows good correlation with PWV measured with Doppler ultrasound [25], and the method has also been found to be a practical approach for the evaluation of arterial stiffness in 799 individuals aged 25–76 years [26]. The median BMI in the study population was 26.3 kg/m$^2$, which corresponds to the average BMI in Finnish men (27.4 kg/m$^2$) and women (26.9 kg/m$^2$) in a large Finriski 2007 survey [56]. The present median office BP (138/89 mmHg) was slightly lower than the reported average BP in the Finnish population (145-148/85-90 mmHg) [57]. Importantly, subjects taking medications with known influences on haemodynamics were excluded from the present study.

## Conclusions

We found that subjects could be classified to 3 phenotypes according to the head-up tilt –induced changes in CV function. Independently of other risk factors, the sustainer and the intermediate phenotypes were associated with increased large arterial stiffness. Evaluation of cardiac autonomic tone showed increased supine and upright sympathovagal balance in the sustainer phenotype. The present results suggest that the functional phenotype of the haemodynamic response to upright posture is associated with arterial stiffness and thus also with the level of CV risk. Future follow-up studies with CV end-points are needed to demonstrate the clinical relevance of the present phenotypic information.

### Abbreviations

BMI, body mass index; BP, blood pressure; CI, confidence interval; CV, cardiovascular; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HF, high frequency; HR, heart rate; HRV, heart rate variability; LDL, low-density lipoprotein; LF, low frequency; LF/HF, low frequency/high frequency ratio; MDRD, modification of diet in renal disease; PWV, pulse wave velocity; SD, standard deviation; SVRI, systemic vascular resistance index.

## Additional files

**Additional file 1:** Figure and Table in pdf-format showing practical classification rule tree based on the head-up tilt –induced changes in systemic vascular resistance index and cardiac index, and contingency table showing the agreement between two clustering procedures (hierarchical clustering and practical rule) in the original data ($n = 470$) and in the validation data ($n = 30$). (PDF 182 kb)

**Additional file 2:** Figure in pdf-format showing the magnitude of the changes in systemic vascular resistance index and cardiac index in response to upright posture in the 3 phenotypes. (PDF 75 kb)

**Additional file 3:** Table in pdf-format containing data about the supine and upright haemodynamics in the three phenotypes. (PDF 80 kb)

**Additional file 4:** Figure in pdf-format showing radial systolic and diastolic blood pressure during the measurement protocol in the 3 phenotypes. (PDF 437 kb)

**Additional file 5:** Table in pdf-format showing the analysis of explanatory factors for supine pulse wave velocity in multivariate analysis using continuous variables without categorization. (PDF 13 kb)

**Additional file 6:** Table in pdf-format showing outcome of adjusted analyses of heart rate variability parameters in supine and upright positions using model 1 presented in Table 2. (PDF 13 kb)

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### Availability of data and materials

The clinical database of the present study contains several indirect identifiers. The informed consent, which was written according to the ethical guidelines of Tampere University Hospital ethics committee, does not allow publication of individual patient data. As this clinical study was started already in 2006, the collection of new informed consents would be laborious and complete coverage of all subjects would be unlikely. Therefore, we cannot share the collected data. However, raw data supporting the obtained results can be requested from the corresponding author, and we are open for collaboration with the haemodynamic data that we have collected.

### Authors'contributions

AMT, AJT, JTM and IHP designed and conducted the study. AMT, AJT, JKK, KN, and IP analysed and interpreted the data, and drafted the first version of the manuscript. AMT, AJT, JKK, MHL, and IHP performed the experiments. JMV, MTU and JV managed the data and performed the HRV analyses. All authors gave critical intellectual input and contributed to the drafting of the revised versions of the manuscript, and approved the final manuscript.

### Competing interests

The authors have no conflicts of interest to disclose.

### Consent to publish

All subjects gave written informed consent to publish the results of the study.

### Ethics

The study complied with the declaration of Helsinki and was approved by the ethics committee of Tampere University Hospital (study code R06086M).

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**References**

1. Ezzati M, Lopez AD, Rodgers A, Vander Hoorn S, Murray CJ. Selected major risk factors and global and regional burden of disease. Lancet. 2002;360:1347–60.
2. Conroy RM, Pyörälä K, Fitzgerald AP, Sans S, Menotti A, De Backer G, et al. Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project. Eur Heart J. 2003;24:987–1003.
3. Wilson PW, Castelli WP, Kannel WB. Coronary risk prediction in adults. The Framingham Heart Study. Am J Cardiol. 1987;59:91G–4G.
4. Smith P, Tuomisto MT, Blumenthal J, Sherwood A, Parkkinen L, Kahonen M, et al. Psychosocial correlates of atrial natriuretic peptide: a marker of vascular health. Ann Behav Med. 2013;45:99–109.
5. Hodgkinson J, Mant J, Martin U, Guo B, Hobbs FD, Deeks JJ, et al. Relative effectiveness of clinic and home blood pressure monitoring compared with ambulatory blood pressure monitoring in diagnosis of hypertension: systematic review. BMJ. 2011;342:d3621.
6. Leino J, Minkkinen M, Nieminen T, Lehtimaki T, Viik J, Lehtinen R, et al. Combined assessment of heart rate recovery and T-wave alternans during routine exercise testing improves prediction of total and cardiovascular mortality: the Finnish Cardiovascular Study. Heart Rhythm. 2009;6:1765–71.
7. Omvik P, Gerdts E, Myking OL, Lund-Johansen P. Long-term central hemodynamic effects at rest and during exercise of losartan in essential hypertension. Am Heart J. 2000;140:624–30.
8. Luke RG. Chronic renal failure–a vasculopathic state. N Engl J Med. 1998;339:841–3.
9. Laurent S, Boutouyrie P, Asmar R, Gautier I, Laloux B, Guize L, et al. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. Hypertension. 2001;37:1236–41.
10. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur Heart J. 2006;27:2588–605.
11. Safar H, Mourad JJ, Safar M, Blacher J. Aortic pulse wave velocity, an independent marker of cardiovascular risk. Arch Mal Coeur Vaiss. 2002;95:1215–8.
12. Tahvanainen A, Leskinen M, Koskela J, Ilveskoski E, Nordhausen K, Oja H, et al. Ageing and cardiovascular responses to head-up tilt in healthy subjects. Atherosclerosis. 2009;207:445–51.
13. Sung SH, Chen ZY, Tseng TW, Lu DY, Yu WC, Cheng HM, et al. Wave reflections, arterial stiffness, and orthostatic hypotension. Am J Hypertens. 2014;27:1446–55.
14. Kasagi F, Akahoshi M, Shimaoka K. Relation between cold pressor test and development of hypertension based on 28-year follow-up. Hypertension. 1995;25:71–6.
15. Tsumura K, Hayashi T, Hamada C, Endo G, Fujii S, Okada K. Blood pressure response after two-step exercise as a powerful predictor of hypertension: the Osaka Health Survey. J Hypertens. 2002;20:1507–12.
16. Avolio A, Parati G. Reflecting on posture. J Hypertens. 2011;29:655–7.
17. Tikkakoski AJ, Tahvanainen AM, Leskinen MH, Koskela JK, Haring A, Viitala J, et al. Hemodynamic alterations in hypertensive patients at rest and during passive head-up tilt. J Hypertens. 2013;31:906–15.
18. Hautaniemi EJ, Tikkakoski AJ, Tahvanainen A, Nordhausen K, Kahonen M, Mattsson T, et al. Effect of fermented milk product containing lactotripeptides and plant sterol esters on haemodynamics in subjects with the metabolic syndrome–a randomised, double-blind, placebo-controlled study. Br J Nutr. 2015;114:376–86.
19. Koskela JK, Tahvanainen A, Haring A, Tikkakoski AJ, Ilveskoski E, Viitala J, et al. Association of resting heart rate with cardiovascular function: a cross-sectional study in 522 Finnish subjects. BMC Cardiovasc Disord. 2013;13:102.
20. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. Ann Intern Med. 1999;130:461–70.
21. Tahvanainen A, Koskela J, Tikkakoski A, Lahtela J, Leskinen M, Kahonen M, et al. Analysis of cardiovascular responses to passive head-up tilt using continuous pulse wave analysis and impedance cardiography. Scand J Clin Lab Invest. 2009;69:128–37.
22. Tahvanainen AM, Tikkakoski AJ, Leskinen MH, Nordhausen K, Kähönen M, Kööbi T, et al. Supine and upright haemodynamic effects of sublingual nitroglycerin and inhaled salbutamol: a double-blind, placebo-controlled, randomized study. J Hypertens. 2012;30:297–306.
23. Kööbi T, Kaukinen S, Ahola T, Turjanmaa VM. Non-invasive measurement of cardiac output: whole-body impedance cardiography in simultaneous comparison with thermodilution and direct oxygen Fick methods. Intensive Care Med. 1997;23:1132–7.
24. Kööbi T, Kaukinen S, Turjanmaa VM, Uusitalo AJ. Whole-body impedance cardiography in the measurement of cardiac output. Crit Care Med. 1997;25:779–85.
25. Kööbi T, Kähönen M, Iivainen T, Turjanmaa V. Simultaneous non-invasive assessment of arterial stiffness and haemodynamics - a validation study. Clin Physiol Funct Imaging. 2003;23:31–6.
26. Koivistoinen T, Kööbi T, Jula A, Hutri-Kähönen N, Raitakari OT, Majahalme S, et al. Pulse wave velocity reference values in healthy adults aged 26-75 years. Clin Physiol Funct Imaging. 2007;27:191–6.
27. Peltola MA. Role of editing of R-R intervals in the analysis of heart rate variability. Front Physiol. 2012;3:148.
28. Malik M, Bigger T, Camm J, Kleiger R, Malliani A, Moss A, et al. (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrofysiology). Heart rate variability. Standards of measurement, physiological interpretation and clinical use. Eur Heart J. 1996;17:354–81.
29. Boer P, Roos JC, Geyskes GG, Mees EJ. Measurement of cardiac output by impedance cardiography under various conditions. Am J Physiol. 1979;237:H491–6.
30. R: A language and environment for statistical computing [http://www.R-project.org/]
31. Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Bohm M, et al. 2013 ESH/ESC Guidelines for the management of arterial hypertension: the Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). J Hypertens. 2013;31:1281–357.
32. Xhyheri B, Manfrini O, Mazzolini M, Pizzi C, Bugiardini R. Heart rate variability today. Prog Cardiovasc Dis. 2012;55:321–31.
33. Aerts AJ. Nitrate stimulated tilt table testing: a review of the literature. Pacing Clin Electrophysiol. 2003;26:1528–37.
34. Oraii S, Maleki M, Minooii M, Kafaii P. Comparing two different protocols for tilt table testing: sublingual glyceryl trinitrate versus isoprenaline infusion. Heart. 1999;81:603–5.
35. de Liefde I, Welten GM, Verhagen HJ, van Domburg RT, Stolker RJ, Poldermans D. Exercise blood pressure response and perioperative complications after major vascular surgery. Coron Artery Dis. 2011;22:228–32.
36. Cruickshank K, Riste L, Anderson SG, Wright JS, Dunn G, Gosling RG. Aortic pulse-wave velocity and its relationship to mortality in diabetes and glucose intolerance: an integrated index of vascular function? Circulation. 2002;106:2085–90.
37. Mitchell GF, Hwang SJ, Vasan RS, Larson MG, Pencina MJ, Hamburg NM, et al. Arterial stiffness and cardiovascular events: the Framingham Heart Study. Circulation. 2010;121:505–11.
38. Bouthier JD, De Luca N, Safar ME, Simon AC. Cardiac hypertrophy and arterial distensibility in essential hypertension. Am Heart J. 1985;109:1345–52.
39. Wildman RP, Mackey RH, Bostom A, Thompson T, Sutton-Tyrrell K. Measures of obesity are associated with vascular stiffness in young and older adults. Hypertension. 2003;42:468–73.
40. The Reference Values for Arterial Stiffness' Collaboration. Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: 'establishing normal and reference values'. Eur Heart J. 2010;31:2338–50.
41. Matsukawa T, Sugiyama Y, Watanabe T, Kobayashi F, Mano T. Gender difference in age-related changes in muscle sympathetic nerve activity in healthy subjects. Am J Physiol. 1998;275:R1600–4.

42. Grassi G, Seravalle G, Cattaneo BM, Bolla GB, Lanfranchi A, Colombo M, et al. Sympathetic activation in obese normotensive subjects. Hypertension. 1995; 25:560–3.
43. Mark AL. The sympathetic nervous system in hypertension: a potential long-term regulator of arterial pressure. J Hypertens. 1996;14(suppl):S159–65.
44. Pal GK, Adithan C, Ananthanarayanan PH, Pal P, Nanda N, Thiyagarajan D, et al. Association of sympathovagal imbalance with cardiovascular risks in young prehypertensives. Am J Cardiol. 2013;112:1757–62.
45. Jaiswal M, Urbina EM, Wadwa RP, Talton JW, D'Agostino Jr RB, Hamman RF, et al. Reduced heart rate variability is associated with increased arterial stiffness in youth with type 1 diabetes: the SEARCH CVD study. Diabetes Care. 2013;36:2351–8.
46. Cardoso CR, Moraes RA, Leite NC, Salles GF. Relationships between reduced heart rate variability and pre-clinical cardiovascular disease in patients with type 2 diabetes. Diabetes Res Clin Pract. 2014;106:110–7.
47. Chandra P, Sands RL, Gillespie BW, Levin NW, Kotanko P, Kiser M, et al. Relationship between heart rate variability and pulse wave velocity and their association with patient outcomes in chronic kidney disease. Clin Nephrol. 2014;81:9–19.
48. Mattace-Raso FU, van den Meiracker AH, Bos WJ, van der Cammen TJ, Westerhof BE, Elias-Smale S, et al. Arterial stiffness, cardiovagal baroreflex sensitivity and postural blood pressure changes in older adults: the Rotterdam Study. J Hypertens. 2007;25:1421–6.
49. Mancia G, Grassi G. The autonomic nervous system and hypertension. Circ Res. 2014;114:1804–14.
50. Kornet L, Hoeks AP, Janssen BJ, Willigers JM, Reneman RS. Carotid diameter variations as a non-invasive tool to examine cardiac baroreceptor sensitivity. J Hypertens. 2002;20:1165–73.
51. Gatzka CD, Kingwell BA, Cameron JD, Berry KL, Liang YL, Dewar EM, et al. Gender differences in the timing of arterial wave reflection beyond differences in body height. J Hypertens. 2001;19:2197–203.
52. Shoemaker JK, Hogeman CS, Khan M, Kimmerly DS, Sinoway LI. Gender affects sympathetic and hemodynamic response to postural stress. Am J Physiol Heart Circ Physiol. 2001;281:H2028–35.
53. Summers RL, Platts S, Myers JG, Coleman TG. Theoretical analysis of the mechanisms of a gender differentiation in the propensity for orthostatic intolerance after spaceflight. Theor Biol Med Model. 2010;7:8.
54. Chen CH, Nevo E, Fetics B, Pak PH, Yin FC, Maughan WL, et al. Estimation of central aortic pressure waveform by mathematical transformation of radial tonometry pressure. Validation of generalized transfer function. Circulation. 1997;95:1827–36.
55. Van Bortel LM, Laurent S, Boutouyrie P, Chowienczyk P, Cruickshank JK, De Backer T, et al. Expert consensus document on the measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity. J Hypertens. 2012;30:445–8.
56. Peltonen M, Harald K, Männistö S, Saarikoski L, Peltomäki P, Lund L, et al. The National FINRISK 2007 Study. Publications of the National Public Health Institute, B34/2008 (with English summary). 2008;https://www.julkari.fi/bitstream/handle/10024/78146/2008b34.pdf.
57. Wolf HK, Tuomilehto J, Kuulasmaa K, Domarkiene S, Cepaitis Z, Molarius A, et al. Blood pressure levels in the 41 populations of the WHO MONICA Project. J Hum Hypertens. 1997;11:733–42.