Tampere University

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Hemodynamic Influences of Major Risk Factors of Cardiovascular Aging

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ACADEMIC DISSERTATION
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Tampere University, Faculty of Medicine and Health Technology
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Dedication

This study is whole-heartedly dedicated to my ever-supportive parents, to my uncles for being my guardians during the educational career, and to my four siblings; you have all been a constant source of support, knowledge, and inspiration.

ABSTRACT

Cardiovascular diseases (CVD) are the leading cause of mortality worldwide. Hypertension is a significant predisposing factor for CVD, while cigarette smoking, and dyslipidemias are one of the most important preventable risk factors for CVD. According to the World Health Organization, the prevalence of elevated blood pressure (BP), high plasma cholesterol concentration, and smoking is continuously rising. A large portion of deaths seem to be attributable to the risk factors raised BP, tobacco use and high cholesterol. The existing data about the influence of smoking on BP, wave reflections in the circulation, and arterial stiffness have been contradictory. Low-density lipoprotein cholesterol (LDL-C) has been linked with elevated BP in some studies but the results about the relationship of LDL-C with arterial stiffness have been inconsistent. The atherogenic index of plasma (AIP), defined as the logarithm of triglycerides to high-density lipoprotein cholesterol ratio, is a strong predictor of future CVD. The association of plasma AIP with hemodynamic variables has not been previously investigated. Also, rather limited information has existed about the detailed hemodynamic features of primary aldosteronism (PA) in the present days.

The aim of the present thesis was to study the hemodynamic features associated with smoking, LDL-C, plasma AIP, essential hypertension (EH) and PA. Therefore, hemodynamics influences of these major risk factors of cardiovascular aging were evaluated. In this thesis we assessed differences in hemodynamics between present, previous and never smokers to expand our understanding about the long-term effects of smoking on the cardiovascular system. We investigated the association of LDL-C with hemodynamic variables and examined the association of AIP with hemodynamic variables and more specifically tested the hypothesis whether AIP is related to large arterial stiffness. We then examined the detailed differences in hemodynamics between patients with medicated PA, medicated EH, never-medicated EH, and normotensive controls.

The study populations consisted of subjects without previously diagnosed CVD (other than hypertension), other forms of secondary hypertension but PA (PA was included in study IV), and BP or lipid lowering medications or other medications that have direct influences on cardiovascular function (Study I-III). Hemodynamics

were recorded non-invasively using whole body impedance cardiography and continuous radial pulse wave analysis, and the results were adjusted, as appropriate.

In Study I, 637 volunteers (19-72 years) without antihypertensive medications were allocated into 3 groups: never smokers (365), present smokers (81) and previous smokers (191). The population of studies II and III consisted of 615 subjects without antihypertensive and lipid-lowering medications. In study IV, 520 subjects were included in the four study groups: medicated PA, medicated EH, never-medicated EH and normotensive controls. The hypertensive groups were matched for age, sex, and body mass index, while the normotensive group was matched to have a similar sex distribution.

In accordance with previous studies, cigarette smoking (Study I) was not associated with change in BP and arterial stiffness, measured via pulse wave velocity (PWV) recordings between the groups. Importantly, augmentation index (AIx) was increased in present smokers, a finding which had been previously reported in association with acute, chronic, and passive smoking. Additionally, smoking was associated with other changes in hemodynamics: I) present smokers presented with increased stroke index and decreased aortic reflection time during upright position versus previous smokers, II) supine and upright cardiac output was higher in present versus previous smokers, III) in spite of the long abstinence previous smokers had lower cardiac output and higher systemic vascular resistance than never smokers, indicating that the magnitude or risk reduction in previous smokers after quitting smoking appears to be longer than previously anticipated. For the first time our study demonstrated by the use of multivariate regression analyses that higher stroke volume index and shorter aortic reflection time were the putative explanations for the higher AIx in present smokers.

Several hemodynamic changes associated with dyslipidemia were observed in this thesis. LDL-C was an independent explanatory factor for BP, PWV, AIx and systemic vascular resistance (Study II). In contrast, AIP was not related with radial or aortic BP, AIx or systemic vascular resistance, but AIP was directly and independently associated with large arterial stiffness (Study III). However, when the results were adjusted for prevailing central BP, LDL-C was no longer an explanatory factor for arterial stiffness (Studies II and III).

When the hemodynamics were compared between medicated PA patients, medicated and never-medicated EH patients and normotensive controls, PA patients present with higher BP than medicated EH patients and normotensive controls. Extracellular water balance was ~4% higher in PA than in all other groups, while cardiac output was ~8% higher in PA than in medicated EH. PWV was higher in

PA than in medicated EH and normotensive controls. Although 82 PA patients were taking concurrent potassium supplements, plasma potassium concentration was lower, while sodium concentration was higher, in the PA patients than in the other groups. In study IV the never-medicated EH patients had the highest PWV when compared with all other groups, indicating long-standing untreated high BP and unawareness of prevailing hypertension.

In conclusion, the examined major risk factors of cardiovascular aging were associated with clear hemodynamic changes and presented with related changes in cardiac function. The present smokers and patients with PA presented with a hyperdynamic circulation. Present smokers also presented with enhanced wave reflection when compared with previous smokers. LDL-C was independently associated with BP via systemic vascular resistance and wave reflection, whereas AIP was directly and independently associated with large arterial stiffness. Altogether, the whole lipid profile is of importance in clinical CVD risk evaluation. The present results also highlighted that in addition to the established methods for the screening and confirmatory testing for PA, the measurement of plasma sodium and potassium concentrations and the evaluation of the detailed hemodynamic features of PA, especially higher extracellular water volume and hyperdynamic circulation in comparison with EH, could be useful in the diagnostics of PA patients in the clinical setting.

CONTENTS

ABBREVIATIONS

ACE	Angiotensin-converting enzyme
AIP	Atherogenic index of plasma
AIx	Augmentation index
ANS	Autonomic nervous system
Ang	Angiotensin
ANOVA	Analysis of variance
AT1	Angiotensin II receptor type 1
BMI	Body mass index
BP	Blood pressure
CI	Confidence interval
CVD	Cardiovascular disease
ECG	Electrocardiography
ECW	Extracellular water
eGFR	Estimated glomerular filtration rate
EH	Essential hypertension
FERHDL	Fractional esterification rate of HDL
FWA	Forward wave amplitude
HDL-C	High-density lipoprotein cholesterol
HF	High-frequency
HRV	Heart rate variability
IMT	Intima-media thickness
LDL-C	Low-density lipoprotein cholesterol
LF	Low-frequency
MAP	Mean arterial pressure
PA	Primary aldosteronism
PP	Pulse pressure
PWV	Pulse wave velocity
Q	Quartile
QUICKI	Quantitative insulin sensitivity check index
RAAS	Renin angiotensin aldosterone system

ROS	Reactive oxygen species
SD	Standard deviation
SEM	Standard error of the mean
SVR	Systemic vascular resistance
SVRI	Systemic vascular resistance index

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following four original publications. In the text these publications are referred to by their Roman numerals I-IV:

Publication I Choudhary MK, Eräranta A, Bouquin H, Tikkakoski A, Kähönen M, Sipilä K, Mustonen J, Pörsti I. Effect of present versus previous smoking on non-invasive haemodynamics. Scientific Reports 2018, 13643.

Publication II Choudhary MK, Eräranta A, Tikkakoski AJ, Koskela J, Hautaniemi EJ, Kähönen M, Mustonen J, Pörsti I. LDL cholesterol is associated with systemic vascular resistance and wave reflection in subjects naive to cardiovascular drugs. Blood Pressure 2019; 28 (1):4-14.

Publication III Choudhary MK, Eräranta A, Tikkakoski AJ, Koskela JK, Nevalainen P, Kähönen M, Mustonen J, Pörsti I. Atherogenic index of plasma is related to arterial stiffness but not to blood pressure in normotensive and never-treated hypertensive subjects. Blood Pressure 2019; 28 (3):157-167.

Publication IV Choudhary MK, Värri E, Matikainen N, Koskela J, Tikkakoski A, Kähönen M, Niemelä O, Mustonen J, Nevalainen P, Pörsti I. Primary aldosteronism: Higher volume load, cardiac output and arterial stiffness than in essential hypertension. Journal of Internal Medicine 2021, 289 (1): 29-41.

1 INTRODUCTION

Cardiovascular diseases (CVD) due to hypertension, atherosclerosis and the associated complications, such as myocardial infarction and stroke, are the leading cause of cardiovascular mortality worldwide representing more than 31% of all deaths worldwide (Benjamin et al., 2017; WHO | Cardiovascular diseases, 2017). Among them more than 13.5 % deaths are due raised blood pressure (BP) (Arima et al., 2011), more than 10% of these deaths are attributed to smoking (Ezzati et al., 2005) and estimated 4.5% due to raised cholesterol (WHO | Raised cholesterol, 2008). In adult males the prevalence of elevated BP in 2015 was around 24% and in females around 20%, affecting more than 1.13 billion people globally, and is forecasted to affect over 1.5 billion by 2025 (WHO | Blood Pressure, 2015). Cigarette smoking is one of the most important preventable risk factors for mortality, accounting for more than 5 million premature deaths globally per year (Mathers & Loncar, 2006). Smoking is also the second most common cause for CVD after elevated BP (Wong, 2014). According to World Health Organization more than one billion people smoke and the prevalence is continuously rising (WHO | Prevalence of tobacco smoking, 2015). Similarly, dyslipidemias are not only a major risk factor for CVD, but can also predict the future development of hypertension (Halperin et al., 2006; Laaksonen et al., 2008; Oparil et al., 2003).

Smoking predisposes to the development of atherosclerosis, shown as increased arterial intima-media thickness (IMT) (Howard et al., 1998), and higher prevalence of atherosclerotic plaques in autopsy studies (Zieske et al., 1999). In a study by Howard et al. the progression of atherosclerosis in present smokers was increased by 50% versus non-smokers, documented using measurements of IMT in the carotid artery (Howard et al., 1998). Smoking is also associated with adverse effects on serum lipids (Huangfu et al., 2017), insulin sensitivity (Kim et al., 2017), and activation of the sympathetic nervous system (Barutcu et al., 2004). Carbon monoxide in the inhaled cigarette smoke increases the levels of carboxyhemoglobin, the proportion of which can exceed 7.5% in smokers, while the average level in non-smokers is 0.32% (Whincup et al., 2006). Although very high levels are uncommon,

symptomatic effects may occur at carboxyhemoglobin levels of 2.5% or more (Whincup et al., 2006).

Dyslipidemia is a major risk factor for CVD, and the primary focus has been on the dominant role of low-density lipoprotein cholesterol (LDL-C) in atherosclerosis. LDL-C has also vasoconstrictor, pro-inflammatory and thrombogenic properties, and it functions as a mitogenic factor that can stimulate vascular hypertrophy via several growth factors (Rosendorff, 2002). The benefits of LDL-C lowering in CVD are well recognized (Cholesterol Treatment Trialists' (CTT) Collaborators, 2012; Heart Protection Study Collaborative Group, 2011; Lamarche et al., 2018). In a systematic review by The Cholesterol Treatment Trialists with individual participant data from 22 trials of statin use versus control (n=134537) reported that each 1 mmol/L reduction in LDL-C produces an absolute reduction in major vascular events of about 11 per 1000 over 5 years in people with low risk of vascular events (Cholesterol Treatment Trialists' (CTT) Collaborators, 2012). LDL-C can reduce nitric oxide bioavailability and blunt the vasodilator response to acetylcholine, and the resulting endothelial dysfunction may be manifested as increased BP (Nickenig et al., 1997; Nickenig & Harrison, 2002; Rajendran et al., 2013; Rosendorff, 2002; Vogel, 1999). In clinical practice, the influence of LDL-C has overridden the significance of high density lipoprotein cholesterol (HDL-C) and triglycerides (Briasoulis et al., 2013; Cholesterol Treatment Trialists' (CTT) Collaborators, 2012; Lamarche et al., 2018; Tocci et al., 2017). LDL-C particles contain esterified cholesterol and triglycerides in their hydrophobic cores (Sommer et al., 1992). Previous studies have reported that not only low, but also very high, levels of HDL-C increase the risk of CVD and mortality (Madsen et al., 2017; Scandinavian Simvastatin Survival Study Group, 1994; Stone et al., 2014). Elevated serum triglyceride level is also a risk factor for CVD (Manninen et al., 1992; Nordestgaard et al., 2007; Sarwar et al., 2007). A meta-analysis of 17 population-based prospective studies with 46,413 men and 10,864 women reported that plasma triglyceride level, independent of HDL-C, was a risk factor for CVD (Hokanson & Austin, 1996).

The atherogenic index of plasma (AIP) is defined as the logarithm of plasma triglyceride to HDL-C ratio (Dobiásová & Frohlich, 2001; Tan et al., 2004). In contrast to plasma triglyceride concentration, AIP shows normal distribution (Holmes et al., 2008), and is therefore well suited for the mathematical modelling of cardiovascular variables. AIP is particularly useful in predicting plasma atherogenicity (Dobiášová, 2004; Dobiásová & Frohlich, 2001). AIP is also a strong marker for the future risk of atherosclerosis and CVD (Cai et al., 2017; Cure et al., 2018; Dobiášová et al., 2011; Dobiásová & Frohlich, 2001; Nam et al., 2020; Onat

et al., 2010; Pletcher et al., 2008; Tan et al., 2004), and the routine calculation of AIP apart from only focusing on LDL-C in clinical CVD risk evaluation would seem warranted.

Several studies have indicated that the prevalence of primary aldosteronism (PA) exceeds 5% among hypertensive patients (Calhoun, 2007; Young, 2019). Aldosterone plays a key role in the homeostatic control and maintenance of BP through regulation of extracellular volume, vascular tone, and cardiac output (Melmed et al., 2011; Schirpenbach & Reincke, 2007; Stowasser & Gordon, 2016). Excessive secretion of aldosterone from the zona glomerulosa of the adrenal cortex leads to enhanced renal sodium reabsorption and potassium and proton secretion, resulting in sodium retention, increased extracellular volume, hypokalemia, alkalosis, and hypertension (Melmed et al., 2011; Schirpenbach & Reincke, 2007; Stowasser & Gordon, 2016).

The suspicion of PA most often arises because of a poor response to antihypertensive medications (Acelajado et al., 2019). Typically, patients with PA have higher BP and they need more antihypertensive medications than patients with essential hypertension (EH) (Acelajado et al., 2019). Independent of the level of BP, increased incidence of myocardial infarction and stroke, and increased prevalence of atrial fibrillation have been reported in patients with PA (Monticone et al., 2018). Thus, higher BP cannot entirely explain the increase in cardiovascular morbidity and mortality in PA patients. According to the German Conn's Registry, individuals with PA are at a higher risk of cardiovascular mortality than patients with EH (Reincke et al., 2012).

Increased pulse wave velocity (PWV) that designates arterial stiffness is a strong predictor of CVD and mortality, independent of the level of BP (Vlachopoulos et al., 2010). The role of unfavorable lipid profile in atherosclerosis is well recognized, but the associations of plasma lipids with arterial stiffness are not straightforward. In spite of the dominant role of LDL-C in atherosclerosis, the relationship of LDL-C with PWV is rather weak (Cecelja & Chowienczyk, 2009). High triglyceride concentration in 1,447 subjects, and low HDL-C levels in 15,302 subjects, were associated with increased PWV (Wang et al., 2013; Wang et al., 2016). However, Wang et al. found that HDL-C was inversely associated with PWV in 2,375 Chinese subjects, while total cholesterol or triglyceride were not associated with PWV (Wang et al., 2011).

Conflicting reports have been published about the effect of smoking on BP (Argacha et al., 2008; Groppelli et al., 1992; Kaplan, 2017; Linneberg et al., 2015; Primatesta et al., 2001). Smoking causes an acute increase in BP, which declines

quickly, and this effect can be missed if BP is measured more than 30 minutes after smoking (Groppelli et al., 1992; Kaplan, 2017). Many studies have reported that chronic smoking is a risk factor for increased arterial stiffness, however, a number of investigations have not found differences in arterial stiffness between smokers and never smokers (Cecelja & Chowienczyk, 2009; Doonan et al., 2010). Higher augmentation index (AIx), a marker of wave reflections, has been found to be independently associated with smoking in several studies (Argacha et al., 2008; Barnoya, 2005; Janner et al., 2011; Markus et al., 2013; Tsuru et al., 2016). Argacha et al. reported an acute smoking-induced increase of AIx in smokers (Argacha et al., 2008). AIx was also increased by 15.7 percentage points at the end of 1-hour exposure to passive smoking (Barnoya, 2005). Polonia et al. found that AIx was reduced by about 9 percentage points in subjects who stopped smoking for 6 months, while there was an increase of 1.7 percentage points in those who continued smoking (Polónia et al., 2009).

According to a review, PA patients had higher aortic PWV than EH patients, whereas no significant difference was found in the variables of wave reflection, AIx and AIx adjusted to heart rate 75 beats per minute (AIx@75) (Ambrosino et al., 2016). Recently, the forward and backward wave amplitudes were reported to be higher in medicated PA than in medicated EH, possibly reflecting vascular damage in PA patients (Hung et al., 2019).

The range of the hemodynamic changes related with various LDL-C concentrations, in patients with PA, and in smokers are still controversial, while the association of AIP with hemodynamics variables has not been previously examined. The study subjects in this project include present, previous and never smokers; normotensive subjects, never-treated and treated hypertensive patients; and patients with PA. The aim of this thesis was to investigate the hemodynamics influences of three major risk factors of cardiovascular aging: smoking, plasma lipids and mineralocorticoid excess, and to evaluate the associations of AIP with the functional hemodynamic variables. In addition, the present investigation examined the detailed hemodynamic alterations in smokers and in patients with PA to add new evidence in the pathophysiological scenario associated with PA and smoking. The methods include the determination of peripheral and central BP, cardiac function, systemic vascular resistance, arterial compliance, and pulse wave reflection. Besides the measurements performed in the supine position, a passive head-up tilt was also utilized in study I.

2 REVIEW OF LITERATURE

2.1 Blood pressure

2.1.1 Basic determinants of blood pressure

The pressure of the blood within the arteries against the arterial wall is produced by the contraction of the left ventricle and the resistance to flow of the arteries and arterioles. Due to the pulsatile function of the heart, the cardiac cycle is divided into two parts: a period of relaxation known as diastole and a period of contraction known as systole. Systolic BP occurs during left ventricular systole, while diastolic BP occurs during ventricular diastole (Guyton & Hall, 2011). The difference in systolic BP and diastolic BP is the pulse pressure (PP). The mean arterial pressure (MAP) is defined as the diastolic BP plus one third of the PP (Guyton & Hall, 2011). Blood flow (F) varies directly due to the change in pressure (ΔP) across a blood vessel and varies inversely with the resistance (R) caused by the viscous drag of the blood against the vessel wall, as illustrated by Ohm's law: $F=\Delta P/R$ (Guyton & Hall, 2011). Normal BP is controlled by cardiac output and the total peripheral resistance (Guyton & Hall, 2011).

BP is dependent on the function of the heart, blood vessels, extracellular volume, the kidneys, the nervous system, humoral factors and events at the membrane and within the cells (Guyton & Hall, 2011). Cardiac output is determined by the stroke volume and heart rate. Stroke volume is dependent on intravascular volume (blood flowing into left ventricle) that is regulated by the kidneys, and on myocardial contractility. Myocardial contractility involves sympathetic and parasympathetic control of heart rate, intrinsic activity of the cardiac conduction system, complex membrane transport and cellular events requiring influx of calcium that leads to myocardial fiber shortening and relaxation, and effects of humoral substances on stimulating heart rate and myocardial fiber tension.

2.1.2 Central wave reflection and arterial stiffness

The circulatory system is a complex entity, regulated by multiple components. Importantly, blood flow in the arteries is not constant, but it is pulsatile. Both the flow wave and the pressure wave should be taken into consideration when evaluating the circulation of blood in the arteries. The two basic physiological functions of arterial tree are: (1) delivery of blood from the left ventricle to the organs and tissues, and (2) moderation of central pulsation so that blood flow in the capillaries is continuous (O'Rourke & Hashimoto, 2007).

After ventricular contraction, pressure wave is generated that travels forward and reaches the branching portion of the arterial tree and the high resistance small arteries, where it strikes, and the wave is reflected backward from the periphery. This phenomenon occurs at the arterial branches and at the point of change between the low resistance arteries and the high resistance arterioles (Laurent et al., 2006; O'Rourke & Hashimoto, 2007). Thus, pressure wave measured from an artery generated from ventricular contraction is a composite of the forward pressure wave and the reflected backward pressure wave. Younger individuals have elastic arteries. Thus, the velocity of the pulse wave is low, and the reflected wave returns to the aortic root during diastole. However, when the arteries stiffen, usually mostly due to aging, the reflected wave arrives back early, increasing the systolic pressure and enlarging the forward wave (Figure 1C and 1D). The additional contribution of the reflected wave to the systolic pressure is called augmentation pressure, while AIx is the ratio between augmentation pressure and PP (Laurent et al., 2006; Vlachopoulos et al., 2011) The difference in the shape of central wave between an elastic artery and a stiffened artery is presented in Figure 1.

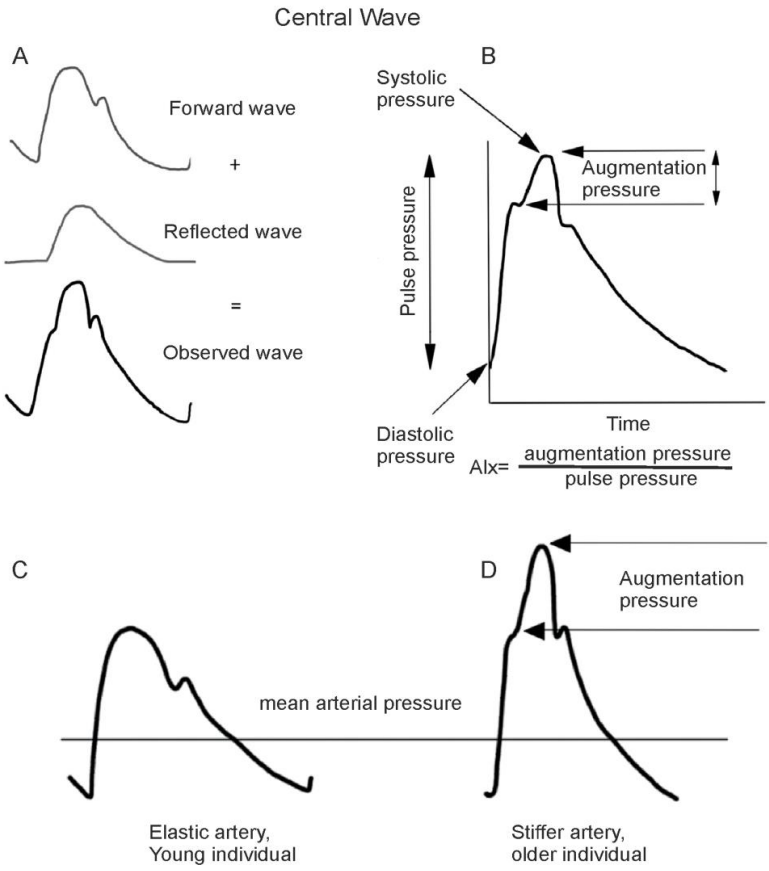

Figure 1. Schematic presentation of the central blood pressure (BP) curve. (**A**) The summation of a forward and a backward wave is responsible for the total BP curve. (**B**). A schematic representation of aortic BP curve with the definition of the augmentation index (AIx). The same mean arterial pressure may correspond to different BP curves in younger (**C**) and older (**D**) individuals. (Modified from Safar et al., 2013).

Amplification

Arterial elasticity varies along the arterial tree. The elastic properties of the thoracic and abdominal aorta are higher when compared to those of the more muscular iliac and femoral arteries (Vlachopoulos et al., 2011). Accordingly, arterial stiffness physiologically starts to increase from the central to the peripheral arteries (Vlachopoulos et al., 2011). Pressure pulse originating from the left ventricle modifies its shape as it travels along the arterial tree from central to peripheral vessels. As demonstrated in Figure 2, the reflection sites of the propagating pulse wave are closer in the peripheral arteries than in central large arteries, due to which the pressure wave amplifies (Latham et al., 1985). Due to these reasons, systolic BP and PP increase in the peripheral arteries when compared to central arteries. This amplification is a physiological phenomenon that is the difference in the systolic BP and PP present between the peripheral and the central arteries (Figure 2). Amplification protects the heart from an increase in post-load by maintaining the central systolic BP and PP low. However due to aging-related increase in large arterial stiffness, the amplification reduces, whereas augmentation increases. The central and peripheral systolic BPs become more and more similar (Safar et al., 2013).

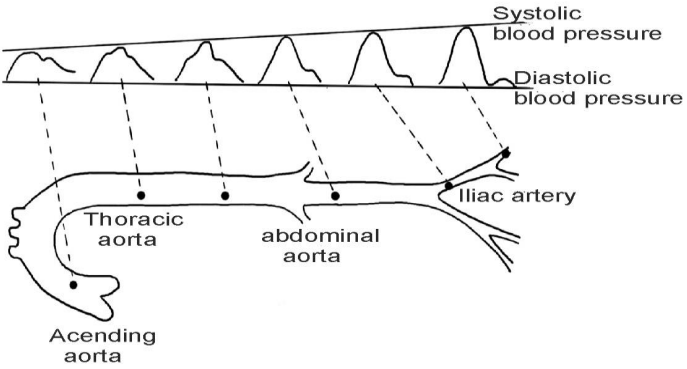

Figure 2. Amplification along the arterial tree (Reproduced from Kangas 2019 and modified from Vlachopoulos et al. 2011).

Arterial Stiffness

Biological aging and arteriosclerosis are especially related to increasing large arterial stiffness. In the development of arteriosclerosis, which is a medial disease, inflammation plays a major role (Lacolley et al., 2017). With aging, dilatation and increased arterial stiffness are the most noticeable physical changes noted in the elastic arteries (O'Rourke & Hashimoto, 2007). These changes are more pronounced in the larger arteries compared to the muscular peripheral arteries, thus making large arteries stiffen more compared to the distal arteries (O'Rourke et al., 2002). One of the major reasons for this phenomenon is that the central arteries are consequently more stressed due to each heartbeat that causes a relative wider dilatation in the aorta and proximal elastic arteries than in the peripheral muscular arteries. Due to repetitive pulsation-induced fatigue over the life span, the structural changes are more pronounced in these arteries (O'Rourke & Hashimoto, 2007). Age-related aortic stiffness leads to an increase in pressure in systole and a decrease in diastole. Increased systolic pressure causes left ventricular hypertrophy, whereas reduced diastolic pressure may interfere with coronary perfusion. Both impaired perfusion pressure and increased oxygen demand predispose to myocardial ischemia (Figure 3) (O'Rourke & Hashimoto, 2007)

Due to reduced arterial distensibility the arterial pulsatile flow is further transferred to the peripheral arteries, which has detrimental influences in the microcirculation. It is more harmful for the kidneys and brain as these organs have naturally high resting flow (O'Rourke & Hashimoto, 2007). Increased PWV that is an acknowledged marker of large arterial stiffness, leads to early wave reflection and thus increases systolic BP and PP. Due to increased arterial stiffness there is more stress in the vessel wall and consequently this results in atherogenesis and increased risk of plaque rupture (Lacolley et al., 2017). It has been reported that atherosclerotic changes increase arterial stiffness (Lacolley et al., 2017). A systematic review reported that with an increase in PWV by 1 m/s there is an increased risk of 14%, 15% and 15% in total cardiovascular events, cardiovascular mortality, and all-cause mortality, respectively (Vlachopoulos et al., 2010).

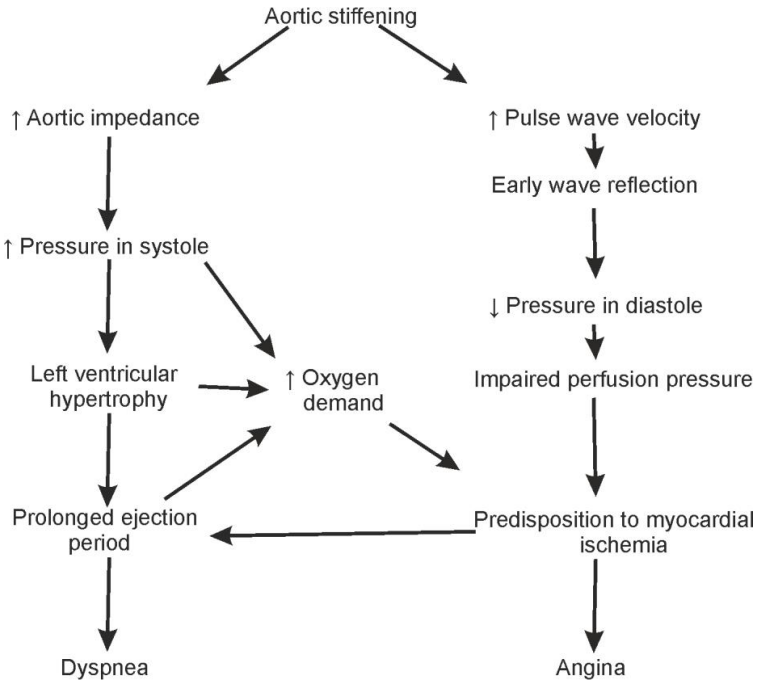

Figure 3. Arterial stiffness mechanism that predisposes the left ventricle to ischemia (Modified from O'Rourke and Hashimoto 2007).

2.1.3 Cardiac function and peripheral vascular resistance

The ability of the heart to meet with the metabolic demands of the human body is the main cardiac function. The principal purpose of heart is to impart energy to blood in order to generate and withstand an arterial BP, which is necessary for blood flow to provide adequate blood perfusion to organs. By contracting heart muscular walls around a closed chamber, it provides appropriate pressure to force blood from the left ventricle through the aortic valve and into the aorta. Similarly, the right ventricle contracts and forces blood via the pulmonic valve into the pulmonary artery to perfuse the lungs. The volume of blood in milliliters pumped from the left ventricle per beat is called stroke volume, while the blood volume pumped into the aorta by the heart during one minute is called cardiac output. In other words, cardiac output is a product of heart rate and stroke volume (Guyton & Hall, 2011).

According to the Frank-Starling law, in response to increases in venous return and preload volume in the ventricles before contraction, the ventricles increase their force of contraction and therefore increase stroke volume. Preload and contractility are positively associated up to a physiological limit (Guyton & Hall, 2011).

Systemic vascular resistance (SVR), also called total peripheral resistance, is the resistance to blood flow by all systemic vasculature except the pulmonary vasculature. The factors that influence the vascular resistance in an individual vasculature can determine SVR. Any mechanism that causes vasoconstriction increases SVR, whereas those mechanisms that cause vasodilation decrease SVR. SVR is calculated as mean aortic pressure minus the mean right atrial pressure divided by cardiac output. Normally the pressure of the right atrium is 2-6 mmHg, but as the pressure in the great veins is very small compared to the aortic pressure, it can be assumed to be zero in the formula (Vlachopoulos et al., 2011). SVR is determined by the changes in the blood vessel diameters primarily, and the main difference in pressure occurs in the arterioles during the blood flow, thus SVR is controlled by the caliber of arterioles. Peripheral resistance in small arteries and arterioles accounts for 45-50%, whereas capillaries account for ≈30% of total peripheral resistance (Guyton & Hall, 2011). However, change in the blood viscosity and other components of the vascular bed can also affect SVR (Vlachopoulos et al., 2011). Several mechanisms including both regional and systemic factors such as humoral, structural, neural and renal regulations can control SVR (Guyton & Hall, 2011). Studies have reported that structural changes are not so profound as in small arteries such arterioles and capillaries than in large arteries with aging (O'Rourke & Hashimoto, 2007). However, SVR increased with aging might be a result from vascular rarefaction and smooth muscle cell hypertrophy, and increased collagen content, i.e. changes that decrease the cross-sectional area of the arterioles (Vlachopoulos et al., 2011). In hypertension, enhanced sympathetic nervous tone, increased alpha-1 receptor activation causing vasoconstriction, and impaired endothelium-mediated vasodilatation, can all increase SVR (Delong & Sharma, 2020).

MAP depends on SVR and cardiac output, therefore any changes in either of the variables can affect MAP. The level of MAP is determined 60% by diastolic pressure and 40% by the systolic pressure, as the diastolic phase is longer than the systolic phase during the cardiac cycle. MAP equals to diastolic pressure plus 1/3 of systolic minus diastolic pressure (i.e. 1/3 of PP).

2.1.4 Cardiac autonomic tone and heart rate variability

The autonomic nervous system (ANS) is involved in numerous physiological bodily functions, such as the regulation of heart rate, digestion, respiratory rate, and pupillary responses. ANS consist of sympathetic and parasympathetic nervous systems. Studies have reported that autonomic imbalance or disturbance is associated with CVD and early mortality (Grassi et al., 2015; Wulsin et al., 2015). By analyzing heart rate variability (HRV), cardiac autonomic one can be evaluated non-invasively ("Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology," 1996). HRV is the variation in the time interval between consecutive heartbeats in milliseconds. HRV can be evaluated via different methods: the time domain method, frequency domain method, rhythm pattern analysis, and nonlinear methods are the most commonly used ("Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology," 1996). The frequency domain method is typically recommended for short-term data recordings (lasting approximately 5 minutes). Short-term recording of HRV is divided into two main components: a high-frequency (HF) component (frequency ranging from 0.15-0.40 Hz) and low-frequency (LF) component (frequency ranging from 0.04-0.15 Hz), respectively, estimating how much of either sympathetic or parasympathetic pathways affect the heart rate. Usually LF/HF ratio is used for evaluation of cardiac autonomic balance, but occasionally very low frequency range is also used for evaluation of cardiac autonomic balance ("Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology," 1996; Xhyheri et al., 2012).

The time domain method of HRV analysis is recommended for long-term (approximately 24-hours) data recording by electrocardiogram (ECG) monitoring (Xhyheri et al., 2012). Time domain methods with a continuous ECG recording can permit determination of instant heart rate or intervals between successive normal QRS complexes (Xhyheri et al., 2012). The standard deviation of normal to normal intervals is a marker of total power (variance) of HRV and it reflects all long-term components that are responsible for variability in the recording period, together with circadian rhythm and physical activity (Xhyheri et al., 2012).

In the evaluation of CVD risk, several studies have reported clinical and prognostic value for HRV. Many factors such as aging and overweight influence HRV, and typically aging decreases parasympathetic cardiac activity. Healthy women have lower 24 hour LF/HF ratio of HRV compared to healthy men (Bonnemeier et

al., 2003; Stein et al., 1997; Xhyheri et al., 2012). Factors such as heart rate (Sacha, 2014), anti-hypertensive medication (Okano et al., 2009), diabetes and ethnicity also influence HRV (Xhyheri et al., 2012). A lower LF/HF ratio of HRV may provide defense against arrhythmias and the early progress of coronary heart disease (Xhyheri et al., 2012). Moreover, circadian rhythm and breathing pattern can also influence HRV (Xhyheri et al., 2012).

2.2 Regulation of blood pressure at rest

Increased arterial BP can be caused by several factors, the basic determinants being cardiac output and SVR (Guyton & Hall, 2011; Navar, 1997, 2014). The ANS is the major short-term regulator of BP, while the kidneys are the main contributors to the long-term maintenance of BP. There are also several other factors involved in the regulation of BP which are controlled by interacting local, neural, humoral and renal factors (Guyton & Hall, 2011; Navar, 2014). Neurogenic or humoral stimuli induce vasoconstriction of blood vessel and cause renal volume retention leading to an increase in cardiac output, tissue blood flow and vascular resistance that causes an increase in arterial BP. A decrease in peripheral compartment capacitance also affects arterial pressure because it causes venous return to transiently exceed cardiac output, thereby increasing central compartment blood volume. Volumes of blood ≤10% of total intravascular volume can be transferred into the central circulation in this fashion (Shoukas & Sagawa, 1973). Increasing blood volume can also lead to an increase in vascular resistance and thus induce an increase in BP (Figure 4) (Guyton & Hall, 2011; Navar, 1997, 2014).

Sodium

Sodium is vital for maintaining fluid balance and many other essential functions. High concentration of sodium is found in the extracellular space, whereas low concentration of sodium and high concentration of potassium are found in the intracellular space. The sodium-potassium pump maintains the electrolyte and water distributions between the intracellular and extracellular fluids. Therefore, the control of sodium balance is essential in maintaining extracellular water volume and BP, and a meta-analysis reported that a modest reduction in sodium intake had a significant lowering effect on BP, assuming that reverse is true dietary sodium intake is associated with increased BP (He & MacGregor, 2004).

The pressure-natriuresis and pressure-diuresis mechanisms play a central role in the long-term control of BP, the importance of which has been long recognized

(Cowley, 1992). In the proximal tubules, sodium reabsorption is directly regulated by renal perfusion pressure. This mechanism explains how kidney can alter the level of sodium and water excretion in reaction to changes in renal arterial pressure (Guyton & Hall, 2011). A reduction in renal BP stimulates juxtaglomerular cells, which synthesize and release renin that activates the renin-angiotensin-aldosterone system, subsequently leading to increased reabsorption of sodium in the renal tubules. Increased oxidative stress in the renal medulla leads to an imbalance in the pressure-natriuresis system, resulting in sodium and volume retention, which can predispose to the development of hypertension. There are many physiological mechanisms that act in response to altered body fluid balance, but normally the regulation of extracellular water (ECW) volume by renal sodium excretion is the main mechanism of bringing arterial pressure back to normal, thus emphasizing the role of the kidneys in the long-term control of BP (Guyton & Hall, 2011).

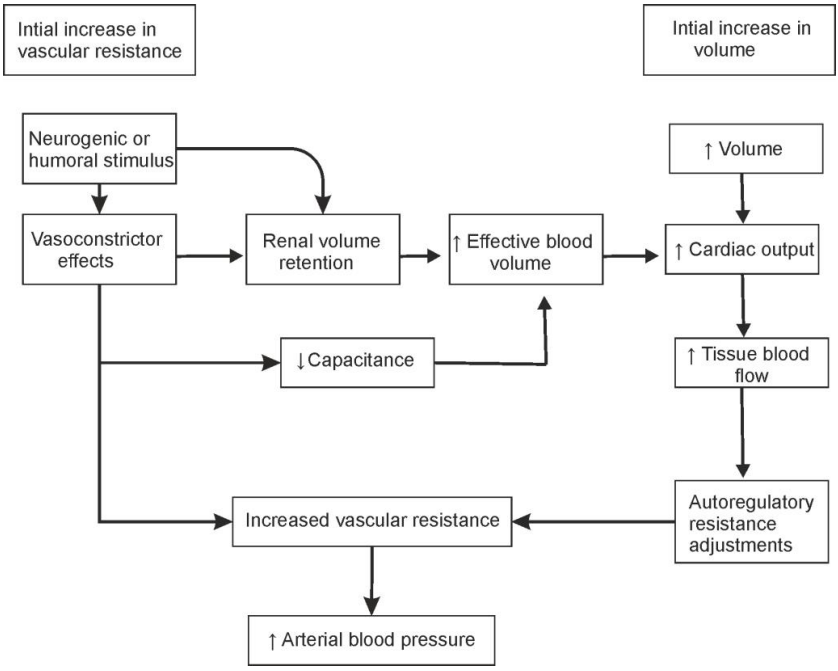

Figure 4. Mechanisms mediating hypertension.

Renin-angiotensin-aldosterone system

Renin-angiotensin-aldosterone system (RAAS) is an important regulator of BP, fluid volume and salt balance via the formation of angiotensin (Ang) II (Figure 5). The binding of Ang II to the Ang II type 1 (AT1) receptor mediates a vast range of processes including vasoconstriction, increase in aldosterone and vasopressin secretion, sodium and water retention, as well as sympathetic activation (Navar, 2014; Riet et al., 2015; Schmieder, 2005). The components of the RAAS are present in both the circulation, as well as locally in various tissues including the kidneys, heart, brain, and arteries (Figure 5) (Riet et al., 2015; Schmieder, 2005). RAAS is considered as a main hormonal regulator of BP together with sodium, potassium and water balance, and it is involved in both the short-term and long-term regulation of BP (Guyton & Hall, 2011; Navar, 2014; Riet et al., 2015; Schmieder, 2005).

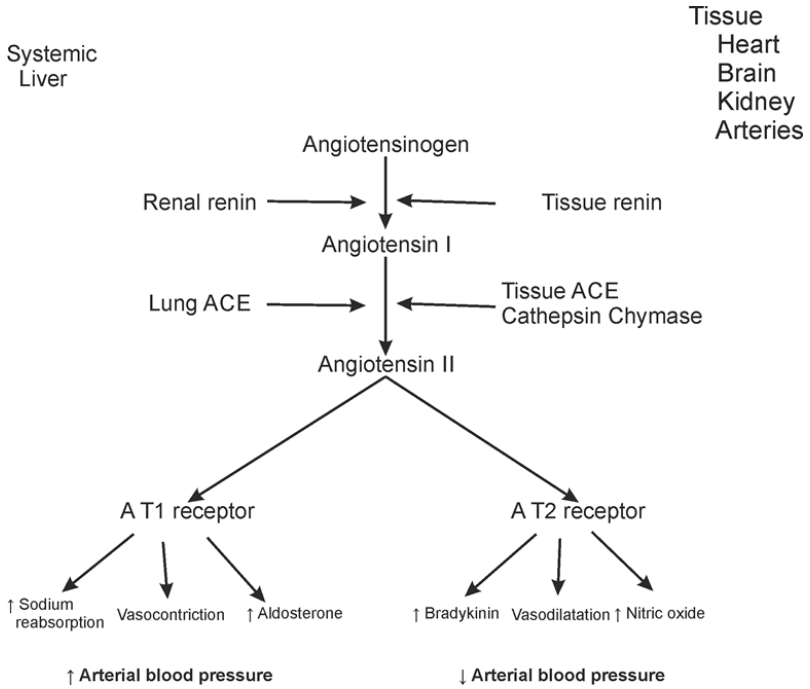

Figure 5. The role of the Renin-Angiotensin-Aldosterone System (RAAS) in the regulation of blood pressure. ACE, angiotensin converting enzyme; AT1, angiotensin II type 1; AT2, angiotensin II type 2. (Adapted from Schmieder 2005).

Kidneys

When the perfusion pressure in the kidney lowered, renin is released from the juxtaglomerular cells into the blood. Circulating renin acts on the protein angiotensinogen, breaks it forming angiotensin I, and this is then converted to Ang II via the angiotensin converting enzyme present in pulmonary endothelial cells and several other tissues (Riet et al., 2015). Circulating Ang II is then transported to various targets such as blood vessels, kidneys, and adrenal glands. It interacts with specific receptors to exert its action, and in the adrenal glands play an important role in aldosteronogenesis. The conversion of Ang II increases following the release of renin, when renal sympathetic activity is increased, or when renal perfusion pressure or arterial pressure are decreased (Navar, 2014). Ang II increases total peripheral resistance through direct constriction of vascular smooth muscle cells and increased sympathetic nerve activity. ECW volume is increased via several mechanisms. Ang II interacts with the AT1 receptors to increase the synthesis and release of aldosterone that in turn will act mainly on the nephron distal tubules to increase sodium reabsorption and excrete potassium and hydrogen into the tubules (Figure 5) (Guyton & Hall, 2011; Riet et al., 2015; Schmieder, 2005). In the gut, intestinal Ang II increases sodium and fluid absorption (Navar, 2014). Posterior pituitary gland secretes a hormone called vasopressin, and Ang II stimulates the hypothalamic-posterior pituitary gland secretory mechanism. Due to the activation, the release of vasopressin leads to enhanced water reabsorption, while at higher concentrations vasopressin is also a powerful vasoconstrictor (Guyton & Hall, 2011; Navar, 2014). In the heart Ang II increases cardiac output, heart rate and cardiac contractility (Navar, 2014).

Endothelium and autacoid bioavailability

Vascular endothelial cells cover the entire inner surface of circulatory system and provide appropriate hemostatic balance (Rajendran et al., 2013). The endothelium synthesizes and releases various autocrine and paracrine agents regulating vascular permeability, thrombosis and thrombolysis, platelet and leukocyte interaction with the vessel wall, smooth muscle cell growth and migration, and maintenance of vascular tone. Appropriate functioning and balancing by the endothelium is necessary for maintaining cardiovascular health (Navar, 2014; Rajendran et al., 2013). Endothelial dysfunction is and early feature and predictor of atherosclerosis, with vascular oxidative stress and inflammation being a major determinants of endothelial dysfunction (Daiber et al., 2017; Navar, 2014; Rajendran et al., 2013).

CVD risks factors such as increased BP, hypercholesterolemia and chronic smoking have all been associated with endothelial dysfunction (Daiber et al., 2017;

Fleming, 2017). Dyslipidemia that causes endothelial dysfunction may lead to the development of hypertension (Oparil et al., 2003; Rajendran et al., 2013). LDL-C has been found to impair endothelium-dependent vasodilatation, the underlying mechanisms being reduced nitric oxide bioavailability through increased vascular production of reactive oxygen species (ROS), and enhanced responses to vasoconstrictors like Ang II (Cominacini et al., 2001; Nickenig, 2002; Nickenig et al., 1997; Vita et al., 1990). Therefore, impaired endothelium-mediated vasomotion in the resistance vessels would lead to elevated BP via increased SVR.

ROS are highly reactive radicals that include superoxide, hydrogen peroxide, hydroxyl radical and peroxynitrite. ROS can cause cellular aging, development of cancer, and diseases such as arteriosclerosis, diabetes, immunodeficiency syndrome and heart disease by promoting oxidative damage to lipids, nucleic acids, proteins, DNA and RNA within cells, thus instigating cellular dysfunction. An imbalance between ROS and antioxidant defense mechanisms of the cells produces tissue damage due to oxidative stress (Birben et al., 2012). ROS play an important role in the pathophysiology of hypertension (Sinha & Dabla, 2015). Higher oxidative stress due to increased levels of Ang II stimulates NADPH oxidase, increases ROS causing vascular inflammation, which plays an important role in renal and vascular dysfunction (Sinha & Dabla, 2015). Endothelial injury due to increased oxidative stress is a main mediator in the pathophysiology of hypertension, which is associated with increased production of superoxide anion hydrogen peroxide, and decrease bioavailability of antioxidants and reduced nitric oxide synthesis (Sinha & Dabla, 2015; Fleming, 2017).

Also the overproduction of the vasoconstrictor peptide endothelin-1 by the endothelium may play a role in the pathophysiology of hypertension (Daiber et al., 2017). Medical therapy by the use of angiotensin-converting enzyme (ACE)-inhibitors, AT_1 receptor antagonists and statins have been shown to have beneficial effects on endothelial dysfunction (Koh et al., 2017). In addition, lifestyle modifications such as healthy diet and exercise may have favorable effects on endothelial function. Regular aerobic exercise in hypertensive patients can prevent impairment of reactive hyperemia, possibly via an exercise-induced increase in the production of nitric oxide (Higashi et al., 1999).

2.3 Hemodynamic response to upright posture and tilt table test

Even though human beings spend most of their time either standing (upright posture) or sitting instead of lying down, a wealth of studies evaluating cardiovascular hemodynamics have been performed in the supine position. When position of the body changes from supine to upright, it causes major changes in blood distribution, autonomic tone and pressure dynamics for maintaining normal BP level (Guyton & Hall, 2011; Saal et al., 2016). Due to the earth gravitational force, blood is transported from the thorax to the lower limbs, due to which there is a decrease in venous return and cardiac output. Due to the subsequent reduction in BP, the autonomic control mechanism is triggered (baroreflex), resulting in heightened sympathetic activity, and an increase in heart rate and SVR (Saal et al., 2016). Because of these mechanisms, arterial waveform morphology is quite different in supine and upright positions.

The tilt table test is used as a diagnostic tool to evaluate syncope and orthostatic hypotension (Saal et al., 2016). The systematic knowledge about the upright hemodynamics is rather limited, and the evaluation of the CVD risk considering the upright posture of the subjects has been long somewhat neglected. A decrease in AIx from supine to upright posture with a parallel increase in SVR has been reported previously, suggesting that reflection and pressure augmentation are not solely dependent on SVR, although the increase in SVR is usually considered to increase wave reflection (Davis et al., 2011).

2.4 Hypertension

Hypertension or high BP is a worldwide problem that affects approximately 15-20% of all adults. Hypertension is also known as a silent killer, and it is a major preventable risk factor for CVD (WHO | Blood Pressure, 2015). According to FinHealth 2017 population study, 48% male and 33% female aged 30-64 years were hypertensive in Finland (Koponen et al., 2018). Hypertension is defined as office systolic BP values ≥140 mmHg and/or diastolic BP values ≥90 mmHg. Home BP mean of systolic BP≥135 mmHg and/or ≥85 mmHg; ambulatory BP daytime (or awake) mean as systolic BP≥135 mmHg and/or ≥85 mmHg; and 24 hour mean as systolic BP≥130 mmHg and/or ≥80 mmHg are other definitions of hypertension according to European Society of Cardiology and the European Society of Hypertension guideline (Williams et al., 2018).

Even though hypertension is rather simple to diagnose and successful reduction of BP can be achieved by following a healthy diet, performing regular exercise, taking the medication prescribed, or a combination of these, hypertension remains a problem. Uncontrolled BP is common in communities worldwide, and since 1990 the disability-adjusted life years lost attributed to hypertension have increased by 40%. (Egan et al., 2013; Forouzanfar et al., 2017; Williams et al., 2018). Hypertension affects the structure and function of small muscular arteries, arterioles and also of larger blood vessels, and can cause damage at a variable rate to several target organs including the kidney, the brain, heart and eye, and hypertension is closely related with the development of end stage of renal disease and genesis of stroke (Lewington et al., 2002; Williams et al., 2018). It is associated with the alterations in the blood vessels wall that affect the endothelium, the media and the adventitia, and especially alterations in the media lead to remodeling of the arterial vessel wall (Harvey et al., 2016).

Essential hypertension

EH is the most prevalent form of hypertension accounting for 95% of all hypertension. EH is a heterogeneous disorder with a multifactorial etiology. The main cause of EH is still unknown but it is considered as the sum of interactions between genetic and multiple environmental factors. Environmental factors including high alcohol intake, high salt intake, low potassium intake, sedentary lifestyle, stress, and low calcium intake in association with aging, obesity, and insulin resistance contribute to the development of hypertension (Carretero & Oparil, 2000). Increased SVR and decreased vascular distensibility are the main pathophysiological changes in EH (Simon, 2004). Central hemodynamic changes that were studied invasively reported high SVR in EH patients (Lund-Johansen, 1991) Indeed, EH is characterized by raised peripheral vascular resistance in association with normal cardiac output (Izzard & Heagerty, 1995).

Primary aldosteronism

PA also known as Conn's syndrome is the most common endocrine origin of secondary hypertension. PA is characterized by increased autonomous aldosterone production, mostly caused by adrenocortical adenoma or bilateral adrenal hyperplasia (Calhoun, 2007). Due to overproduction of aldosterone, it results in sodium reabsorption, potassium excretion, and fluid retention, which cause an increase in systolic and diastolic BP (Stowasser & Gordon, 2016).

Today, PA is considered one of the most common causes of secondary hypertension with a prevalence of ≈10% among all hypertensive patients, and ≈20%

among patients with resistant hypertension (Calhoun, 2007). The suspicion of PA often arises in patients due to their poor response to antihypertensive medication and subsequently, the clinicians carry out the diagnostics of PA in hypertensive patients who are already ingesting BP-lowering agents (Acelajado et al., 2019). Typically, patients diagnosed with PA have also higher mean BP and they need more antihypertensive medications than patients with EH (Acelajado et al., 2019; Clark et al., 2012; Lin et al., 2012; Turchi et al., 2014).

Increased incidence of myocardial infarction and stroke, and increased prevalence of atrial fibrillation have been reported in patients with PA, independent of the level of BP (Monticone et al., 2018). According to the German Conn's Registry individuals with PA are at a higher risk of cardiovascular mortality than patients with EH (Reincke et al., 2012). Increased in BP cannot entirely explain the increase in cardiovascular morbidity and mortality in PA patients and the underlying mechanisms are not yet clearly understood.

Patients with PA have increased carotid intima media thickness measured via ultrasound compared with EH, suggesting that aldosterone excess contributes to fibrosis and thickening of the arterial wall (Holaj et al., 2007). The potential consequences of increased arterial stiffness and atherosclerosis may impede conduit arterial function and worsen cardiovascular outcome. Excess of aldosterone, by enhancing oxidative stress and inflammation, has also been shown to adversely affect endothelial function (McCurley & Jaffe, 2012).

Hemodynamics of hypertensive patients

The hemodynamic profiles of hypertensive patients are greatly influenced by age. Over time the wall of large conduit arteries, especially aorta, thickens and loses elasticity, and this result in an increase in PWV, an important and reliable measure of arterial stiffness. The increased arterial stiffness that takes place due to multifactorial causes (Lacolley et al., 2017), reduces the buffering function of the conduit arteries near the heart and increases PWV, which increase systolic and PP. Consequently, age-related hypertension is characterized by a significant rise in systolic BP with no change or even a lower diastolic BP, a condition also called isolated systolic hypertension. Age and BP are both important determinants of PWV (AlGhatrif et al., 2013; Townsend et al., 2015). The development of increased large arterial stiffness is a complex process that comprises influences mediated via mechanical pulsatile stress, inflammatory cells, growth factors, and alterations in endothelial function, enzymes that degrade elastin, changes in smooth muscle cells from the contractile to the synthetic phenotype, and increased extracellular matrix production by fibroblasts (Lacolley et al., 2017).

Elevated resting heart rate is also an independent risk for the development of hypertension (Aladin et al., 2016). Particularly, there is a direct relationship between elevated resting heart rate and peripheral BP, while there is an inverse association between elevated resting heart rate and central BP (Messerli et al., 2016; Stergiou et al., 2016). Additionally, elevated heart rate has been directly associated increased PWV (Mangoni et al., 1996). A study examining young Finnish adults reported that PWV was directly and independently associated with an increase in BP. It also reported that PWV was an independent predictor of incident hypertension (Koivistoinen et al., 2018).

Many studies have confirmed the beneficial effects of reducing BP on cardiovascular risk. A meta-analysis which included 123 randomized controlled trials with a minimum of 1000 patients-years of follow-up in each study group, reported that lowering of BP significantly reduced the risk of major CVD events by 20%, coronary heart disease by 17%, stroke by 27%, and heart failure by 28%, irrespective of the starting level of BP (Ettehad et al., 2016). Lifestyle modifications such as reducing weight, aerobic exercise, eating a healthy diet with more fruit, vegetables, and less saturated and total fats, quitting smoking, and reducing or stopping alcohol consumption are some of the important aspects for both prevention and treatment of hypertension. In patients with grade 1 hypertension (office systolic BP 140-159 and/or diastolic BP 90-99), lifestyle modifications can delay or even prevent the requirement for medical therapy (Williams et al., 2018). Of note, lifestyle changes are also beneficial for cardiovascular health improvement by improving lipid profiles. Hypertension awareness, treatment and control are the three pillars for the prevention and adequate control of hypertension.

2.5 Smoking

Cigarette smoking, either active or passive, can cause CVDs via a series of interdependent processes, such as enhanced oxidative stress, hemodynamic and autonomic alterations, endothelial dysfunction, thrombosis, inflammation, hyperlipidemia, and insulin resistance (Ambrose & Barua, 2004). Even passive or occasional smoking, only few cigarettes per day, can have deleterious consequences (Lim et al., 2012). Cigarette smoke contains more than 4000 chemical substances that have harmful effects on cardiovascular function (Ambrose & Barua, 2004). Of these only few components have been examined in isolation that are specific and

also known to be damaging to the health: nicotine and carbon monoxide (Ambrose & Barua, 2004).

Nicotine

Nicotine is categorized as an alkaloid. One cigarette delivers 1.2-2.9 mg of nicotine, and the typical one pack-per-day smoker absorbs 20-40 mg of nicotine each day (Lande, 2019). Nicotine deregulates cardiac autonomic function, boosts sympathetic activation, raises heart rate, causes coronary and peripheral vasoconstriction, increases myocardial workload, and stimulates adrenal and neuronal catecholamine release (Benowitz & Gourlay, 1997). In addition, nicotine is associated with insulin resistance, increased serum lipid levels, and intravascular inflammation that contribute to the development of atherosclerosis (Figure 6) (Papathanasiou et al., 2014)

Cigarette smoking affects the endothelium through an increase in oxidative stress, with effects on both endothelial cell function and structure (Messner & Bernhard, 2014). The scavenging activity of superoxide due to its increased level, and other reactive oxygen species produced by smoking, along with uncoupling of endothelial nitric oxide synthase, lead to nitric oxide inactivation. Reduced bioavailability of nitric oxide interferes with its vasodilatory, antithrombotic, anti-inflammatory and antioxidant effects, as well as with its influence on endothelial permeability and myocardial function (Gusarov et al., 2009; Kietadisorn et al., 2012).

Chronic smoking is associated with dysfunction of the autonomic nervous system (Narkiewicz et al., 1998), and the increased heart rate responses to tobacco may be implicated in the link between smoking and cardiovascular disease (Argacha et al., 2008; Linneberg et al., 2015; Savonen et al., 2006). Although the precise mechanism of action of smoke components are still under investigation, many proposed hypotheses state that the main effects of smoking on cardiovascular function are associated with the direct or indirect actions of nicotine on the neuro-regulation of the circulatory system, wherein sympathetic activity is increased and parasympathetic activity is reduced (Figure 6) (Papathanasiou et al., 2014).

The nicotine-induced sympathetic overdrive causes the adrenal medulla to increase the secretion of both epinephrine and norepinephrine into the circulating blood (Guyton & Hall, 2011). In addition, nicotine stimulates the vasomotor center of the medulla, causing secretion of norepinephrine from local deposits. Subsequently, secretion of catecholamines from the free nerve endings of the sympathetic nerves, and the local release of epinephrine and norepinephrine, are increased. The stimulation of catecholamine secretion, in combination with the

depressed production of prostacyclin (a potent vasodilator), result in an acute rise in blood pressure, a significant rise in heart rate, an increase in cardiac contractility, and a significant increase in myocardial work (Kalkhoran et al., 2018; Papathanasiou et al., 2014).

Cotinine is an alkaloid found in tobacco and is also the predominant metabolite of nicotine. Lower BP in pipe smokers than in non-smoker suggested that nicotine or its metabolites might be involved (Gyntelberg., & Meyer, 1974). The levels of nicotine and cotinine in pipe smokers were as high as in cigarette smokers; however, pipe smokers inhaled much less tar and carbon monoxide (Wald et al., 1981). Nicotine is absorbed through the buccal mucosa. Thus, the putative BP-lowering effect observed in pipe smokers argues that the vasodilator effect of the nicotine metabolite cotinine rather than tar or gaseous components of tobacco smoke were responsible (Gyntelberg., & Meyer, 1974; Benowitz & Sharp, 1989).

Smoking increases insulin release and causes insulin resistance (Kim et al., 2017). However, smoking may also reduce insulin production, slow glucose catabolism and lead to glucose accumulation in the body (Papathanasiou et al., 2014). The smoking-induced insulin resistance is also associated with an increase in triglyceride concentration (Ambrose & Barua, 2004; Axelsen et al., 1995), because in fat tissue glucose is converted to triglycerides. Similarly, tobacco smoke has significant effects on lipid metabolism (Axelsen et al., 1995; Nakanishi et al., 2014). Smoking elevates total cholesterol, LDL-C and triglycerides, while it lowers HDL-C (Ambrose & Barua, 2004; Nakanishi et al., 2014). Cigarette smoking also increases oxidative modification of LDL (Papathanasiou et al., 2014). The triglycerides and/or HDL abnormalities related to insulin resistance might also be a potential key link between cigarette smoking and CVD (Ambrose & Barua, 2004; Nakanishi et al., 2014).

Carbon monoxide

Carbon monoxide exposure has long been implicated in the process of atherosclerosis, contributing to the accumulation of cholesterol in the aorta and coronary arteries (Astrup et al., 1970; Thomsen, 1974). Epidemiological evidence suggests that subjects exposed to high carbon monoxide concentrations have higher cardiovascular morbidity and mortality compared to the expected rate in the general population (Koskela, 1994). The main mechanism by which carbon monoxide causes heart disease is through hypoxia. Inhalation of cigarette smoke, by either active or passive smokers, increases the levels of carboxyhemoglobin in the blood, decreasing the supply of oxygen to the tissues. In addition, myoglobin binds with carbon monoxide so that the heart muscle does not take up the necessary oxygen and does not perform optimally. The reduced oxygen uptake as a result of smoking, together

with an increase in serum lactic acid levels (lactic acidosis), leads to a reduction in peak aerobic capacity and to a significant decrease in maximum oxygen uptake (Figure 6) (Papathanasiou et al., 2014).

The free radicals and reactive oxygen species from cigarette smoke cause endothelial dysfunction and platelet activation, and promote atherosclerosis through oxidization of low density-lipoprotein (Reilly, 2013). The particulate matter inhaled from cigarette smoking can also cause oxidative stress, endothelial dysfunction, and platelet activation, and has effects on the ANS (Figure 6) (Brook et al., 2010).

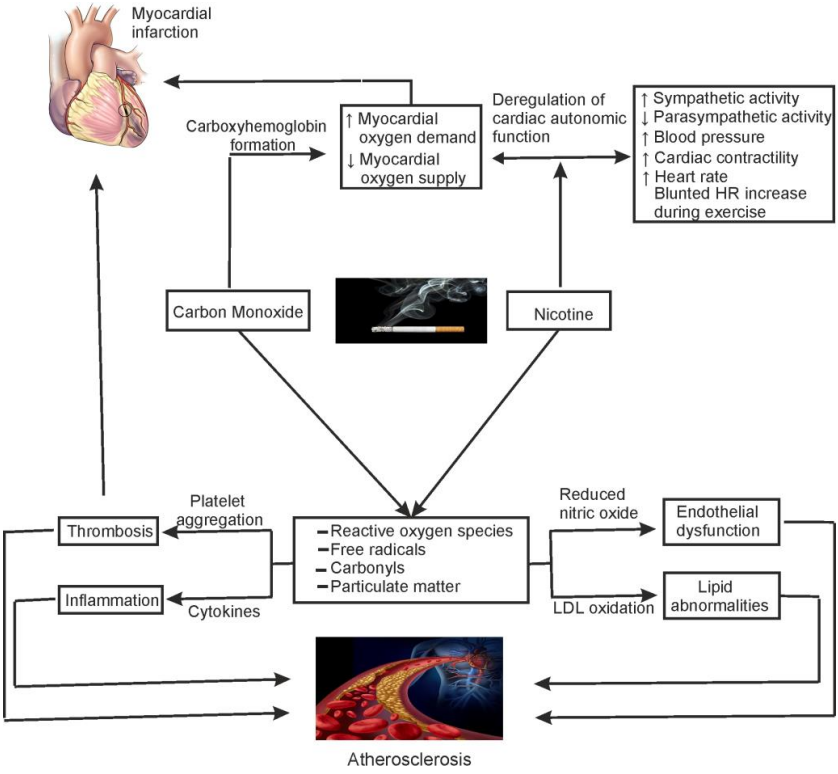

Figure 6. Mechanisms by which smoking causes cardiovascular diseases (Adapted from MacDonald and Middlekauff 2019 and Papathanasiou et al. 2014).

Smoking and hemodynamics

The effect of smoking on BP has been controversial (Argacha et al., 2008; Groppelli et al., 1992; Linneberg et al., 2015). It has been reported that chronic smoking is a risk factor for increased arterial stiffness, however many studies did not find any difference in arterial stiffness between smokers and never smokers (Cecelja & Chowienczyk, 2009; Doonan et al., 2010).

A study reported that 1 hour of environmental tobacco smoke acutely increases AIx by 16% (Barnoya, 2005). Environmental tobacco smoke exposure also produced a marked change in the aortic waveform, probably through a primary toxicity induced by tobacco in the vascular tree. Enhanced arterial wave reflection, because of a change in peripheral vascular reflection site, and/or augmented PWV, can generate an increase in AIx (Argacha et al., 2008). A study reported that pulse transit time was decreased by tobacco use, suggesting that the intensified arterial wave reflection in the aorta could be explained by a reduction in vessel compliance after tobacco smoke exposure (Argacha et al., 2008). Although many studies have demonstrated a positive and independent association between AIx and smoking (Argacha et al., 2008; Barnoya, 2005; Janner et al., 2011; Markus et al., 2013; Tsuru et al., 2016), the underlying mechanism are not well understood.

2.6 Lipids

Lipids pathogenesis and hypertension

Dyslipidemia is a major risk factor for CVD, while several studies have reported that low levels of HDL-C, or also extremely high level, carry a high risk of CVD and high mortality (Madsen et al., 2017; Scandinavian Simvastatin Survival Study Group, 1994; Stone et al., 2014). An elevated serum triglyceride level is also a risk factor for CVD (Nordestgaard et al., 2007; Sarwar et al., 2007). Hypertension and dyslipidemia are both indicators of the metabolic syndrome, a condition that includes increased BP, elevated blood glucose level, excess abdominal body fat, and abnormal cholesterol and triglyceride levels. The pathogenesis of metabolic syndrome and hypertension are not well understood, but endothelial dysfunction may play a major role in their pathophysiology (Oparil et al., 2003; Rajendran et al., 2013). A meta-analysis reported that plasma triglyceride level, independent of HDL-C, is a risk factor for CVD in a population-based prospective studies (Hokanson & Austin, 1996). Usually, the primary focus in the research on dyslipidemia has been on the dominant role of LDL-C. Unquestionably, the benefits of lowering LDL-C in the

prevention of CVD are well documented (Cholesterol Treatment Trialists' (CTT) Collaborators, 2012; F. Lamarche et al., 2018). LDL contains esterified cholesterol and triglycerides in their hydrophobic cores (Sommer et al., 1992). Laaksonen et al. reported that the odds ratio of developing hypertension during 11 years of follow-up was 1.29 for LDL-C, 0.68 for HDL-C, and 1.47 for triglyceride content, respectively, in 311 middle-aged men who were not hypertensive at baseline (Laaksonen et al., 2008). A follow-up study with 1039 subjects who were initially nondiabetic and nonhypertensive suggested that the risk factors for atherosclerosis, such as triglycerides, also predicted the development of hypertension (Haffner et al., 1996).

Previous studies have reported well-established pathophysiologic pathways connecting hypertension and dyslipidemia to increased risk of atherothrombosis. The above disease states may share common mechanisms partially overlapping to other cardiovascular risk factors (Figure 7). These views have been supported by many lipid-lowering trials, reporting that following treatment with statins, there has also been a moderate but statistically significant reduction in BP (Lamarche et al., 2018; Tocci et al., 2017), reduced arterial stiffness (Upala et al., 2017) and improved endothelial function (Katsiki et al., 2018). The heightened activity of the renin-angiotensin system that plays an important role in hypertension, might also be activated in dyslipidemia, leading to endothelial dysfunction, inflammation, and thrombosis (Oparil et al., 2003; Rajendran et al., 2013).

As mentioned above in section 2.1.2, LDL-C can impair endothelium-dependent vasodilatation, and the underlying mechanisms being reduced nitric oxide bioavailability due to both a decrease in synthesis and an increase in degradation. LDL-C also increases vascular production of reactive oxygen species and enhances responses to vasoconstrictors like Ang II (Cominacini et al., 2001; Nickenig, 2002; Nickenig et al., 1997; Vita et al., 1990). Hence, impaired endothelium-mediated vasomotion in the resistance vessels would lead to elevated BP via increased SVR. Another key feature of atherosclerosis pathophysiology is recruitment of inflammatory cells into the vascular wall. Following damage to the arterial wall, platelet adhesion and aggregation lead to the activation of the coagulation cascade. The first step of the coagulation cascade in thrombosis is activation of tissue factor, and this factor is present in the lipid-rich component of the atherosclerotic plaques in humans (Toschi et al., 1997).

Atherogenic index of plasma and cardiovascular risk

The logarithm of the plasma concentration of triglyceride to HDL-C ratio is called the atherogenic index of plasma (AIP) (Dobiásová & Frohlich, 2001; Tan et

al., 2004). Previous studies have reported that AIP is a strong marker to predict the risk of atherosclerosis and CVD (Cure et al., 2018; Dobiášová et al., 2011; Dobiásová & Frohlich, 2001; Tan et al., 2004). Dobiasova et al. investigated subjects with various risks of atherosclerosis and reported that AIP directly correlated with the fractional esterification rate of HDL (FERHDL) (r = 0.803), and inversely correlated with LDL particle size (r= –0.776). FERHDL strongly predicted particle size in LDL (r = –818), and the use of lg10(triglycerides/HDL-C) ratio was considered as a useful predictor of plasma atherogenicity, as it reflected the metabolic interactions within the whole lipoprotein complex (Dobiásová & Frohlich, 2001). Remnants of triglycerides-rich lipoproteins (fasting and postprandial) are capable of transferring cholesterol to the arterial intima. The related small dense LDL has enhanced atherogenicity. Cholesterol ester transfer protein-mediated lipid transfer results in cholesterol depletion of HDL, hence resulting in low HDL-C. Insulin resistance, dysglycemia and low-grade inflammation may play a role as well, and all of the above are components of the metabolic syndrome (Chapman et al., 2010; Masuda & Yamashita, 2017).

Though LDL-C has been in the major focus of the connection between plasma lipids and CVD, previous studies have clearly recognized the important roles of low HDL-C and elevated triglycerides in CVD risk, calling this combination an atherogenic dyslipidemia (Kutkiene et al., 2018; Mach et al., 2020). Furthermore, atherogenic dyslipidemia that is accompanied by increased CVD risk, is also often associated with decreased insulin sensitivity beside increased triglyceride and decreased HDL-C concentrations (Alberti et al., 2009; Kutkiene et al., 2018; Valensi et al., 2016).

AIP reflects the actual composition of the lipoprotein spectrum and thus predicts both the cardiovascular risk and effectiveness of therapy. It has been suggested that an AIP value of less than 0.11 is associated with low risk, while values above 0.11 are associated with intermediate risk and value above 0.24 are associated with high risk for CVD (Dobiásová, 2006). AIP can be used in clinical practice as it can be readily calculated from the routine lipid profiles and is associated with the atherogenic lipoprotein size and correlates with findings from coronary angiography (Dobiášová et al., 2011).

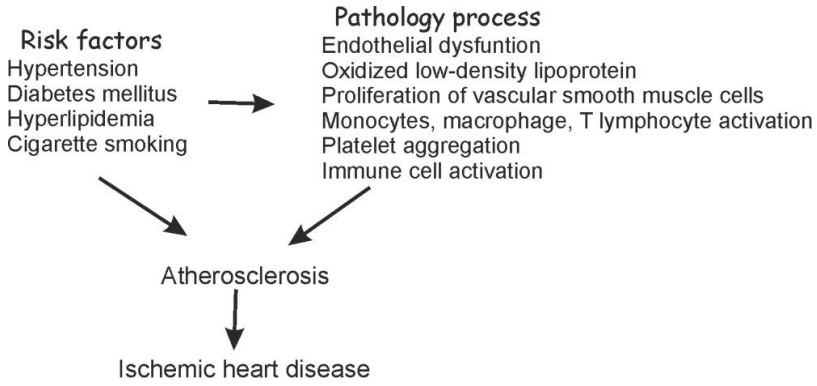

Figure 7. Risk factors and pathological processes of atherosclerosis.

2.7 Non-invasive assessment of arterial stiffness

2.7.1 Pulse wave velocity

The velocity at which the pressure wave, which is generated by the systolic contraction of the heart, transmits along the arterial tree is defined as PWV. The distance travelled by the pulse wave, divided by the travelling time of the pulse wave between the two measurement sites, is the method to calculate PWV (Figure 8). The measurement of PWV is a simple, highly reproducible, non-invasive method to evaluate large arterial stiffness. Large arterial stiffness is also an independent predictor of CVD risk (Vlachopoulos et al., 2010).

The measurement of carotid-femoral PWV is considered the gold standard for the assessment of large arterial stiffness. It provides information about the elastic properties of the arterial system (Vlachopoulos et al., 2010). PWV can be measured from numerous locations, but the measurement of aortic PWV is clinically applicable, since the large arteries are responsible for most of the pathophysiological events related to arterial stiffening (Laurent et al., 2006). However, in rather recent study it has also been reported that brachial-ankle PWV reflects similar characteristics to those of aortic PWV (Tsuchikura et al., 2010).

By the help of a measuring tape over the body surface of the subject, the distance between the two-pulse assessment points is usually measured. To determine the pulse transit time, pulse waveforms are obtained from two locations, which are usually carotid and femoral arteries to define the aortic PWV (Figure 8). The foot of the pulse waveform, which is end of the diastole, is usually acknowledged when determining the pulse transit time, as this part of the waveform is least affected by the wave reflection.

The pulse waveform can be obtained using numerous methods. When placed on the skin, mechano-transducers assess the arterial pressure pulse at different sites simultaneously, and a correlation algorithm is applied to determine the transit time (Asmar et al., 1995). Pressure waveforms can also be subsequently determined from different sites using applanation tonometry, and the timing between the waveform foots is defined using a parallel recorded electrocardiogram. Echo-tracking device or continuous Doppler probes can also be used to determine the distension waveforms. Aortic-to-popliteal PWV can also be determined using CircMon®, which is a whole-body impedance cardiography (ICG_{WB}) device. From the popliteal artery at the knee joint level, the distal impedance is recorded and on the lateral side of the knee, the active electrode is fixed. The reference electrode is placed on the calf below the knee. To measure PWV, the CircMon® software records the time difference between the onset of the decrease in the impedance of the whole-body signal and the signal from the popliteal artery region (Kööbi et al., 2003).

PWV estimation includes potential errors in waveform determination and distance travelled. In obese subjects, the measurement of the distance travelled over the body surface area is usually overestimated, assuming that the aorta is straight. Whereas, for the time determination, the credentials of pulse waveform foot may fail, thus several different points can be picked to indicate the start of the pulse (Chiu et al., 1991). The femoral pressure waveform may be challenging to determine in subjects with the metabolic syndrome, obesity, diabetes or peripheral arterial disease (Van-Bortel, 2002).

The PWV evaluation provides understanding about the elastic properties of the arterial system. The higher PWV corresponds to lower vessel distensibility and compliance and, therefore, to higher arterial stiffness. As the mechanical properties of the arterial walls change along the arterial system, from the large arteries to the periphery, PWV is also affected by these changes. When the pulse wave travels through the arteries, its velocity depends on the vessel. In the case of cardio-popliteal PWV, pressure wave travels along the thoracic aorta, abdominal aorta, iliac artery and the femoral artery. The elastic properties of the thoracic and abdominal aorta

are higher than those compared to the more muscular iliac and femoral arteries (Vlachopoulos et al., 2011). According to an expert consensus on arterial stiffness, PWV is normally 4-5 m/s in the ascending aorta, 5-6 m/s in the abdominal aorta and 8-9 m/s in the iliac and femoral arteries (Laurent et al., 2006). Due to the above reasons, the cardio-popliteal PWV readings are higher than those compared to carotid-femoral PWV.

In the aorta, PWV increases progressively with increasing age due to the loss of elasticity. In contrast, PWV in the femoral artery does not increase markedly with aging (O'Rourke et al., 2002). However, both the aorta and the femoro-popliteal arteries are often affected by atherosclerosis. Due to this fact both these regions are relevant in the study of large arterial pathophysiology (Lowry et al., 2018). Furthermore, the convenient approach to analyze PWV on an artery segment avoids coarse approximations of the distance between the test points, constituting an important advance in PWV assessment. In fact, the carotid–femoral PWV assessment contains the measurement of the distance between carotid and femoral sites over the body surface. The accuracy of this measurement is markedly influenced by either the distance determination protocols or the presence of abdominal obesity. A local PWV measurement technique may hence provide more accurate readings (Laurent et al., 2006; Vlachopoulos et al., 2011).

Increased PWV has been associated with cardiovascular mortality and morbidity. However, the predictive value is significantly lower in the general population when compared to high risk groups such as patients with hypertension, coronary artery disease, or renal disease (Vlachopoulos et al., 2010).

2.7.2 Augmentation index

The AIx is the ratio of the augmentation pressure to PP (Figure 8), and this variable is highly influenced by the large arterial stiffening (O'Rourke & Hashimoto, 2007). AIx is mostly used to express the wave reflection in the central circulation. It is usually recorded non-invasively from the radial artery at the wrist region using applanation tonometry, and the aortic pressure waveform is derived using a pulse wave analysis software. AIx is a widely used variable for the evaluation of central hemodynamics and it is also a moderate indicator of cardiovascular risk (Vlachopoulos et al., 2010).

AIx is influenced by the pressure wave produced from the left ventricle during ventricular ejection, stroke volume, heart rate, and SVR. In addition to the above

factors, other determinants such as height, gender, and age have impact on AIx (Kingwell & Gatzka, 2002; Laurent et al., 2006; Vlachopoulos et al., 2011; Wilenius et al., 2016). Due to these factors, AIx cannot truly be regarded as an indicator of arterial stiffness, but it is more an index of wave reflection that is influenced by arterial compliance. Importantly, Wilkinson et al. reported that there is a linear relationship between AIx and heart rate, with approximately 4% fall in AIx for every 10 beats/min increase in heart rate (Wilkinson et al., 2000). Therefore, AIx corrected to heart rate 75 beats/min has been widely used (Vyssoulis et al., 2010).

The composition of the central wave and PP is presented in Figure 8.

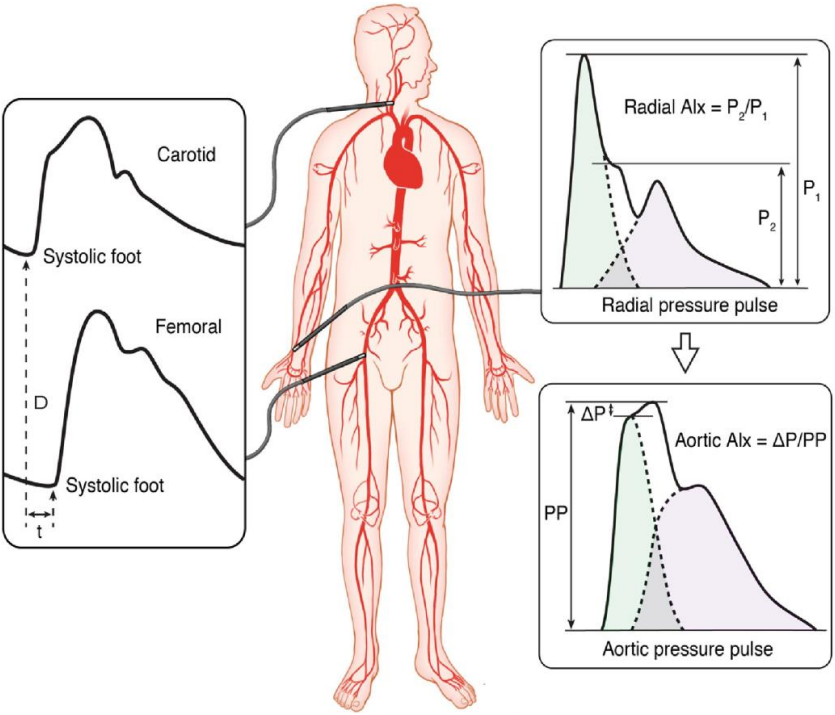

Figure 8. Carotid-femoral pulse wave velocity (cfPWV) and central arterial waveform assessment using radial arterial applanation tonometry. (Left) Waveforms are consecutively recorded from right carotid and femoral arteries using arterial applanation tonometry. cfPWV is estimated as the distance (D) between carotid and femoral sampling sites, divided by the time delay between 2 waveforms. CfPWV=D/t, (m/s). (Right) Central aortic pressure waveform can be derived from arterial applanation tonometry. (1) Use of carotid artery waveform as a surrogate of central pressure due to its proximity to the aorta; or (2) a mathematical transfer function is applied to the radial artery waveform (shown on the right side of the figure), which derives an aortic waveform from the radial waveform. AIx, augmentation index; P1, first systolic pressure peak; P2, second systolic pressure peak; PP, pulse pressure. Reproduced by permission of Canadian Journal of Cardiology (Coutinho, 2014).

2.7.3 Pulse pressure

PP is defined as the difference between systolic and diastolic BP and it is highly influenced by stroke volume and arterial compliance (Dart & Kingwell, 2001).

Although PP is considered as a simple measurement of arterial stiffness, many other factors such as increased wave reflection, play a role influencing the level of PP. The rise in PP can also be secondary to an increase in stroke volume without a change in arterial distensibility (Alfie et al., 1999). PP is directly related to MAP, and with reduction in BP, there is decrease in PP without a parallel decrease in arterial stiffness. PP is also correlated to amplification due to the backward pressure wave. Because of the amplification phenomenon, the amplitudes of PP measured in peripheral arteries are higher when compared to central arteries (Laurent et al., 2006). Use of drugs and other pathophysiological conditions might also play a role in changing the central PP without any alterations in peripheral PP (Laurent et al., 2006; Safar et al., 2013). It is also a well-known fact that young subjects have more compliant arteries than older ones, while arterial compliance diminishes with aging and due to arterial wall stiffening (Safar et al., 2013).

Both peripheral and central PP predict cardiovascular morbidity and mortality, probably due to their close relation with large arterial stiffness (Dart & Kingwell, 2001). An increase in PP has been shown to induce endothelial dysfunction (Ryan et al., 1995), and endothelial dysfunction in turn predisposes to atherosclerosis.

A possible additional clarification for the relationship between PP and cardiovascular end-points is provided by the concept of bidirectionality - this means that elevated PP is both a cause and a consequence of atherosclerosis (Figure 9) (Dart & Kingwell, 2001).

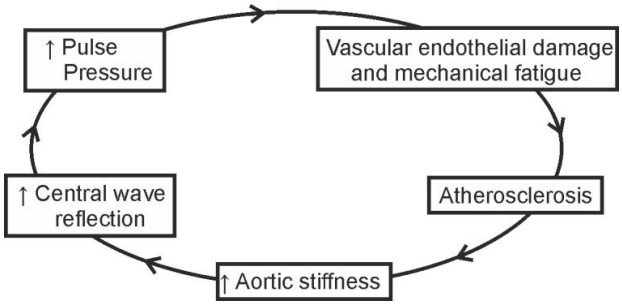

Figure 9. Schematic diagram illustrating the bidirectional relationship between pulse pressure and atherosclerosis. Increased pulse pressure promotes vascular damage, which leads to atherosclerosis, which results in increased stiffness and increased wave reflection, which further amplify pulse pressure. It is unclear which is the initial event in this cycle (Adapted from Dart and Kingwell 2001).

3 AIMS OF THE STUDY

The existing data about the influence of smoking on BP, wave reflections and arterial stiffness has been contradictory (Argacha et al., 2008; Groppelli et al., 1992). Although LDL-C has been linked with elevated BP in some studies, results about the relationship of LDL-C with arterial stiffness have been inconsistent. To my knowledge, the association of plasma AIP with hemodynamic variables has not been previously investigated. Also, rather limited information has existed about the detailed hemodynamic features of PA in the present days. Previous studies in patients with PA have mainly focused on the features of treatment-resistant hypertension, large arterial stiffness, and carotid artery IMT. In addition, only few studies have examined the hemodynamic differences between PA and EH patients, who were carefully matched for confounding factors such as age, sex, body mass index (BMI), and intake of various medications. Here we examined the hemodynamic features associated with smoking, LDL-C, plasma AIP, EH and PA.

The specific aims of the study were:

1. To study differences in hemodynamics between present, previous, and never smokers and expand understanding about the long-term effects of smoking on the cardiovascular system in the study groups. A passive head-up tilt was included in the study, as possible changes in the hemodynamic profile might be more apparent during upright posture (Study I).
2. To investigate the association of LDL-C with hemodynamic variables that could potentially explain possible deviations in BP related with different plasma concentrations of LDL-C (Study II).
3. To examine the association of AIP with hemodynamic variables and more specifically test the hypothesis whether AIP is related to arterial stiffness (Study III).
4. To examine putative differences in hemodynamics between patients with medicated PA, medicated EH, never-medicated EH, and normotensive controls. An additional focus was on the hemodynamic significance of unawareness of hypertension (Study IV).

4 SUBJECTS AND METHODS

4.1 Study subjects

All the participants were volunteers from an ongoing study with a primary aim of investigating the hemodynamic changes in primary and secondary hypertensive subjects versus normotensive controls (DYNAMIC study; Eudra-CT 2006-002065-39, ClinicalTrials.gov identifier NCT01742702). The participants for the study were enrolled by announcements distributed to the personnel of Tampere University and Tampere University Hospital, patients treated at Tampere University Hospital, to the clients of Varala Sports Institute, and local occupational healthcare providers. In addition, two announcements were published in a local newspaper. Those volunteers who agreed to participate were recruited in the order in which they contacted the research nurses. The participant recruitment for study I, II and III are also presented in the form of a study flow-chart (Figure 10).

By the time of study IV analyses, patients with confirmed biochemical aldosteronism from all five University clinics in Finland were subjected to adrenal vein sampling in Tampere University Hospital. These patients, too, were invited to participate in non-invasive hemodynamic recordings. The most important exclusion criteria for study I-III were subjects using BP- or lipid lowering medications, other forms of secondary hypertension, and subjects with history of coronary artery disease, stroke, heart failure, valvular heart disease, chronic kidney disease, alcohol or substance abuse, psychiatric illnesses, or heart rhythm other than sinus. Thus, in study IV secondary hypertension forms other than PA were excluded.

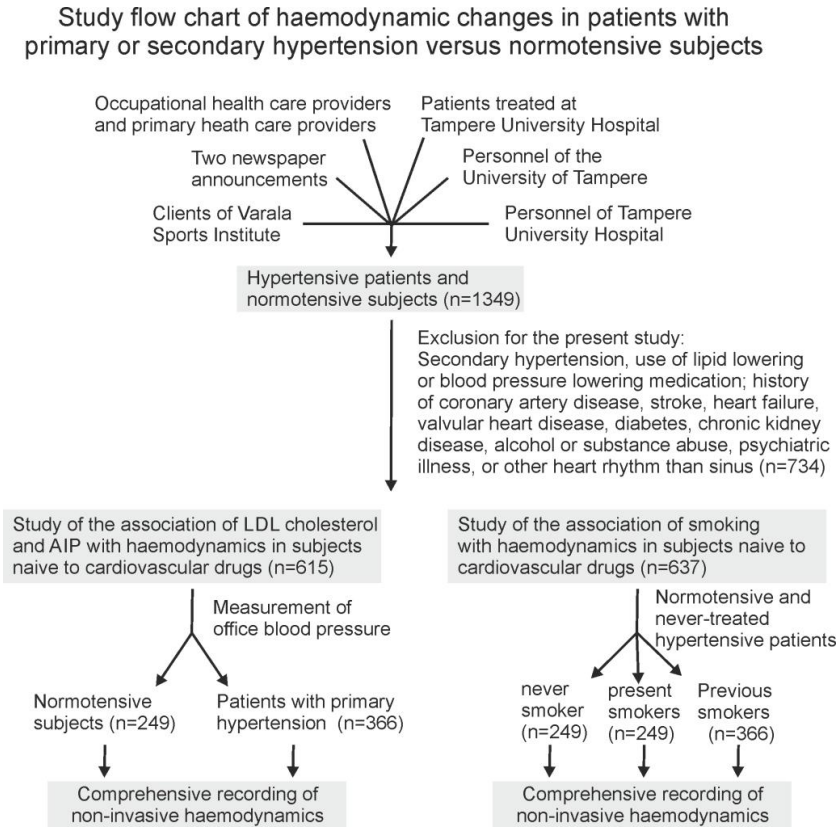

Figure 10. Flow chart of studies I, II and III.

According to the guidelines of the European Society of Hypertension, office BP measurements and laboratory analyses for elevated BP were performed (Williams et al., 2018). Never-medicated EH patients (study I-IV) were defined as elevated office BP (≥140/90 mmHg) (Williams et al., 2018). In study IV, the diagnosis of PA was based on screening and confirmatory testing (Young, 2019). Screening of aldosteronism (n=130) was defined as: 1) serum aldosterone (pmol/l) to plasma renin activity (ng/ml/h) ratio >750, with serum aldosterone concentration ≥280 pmol/l (Funder et al., 2016; Young, 2019); 2) or serum aldosterone (pmol/l) to plasma renin concentration (mU/l) ratio >30, with serum aldosterone concentration ≥280 pmol/l (Juutilainen et al., 2014; Ma et al., 2018; Young, 2019).

Most of the patients (n=82) enrolled in study IV were hypokalemic at the time of recruitment (Table 1). Confirmatory testing was performed in the majority (n=113) of the PA patients, showing urine aldosterone excretion >33 nmol/day during oral sodium loading (Funder et al., 2016; Young, 2019). Seven subjects with borderline screening tests for PA were included, as they were hypokalemic (plasma potassium <3.3 mmol/l), had elevated serum aldosterone (range 513-1290 pmol/l) in control samples, and showed elevated 24-hour urine aldosterone excretion (range 44-132 nmol/day) during oral sodium loading (Table 1) (Young, 2019).

A physician examined all study participants. Medical history, lifestyle habits, dietary supplements, medicines, smoking status, and alcohol consumption as standard drinks (~12 grams of absolute alcohol) per week were documented. Leg edema was classified clinically as no edema, mild, moderate, and edema extending to the proximal parts of the calves (severe).

In study I, with age range of 19-72 years, altogether 637 normotensive subjects and never-medicated hypertensive patients were included. These participates were divided into three groups: 1) never smokers, n=365, 2) present smokers, n=81, and 3) previous smokers, n=191.

In studies II and III, 615 normotensive and never-medicated EH subjects, aged 19-72 years, were included. In these two studies, as based on office BP measurements on a single occasion, 249 (40.5%) of the subjects were normotensive and 366 (59.5%) were hypertensive. For the graphic illustrations, subjects were divided into age- and sex-adjusted LDL-C quartiles (Q): Q1, n =153; Q2, n=158; Q3, n=148; and Q4, n=156 in study II. Similarly, in study III the participants were divided into age- and sex-adjusted AIP tertiles: Tertile 1, n=202; Tertile 2, n=208; and Tertile 3, n=205.

Table 1. Laboratory characteristics of 130 patients with primary aldosteronism

	Mean	95% confidence interval		Number*	Normal range
		Lower bound	Upper bound		
Lowest plasma potassium (mmol/l)	3.14	3.06	3.21	130	3.3-4.8
Serum aldosterone (pmol/l)	822	720	925	130	<520
Plasma renin activity (ng of Ang I/ml/h)	0.32	0.24	0.42	79	1.5-5.7
Plasma renin concentration (mU/l)	13.3	9.2	17.4	51	4.4–46
Ratio of aldosterone to renin activity	3234	2727	3740	79	<750
Ratio of aldosterone to renin concentration	119	79	158	51	<30
Urinary aldosterone (nmol/24h)	100.5	55.3	145.6	113	<40
Urinary sodium (mmol/24h)	223	205	242	89	130-240
Urinary potassium (mmol/24h)	113	104	122	78	60-90

*Number of subjects with available result of the laboratory determination.

Table 2. Number of subjects using beta blockers or beta+alpha blockers

Medication	Medicated essential hypertension (n=130)		Medicated primary aldosteronism (n=130)	
	Number (n=69)	Mean dose (mg)	Number (n=70)	Mean dose (mg)
Bisoprolol	38	10 (3)	49	7 (1)
Metoprolol	19	89 (12)	11	140 (19)*
Propranolol	3	37 (22)	1	10
Betaxolol	2	15 (5)	0	0
Nebivolol	2	5 (0)	2	5 (0)
Atenolol	1	100	1	100
Carvedilol	4	25 (0)	5	30 (5)
Labetalol	0	0	1	100

Doses are shown as mean (standard error of mean); *P<0.05 vs. medicated essential hypertension.

In study IV, 520 subjects recruited during 2006-2019, were included from the ongoing DYNAMIC study. The study groups were: 1) normotensive controls; 2) never-medicated EH; 3) medicated EH; and 4) medicated PA. In this study, the three hypertensive groups were matched for age (53 years), sex (84 male/ 46 female) and BMI (30kg/m^2). Additionally, the medicated EH and medicated PA groups were matched for the use of beta blockers and/or beta+alpha blockers (Table 2). The normotensive control groups were matched for sex and had a mean age of 48 years and BMI of 27 kg/m^2.

Some medications were regularly used among the study participants. Altogether 247 participants (39%) in study I, 230 (37.4%) in studies II and III, and 362 (69.7%) participants in study IV used some medications. The most used medications were systemic estrogen, progestin, or their combination (for contraception or hormone replacement therapy) by 78 females in study I, and by 76 females in studies II and III. Forty-one subjects were taking antidepressants, 18 antihistamines, 17 inhaled corticosteroids, while 22 euthyroid subjects were on a stable dose of thyroid hormone in studies I-III. In study IV, the medications used among the participants were specifically listed, and these are presented in Table 3 and Table 4.

Table 3. Number of subjects using anti-hypertensive or lipid-lowering medications

	Normotensive controls (n=130)	Never-medicated essential hypertension (n= 130)	Medicated essential hypertension (n= 130)	Medicated primary aldosteronism (n= 130)
Number of antihypertensive medications (median)	0	0	2	3
ACE inhibitor	0	0	42	22*
Angiotensin II receptor blocker	0	0	52	65
Beta blocker	0	0	65	64
Beta and alpha blocker	0	0	4	6
Calcium channel blocker	0	0	60	115*
Thiazide	0	0	53	15*
Furosemide	0	0	6	5
Spironolactone	0	0	7	4
Amiloride	0	0	8†	0
Nitrate	0	0	2	0
Moxonidine	0	0	5	18*
Minoxidil	0	0	0	1
Potassium supplement	0	1	4	82*
Prazosin	0	0	6	23*
Statin	5	3	46	36
Ezetimibe	1	0	1	0
Fibrate	0	0	0	1

Statistic is only about the differences between primary aldosteronism vs. medicated essential hypertension; spironolactone was previously used by 51 patients with PA, and this medication was discontinued in 47 of them 6 weeks before the recordings; *$p<0.05$; †in 7/8 combination with hydrochlorothiazide.

Table 4. Number of subjects using other than anti-hypertensive or lipid-lowering medications in study IV

	Normotensive controls (n= 130)	Never-medicated essential hypertension (n= 130)	Medicated essential hypertension (n= 130)	Medicated primary aldosteronism (n= 130)
Estrogen, progestin, or combination (contraception or hormone replacement)	12	8	6	2*
Acetylsalicylic acid	3	6	22*†	25*†
Other platelet inhibitors	0	0	2	4
Vitamin D	8	8	16	18
Calcium supplements	2	3	10	8
Proton pump inhibitors	3	9	7	10
Asthma medications	5	5	4	6
Metformin	1	1	15*†	21*†
Sulfonylureas	0	0	2	1
Dipeptidyl peptidase 4 inhibitors	0	1	3	10†
Incretin mimetics	1	0	2	4
Sodium/glucose cotransporter 2 inhibitors	0	0	2	2
Insulin	0	1	6	10†
Selective serotonin or serotonin–norepinephrine reuptake inhibitors	3	12	8	9
Other antidepressants	1	2	7	2
Antirheumatic agents	5	4	4	9
Non-steroidal anti-inflammatory drugs	2	1	2	4

Antihistamines	2	3	5	4
Alpha-adrenergic blocking agents	2	1	4	3
5α-reductase inhibitors	0	0	2	4
Warfarin	0	0	6	4
Allopurinol	0	2	6	1
Benzodiazepines	1	4	2	2
Urinary incontinence medications	0	0	2	4
Pregabalin or gabapentin	1	1	0	3
Antiepileptics	1	0	1	2
Low-dose testosterone	0	0	1	2
Timolol, topical for glaucoma	2	2	0	0
Other topical glaucoma medications	2	2	1	0
Coxibs	0	1	1	1
Varenicline or bupropion	0	1	2	0
Antiarrhythmic agents	1	0	1	0
Bisphosphonate	0	0	0	1
Thyroxin	5	5	11	8

*$p<0.05$ vs. normotensive controls; †$p<0.05$ vs. never-medicated essential hypertension.

4.2 Hemodynamic measurements

4.2.1 Measurement protocol

Trained nurses in a temperature-controlled laboratory performed hemodynamic recordings. All the participants were instructed to abstain from use of caffeine-containing products, smoking, or eating heavy meals for ≥4 hours, and from alcohol consumption for at least >24 hours prior to the investigation. All the participants were rested in supine position on a tilt-table with the electrodes placed on the body surface, the tonometric sensor on the left radial pulsation, and the oscillometric brachial cuff to the right upper arm. The left arm with the tonometric wrist sensor was abducted to 90 degrees in an arm support, which held the extended arm steady and kept the measurement probes at the heart level both supine and upright.

To familiarize the participants with the tilt protocol, an introductory head-up tilt was performed before the actual measurements. For study I, the measurement protocol consisted of one 5-minute period continuous data capturing in supine position and second 5-minute period continuous data capturing in upright position. Whereas for studies II-IV, 5-minute periods with continuous data capturing in supine positions were included. The mean values of each 1-minute period of recording were calculated for the statistical analyses. In previous study, the good repeatability and reproducibility of the supine and upright measurement protocol has been demonstrated (Tahvanainen et al., 2009).

4.2.2 Pulse wave analysis

Continuous pulse waveform and radial BP were captured from the radial artery pulsation by an automated tonometric sensor (Colin BP-508T, Colin Medical Instruments Corp., USA). Radial BP signal was calibrated twice during the 5-minute period by contralateral right brachial BP measurements. Aortic BP, AIx (augmented pressure/pulse pressure* 100) and AIx@75 were determined using the SphygmoCor software (SpygmoCor PWMx®, AtCor medical, Australia) (Chen et al., 1997; Wilenius et al., 2016).

4.2.3 Whole-body impedance cardiography

A whole-body impedance cardiography device (CircMon®, JR Medical Ltd., Tallinn, Estonia) recorded changes in body impedance during cardiac cycles. This device was used as described previously to determine beat beat-to-beat heart rate, stroke volume, cardiac output, ECW, and PWV (Kööbi et al., 1997, 2003; Tahvanainen et al., 2012; Tikkakoski et al., 2013). When appropriate, the values were normalized to body surface area and they were expressed as stroke index, cardiac index, systemic vascular resistance index (SVRI). SVR was calculated from the tonometric BP and cardiac index measured by CircMon®: Normal central venous pressure (4 mmHg) was subtracted from mean arterial pressure, and the value was divided by cardiac output. The stroke volume values were measured using CircMon® and previous results obtained with this method correlated well with 3 dimensional ultrasound (Koskela et al., 2013). The cardiac output values also correlate well with values measured using thermodilution (bias 0.00 l/min, 95% confidence interval (CI) -0.26 to 0.26) and direct oxygen Fick method (bias -0.32 l/min, 95% CI -0.69 to 0.05) (Kööbi et al., 1997).

The ECW volume measured by the CircMon® was evaluated by the formula ECW = k*(H^2/Z), coefficient k (Ω*cm) derived from blood resistivity and the relation between the distance of voltage electrodes. H is body height (cm), and Z is the recorded impedance of the body. The bioimpedance-derived ECW volume correlates well with ^{51}Cr-EDTA dilution based ECW measurement (n=15, r=0.74, bias 0.2±1.1 l, mean±SD) (Kööbi et al., 2000). The ECW balance is calculated as $ECW/ECW_{predicted}$. The formula for predicted ECW is 2.4 * (0.0236 * $H^{0.725}$ * $Weight^{0.423}$ – 1.229) in males and 2.6 * (0.0248 * $H^{0.725}$ * $Weight^{0.423}$ – 1.9549) in females (Albert, 1971; Nadler et al., 1962; Tishchenko, 1973). In study IV, the ECW balance of the normotensive groups was adjusted to 1.0.

To measure PWV, the CircMon® software records the time difference between the onset of the decrease in the impedance of the whole-body signal and the signal from the popliteal artery region (Kööbi et al., 2003). PWV was measured between aortic and popliteal level. PWV is calculated from the time difference and the distance between the electrodes. The placements of the electrodes are presented in Figure 11.

The whole-body impedance cardiography systematically overestimates PWV, thus a validated equation was applied to calculate values that correspond to the ultrasound method (PWV = PWVimpedance * 0.696 + 0.864) (Kööbi et al., 2003).

With the above equation, the PWV values recorded using CircMon® show good correlations with values measured using SphygmoCor® (r=0.82, bias 0.02 m/s, 95% CI -0.21 to 0.25) (Wilenius et al., 2016) or ultrasound (r=0.91) (Kööbi et al., 2003).

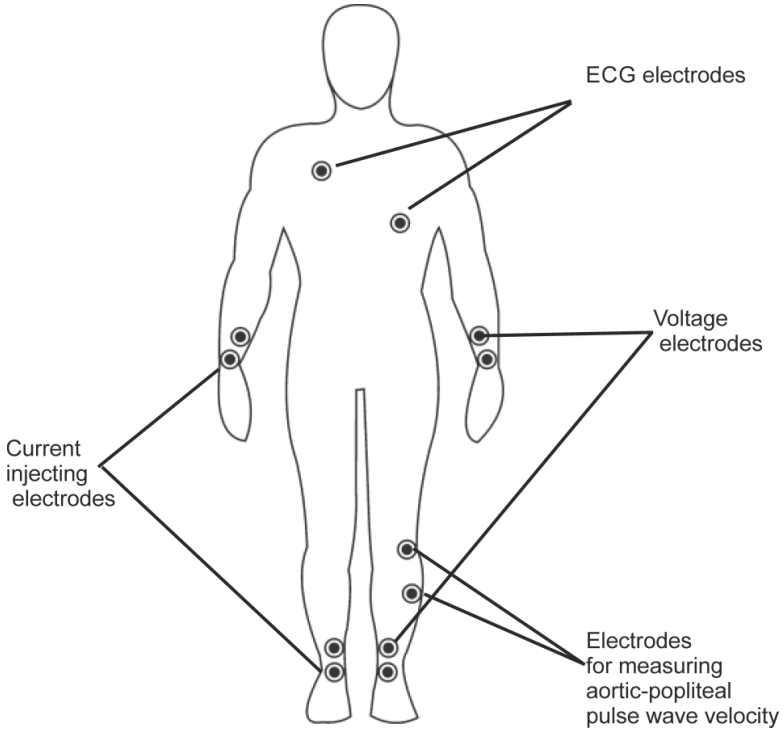

Figure 11. Placements of the electrodes for the impedance cardiography device recordings (Modified from CircMon® manual).

4.3 Laboratory tests

Blood and urine sampling were obtained from the antecubital vein after about 12 hours of fasting. Plasma sodium, potassium, glucose, cystatin-C, total cholesterol, HDL-C, LDL-C, triglyceride, C-reactive protein (CRP), uric acid, and creatinine concentrations, and urine sodium and potassium concentrations, were determined using Cobas Integra 700/800 (F. Hoffmann-Laroche Ltd, Basel; Switzerland) or

Cobas 6000, module c501 (Roche Diagnostics, Basel, Switzerland), and blood cell count by ADVIA 120 or 2120 (Bayer Health Care, Tarrytown, NY, USA). In some cases LDL-C was calculated using the Friedewald formula (Friedewald et al., 1972). Insulin was determined using electrochemiluminescence immunoassay (Cobas e 411, Roche Diagnostics).

Initially, plasma renin activity was determined using radioimmunoassay (DiaSorin, Saluggia, Italy), but this method was replaced by the analysis of plasma direct renin concentration (LIAISON immunoanalyzer, DiaSorin, Saluggia, Italy) (Juutilainen et al., 2014). As described in previous study, plasma and urine aldosterone was quantified using liquid chromatography–mass spectrometry (LC–MS/MS) on API 4000 (Turpeinen et al., 2008). To exclude patients with renal diseases, automated urine dipstick refractometer analysis was performed (Siemens Clinitec Atlas or Advantus, Siemens Healthcare GmbH, Erlangen, Germany). For evaluation of insulin sensitivity, the quantitative insulin check index (QUICKI)(Katz et al., 2000) was calculated. Glomerular filtration rate (eGFR) was estimated using the CKD-EPI creatinine-cystatin C formula (Inker et al., 2012). Left ventricular mass was estimated using the Cornell voltage QRS duration product from a standard 12-lead ECG, and the cut-point for left ventricular hypertrophy was 2440 mm x ms (Mancia et al., 2013).

4.4 Statistical analyses

The demographic and laboratory data were analyzed using analysis of variance (ANOVA), and the Bonferroni correction was applied in the post-hoc analyses for studies I-IV. The homogeneity of variances was tested with the Levene's test. Spearman's correlations (rS) were calculated, and the variables that correlated with the variable of interest with $p<0.1$ were included in the regression analyses, as appropriate (Study I-IV).

Due to skewed distribution, PWV and triglycerides for study I, CRP and PWV for study II, PWV for study III were corrected by lg_{10}-transformation before statistical analyses. Alcohol consumption was treated as a series of discrete variables and were assigned with a score of either 0 or 1; cut-points for women 0, 1–7, 8–14, and above 15 doses per week; for men 0, 1–14, 15–24, and above 25 doses per week, according to the prevailing Finnish Guidelines (Treatment of Alcohol Abuse. Current Care Guideline by the Finnish Medical Society Duodecim and the Finnish Society of Addiction Medicine, 2015).

In study I, the variable values were expressed as means and standard error of the mean (SEM), or median [25th to 75th percentile]. The hemodynamic differences between the groups in either supine or upright position were examined using ANOVA for repeated measures. One-minute means of minutes 1 to 5 in each phase were used in ANOVA for repeated measures. The analyses were adjusted for age, sex, BMI, use of alcohol as standard doses per week, LDL-C. In analyses concerning PWV, systolic BP was also included for the adjustment. Linear regression analysis with the enter method was used to examine the effect of gender and the hemodynamic variables on the level of AIx in supine and upright positions.

In Study II and III, continuous variables were expressed as the mean and standard deviation (SD), SEM, or 95% CI of the mean. In study II, baseline characteristics were shown as age- and sex-adjusted quartiles of LDL-C. For the illustrations, the hemodynamic differences between the quartiles were examined using ANOVA for repeated measures adjusted for age and sex. In study the III baseline characteristics were presented as age- and sex-adjusted tertiles of AIP and for graphics, the hemodynamic differences between the tertiles were examined using one-way ANOVA. To evaluate the associations between age, sex, BMI (for systemic vascular resistance index, BMI was substituted by height and weight), smoking status, alcohol consumption, QUICKI, plasma CRP, sodium, uric acid, LDL-C, eGFR for study II and III as an independent variables, a multiple regression analysis with stepwise elimination was applied. Additionally, to the above variables, HDL-C and triglycerides were included for study II, and AIP for study III as independent variables.

In study II, the dependent variables in model 1 were aortic systolic and diastolic BP, aortic pulse pressure, AIx, PWV and SVRI. For model 2, the variables used were model 1+PWV (independent variables) for aortic systolic and diastolic BP, aortic pulse pressure (dependent variables); for AIx (dependent variable): model 1+heart rate, SVRI, and PWV (independent variables); for PWV (dependent variable): model 1+aortic systolic BP (independent variables).

In study III, for model 1 the dependent variables were radial systolic and diastolic BP, heart rate, and PWV. Heart rate was also included as an independent variable in the case of PWV. The variables in the model 2 comprised of model 1+PWV (independent variables) for radial systolic and diastolic BP and heart rate (dependent variables), and model 1+aortic systolic BP (independent variables) for PWV (dependent variable).

In study IV, the results were presented as mean and SEM, or as mean and 95% CI of the mean. Generalized estimating equation analysis was used for studying the

hemodynamic differences between the groups. In this method, linear scale response was applied, and the autoregressive option was chosen for the correlation matrix, as successive serial measures of hemodynamic variables in individual participants are autocorrelated. The groups presented with differences in age, BMI, proportions of diabetic subjects, eGFR; plasma uric acid, triglycerides, HDL-C, LDL-C, and glucose. The PWV analyses were additionally adjusted for mean aortic pressure (Townsend et al., 2015). No adjustments were performed for plasma sodium, potassium and CRP, as these variables probably reflect true effects of aldosterone (Monticone et al., 2018; Young, 2019). In analyses concerning ECW volume and balance, lean body mass was used instead of BMI , as lean body mass is more suitable for normalization of body fluid volumes (Boer, 1984).

The statistical analyses were performed utilizing SPSS version 22.0 (Study I), version 25.0 (Study II and III) and version 26.0 (study IV) (IBM SPSS Statistics, Armonk, NY, USA), and $p<0.05$ was considered statistically significant.

4.5 Ethical aspects

All the participants gave written informed consent. The study complies with the declaration of Helsinki and was approved by the ethics committee of the Tampere University Hospital (study code R06086M). The studies I-IV are part of an ongoing investigation on hemodynamics with the primary aim to examine hemodynamics in primary and secondary hypertension (Eudra-CT 2006-002065-39, ClinicalTrails.gov NCT01742702).

5 RESULTS

5.1 Hemodynamic response to passive head up tilt, study I

5.1.1 Population and laboratory characteristics

In study I, the mean age did not differ between the study groups, while in the previous smokers there was slightly lower proportion of female subjects compared to never smokers (Table 5). BMI was higher in previous smokers when compared to never and present smokers. Office systolic BP was about 6 mmHg higher in previous smokers than present smokers, while diastolic BP was higher in previous smokers compared to never and present smokers.

The median number of cigarettes consumed by present and previous smokers were 21900, and 10 years was the median abstinence from smoking in previous smokers. Present smokers consumed slightly higher amount of alcohol per week than the other groups (Table 5). Levels of LDL-C and triglycerides were higher in present and previous smokers than never-smokers. Fasting plasma glucose concentration was higher in the previous smokers compared to never smokers (Table 5.).

Table 5. Basic Clinical Characteristics and Laboratory Results (Study I)

	Never smoker (n=365)	Present smoker (n=81)	Previous smoker (n=191)
Male / female	164 / 201	43 / 38	107 / 84*
Age (years)	44.2 (0.6)	44.2 (1.3)	46.7 (0.8)
Body mass index (kg/m^2)	26.2 (0.2)	26.4 (0.5)	28.0 (0.3)*†
Office systolic BP (mmHg)	139.6 (1.1)	136.9 (2.4)	144.4 (1.6)†
Office diastolic BP (mmHg)	88.7 (0.6)	87.5 (1.4)	91.8 (0.9)*†
Cigarettes / day	0	5 [2-12]*	10 [3-19]*†
Smoking duration (years)	0	15 [7-25]*	10 [3.0-16.5]*†
Total number of cigarettes	0	21900 [7300-87600]*	21900 [4562-79387]*
Smoking abstinence (years)	n.a.	0	10 [3-20]†
Alcohol (standard drinks/week)	2.0 [0.0-4.0]	5.5 [2.0-13.0]*	3.0 [1.0-9.5]*†
Estimated GFR (ml/min/1.73m^2)	99.3 (0.8)	98.5 (1.6)	96.4 (1.0)
Hemoglobin (g/L)	143.0 (0.7)	146.0 (1.2)	145.6 (0.8)
Sodium (mmol/l)	140.4 (0.1)	140.5 (0.2)	140.3 (0.1)
Potassium (mmol/l)	3.81 (0.01)	3.79 (0.02)	3.81 (0.02)
C-Reactive Protein (mg/l)	1.5 (0.1)	1.8 (0.3)	2.0 (0.3)
Triglycerides (mmol/l)	0.97 [0.68-1.34]	1.18 [0.86-1.75]*	1.18 [0.86-1.58]*
HDL cholesterol (mmol/l)	1.60 (0.02)	1.52 (0.04)	1.54 (0.03)
LDL cholesterol (mmol/l)	2.91 (0.05)	3.21 (0.11)*	3.26 (0.07)*
Glucose (mmol/l)	5.39 (0.03)	5.54 (0.08)	5.54 (0.04)*
Insulin (mU/l)	9.04 (1.15)	8.86 (0.84)	9.02 (0.47)
QUICKI	0.361 (0.002)	0.355 (0.004)	0.352 (0.003)#

Results shown as mean (standard error of mean) or median [25th to 75th percentile]; n.a., not applicable; GFR, glomerular filtration rate (CKD-EPI cystatin-C creatinine formula); QUICKI, quantitative insulin sensitivity check index; *P<0.05 vs. never smoker; †P<0.05 vs. present smoker (#p=0.059 vs. never smokers).

5.1.2 Blood pressure and effects of smoking

Radial and aortic systolic and diastolic BP was higher in previous smokers than present smokers and never smokers in unadjusted analyses ($p \leq 0.023$ for all comparisons) (Figures 12A-D). However, when adjusted for confounding factors such age, sex, BMI, LDL-C, and use of alcohol, the differences in BP values between the groups were not significant (adjusted figure presented as Figures 12E-F).

5.1.3 Hemodynamic effects associated with smoking

In the text below regarding study I, only the results of the adjusted analyses are referred to, while the unadjusted statistics are also shown in the figures.

Heart rate (Figure 13A) and ejection duration (Figure 13B) were not different between the study groups. Both supine and upright forward wave amplitude (FWA) was lower in present smokers than in never smokers ($p \leq 0.042$) (Figure 13C). Supine aortic reflection time was not different between the groups, but during upright position aortic reflection time was shorter in present smokers than in previous smokers ($p=0.049$) (Figure 13D).

In the supine position, the deviations between individual groups were not significant for AIx (Figures 13E). However, in the supine position the heart rate adjusted AIx@75 was increased ($p=0.045$) in present smokers versus never smokers (Figure 13F). In the upright position, both AIx and AIx@75 were higher in present smokers than in never smokers ($p \leq 0.003$), whereas AIx@75 was also higher in present smokers than in previous smokers ($p=0.031$, Figures 13E-F).

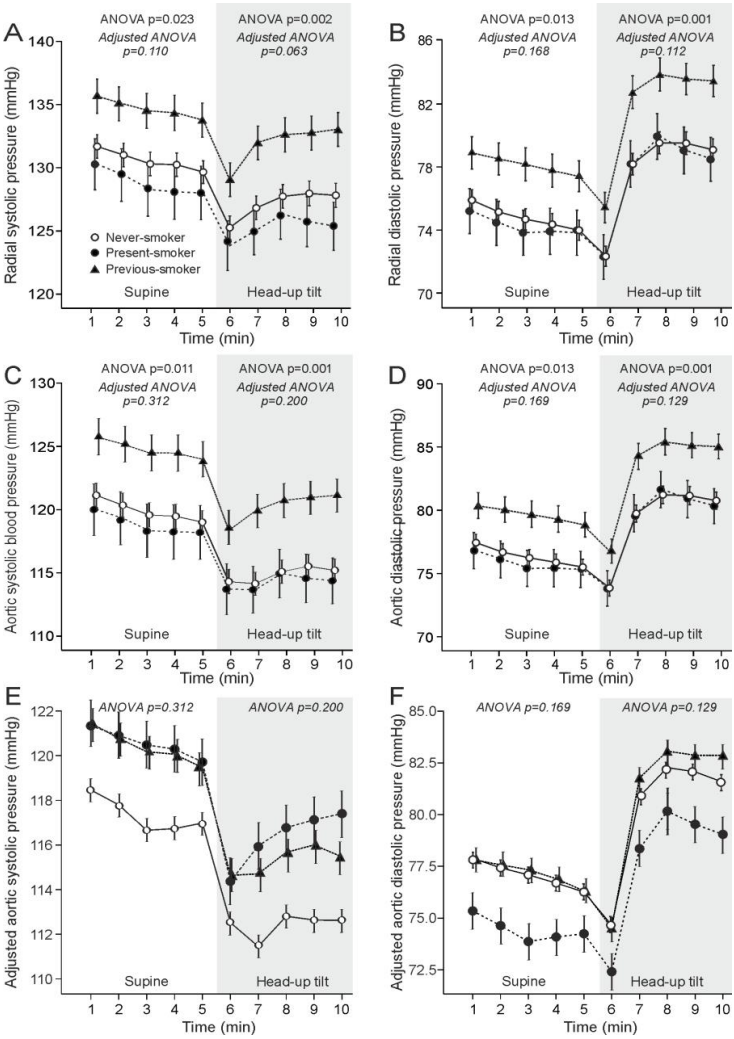

Figure 12. Supine and upright radial systolic (**A**) and diastolic (**B**) blood pressure, and aortic systolic (**C**) and diastolic (**D**) blood pressure; adjusted aortic systolic (**E**) and diastolic (**F**) blood pressure in never smokers (n = 365), present smokers (n = 81), and previous smokers (n = 191); mean ± standard error of the mean; ANOVA results from unadjusted analyses (plain text) and from analyses adjusted for age, sex, BMI, LDL-C, and alcohol use (italic) are shown (Study I).

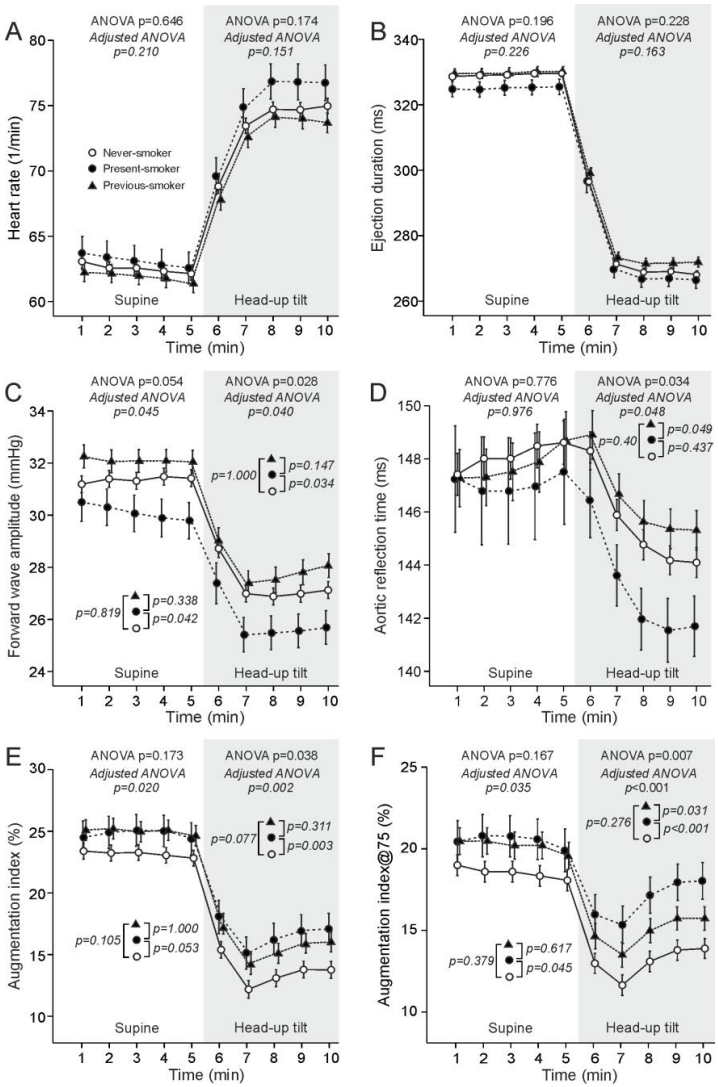

Figure 13. Heart rate (**A**), ejection duration (**B**), forward wave amplitude (**C**), aortic reflection time (**D**), augmentation index (**E**), and augmentation index adjusted to heart rate of 75 beats per minute (**F**) in never smokers (n=365), present smokers (n=81), and previous smokers (n=191); mean ± standard error of the mean; ANOVA results from unadjusted (plain text) and adjusted (italic) analyses are shown (Study I).

Stroke index in the supine position was higher in present smokers when compared with never smokers ($p=0.009$) and previous smokers ($p=0.001$). In the upright position the values were higher in present smokers that previous smokers (Figure 14A). Cardiac index was increased in present smokers versus previous smokers both supine and upright ($p\leq0.016$), while cardiac index was lower in previous smokers than in never smokers in the upright position ($p=0.032$, Figure 14B).

Supine but not upright SVRI was lower in present smokers ($p=0.041$) versus never smokers. Upright but not supine SVRI was increased in previous smokers versus never smokers ($p<0.014$). Both supine and upright SVRI were higher in previous smokers versus present smokers ($p\leq0.001$) (Figure 14C).

When adjusted for confounding factors such as age, sex, BMI, LDL-C, use of alcohol, and systolic BP, no significant differences were found in PWV between the individual study groups (Figure 14D).

5.1.4 Relation of augmentation index with smoking in supine and upright positions

In study I, there was an increase in AIx among present smokers although in this study smoking did not reduce heart rate, ventricular ejection duration, stroke volume, elevate PWV, or increase SVR (Figures 13 and 14). The above variables induce changes that are most often related to an increase in AIx (Laurent et al., 2006; Sakurai et al., 2007; Vlachopoulos et al., 2011; Wilenius et al., 2016). Therefore, linear regression analysis was performed to examine the relations of the hemodynamic variables with AIx (Table 6). These analyses revealed that sex and the hemodynamic variables such as SVRI, PWV, heart rate, ejection duration, and aortic reflection time were significant explanatory variables for AIx ($p \leq 0.027$) in both supine and upright positions. Stroke index was a significant explanatory variable for AIx in supine ($p < 0.001$) but not in the upright position ($p = 0.137$). The overall R^2 values for this analysis were 0.609 in the supine and 0.733 in the upright position (Table 6).

Additionally, the relations between demographic variables, smoking status, alcohol intake, laboratory variables, hemodynamic variables, and AIx were examined using regression analysis. These analyses also showed that the variables that could explain an increase in AIx in present smokers, i.e. elevated supine stroke index and shorter upright aortic reflection time, were independently associated with AIx. Moreover, present smoking was related with elevated AIx in both supine and upright positions.

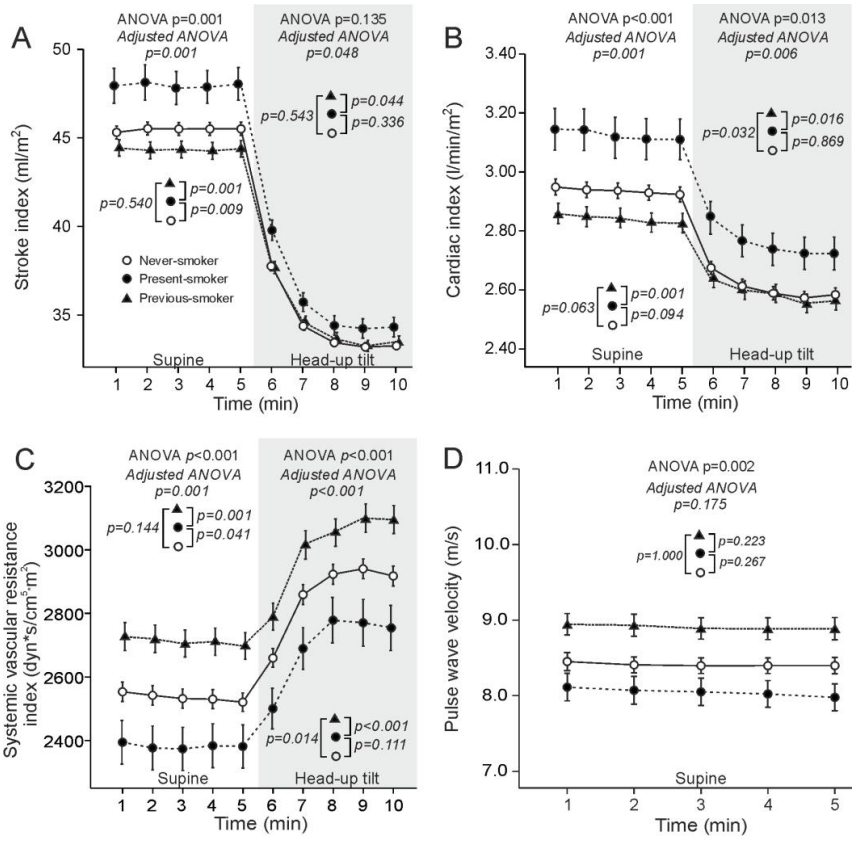

Figure 14. Stroke index (**A**), cardiac index (**B**), systemic vascular resistance index (**C**), and pulse wave velocity (**D**) in never smokers (n = 365), present smokers (n = 81), and previous smokers (n = 191); mean ± standard error of the mean; ANOVA results from unadjusted analyses (plain text) and from analyses adjusted for age, sex, BMI, LDL-C, and alcohol use (italic) are shown (Study I).

Table 6. Linear regression analysis with the enter method: hemodynamic variables and sex as explanatory variables for augmentation index

Augmentation index	b	beta	95% confidence interval for b		P value
			Lower	Upper	
Supine, $R^2 = 0.609$, $p < 0.001$					
Constant	7.069		-16.932	31.069	0.563
Male sex	-6.936	-0.291	-8.423	-5.450	< 0.001
Systemic vascular resistance index	0.005	0.245	0.003	0.007	< 0.001
Lg_{10} of pulse wave velocity	36.760	0.290	28.668	44.851	< 0.001
Stroke index	0.230	0.138	0.106	0.353	< 0.001
Heart rate	-0.232	-0.187	-0.337	-0.127	< 0.001
Ejection duration	0.093	0.154	0.048	0.138	< 0.001
Aortic reflection time	-0.361	-0.474	-0.406	-0.317	< 0.001
Upright, $R^2 = 0.733$, $p < 0.001$					
Constant	-10.109		-28.205	7.987	0.273
Male sex	-1.867	-0.076	-3.286	-0.448	0.010
Systemic vascular resistance index	0.002	0.086	0.001	0.003	0.005
Lg_{10} of pulse wave velocity	21.012	0.161	14.842	27.183	< 0.001
Stroke index	-0.127	-0.047	-0.295	0.041	0.137
Heart rate	-0.089	-0.080	-0.167	-0.010	0.027
Ejection duration	0.328	0.600	0.292	0.365	< 0.001
Aortic reflection time	-0.532	-0.454	-0.585	-0.478	< 0.001

Variables used: Systemic vascular resistance index, the common logarithm of PWV, heart rate, stroke volume index.

Lg_{10}, the common logarithm; n=631 subjects (Study I).

5.2 Lipids, blood pressure and arterial stiffness, studies II-III

5.2.1 Population and laboratory characteristics

In studies II and III, in total 615 subjects were included in the analyses, among whom 314 (51%) were male and 301 (49%) were female (Tables 7 and 8). The age range in both studies was 19-72 years, and the mean (SD) age and BMI were 44.9 (11.9) years and 26.8 (4.4) kg/m², respectively. The average office systolic/diastolic BP in studies II and III were 140.5 (21.1)/89.3 (12.5) and 140.6 (20.6)/ 89.5 (12.3) mmHg, respectively.

For graphic illustration, the participants were divided into age- and sex-adjusted LDL-C quartiles and AIP tertiles. The average LDL-C in the quartiles ranged from 2.05 (0.51) (Q1) to 4.15 (0.75) (Q4) mmol/l and the average AIP in the tertiles ranged from -0.44 (0.17) (Terile 1) to 0.15 (0.23) (Tertile 3) (Tables 7 and 8).

The age- and sex-adjusted LDL-C quartiles and AIP tertiles presented with differences in BMI, office systolic and diastolic BP, eGFR, QUICKI: and plasma cystatin C, total cholesterol, triglycerides, HDL-C, and glucose concentrations (Tables 7 and 8). In study III, uric acid, CRP, LDL-C also presented with differences between the tertiles (Table 8). Age, alcohol intake, smoking status, and plasma creatinine, sodium, potassium, and insulin concentrations were not different between the quartiles and tertiles of studies II and III.

Table 7. Age and sex adjusted characteristics of the study population in quartiles of LDL-C (Study II)

	Overall	Q1	Q2	Q3	Q4
Number	615	153	158	148	156
Male / female	314 / 301	80 / 73	79 / 79	75 / 73	80 / 76
Age (years)	44.9 (11.9)	44.6 (11.8)	44.1 (12.6)	45.1 (12.6)	45.7 (10.7)
Age range (years)	19-72	20-67	19-71	20-72	21-72
BMI (kg/m^2)	26.8 (4.4)	25.6 (4.5)	26.4 (4.5)	27.2 (4.1)*	28.1 (4.2)*†
Alcohol (standard doses/week)	4.5 (5.7)	3.8 (4.9)	4.1 (5.1)	4.9 (6.5)	5.0 (6.3)
Smokers (number/percentage)	70 / 11.4%	14 / 9.1%	16 / 10.1%	21 / 14.2%	25 / 16.0%
Office systolic BP (mmHg)	140.5 (21.1)	134.7 (19.9)	138.8 (20.9)	139.4 (19.8)*	149.0 (20.9)*†
Office diastolic BP (mmHg)	89.3 (12.5)	85.3 (12.0)	88.6 (12.9)	90.0 (11.4)*	94.0(12.1)*†
eGFR (ml/min per 1.73 m^2)	98.8 (18.1)	102.0 (18.1)	101.3 (18.3)	97.7 (17.1)	94.2 (18.0)*†
QUICKI	0.358 (0.042)	0.369 (0.041)	0.365 (0.056)	0.349 (0.032)*†	0.349 (0.032)*†
Creatinine (µmol/l)	73.7 (13.5)	72.2 (12.3)	74.8 (14.1)	74.1 (13.5)	73.6 (13.9)
Cystatin C (mg/l)	0.85 (0.15)	0.82 (0.15)	0.83 (0.15)	0.85 (0.14)	0.89 (0.14)*†
Sodium (mmol/l)	140.3 (2.0)	140.3 (2.1)	140.4 (2.0)	140.5 (2.0)	140.3 (1.7)
Potassium (mmol/l)	3.81 (0.28)	3.82 (0.28)	3.81 (0.28)	3.84 (0.26)	3.77 (0.31)
Uric acid (µmol/l)	303 (76)	291 (78)	297 (79)	310 (76)	312 (71)
CRP (mg/l)	1.7 (2.9)	1.6 (3.0)	1.7 (4.1)	1.5 (1.5)	2.0 (2.2)
Total cholesterol (mmol/l)	5.15 (1.02)	4.23 (0.68)	4.78 (0.64)*	5.33 (0.63)*†	6.25 (0.85)*†‡
Triglycerides (mmol/l)	1.23 (0.76)	1.05 (0.96)	1.17 (0.69)	1.23 (0.62)	1.49 (0.70)*†‡
HDL-C (mmol/l)	1.58 (0.44)	1.71 (0.488)	1.54 (0.41)*	1.56 (0.41)*	1.52 (0.43)*
LDL-C (mmol/l)	3.05 (0.95)	2.05 (0.51)	2.75 (0.48)*	3.26 (0.50)*†	4.15 (0.75)*†‡
Insulin (mU/L)	8.9 (17.0)	7.2 (5.6)	7.9 (6.1)	11.6 (33.3)	9.1 (5.4)
Glucose (mmol/l)	5.44 (0.58)	5.34 (0.69)	5.40 (0.59)	5.49 (0.48)	5.55 (0.54)*

Mean (standard deviation), *p<0.05 vs Q1; †p<0.05 vsQ2; ‡p<0.05 vs Q3; BMI, body mass index; BP, blood pressure; eGFR, cystatin C based CDK-EPI formula for estimated glomerular filtration rate; QUICKI, quantitative insulin sensitivity check index; CRP, C-reactive protein; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol.

Table 8. Age and sex adjusted characteristics of the study population in tertiles of atherogenic index of plasma (Study III)

	Overall	Tertile 1	Tertile 2	Tertile 3
Male / female (n/n)	314 /301	104 / 98	106 / 102	104 / 101
Age (years)	44.9 (11.9)	44.7 (12.3)	44.1 (11.9)	44.9 (11.6)
BMI (kg/m^2)	26.8 (4.4)	25.1 (3.7)	26.4 (4.0) *	28.9 (4.7) *†
Alcohol (standard doses/week)	4.5 (5.7)	3.9 (5.4)	4.2 (5.3)	5.2 (6.4)
Smokers (number/percentage)	76 / 12.4%	19 / 9.4%	27 / 13%	30 / 14.6%
Office systolic BP (mmHg)	140.6 (20.6)	135.7 (19.8)	140.2 (20.3)	145.7 (20.7) *†
Office diastolic BP (mmHg)	89.5 (12.3)	86.1 (12.1)	89.5 (12.7) *	93.0 (11.4) *†
eGFR (ml/min per 1.73 m^2)	98.8 (18.1)	102.6 (16.8)	97.9 (18.2) *	95.1 (18.7) *
QUICKI	0.360 (0.042)	0.375 (0.048)	0.360 (0.037) *	0.341 (0.040) *†
Creatinine (µmol/l)	74.0 (13.5)	72.4 (13.2)	75.0 (13.0)	73.8 (14.3)
Cystatin C (mg/l)	0.85 (0.15)	0.81 (0.14)	0.85 (0.14) *	0.87 (0.15) *
Sodium (mmol/l)	140.4 (2.0)	141.0 (2.0)	140.3 (1.8)	140.3 (2.1)
Potassium (mmol/l)	3.81 (0.28)	3.80 (0.29)	3.78 (0.27)	3.83 (0.28)
Uric acid (µmol/l)	303 (76)	280 (71)	300 (70) *	327 (81) *†
CRP (mg/l)	1.7 (2.9)	1.3 (2.3)	1.4 (1.7)	2.4 (4.0) *†
Total cholesterol (mmol/l)	5.2 (1.0)	4.83 (1.0)	5.17 (1.0) *	5.44 (1.0) *†
Triglycerides (mmol/l)	1.23 (0.77)	0.70 (0.21)	1.09 (0.33) *	1.92 (0.92) *†
HDL-C (mmol/l)	1.58 (0.44)	1.90 (0.40)	1.58 (0.37) *	1.30 (0.34) *†
LDL-C (mmol/l)	3.1 (1.0)	2.70 (0.90)	3.12 (0.90) *	3.40 (1.0) *†
Atherogenic index	-0.15 (0.31)	-0.44 (0.17)	-0.17 (0.16) *	0.15 (0.23) *†
Insulin (mU/L)	8.89 (17.0)	8.1 (28.4)	7.6 (4.7)	10.9 (7.10)
Glucose (mmol/l)	5.44 (0.59)	5.33 (0.62)	5.40 (0.52)	5.60 (0.58) *†

Mean (standard deviation), *p<0.05 vs Tertile 1; †p<0.05 vs Tertile 2; BMI, body mass index; BP, blood pressure; eGFR, cystatin C based CDK-EPI formula for estimated glomerular filtration rate; QUICKI, quantitative insulin sensitivity check index; CRP, C-reactive protein; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol.

5.2.2 Relations of lipids with blood pressure

In study II, radial and aortic systolic and diastolic BP were significantly different in comparisons between the quartiles but were not significantly different in the adjacent quartiles. The highest LDL-C quartile (Q4) presented with the highest BP (Figure 15). Similarly, in study III, radial and aortic systolic and diastolic BP were higher in the highest AIP tertile than in the lowest AIP tertile (Figure 16). Radial systolic BP was also higher in the highest compared to the middle AIP tertile (Figure 16).

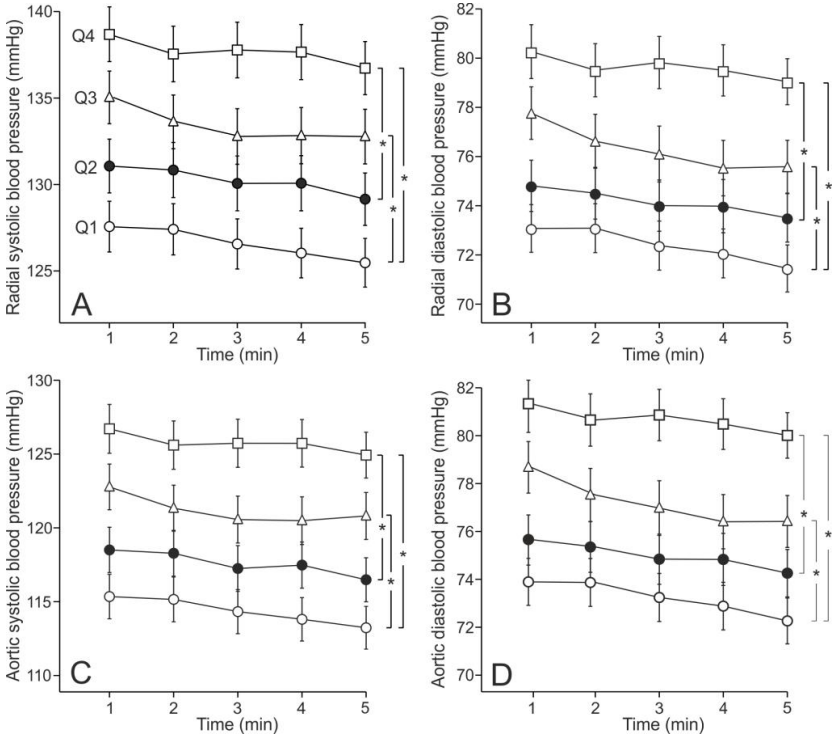

Figure 15. Supine radial systolic (**A**) and diastolic (**B**) blood pressure and aortic systolic (**C**) and diastolic (**D**) blood pressure in age- and sex-adjusted quartiles (Q1-Q4) of LDL-cholesterol during 5-minute recordings. Q1 (n=158), Q2 (n=158), Q3 (n=148) and Q4 (n=156); mean ± standard error of the mean; *p<0.05, ANOVA for repeated measurements (Study II).

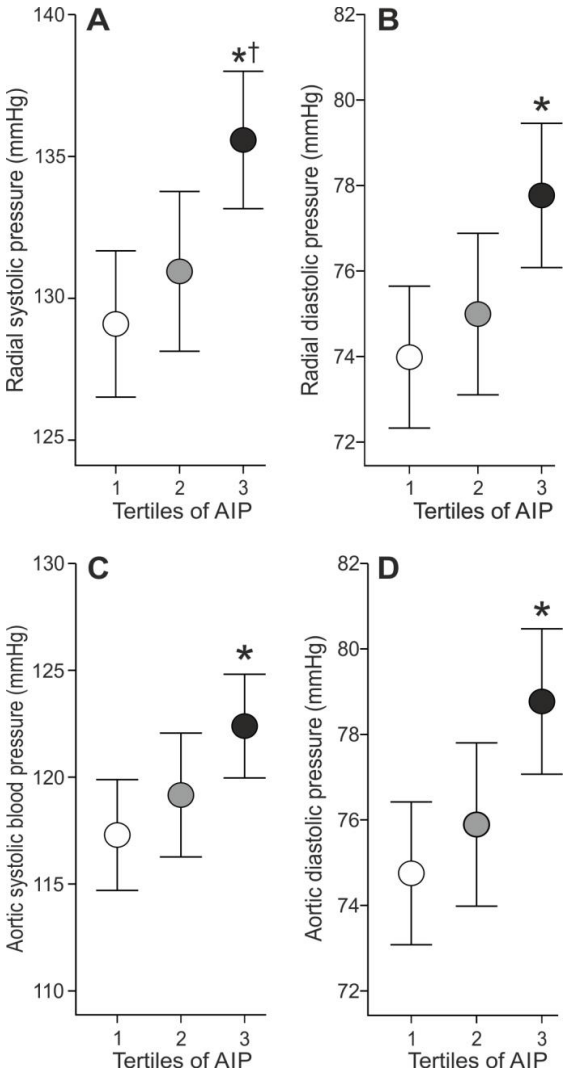

Figure 16. Averages of supine radial systolic (**A**) and diastolic (**B**) blood pressure and aortic systolic (**C**) and diastolic (**D**) blood pressure in age- and sex-adjusted tertiles of atherogenic index of plasma (AIP) during 5-minute recordings. Tertile 1 (n=202), Tertile 2 (n=208), and Tertile 3 (n=205); mean and 95% confidence interval; *p<0.05 vs Tertile 1; †*p*<0.05 vs Tertile 2, one-way ANOVA (Study III).

5.2.3 Hemodynamic variables according to quartiles of LDL-C and tertiles of AIP

In study II, aortic pulse pressure differed between the highest (Q4) and lowest LDL-C quartile (Q1), whereas AIx and AIx@75 were not significantly different between the quartiles. The more detailed results and figures are presented in the original publication (Study II). Heart rate and cardiac index were not significantly different between the quartiles (Figures 17A-B). SVRI differed between Q4 and Q1 (Figure 17C); while PWV was higher in Q4 than in Q1 and Q2 (Figure 17D).

In study III, heart rate was higher in the highest tertile than in the lowest AIP tertile. PWV was higher in the highest and the middle tertile than in the lowest AIP tertile, whereas SVRI was not different between the tertiles (Figure 18). AIx@75 and cardiac index were not different between the tertiles, figures are presented in the original publication (study III).

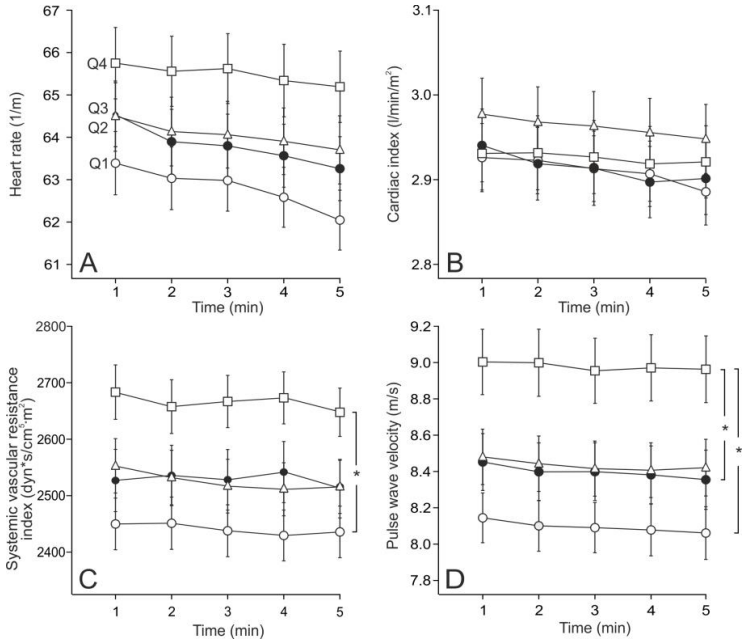

Figure 17. Heart rate (**A**), cardiac index (**B**), systemic vascular resistance index (**C**), and pulse wave velocity (**D**) in age- and sex adjusted quartiles of LDL-cholesterol during 5-minute recordings. Quartiles as in Figure 1; mean ± standard error of the mean; *p<0.05, ANOVA for repeated measurements (Study II).

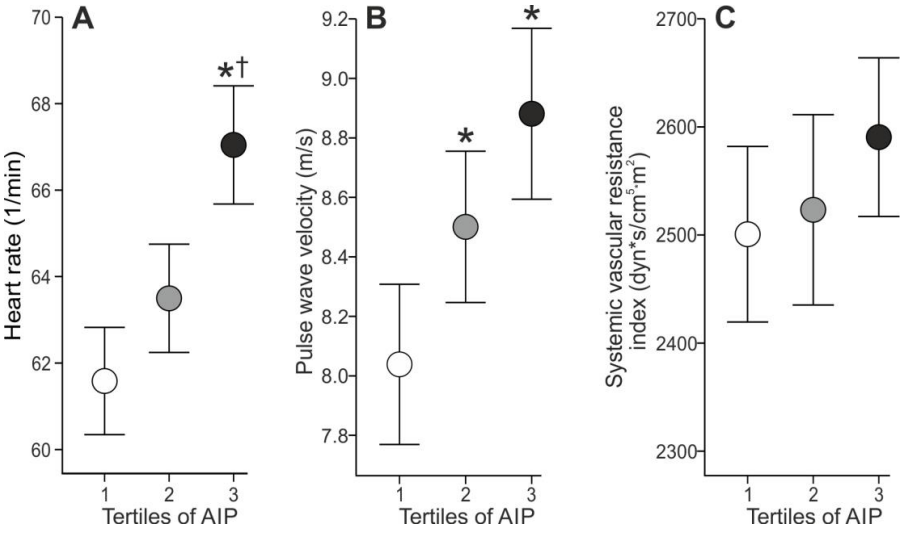

Figure 18. Averages of heart rate (**A**), pulse wave velocity (**B**), and systemic vascular resistance index (**C**) in age- and sex-adjusted tertiles of atherogenic index of plasma (AIP) during 5-minute recordings in the supine position; mean and 95% confidence interval; *p<.05 vs Tertile 1; †p<.05 vs Tertile 2, one-way ANOVA (Study III).

5.2.4 Association of LDL-C and AIP with blood pressure in supine position

In studies II and III, we performed linear regression analyses to examine the relationships between the hemodynamic variables and LDL-C and AIP with two applied models (see methods). The hemodynamic variables have been described in the methods above and in the original publications (Study II and III). These analyses showed that LDL-C was a significant independent explanatory factor for aortic systolic and diastolic BP ($p<0.05$ for all) (model 1, Table 9). The model 2 for aortic systolic and diastolic BP included PWV in addition to the above variables of model 1 (model 2, Table 9). These results showed that LDL-C was still a significant independent explanatory factor for aortic systolic and diastolic BP ($p<0.05$ for all).

In the regression analyses of study III, AIP was not an explanatory factor for radial systolic and diastolic BP in either model (Table 10).

TABLE 9. Significant explanatory factors for blood pressure in linear regression analyses with stepwise elimination (Study II)

Aortic systolic blood pressure: model 1					Aortic systolic blood pressure: model 2				
	B	Beta	R squared	*p*		B	Beta	R squared	*p*
(Constant)	-16.615			0.738	(Constant)	-98.362			0.044
eGFR	-0.222	-0.208	0.205	<0.001	PWV	77.269	0.377	0.306	<0.001
LDL-C	3.343	0.165	0.259	<0.001	eGFR	-0.187	-0.175	0.352	<0.001
Age	0.332	0.204	0.285	<0.001	LDL-C	2.586	0.128	0.366	0.001
BMI	0.531	0.122	0.304	0.003	Sodium	1.077	0.109	0.374	0.001
Sodium	0.945	0.096	0.312	0.006	QUICKI	-34.232	-0.076	0.380	0.036
QUICKI	-38.761	-0.086	0.317	0.026	HDL-C	4.509	0.103	0.384	0.005
					BMI	0.434	0.099	0.389	0.014

Aortic diastolic blood pressure: model 1					Aortic diastolic blood pressure: model 2				
	B	Beta	R squared	*p*		B	Beta	R squared	*p*
(Constant)	19.283			0.588	(Constant)	-32.811			0.316
eGFR	-0.204	-0.286	0.170	<0.001	PWV	54.974	0.401	0.244	<0.001
LDL-C	2.265	0.168	0.217	<0.001	eGFR	-0.155	-0.218	0.284	<0.001
QUICKI	-33.714	-0.112	0.238	0.005	QUICKI	-35.114	-0.116	0.306	0.001
Sex	2.564	0.099	0.252	0.008	Alcohol amount	0.198	0.088	0.317	0.011
BMI	0.283	0.097	0.257	0.021	Sodium	0.628	0.095	0.324	0.005
Sodium	0.525	0.080	0.261	0.032	Age	-0.149	-0.137	0.330	0.006
					LDL-C	1.466	0.109	0.337	0.006

Variables in **model 1**: age, sex, body mass index (BMI), smoking status, consumption of standard drinks of alcohol per week, quantitative insulin sensitivity check index (QUICKI), plasma C-reactive protein (CRP), sodium, uric acid, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triglycerides, and cystatin C based CDK-EPI formula for estimated glomerular filtration rate (eGFR). Variables in **model 2**: **model 1** + PWV.

TABLE 10. Explanatory factors for blood pressure in linear regression analyses with stepwise elimination (Study III)

Radial systolic BP: model 1 (R squared 0.278)				**Radial systolic BP: model 2** (R squared 0.338)			
	B	Beta	*p*		B	Beta	*p*
(Constant)	5.667		0.915	(Constant)	-54.968		0.281
eGFR	-0.265	-0.251	<0.001	PWV	81.530	0.396	<0.001
BMI	0.613	0.142	0.001	QUICKI	-53.779	-0.120	0.001
Male sex	5.257	0.137	<0.001	eGFR	-0.207	-0.196	<0.001
LDL-C	3.242	0.162	<0.001	Male sex	3.270	0.086	0.021
Sodium	1.000	0.102	0.006	Sodium	1.089	0.111	0.002
QUICKI	-45.295	-0.101	0.010	LDL-C	2.220	0.111	0.006
Present smoker	-4.690	-0.081	0.025	Age	-0.209	-0.130	0.011

Radial diastolic BP: model 1 (R squared 0.274)				**Radial diastolic BP: model 2** (R squared 0.333)			
	B	Beta	*p*		B	Beta	*p*
(Constant)	21.741		0.540	(Constant)	-37.667		0.254
eGFR	-0.199	-0.282	<0.001	PWV	54.136	0.393	<0.001
LDL-C	2.227	0.166	<0.001	eGFR	-0.157	-0.222	<0.001
QUICKI	-31.547	-0.105	0.008	QUICKI	-33.656	-0.112	0.002
Male sex	2.851	0.112	0.003	LDL-C	1.622	0.121	0.003
High alcohol intake	8.612	0.096	0.008	Sodium	0.661	0.101	0.004
Present smoker	-3.173	-0.082	0.024	Age	-0.150	-0.140	0.006
BMI	0.276	0.096	0.024	High alcohol intake	6.602	0.074	0.033
Sodium	0.495	0.076	0.042				

Variables in **model 1**: age, sex, BMI, smoking status, categorized alcohol consumption, QUICKI, Lg_{10} of plasma CRP, sodium, uric acid, LDL-C, atherogenic index, and eGFR. **Model 2**: model 1 + PWV.

5.2.5 Association of LDL-C and AIP with hemodynamic variables

In study II, linear regression analyses showed that LDL-C was a significant independent explanatory factor for aortic pulse pressure, AIx, and SVRI ($p<0.05$ for all) (model 1, Table 11). While in model 2, LDL-C was no more an explanatory factor for aortic pulse pressure. The model 2 for AIx contained the variables including heart rate, SVRI and PWV in addition to the variables of model 1. In model 2, LDL-C, smoking status, sex, age, BMI, heart rate, and SVRI were independent significant explanatory factors for AIx (model 2, Table 11).

In study III, AIP was a moderate explanatory factor for heart rate in model 1, in addition to QUICKI, CRP, sex, and moderate alcohol consumption. However, when PWV was included in the model, AIP was no longer an explanatory factor for heart rate (Table 12).

Table 11. Significant explanatory factors for hemodynamic variables in linear regression analyses with stepwise elimination

Systemic vascular resistance index: model 1				
	B	Beta	R squared	*p*
(Constant)	3417.956			<0.001
eGFR	-5.613	-0.172	0.106	<0.001
Weight	9.149	0.246	0.142	<0.001
Age	5.122	0.103	0.159	0.031
Smoker	-220.835	-0.123	0.171	0.001
Height	-8.445	-0.133	0.181	0.005
LDL-C	64.294	0.104	0.188	0.016

Aortic pulse pressure: model 1					Aortic pulse pressure: model 2				
	B	Beta	R squared	*p*		B	Beta	R squared	*p*
(Constant)	-46.757			0.092	(Constant)	-50.479			0.076
Age	0.307	0.354	0.206	<0.001	Age	0.190	0.219	0.206	<0.001
BMI	0.355	0.152	0.223	<0.001	PWV	24.365	0.222	0.238	<0.001
LDL-C	1.331	0.123	0.230	0.003	eGFR	-0.047	-0.083	0.244	0.064
HDL-C	2.398	0.102	0.235	0.012	BMI	0.291	0.125	0.248	0.003
Sodium	0.417	0.079	0.240	0.032	HDL-C	2.677	0.114	0.255	0.005
					Sodium	0.393	0.074	0.259	0.041

Augmentation index: model 1					Augmentation index: model 2				
	B	Beta	R squared	*p*		B	Beta	R squared	*p*
(Constant)	-1.268			0.414	(Constant)	7.678			0.016
Age	0.576	0.570	0.350	<0.001	Age	0.532	0.527	0.350	<0.001
Sex	-9.060	-0.378	0.483	<0.001	Sex	-9.687	-0.404	0.483	<0.001
LDL-C	0.841	0.067	0.485	0.043	SVRI	0.005	0.247	0.549	<0.001
					Heart rate	-0.205	-0.164	0.576	<0.001
					Smoker	2.930	0.080	0.583	0.003
					BMI	-0.240	-0.088	0.587	0.004
					LDL-C	0.774	0.062	0.589	0.047

For all these analyses, the skewed distribution of pulse wave velocity (PWV) was Lg10 transformed.
Variables in **model 1**: age, sex, body mass index (BMI) (for systemic vascular resistance BMI was replaced by height and weight), smoking status, consumption of standard drinks of alcohol per week, quantitative insulin sensitivity check index (QUICKI), plasma C-reactive protein (CRP), sodium, uric acid, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triglycerides, and cystatin C based CDK-EPI formula for estimated glomerular filtration rate (eGFR).
Variables in **model 2**: **model 1** + PWV; for augmentation index: **model 1** + heart rate, systemic vascular resistance index (SVRI), and PWV; for PWV: **model 1** + aortic systolic blood pressure.

Table 12. Significant explanatory factors for hemodynamic variables in linear regression analyses with stepwise elimination (Study III)

Heart rate: model 1 (R squared 0.108)				**Heart rate: model 2** (R squared 0.171)			
	B	Beta	*p*		B	Beta	*p*
(Constant)	80.017		<0.001	(Constant)	51.106		<0.001
QUICKI	-38.761	-0.172	<0.001	PWV	43.525	0.421	<0.001
CRP	2.310	0.096	0.023	QUICKI	-42.282	-0.188	<0.001
Male sex	-3.532	-0.184	<0.001	Male sex	-4.161	-0.217	<0.001
Atherogenic index	4.485	0.143	0.003	Age	-0.215	-0.266	<0.001
Moderate alcohol intake	3.798	0.111	0.006	Moderate alcohol intake	4.584	0.134	0.001
				Previous smoker	-1.709	-0.082	0.037

Variables in **model 1**: age, sex, BMI, smoking status, categorized alcohol consumption, QUICKI, Lg_{10} of plasma CRP, sodium, uric acid, LDL-C, atherogenic index, and eGFR, and for PWV also heart rate. **Model 2**: model 1 + PWV; for PWV **model 2:** model 1 + aortic systolic BP.

5.2.6 Relations of LDL-C and AIP with arterial stiffness

In study II, linear regression analyses showed that LDL-C was a significant independent explanatory for PWV (p=0.021) in model 1. In model 2, PWV also contained aortic systolic BP in addition to the variables of model 1. In this model, LDL C was no more an explanatory factor for PWV. The significant explanatory factors for PWV were age, aortic systolic BP, uric acid, triglycerides, HDL-C, smoking status, and sodium (model 2, Table 13). If aortic mean BP or aortic diastolic BP was used in the model 2 instead of aortic systolic BP, LDL C was not an independent explanatory factor for PWV, either (data not shown).

In study III, in both of the applied models, AIP was a significant independent explanatory factor for PWV (Table 14). The other significant explanatory factors for PWV were age, aortic systolic BP, heart rate, plasma uric acid and present smoking (Table 14, model 2). If aortic systolic BP was replaced by aortic mean BP or aortic diastolic BP in the model 2, AIP remained as an independent explanatory factor for PWV (data not shown).

Table 13. Significant explanatory factors for hemodynamic variables in linear regression analyses with stepwise elimination (Study II)

Pulse wave velocity: model 1					Pulse wave velocity: model 2				
	B	Beta	R squared	*p*		B	Beta	R squared	*p*
(Constant)	0.566			<0.001	(Constant)	0.916			<0.001
Age	0.004	0.544	0.419	<0.001	Age	0.004	0.501	0.419	<0.001
Uric acid	0.0002	0.172	0.493	<0.001	Aortic systolic blood pressure	0.001	0.281	0.510	<0.001
Triglycerides	0.013	0.102	0.509	0.002	Uric acid	0.0002	0.154	0.555	<0.001
LDL-C	0.011	0.116	0.518	0.001	Triglycerides	0.012	0.095	0.568	0.003
Smoker	-0.023	-0.081	0.525	0.005	Smoker	-0.019	-0.067	0.571	0.013
BMI	0.002	0.080	0.528	0.019	HDL-C	-0.019	-0.088	0.575	0.006
					Sodium	-0.003	-0.056	0.578	0.045

For all these analyses, the skewed distribution of pulse wave velocity (PWV) was Lg10 transformed.

Variables in **model 1**: age, sex, body mass index (BMI) (for systemic vascular resistance BMI was replaced by height and weight), smoking status, consumption of standard drinks of alcohol per week, quantitative insulin sensitivity check index (QUICKI) (Katz et al., 2000), plasma C-reactive protein (CRP), sodium, uric acid, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triglycerides, and cystatin C based CDK-EPI formula for estimated glomerular filtration rate (eGFR) (Inker et al., 2012).

Variables in **model 2**: **model 1** + PWV; for augmentation index: **model 1** + heart rate, systemic vascular resistance index (SVRI), and PWV; for PWV: **model 1** + aortic systolic blood pressure.

Table 14. Significant explanatory factors for hemodynamic variables in linear regression analyses with stepwise elimination (Study III)

Pulse wave velocity: model 1 (R squared 0.582)				**Pulse wave velocity: model 2** (R squared 0.616)			
	B	Beta	*p*		B	Beta	*p*
(Constant)	0.482		<0.001	(Constant)	0.444		<0.001
Age	0.004	0.567	<0.001	Age	0.004	0.508	<0.001
Atherogenic index	0.030	0.100	0.007	Aortic systolic BP	0.001	0.239	<0.001
Heart rate	0.002	0.197	<0.001	Atherogenic index	0.044	0.145	<0.001
Uric acid	0.0002	0.129	0.001	Heart rate	0.002	0.174	<0.001
Present smoker	-0.024	-0.086	0.002	Uric acid	0.0002	0.161	<0.001
LDL-C	0.007	0.076	0.021	Present smoker	-0.021	-0.076	0.004
BMI	0.002	0.079	0.020				
Male sex	0.015	0.080	0.023				

Variables in **model 1**: age, sex, BMI, smoking status, categorized alcohol consumption, QUICKI, Lg_{10} of plasma CRP, sodium, uric acid, LDL-C, atherogenic index, and eGFR (Inker et al., 2012), and for PWV also heart rate. **Model 2**: model 1 + PWV; for PWV **model 2:** model 1 + aortic systolic BP.

Blood pressure (BP), coefficient of regression (B), standardized coefficient of regression (Beta), cystatin C based CDK-EPI formula for estimated glomerular filtration rate (eGFR) (Inker et al., 2012), body mass index (BMI), low density lipoprotein cholesterol (LDL-C), quantitative insulin sensitivity check index (QUICKI) (Katz et al., 2000); the skewed distributions of C-reactive protein (CRP) and pulse wave velocity (PWV) were Lg_{10} transformed.

5.3 Hemodynamics of essential hypertension versus primary aldosteronism, study IV

5.3.1 Population and laboratory characteristics

In study IV, 336 (65 %) male and 184 (35%) female subjects participated, and the age range of the subjects was 21-80 years (Table 15). As per the inclusion protocol, the three hypertensive groups were well matched for age (53 years), sex (84 male/46 female), and BMI (30 kg/m^2). In addition, the PA and medicated EH group were also matched for the use of beta blocker or beta and alfa blocker; while the normotensive controls (n=130) were matched for sex (age 48 years, BMI 27 kg/m^2). The mean age between the PA and EH groups did not differ but was higher than in the normotensive controls. The normotensive controls were about 5 years younger with about 3 kg/m^2 lower BMI compared to the other groups. The medicated EH and PA groups had a higher number of subjects with type 2 diabetes compared to the never-medicated EH and normotensive control groups. In patients with PA, office systolic BP was about 10 mmHg higher compared to medicated EH, although diastolic BP was not different. Office systolic and diastolic BP were highest in the never-medicated EH group (Table 15). Clinically evaluated lower extremity edema was not different between the groups.

PA patients presented with the longest known hypertension history (Table 15). There was no difference in median number of antihypertensive medications between PA and medicated EH (p=0.135, Table 3). Plasma potassium concentration was lowest, whereas plasma sodium concentration was highest, in the PA group, even though 82 PA patients were on potassium supplements (Tables 1 and 3). Among the study groups, plasma total cholesterol was lowest in the PA group, while LDL-C was highest in the never-medicated EH group (Table 15). In the three-hypertensive groups, plasma triglycerides and glucose were higher, and HDL-C was lower when compared to normotensive controls. Fasting plasma glucose was slightly higher in the PA group and the medicated EH group than in the never-medicated EH group. The three hypertensive groups had correspondingly higher Cornell voltage products than the normotensive controls (Table 15).

Table 15. Basic clinical characteristics and laboratory results

	Normotensive controls	Never-medicated EH	Medicated EH	Medicated PA
Age (years)	47.9 (0.9)	53.5 (0.8)*	52.9 (1.1)*	53.0 (1.0)*
Body mass index (kg/m^2)	26.7 (0.3)	30.1 (0.5)*	29.5 (0.4)*	30.3 (0.5)*
Number of type 1 diabetics	0	0	1	1
Number of type 2 diabetics	1	3	21*†	31*†
Office systolic BP (mmHg)	126.3 (0.8)	161.7 (1.6)*	145.2 (1.8)*†	154.1 (1.5)*†‡
Office diastolic BP (mmHg)	81.9 (0.5)	99.3 (0.8)*	90.9 (1.2)*†	91.6 (1.0)*†
Hypertension duration (years)	0.0	1.6 (0.5)	11.2 (0.9)*†	14.9 (0.9)*†‡
Office heart rate	65 (1)	70 (1)*	66 (1)	69 (1)*
eGFR (ml/min/1.73m^2)	96.2 (1.3)	89.5 (1.1)*	88.5 (1.6)*	86.8 (1.8)*
Sodium (mmol/l)	140.5 (0.2)	140.7 (0.2)	140.0 (0.2)†	142.8 (0.2)*†‡
Potassium (mmol/l)	3.80 (0.02)	3.82 (0.03)	3.75 (0.03)	3.48 (0.03)*†‡
C-Reactive protein (mg/l)	1.5 (0.2)	2.5 (0.4)	2.5 (0.3)	3.1 (0.6)*
Creatinine (µmol/l)	76.6 (1.2)	75.7 (1.2)	77.1 (1.3)	80.7 (2.9)
Cystatin C (mg/l)	0.87 (0.01)	0.93 (0.01)*	0.95 (0.02)*	1.0 (0.02)*
Uric acid (µmol/l)	303 (6)	336 (6)*	349 (8)*	327 (7)
Total cholesterol (mmol/l)	5.1 (0.1)	5.6 (0.1)*	5.1 (0.1)†	4.7 (0.1)*†‡
Triglycerides (mmol/l)	1.07 (0.05)	1.53 (0.08)*	1.49 (0.07)*	1.48 (0.09)*
HDL cholesterol (mmol/l)	1.60 (0.04)	1.45 (0.04)*	1.41 (0.04)*	1.36 (0.04)*
LDL cholesterol (mmol/l)	3.04 (0.09)	3.54 (0.08)*	3.20 (0.09)†	2.96 (0.08)†
Glucose (mmol/l)	5.42 (0.05)	5.81 (0.06)*	6.33 (0.14)*†	6.55 (0.13)*†
Cornell voltage-duration product (mm*ms)	1569 (50)	1917 (47)*	2017 (98)*	2163 (75)*

Results shown as mean (standard error of mean); eGFR, estimated glomerular filtration rate (CKD-EPI cystatin-C creatinine formula); EH, essential hypertension; PA, primary aldosteronism; *P<0.05 vs. normotensive; †P<0.05 vs. never-medicated EH, ‡p<0.05 vs. medicated EH.

5.3.2 Hemodynamics in normotensive controls, and in patients with medicated and never-medicated essential hypertension and primary aldosteronism

Radial systolic and diastolic BP were similar in PA and never-medicated EH, and higher than in medicated EH and normotensive controls. In addition, medicated EH patients had higher radial BP than normotensive controls ($p<0.001$ for all comparisons) (Figure 19A-B). Adjusted ECW volume was higher in PA patients compared to medicated EH and controls ($p<0.05$ for all). ECW balance derived from bioimpedance device was ~4% higher in PA compared to hypertensive and normotensive control groups ($p\leq0.009$ for all) (Figure 19C-D).

Heart rate was higher in never-medicated EH compared to PA, medicated EH, and controls ($p<0.05$ for all) (Figure 20A). Stroke index was not different between PA and normotensive controls, while it was significantly higher in normotensive controls and PA patients compared to medicated and never-medicated EH groups ($p\leq0.033$ for all) (Figure 20B). Compared to medicated EH, cardiac index was ~8% higher in PA ($p=0.012$) despite similar use of beta-blockers (Figure 20C). When compared to controls, SVRI was higher in PA, medicated and never-medicated EH groups ($p<0.001$ for all) (Figure 20D).

The FWA was not different between PA and never-medicated EH, and was higher in PA than in medicated EH and normotensive controls. Never-medicated EH patients had also higher FWA than normotensive controls ($p\leq0.002$) (Figure 21A). AIx@75 was corresponding in all hypertensive groups, and higher than in normotensive controls ($p<0.001$ for all) (Figures 21B). Aortic pulse pressure was higher in PA and EHs groups compared to normotensive controls ($p<0.001$ for all). Aortic pulse pressure was also higher in PA versus medicated EH ($p=0.008$) (Figure 21C).

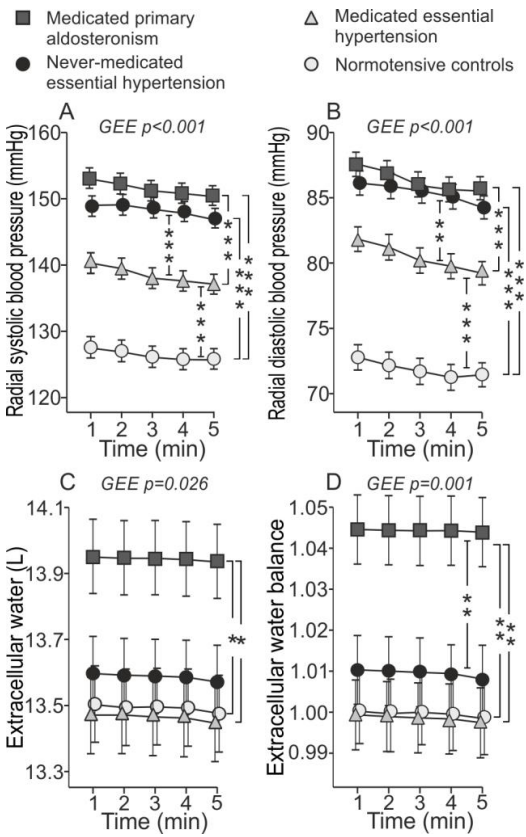

Figure 19. Radial systolic (**A**) and diastolic (**B**) blood pressure, extracellular water volume (**C**), and extracellular water balance (**D**) in medicated primary aldosteronism (n=130), never-medicated essential hypertension (n=130), medicated essential hypertension (n=130), and normotensive controls (n=130) during 5-minute recordings in supine position; mean±standard error of the mean; statistics by generalized estimating equations (GEE) adjusted for age, BMI (for extracellular water analyses BMI was replaced with lean body mass), presence of diabetes, eGFR; and plasma triglycerides, HDL cholesterol, LDL cholesterol, uric acid, and glucose (see Methods); *p<0.05, **p<0.01, ***p<0.001.

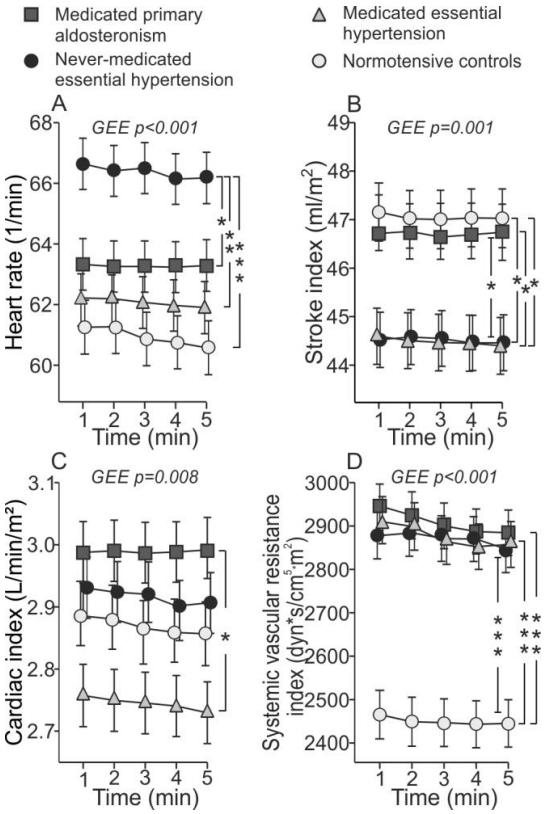

Figure 20. Heart rate (**A**), stroke index (**B**), cardiac index (**C**), and systemic vascular resistance index (**D**). Groups and statistics as in Figure 19; **p*<0.05, ***p*<0.01, ****p*<0.001.

5.3.3 Arterial stiffness in primary aldosteronism versus essential hypertension

When adjusted for mean aortic pressure in addition to demographic and metabolic factors, aortic-to-popliteal PWV was higher in medicated PA than in medicated EH and normotensive subjects ($p \leq 0.033$). However, PWV was highest in never-medicated EH ($p \leq 0.004$ for all comparisons) (Figure 21D).

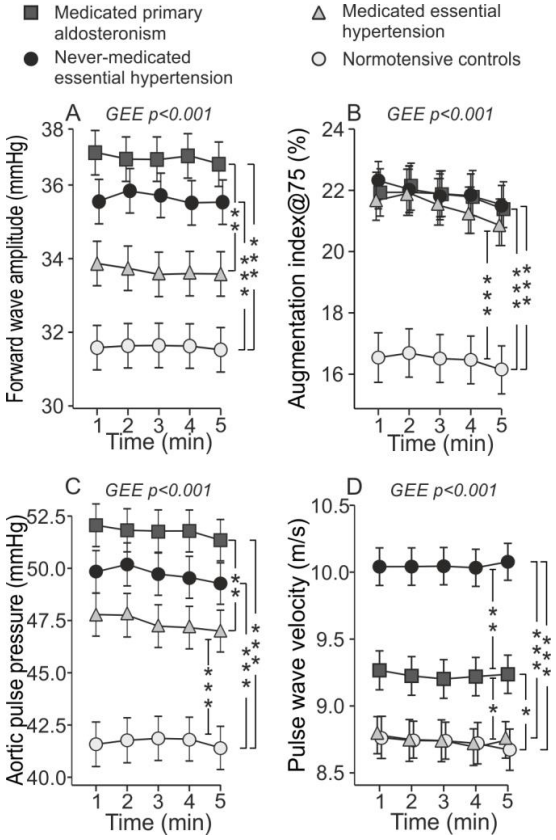

Figure 21. Forward wave amplitude (**A**), augmentation index adjusted to heart rate of 75 beats per minute (**B**), aortic pulse pressure (**C**), and aortic-to-popliteal pulse wave velocity (PWV) (**D**). Groups and statistics as in Figure 19 and in PWV analyses the results were also adjusted for mean aortic blood pressure; **p*<0.05, ***p*<0.01, ****p*<0.001.

6 DISCUSSION

6.1 Cardiovascular risk stratification

CVD is a major cause of disability and premature death worldwide. The predominant underlying pathology is related to atherosclerosis, which develops over a period of time. Atherosclerosis is a multifactorial, immunoinflammatory disease of medium- and large size arteries driven by lipids and high BP. Development of atherosclerosis mainly involves atherogenic lipoprotein deposition, maladaptive inflammation, apoptosis/necrosis, calcification, and fibrosis of the vascular wall (Falk, 2006). The detection of increased CVD risk factors is important to prevent morbidity and mortality. Subsequently, risk factor identification and modification can reduce clinical events and premature death (WHO | Cardiovascular diseases, 2017). The WHO/ISH risk prediction charts are crafted to indicate 10-year risk of a fatal or nonfatal major cardiovascular event, corresponding to conventional and generally known cardiovascular risk markers such as age, sex, elevated BP, smoking status, or increased cholesterol level (WHO | Risk prediction, 2007). According to the INTERHEART study conducted in 52 countries, modifiable risk factors such as high BP, dyslipidemia, smoking, diabetes, and inactive lifestyle account for an overwhelmingly large proportion (over 90%) of the risk of an acute myocardial infarction. The effects of these risk factors were similar between both genders, across different ethnic groups, and geographic regions (Yusuf et al., 2004). In 2004, two important risk factors, smoking and abnormal lipids, together account for two-thirds of the population-attributable risk of an acute myocardial infarction, while hypertension was the next most important risk factor in men and women (Yusuf et al., 2004). However, in the Global Burden of Disease study in 2012, high BP was found to be the most important risk factor for disability-adjusted life years worldwide (Lim et al., 2012).

Hypertension, smoking and abnormal lipid levels are the most important modifiable risk factors for CVDs. In addition, several studies have reported that hypertension, dyslipidemia, and other cardiovascular risk factors are often interrelated (Figure 7). It has been reported that in individuals aged 40 to 70 years, for every 20 mmHg increase in systolic BP, or 10 mmHg increase in diastolic BP, the risk for CVD doubles. The World Health Report in 2002 found that about 62% of cerebrovascular disease and 49%

of ischemic heart disease were attributable to suboptimal BP (systolic>115 mmHg), with little variation by sex (WHO | The world health report, 2002).

Cortisol or hydrocortisone is the major human glucocorticoid that is essential for maintenance of normal BP, while cortisol excess can result in the development of hypertension (Kelly et al., 1998). Mineralocorticoids are steroid hormones that cause sodium retention and potassium excretion, and mineralocorticoid excess causes hypertension that is characterized by sodium retention and hypokalemia. Aldosterone is the main mineralocorticoid, and the most frequent causative factor for the genesis of secondary hypertension. PA is the classic form of mineralocorticoid hypertension (Fuller & Young, 2005). Aldosterone excess is associated with increased incidence of various cardiovascular complications. Patients with PA are at a higher risk of CVD than patients with EH (Monticone et al., 2018; Ohno et al., 2018). A study done in 2582 PA patients reported that the prevalence of CVD was higher among PA patients compared to that of age-, sex-, and BP -matched EH population. Patients with unilateral aldosterone excess present more often with hypokalemia and are at greater risk of CVD than patients with bilateral aldosterone excess (Ohno et al., 2018). In a retrospective case-control study from Germany, cardiovascular mortality was the main cause of death in PA (50% versus 34% in hypertensive controls) (Monticone et al., 2018)

A prospective study of 188,167 individuals without CVD and cancer reported that smoking increases the risk of almost all CVD subtypes, doubling the risk of acute myocardial infraction, cerebrovascular disease, and heart failure (Banks et al., 2019) However, the extent of the increase in risk varies according to the other risk factors. Not surprisingly, smoking-associated CVD risks are found to be highest in present smokers when compared with previous smokers who have quit smoking for quite some time, and never smokers (Banks et al., 2019; Pirie et al., 2013). Studies have also shown that smoking amplifies the cardiovascular risk in patients with hypertension (Fagard, 2009).

A 50% reduction in heart disease within 5 years was reported with 10% reduction in serum cholesterol among men aged 40 years (Law et al., 1994). In Ireland, a 30% reduction in death due to heart disease has been attributed to a 4.6% reduction in the population mean concentration of total cholesterol. Similarly, in Finland a 50% reduction in ischemic heart disease mortality was explained by the reduction of population blood cholesterol level (WHO | Raised cholesterol, 2008). Across all age and sex groups, a major decline was observed in mean serum cholesterol levels from 6.2 mmol/l to 5.6 mmol/l in Finland from 1982 to 1997, but the parallel decline in mean BP and smoking prevalence was smaller (Laatikainen et al., 2005).

As also mentioned chapter 2.6, dyslipidemia is a major risk factor for CVD, and the primary focus has been on the central role of LDL-C in the process of atherosclerosis overriding the significance of HDL-C and triglycerides (Cholesterol Treatment Trialists' (CTT) Collaborators, 2012; Lamarche et al., 2018). However, studies have also recognized the important role of HDL-C and triglycerides in CVD. Due to the normal distribution of AIP, in contrast to plasma triglycerides concentration (Holmes et al., 2008), AIP is well suited for the mathematical modelling of cardiovascular variables. AIP is a strong marker for the future risk of atherosclerosis and CVD (Dobiášová, 2004; Dobiásová & Frohlich, 2001; Tan et al., 2004). The ICEBERG study reported that serum lipid levels are useful in stratifying hypertensive patients into cardiovascular risk groups more accurately, and also for appropriate antihypertensive treatment (Kabakci, 2008). Taken together, the whole lipid profile is of importance in clinical CVD risk evaluation.

In addition to seated BP, the association of various other hemodynamic variables with cardiovascular risk, measured at rest or during a physical challenge like a change in posture, should be more extensively studied. The concept that routine CVD risk evaluation of an individual should be based on more extensive risk models that are integrated to the routine clinical practice should be considered. Regardless of the risk model chosen, patients are usually categorized in a low, intermediate, and high estimated 10-year risk for developing CVD. Different population groups also influence risk prediction, and thus potential risk factors should be studied within various cohorts of subjects. Only thoroughly investigated risk factors provide a worthwhile addition to prediction models. Based on the patient's category, lifestyle changes, initiation of treatments for primary prevention, and reassessment can be done. Altogether, the pathophysiology of potential cardiovascular risk factors, their associations with other risk markers, and effects on morbidity and mortality should be carefully studied in different populations before the factors can be widely applied for risk stratification.

6.2 Study populations

The subjects in this thesis were all taking part in an ongoing clinical study on human hemodynamics with the primary aim to examine hemodynamics in primary and secondary hypertension at Tampere University (DYNAMIC study, Eudra-CT 2006-002065-39, ClinicalTrials.gov NCT01742702). The age of the participants in this thesis varied from 19-80 years. In all four included studies the study population varied slightly due to the exclusion criteria, the timing of the analyses, and the setting of the study. In

contrast to many previous hemodynamic reports (Kovács et al., 2010; Smith et al., 2006), the participants included in the studies I-III were all without anti-hypertensive medicines that could have had direct cardiovascular influences, and the studies also included never-treated hypertensive subjects. In study IV apart from medicated PA and medicated EH subjects, also patients without anti-hypertensive medications and normotensive subjects were included. Due to the inclusion of subjects without anti-hypertensive medication, the study allowed the evaluation of the primary hemodynamic changes in hypertensive subjects without the confounding effects of anti-hypertensive medications on the hemodynamic measurements. This approach can be considered as one of the major strengths of these studies.

In the DYNAMIC study, participant recruitment was from many different organizations. For example, patients treated at Tampere University Hospital, or personnel of Tampere University Hospital and Tampere University who were willing to participate were recruited. Additionally, patients from occupational health care providers, primary health care workers, and clients of Varala Sports Institute also participated. Announcements were even published in a local newspaper, and all of the voluntary subjects participated in the ongoing study.

For adrenal vein sampling, patients with confirmed aldosteronism from all five university clinics in Finland are referred to Tampere University Hospital. These patients were also invited to participate in the non-invasive hemodynamic recordings. As the participants in the study were voluntary, we can assume that these subjects enrolled in the studies were more concerned about their own health than those few who rejected our invitation. In addition, due to the primary aim of the DYNAMIC study, subjects with high BP may be overrepresented. As the hemodynamic recordings were performed in voluntary subjects, it is likely that the selection bias cannot be completely avoided in this study.

However, none of the present participants had diagnosed coronary artery disease or other atherosclerotic vascular disease, cardiac insufficiency, or renal disease. Due to the strict exclusion criteria, confounding factors were minimized in all studies, and the study groups were as comparable to each other as possible.

6.3 Cardiovascular aging and hemodynamics

Aging is a very important risk factor for the development of CVD. However, the question arises that how unchangeable is chronological age as a risk factor and whether all the effects of aging immutable? (Sniderman & Furberg, 2008). The common perception is that at the same chronological age, an individual's general health status can be noticeably different from most people. In the seventeenth century Thomas Sydenham, the "English Hippocrates", provided the theoretical paradigm that "a man is as old as his arteries" (Leonard, 1990). This hypothesis has been recently reexamined through the early vascular aging concept (Nilsson, 2008). Some of the known and emerging biomarkers that are associated with pathological aging are increased SVR, increased large artery stiffness, endothelial dysfunction, RAAS activation, vascular classification, decreased nitric oxide production, and increase oxidative stress (Nilsson et al., 2014). Such biomarker processes can often co-exist in an individual, but it is difficult to establish causative pathways and links between these entities. This line of thinking has led to the concept of cardiovascular atherosclerotic continuum (O'Rourke et al., 2010). The cardiovascular disease continuum is a chain of events triggered by several cardiovascular risk factors, which if left untreated, inevitably culminates in the end-stage heart failure and death (Figure 22) (O'Rourke et al., 2010).

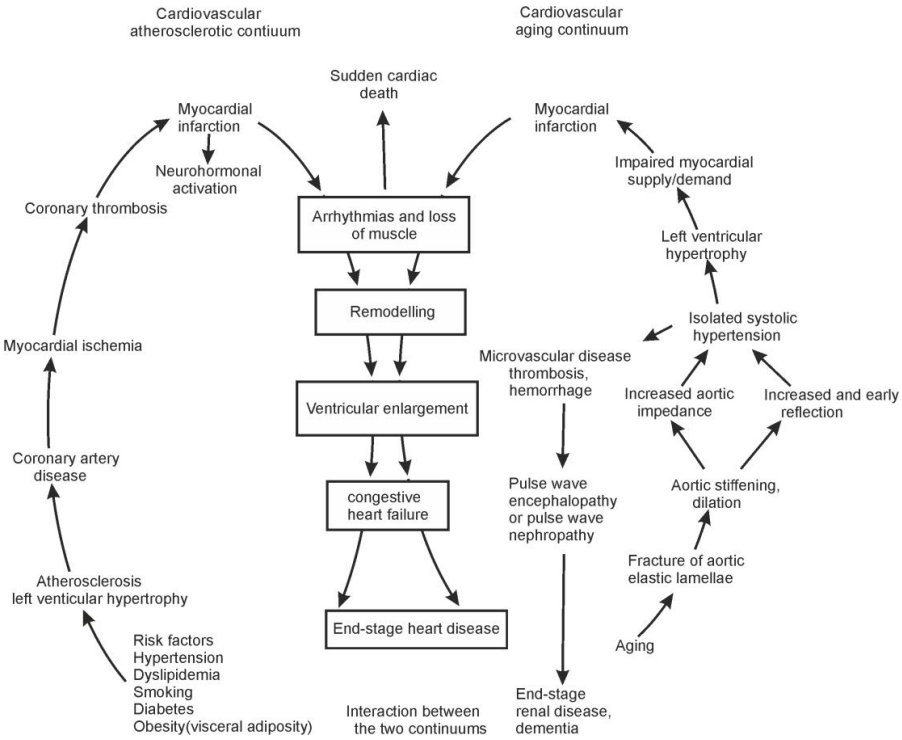

Figure 22. The interaction between the two continuums: the cardiovascular continuum (left) and the aging continuum (right) (Adapted and modified from O'Rourke et al. 2010).

Pathologic aging often starts already in adolescence, though CVD events may only appear after reaching the middle age or above (Figure 23). Pathologic arterial aging occurs principally with increased SVR in the early adulthood and predominantly with increased stiffness in the later adulthood (Nilsson, 2014). Not all arteries become stiff with age: long-term structural changes due to aging cause increased stiffness of the elastic arteries, while the peripheral muscular arteries retain their normal properties with aging. Normal vascular aging is associated with a gradual change in the vascular structure and function that results in decreased arterial compliance and increased large arterial stiffness (Laurent et al., 2006). Central arterial elasticity depends on normal content and function of the matrix protein elastin. Long standing cyclic stress in the media of elastin-

containing arteries results in fracturing and disarray of elastin together with structural changes of the extracellular matrix including proliferation of collagen and deposition of calcium. Humoral factors, cytokines and oxidative metabolites also play a role (Vlachopoulos et al., 2011), leading to increased stiffness of the aortic wall, classically termed as arteriosclerosis. In the CARDIA study (Coronary Artery Risk Development in Young Adults), subjects with prehypertension exposure, matched for age with normotensive subjects prior to age of 35, developed a significantly higher coronary calcium score 20 years later as a sign of coronary atherosclerosis (Pletcher et al., 2008). This study suggested that young age is a critical period where even a small elevation in BP can result in pathological arterial aging and increased arterial stiffness when the individual reaches the middle age. A recent study reported that children with elevated BP showed signs of accelerated vascular aging. These changes indicated that their biological age was 4-5 years older than in their normotensive control subjects (Litwin & Feber, 2020). Thus, BP can be a good measure for vascular aging (Bruno et al., 2020; Litwin & Feber, 2020; Nilsson et al., 2014).

Early, pathological arterial stiffness can occur prior to, or in relationship with, elevated BP or with common clusters of other CVD risk factors such as dyslipidemia or smoking (Nilsson, 2014). In large elastic arteries, increased SVR and arterial stiffness are two major hemodynamic abnormalities that accelerate pathological aging (Nilsson, 2014). Early detection and treatment of the risk factors that initiate the cardiovascular atherosclerotic continuum could stop or delay its further progression (Safar et al., 2012). As arteries are damaged over a long period of time, the emphasis should be on the mitigation of the process of pathological aging with early and effective lifestyle and other therapeutic interventions as an alternative to waiting for the disease to develop and then treating it. Data from four prospective population studies conducted in the United States, Finland and Australia reported that the individuals who had elevated BP in childhood or adolescence, but subsequently normalized BP in their adulthood, did not present with increased carotid IMT and PWV in their fourth decade of life (Juhola et al., 2013).

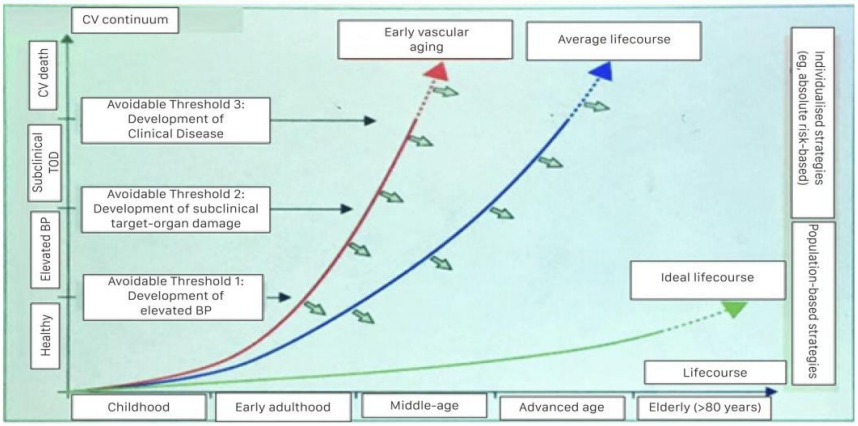

Figure 23. Ideal life course and vascular aging (Reproduced from Campana et al. 2020). BP, blood pressure; CV, cardiovascular; TOD, target organ damage.

MAP and PP are two principle components of BP. Ventricular ejection and SVR are the two major components of MAP, whereas ventricular ejection, large artery stiffness and wave reflection are components of PP. Elevated SVR has been considered as the cardinal hemodynamic manifestation of EH, as SVR increases in proportion to the elevation of BP (Delong & Sharma, 2020). Peripheral PP, central PP, and AIx provide additional information about wave reflection, and they are considered as surrogate measurements of central arterial stiffness and play a role in the interface between the traditional cardiovascular continuum and aging continuum, representing the pathophysiology of CVDs (O'Rourke et al., 2002; Van-Bortel et al., 2002). Other physiological approaches based on pulse wave analysis such as recording of PWV can be used as an applicable method to measure the early vascular aging process. As also mentioned above in chapter 2.7.1, PWV in different populations including patients with EH, has an independent predictive value for cardiovascular events (Laurent et al., 2001). The application of the above methods could provide clinically significant insights into the panorama of hemodynamic changes in arteries with aging, and ultimately the connection of the related changes in cardiac function.

6.4 Main results of the study

6.4.1 Association of smoking with alterations in cardiovascular function

Although smoking has many detrimental effects on health, controversies still exist about the effect of smoking on BP and arterial stiffness (Argacha et al., 2008; Cecelja & Chowienczyk, 2009; Doonan et al., 2010; Groppelli et al., 1992; Linneberg et al., 2015; Primatesta et al., 2001). It has been reported that heavy smoking is associated with an acute rise in systolic BP (15-20 mmHg), but these effects may escape clinical BP measurement as the effect starts to decline after 10 minutes and can be missed if the BP is measured more than 30 minutes after smoking (Groppelli et al., 1992; Kaplan, 2017). Some reports have found that male smokers have increased BP (Primatesta et al., 2001), in contrast, some reports found that present smokers have lower BP than non-smokers (Li et al., 2017; Linneberg et al., 2015; Mikkelsen et al., 1997; Okubo et al., 2004). The putative reduction of BP in smokers may be related to a decrease in body weight, and support for this observation is provided by the higher body weight and increased BP among previous smokers versus never smokers (Halimi et al., 2002; Poulter, 2002). The vasodilator effect of the nicotine metabolite cotinine may contribute to the reduction of BP in smokers (Benowitz & Sharp, 1989)

A 4-year prospective study found that the risk of hypertension was increased in previous smokers with increasing number of abstinence in years (Lee et al., 2001). In study I, functional approach including the head-up tilt protocol was used since the upright cardiovascular influence of smoking is not well understood and limited information exists about the hemodynamics in smokers in response to passive head-up tilt. Measurement performed only at rest may underestimate the hemodynamic effects of smoking. Passive head-up tilt may also show more clearly possible differences in hemodynamic responses, as reported in few previous studies (Hautaniemi et al., 2017; Kangas et al., 2016).

In this thesis (Study I), the role of smoking on hemodynamic was evaluated by allocating the participants to different groups according to their smoking status. We found that higher BP was observed in previous smokers, but the rise in BP was attributed to the common confounding factors such as age, sex, BMI, alcohol use and LDL-C and not directly to smoking habits. When adjusted for these confounding factors neither present nor previous smoking influenced BP. In concert, a Mendelian randomization meta-analysis with a total of 141,317 subjects reported that present smoking was not associated with BP when adjusted for confounding factors (Linneberg

et al., 2015). In this study, previous smokers had also higher BMI than present and never smokers. Smoking cessation predisposes to weight gain, resulting in an increase in BP (Halimi et al., 2002), and the risk of hypertension in previous smokers increases with the number of years of abstinence (Lee et al., 2001). Excess visceral fat accumulation is associated with vast metabolic changes in glucose and lipid homeostasis, which may eventually lead to hypertension and other cardiovascular complications. Secondary mechanisms such as RAAS upregulation and disturbances of the sympathovagal balance are often reported as the main culprits behind the obesity-related increase in BP (Hall et al., 2015)

The influence of smoking on arterial stiffness are not consistent in all investigations, and it is somewhat surprising that PWV is not always increased in smokers (Cecelja & Chowienczyk, 2009; Doonan et al., 2010). In study I, PWV was highest in previous smokers, but after adjustment for confounding factors including systolic BP, PWV did not differ between the study groups. Arterial wall stiffening and thickening are two important components of atherosclerosis. There have been studies reporting that PWV was strongly associated with both carotid IMT and plaque in crude analysis, but this relationship was no longer present when adjusting for potential confounders including age, gender, heart rate, BP, body height and other cardiovascular risk factors including smoking and diabetes (Gómez-Marcos et al., 2011; Robustillo-Villarino et al., 2017). Moreover, the lack of the effect of risk factors on arterial stiffness may also be explained by the fact that early stages of atherosclerosis do not influence the stiffness of the arterial wall. In contrast, advanced plaques, particularly calcified plaques, are associated increased aortic stiffness (Zureik et al., 2003). These studies demonstrated that there is a poor agreement between PWV and carotid IMT, confirming that the measurements used to assess arterial stiffness and thickening in clinical practice are not interchangeable. Therefore, it is important to consider that assessment of aortic stiffness cannot always predict the severity of large arterial atherosclerosis (Gómez-Marcos et al., 2011; Megnien et al., 1998; Robustillo-Villarino et al., 2017).

Previously, multiple reports have almost unanimously shown that acute, chronic, or passive smoking are all associated with an increase in AIx (Argacha et al., 2008; Barnoya, 2005; Janner et al., 2012; Markus et al., 2013; Polónia et al., 2009; Tsuru et al., 2016). As described in chapter 2.7.2, multiple hemodynamic factors such as arterial stiffness, heart rate, ventricular ejection duration, travelling distance of pressure waves (body height), BP, SVR, and stroke volume influence AIx (Laurent et al., 2006; Vlachopoulos et al., 2011; Wilenius et al., 2016). In study I the present smokers had higher AIx in the absence of changes in BP, heart rate, ejection duration, PWV, FWA, and SVR that could explain such an increase in AIx. Actually, present smokers had lower SVR than never

smokers in the supine position. Nevertheless, present smokers presented with increased stroke index and decreased aortic reflection time during upright position versus previous smokers. Both of these factors are associated with higher AIx (Baksi et al., 2009; O'Rourke et al., 2011; Wilenius et al., 2016). Regarding the mechanisms of higher AIx with smoking, my study demonstrated with multivariate analyses that stroke index was an independent determinant of AIx in supine position, while shorter aortic reflection time was linked with higher AIx both supine and upright. There have been studies reporting that cessation of smoking reduces AIx@75, which is visible in my study too, with borderline nonsignificant supine but highly significant on upright reduction of AIx@75 in previous smokers when compared with present smokers (Polónia et al., 2009; Takami & Saito, 2011). Also, the level of AIx did not differ between previous smokers and never smokers.

Carbon monoxide inhaled by smoking increases the level of carboxyhemoglobin in the red blood cells, which impairs oxygen transport to the tissues (Papathanasiou et al., 2014; Whincup et al., 2006). Studies have also reported that carbon monoxide has vasodilatory properties (Leffler et al., 2011). In study I, we found that SVR in the present smokers was lower than in never smokers in the supine position, and lower than in previous smokers in both supine and upright positions. In addition to the putative changes in the metabolic rate of tissues (Perkins et al., 1989), these hemodynamic changes may be related to the impaired oxygen transport properties of blood during smoking, as carbon monoxide in cigarette smoke increases the levels of carboxyhemoglobin in red cells (Puente-Maestu et al., 1998). Corresponding to my findings, young healthy male smokers presented with complex disruption of peripheral microcirculatory regulation including vasodilatation in the palmar microvasculature when compared with non-smokers (Dalla-Vecchia et al., 2004)

Nicotine and other substances in tobacco smoke can stimulate the sympathetic nervous system (Barutcu et al., 2004; Perkins et al., 1986). In 10 male smokers, nicotine was found to increase the metabolic rate at rest by ~5%, and to increase energy expenditure during light exercise by ~12% (Perkins et al., 1989). There has been very little to no information regarding the effects of smoking on stroke index. Thus, my study provides insight that smoking indeed causes an increase in stroke volume in the absence of changes in heart rate. Smoking stimulated the contractile properties of the heart, probably via similar mechanisms that increase the metabolic rate in the body (Perkins et al., 1989) and elevate the sympathetic tone (Barutcu et al., 2004; Perkins et al., 1986). Increased stroke index was also translated to higher cardiac output in the present smokers versus previous smokers. Previously, a rather large comprehensive study based on evaluation using cardiac ultrasound reported that 172 present smokers

had ~8% higher cardiac output than 81 never smokers (Kraen et al., 2017). This study corresponds to my results using whole-body impedance cardiography and supports the view that smokers indeed have a hyperdynamic circulation.

In a systematic review subjects who continuously smoked had twice the death rate due to coronary events versus never-smokers, while in patients with coronary heart disease the risk of mortality was reduced after 2 years of abstinence from smoking (Critchley & Capewell, 2003). There is some reduction in CVD risk immediately after smoking cessation (Mahmud & Feely, 2003), but the extent and period of the remaining risk are not clear. Some studies reported that the level of CVD risk in previous smokers reverts to the level of never smokers only after more than 5 to 10 years following quitting of smoking (Honjo et al., 2010; Mannan et al., 2010). In study I, the hemodynamics in previous-smokers, despite 10 years of abstinence, had not returned to the level of never-smokers. Reduced upright cardiac output and increased SVR in the previous smokers versus never smokers may represent persistent changes in hemodynamics induced by tobacco smoke in cardiovascular regulation even after 10 year-long abstinence.

The findings of study I further strengthen the previously reported significance of increased AIx in smokers. Importantly, for the first time in this study we explained the mechanism why AIx is almost always higher in smokers: My findings indicate that increased AIx in present smokers can be attributed to an increase in stroke volume and shortening of the aortic reflection time. The present smokers also presented with hyperdynamic circulation. In this study the kinetics and the magnitude of risk reduction in previous smokers after quitting smoking appear to be longer than previously anticipated.

6.4.2 Association of plasma lipids with alterations in cardiovascular function

One of the great biomedical findings of the 20th century is the multifaceted pathway by which cholesterol is linked to coronary heart disease. From a century of research studies LDL-C has been revealed as the essential causative agent and the instigator of atherosclerotic plaques, while high dietary saturated fat has been recognized as a major cause of pathologic LDL levels. Currently available guidelines recommend that LDL-C levels should be used as the primary target to initiate and also titrate lipid-lowering therapy (Grundy et al., 2019; Mach et al., 2020).

Among various other risk factors for cardiovascular disease, dyslipidemias can also predict future development of hypertension (Castelli & Anderson, 1986; Halperin et al.,

2006; Laaksonen et al., 2008; Oparil et al., 2003; Selby et al., 1991; Shieh et al., 1987) and cause endothelial dysfunction (Kim et al., 2012; Steinberg et al., 1997). In a study group of 20,074 subjects the incidence of new onset hypertension, as based on the initiation of drug therapy, was significantly lower in subjects with better control of LDL-C, a finding suggesting the view that plasma LDL-C may be a significant risk factor for new onset hypertension (Borghi et al., 2014). Few previous studies have reported a relationship between LDL-C or very low LDL-C and arterial stiffness (Takahashi et al., 2010; Wang et al., 2011). These results have also been seen in a group of 315 children aged 8–9 years, showing a positive correlation between PWV and total cholesterol, LDL-C, triglycerides (Correia-Costa et al., 2016). While, some other studies have reported an inverse relationship between either LDL-C or total cholesterol with aortic compliance using ultrasound measurements (Dart et al., 1991; Giannattasio et al., 1997; Toikka et al., 1999). However, a systematic review reported that less than 10% of studies have found a positive correlation between serum lipids and arterial stiffness (Cecelja & Chowienczyk, 2009) and the influence of risk factors other than age and BP on large arterial stiffness were either very small or insignificant. In study II, the relation of dyslipidemia for the prediction of hypertension and arterial stiffness was evaluated in age- and sex- adjusted LDL-C quartiles. The most important result in this study was the finding that LDL-C was independently associated with BP, AIx and SVRI. LDL-C was also associated with increased PWV, but the association between LDL-C and PWV was no more significant when BP was also included as a confounding factor in the model. The background mechanisms for the association between dyslipidemia and hypertension are not completely understood, but they probably share common mechanisms partially overlapping with other cardiovascular risk factors.

Endothelial dysfunction likely plays an important role in the pathogenesis of primary hypertension (Fleming, 2017; Hadi et al., 2005; Oparil et al., 2003). Studies have shown that factors such as dyslipidemia that cause endothelial dysfunction may also lead to the genesis of hypertension (Hadi et al., 2005; Rajendran et al., 2013). Oxidative stress and endothelial dysfunction are consistently observed in hypertensive subjects (Schulz et al., 2011). Blood cholesterol, including oxidized LDL-C, have numerous direct non-atheromatous effects on the arterial wall. They increase oxidative stress and inflammation, promote elastin damage and calcium deposition within the arterial wall (Abedin et al., 2004; Wilkinson & Cockcroft, 2007). Studies have found that LDL-C impairs endothelium-dependent vasodilatation, the primary mechanisms being reduced nitric oxide bioavailability through increased vascular production of reactive oxygen species and enhanced responses of arterial smooth muscle cells to vasoconstrictors like Ang II (Cominacini et al., 2001; Nickenig, 2002; Nickenig et al., 1997). The observed

impaired endothelium-mediated vasomotion in the resistance blood vessels would lead to elevated BP via increased SVR.

Several clinical studies looking into the effects of statins on hemodynamic variables have shown small but significant reductions in BP after treatment with statins (Lamarche et al., 2018; Tocci et al., 2017; Upala et al., 2017). One year treatment with atorvastatin and rosuvastatin has been reported to improve flow-mediated dilatation in the brachial artery measured using ultrasonography (Demir et al., 2018). A meta-analysis comprising 6 studies with 7 treatment arms reported a significant increase of flow-mediated dilatation induced by pitavastatin (Katsiki et al., 2018). Statins can reverse the deleterious effects of LDL-C on endothelial dysfunction, which also provides a potential explanation for the beneficial effects of statins on BP (Canepa et al., 2018; Demir et al., 2018; Upala et al., 2017). Experimental studies by Nickenig et al. suggest that LDL-C can increase the Ang II type 1 receptor density in the arterial wall (Nickenig, 2002; Nickenig et al., 1997), while lipid-lowering therapy with high dose statins *in vivo* can reduce the vasoconstrictor responses caused by Ang II in isolated human internal thoracic artery segments obtained during coronary bypass surgery and studied *in vitro* (Harst et al., 2005). Together, the findings presented above support the results of study II that LDL-C is a significant explanatory factor for higher BP. It seems understandable that the loss of physiological vasodilator activity resulting from endothelial dysfunction caused by LDL-C may be displayed as elevated BP due to increased SVR.

PWV is strongly dependent on age and prevailing BP (Cecelja & Chowienczyk, 2009; Laurent et al., 2006). In study II, we found an association between LDL-C and PWV, but the association was lost when BP was considered in the regression model. Higher triglyceride, lower HDL-C and higher uric acid, the characteristic components of the metabolic syndrome were all associated with large arterial stiffness. Kangas et al. have previously reported a direct association between PWV and the metabolic syndrome even in the absence of hypertension (Kangas et al., 2013).

As discussed also above, HDL-C and triglycerides are associated with arterial stiffness, but controversies exist. It has been also reported that higher triglyceride and lower HDL-C levels are associated with increased PWV (Wang et al., 2013; Wang et al., 2016). Wang et al. reported that PWV was inversely associated with HDL-C, however total cholesterol or triglycerides were not associated with aortic or peripheral stiffness (Wang et al., 2011). Moreover, in a total population of 12,900 Chinese adults, HDL-C was not correlated with PWV in different age and gender and metabolic syndrome components groups, while elevated BP was positively related with PWV (Weng et al., 2012). Even after adjustment for confounding factors such as BP and all metabolic syndrome components, one study failed to find any correlation between the level of

triglycerides or HDL-C and arterial stiffness (Czernichow et al., 2005). Altogether, few studies have reported an association of triglyceride levels with arterial stiffness (Kärkkäinen et al., 2014; Wang et al., 2016). The association of each component of the metabolic syndrome with arterial stiffness may differ between men and women (Gomez-Sanchez et al., 2016; Kim et al., 2015). A study reported that an increase in triglyceride levels of 48 mg/dL resulted in a 1.0% increased PWV, but did not identify a significant relationships between baseline BP, HDL-C and arterial stiffness as measured using PWV over time with type I diabetes (Dabelea et al., 2013).

Due to the central role of LDL-C in atherosclerosis the influences of LDL-C outweigh the importance of HDL-C and triglycerides in clinical practice. Nevertheless, study II found that LDL-C was not associated with PWV when the level of BP was taken into account and my finding is in fact concordant to the majority of previously published papers (Cecelja & Chowienczyk, 2009). Ridker et al. reported that even after lowering LDL-C to the recommended levels in an individual, residual cardiovascular risk remains, therefore recommending studies to find new CVD predictors (Ridker et al., 2002). Even at the LDL-C target goal, patients with cardiometabolic abnormalities remain at high risk of cardiovascular events (Genest Jr et al., 1992; Superko, 1995). An unfavorable shift in the levels of any lipid profile component can make an individual more prone to atherosclerotic complications. The roles of HDL-C and triglycerides in combination have also been acknowledged as atherogenic dyslipidemia (Mach et al., 2020; Kutkiene et al., 2018). Several markers that can be calculated from the concentrations of lipoprotein fractions have been established in an attempt to make more accurate prediction of cardiovascular mortality and morbidity and one of the promising markers is AIP (Kutkiene et al., 2018).

Due to the rather weak association of LDL-C with PWV in study II, we evaluated the relationship of AIP with PWV and other hemodynamic variables in study III. As AIP shows normal distribution (Holmes et al., 2008; Holmes & Buhr, 2007), in contrast to plasma triglyceride concentration, it is well suited for mathematical modelling of the cardiovascular variables. By use of normal probability plots and correlations between residual error and expected residual error terms Tan et al. demonstrated that AIP was preferable to the triglycerides/HDL-C ratio for use in statistical analyses (Tan et al., 2004). The AIP has not been previously been related with hemodynamic variables, but it might be a useful tool for a more accurate identification of risk factors for large arterial stiffness (Dobiásová & Frohlich, 2001; Tan et al., 2004). Many studies has reported that AIP is a strong marker to predict the risk of atherosclerosis and CVD (Cure et al., 2018; Dobiášová et al., 2011; Dobiásová & Frohlich, 2001; Onat et al., 2010; Tan et al., 2004). In the study III of this thesis, we for the first time reported that AIP was directly and

independently associated with large arterial stiffness, e.g. increased PWV, a variable which is strongly related to cardiovascular risk.

The ratio of triglycerides to HDL-C may be related to the processes involved in LDL size pathophysiology, which might be applicable for evaluating the clinical vascular disease risk (Boizel et al., 2000). Higher triglyceride to HDL-C ratio can identify overweight adolescents of less than 18 years with abundance of atherogenic LDL particles (Burns et al., 2012). An increased proportion of small, dense LDL particles has also been associated with marked alterations in plasma lipoprotein and lipid levels, such as elevated triglycerides, age, total cholesterol, apolipoprotein B concentrations, and reduced HDL-C levels, all of which are predictive of an increased risk for CVD (Griffin et al., 1994; Hirayama & Miida, 2012; McNamara et al., 1987; Moin & Rohatgi, 2011). Small dense LDL particles have strong atherogenic characteristics, and they increase the process of lipid peroxidation and generate reactive oxygen species. The mean LDL particle size in stroke patients was found to be smaller than in control subjects, in spite of similar total LDL-C concentrations (M. C. Cure et al., 2013). Other studies have advocated a direct measurement of the LDL particle size as the only definite way to assess potential atherogenicity of LDL particles (Lamarche et al., 1997; Superko, 1996).

As described in chapter 2.6, In a total of 1433 subjects from 35 cohorts with various risk of atherosclerosis, Dobiasova et al. reported that the AIP correlated positively with the FER-HDL, and inversely with LDL particle size. As, FER-HDL predicts particle size in HDL and LDL, which in turn can predict CVD risk, the simultaneous use of $\log_{10}$(triglycerides/HDL-C) ratio as AIP may be useful in predicting plasma atherogenicity. These results proposed that AIP reflects the metabolic interactions within the whole lipoprotein complex (Dobiášová, 2004; Dobiásová & Frohlich, 2001).

The ratio of fasting triglycerides/HDL-C has also been reported to have a strong association with insulin resistance (McLaughlin et al., 2003). Several studies have confirmed that elevated AIP, or reduced HDL-C simultaneously with elevated triglycerides, are all associated with decrease insulin sensitivity (Kutkiene et al., 2018; Tan et al., 2004; Valensi et al., 2016). A meta-analysis suggested that lipid parameters have ability to reflect the risk of type 2 diabetes, while especially AIP may be more closely associated with the risk of type 2 diabetes (Zhu et al., 2015). A cross-sectional study showed significant correlations between AIP and the CVD risk factors total cholesterol, LDL-C, triglycerides, HDL-C, and glucose (Bo et al., 2018). The above views correspond to the finding of study III where insulin sensitivity, evaluated by the calculation of QUICKI, was different in all AIP tertiles and was found to be lowest in the highest AIP tertile.

The triglyceride/HDL-C ratio was a strong predictor of myocardial infarction, and the ratio may be a marker for abnormal metabolic interaction between the triglycerides- and cholesterol ester-rich lipoproteins that increases the risk of myocardial infarction, although further basic and epidemiological studies are warranted (Gaziano et al., 1997). A study conducted in a Chinese population reported that AIP was higher in 2936 patients with coronary artery disease versus 2451 controls (Cai et al., 2017), while AIP has also been suggested as an strong independent marker predicting the risk of coronary artery disease (Cai et al., 2017; Wu et al., 2018). In study III, AIP in the highest tertile presented with highest PWV with a mean AIP of 0.15. Previously it has been postulated that an AIP value of less than 0.11 is associated with low risk, while the values above 0.11 are associated with intermediate risks for CVD (Dobiášová, 2004; Frohlich & Dobiášová, 2003).

In this thesis we could not provide an explanation why AIP is better correlated with arterial stiffness than LDL-C. The pathophysiology leading to increased large arterial stiffness is a complex process. It comprises effects mediated via mechanical pulsatile stress, inflammatory cells, growth factors, alterations in endothelial function, enzymes that damage elastin, changes in smooth muscle cells, and enhanced extracellular matrix production by fibroblasts (Lacolley et al., 2017). As plasma triglycerides and HDL-C are opposite in direction with respect to oxidative stress/systemic low-grade inflammation, and in relation with phenotypic changes in vascular smooth muscle from contractile to synthetic, changes in extracellular matrix, a single index such as AIP might of greater practical value than the two individual concentrations taken apart for these influences (Lacolley et al., 2017; O'Rourke et al., 2002) .

The results of studies II and III of this thesis stress the importance of abnormal lipid profiles as cardiovascular risk factors, as clear associated hemodynamic changes were observed. The results of study III may be of high importance, as AIP can be used in daily clinical practice as it can be easily calculated from routine lipid profiles. The strong correlation of AIP with lipoprotein particle size (Dobiášová et al., 2011; Dobiásová & Frohlich, 2001) may explain its high predictive value for the occurrence of CVDs. Arterial stiffness is a manifestation of arteriosclerosis, which in turn is associated with aging, diabetes, and chronic kidney diseases, while several previous studies have shown strong correlations between AIP and arteriosclerosis diseases (Frohlich & Dobiášová, 2003; Gaziano et al., 1997; Lacolley et al., 2017; Zhu et al., 2015). Altogether, the link between AIP and large arterial stiffness in study III supports the more widespread use of AIP in standard clinical practice for cardiovascular risk evaluation.

6.4.3 Hemodynamic characteristics of primary aldosteronism

PA is a secondary, endocrine-mediated form of hypertension characterized by an autonomous aldosterone overproduction, which is caused in most cases by bilateral adrenal hyperplasia or adrenocortical adenoma (Calhoun, 2007). Recent studies from various geographic populations reported considerably higher prevalence of PA in hypertensive patients, ranging from 5% to 20%, than that previously reported (Calhoun, 2007; Rossi et al., 2006; Stowasser et al., 2001). Previous studies have demonstrated that hypertension is not a hemodynamically consistent entity that is always characterized by a normal cardiac output and elevated peripheral resistance (Mujais et al., 1982). The phenotypic differences are related to factors such as neural, hemodynamic, or intravascular volume, which might be interrelated and not attributed to a disturbance of any single factor only. Aldosterone plays a key role in the homeostatic control and maintenance of BP through regulation of ECW volume, vascular tone, and cardiac output (Melmed et al., 2011; Schirpenbach & Reincke, 2007; Stowasser & Gordon, 2016). Due to excessive autonomous aldosterone secretion from the zona glomerulosa of the adrenal cortex, there is increased stimulation of the mineralocorticoid receptor in distal tubular kidney cells, resulting in sodium and water retention with subsequent potassium loss. Increased sodium reabsorption paired with water reabsorption results in isotonic volume expansion, which increases glomerular hyperfiltration, and induces a vicious and self-propagating cycle of distal sodium delivery and reabsorption, and further volume expansion. Any impairment in vascular compliance and/or the renal handling results in sodium retention and increase in ECW volume that will manifest as an increase in arterial BP and, ultimately, hypertension (Melmed et al., 2011; Schirpenbach & Reincke, 2007; Stowasser & Gordon, 2016; Vaidya et al., 2018).

From the clinical perspective, the prevalence of PA increases with increasing severity of hypertension. A study reported that in patients with stage 1 hypertension (systolic 140-159 and diastolic 90-99 mmHg), the prevalence of PA was not different from that of normotensive patients. However the prevalence of PA increased to 8% with stage 2 (systolic 160-179 and diastolic 100-109 mmHg) and to 13% in subjects with stage 3 (≥systolic 180 and diastolic 110 mmHg) hypertension, respectively (Calhoun, 2007). Classically, excessive aldosterone secretion results in difficulty in managing high BP in the majority of patients and is a common cause of resistant hypertension. Multiple studies have indicated that the incidence of resistant hypertension in PA patients is higher compared to that in the general hypertensive population (Acelajado et al., 2019; Calhoun, 2007). The PATHWAY-2 sub-study suggested that resistant hypertension is attributable in a large part to excess fluid retention mediated by aldosterone excess

(Williams et al., 2015). In the real-world scenario, the suspicion of PA most often arises following a lengthy history of hypertension and a poor response to antihypertensive medications. Characteristically, patients diagnosed with PA have higher mean BP than those without PA and they are also taking more antihypertensive medications than patients with EH (Acelajado et al., 2019).

A meta-analysis reported that aldosterone excess was responsible for an increased incidence of myocardial infarction and stroke, and increased prevalence of atrial fibrillation, independent of the level of BP (Monticone et al., 2018). Increased BP cannot entirely explain the increase in cardiovascular morbidity and mortality in PA patients, but the underlying mechanisms are not yet clearly understood. As also illustrated in Table 16, a multitude of studies have reported that PA patients have higher BP compared to EH patients and normotensive controls (Table 16). In agreement, study IV of this thesis too reported that medicated PA patients had higher BP than medicated EH patients and normotensive controls. Patients with PA had the longest known history of hypertension, but median number of antihypertensive medications were not different from medicated EH patients (Table 15).

As mentioned in chapter 2.7.2, AIx is an index of wave reflection occurring at the branching of the arteries and from the resistance arterioles, and this variable is influenced by several confounding factors. Previous studies have reported that patients with PA have increased AIx and AIx@75 when compared with normal BP controls; however, the values were reported to be similar between PA and EH patients (Ambrosino et al., 2016; Mark et al., 2014; Strauch et al., 2008). This view from above studies corresponds to my finding in study IV, where AIx@75 was similar in all hypertensive groups and was higher than in normotensive controls. In study IV, SVRI was also comparable in all hypertensive groups and higher than in normotensive controls. Hemodynamic imbalances between SVR and cardiac output are the traditional underlying characteristics of increased BP. Previously the known hemodynamic features of PA include treatment resistant hypertension, increased large arterial stiffness, and increased carotid artery IMT (Acelajado et al., 2019; Bernini et al., 2008). To my knowledge, very limited information exists about other hemodynamic features of PA. A comprehensive summary of hemodynamic findings in primary aldosteronism from study IV and previous studies is presented in Table 16.

Of note, PA patients with hypokalemia had higher cardiovascular morbidity than PA patients with normokalemia (Born-Frontsberg et al., 2009). This implies that in PA patient's hypokalemia plays a significant role in the incidence of cardiovascular comorbidities. In the current study IV, PA patients had more frequently presented with hypokalemia and 82 patients were taking potassium supplements. However, mean

plasma potassium concentration was still lowest in the PA group when compared with the other groups (Table 15). This finding in study IV of this thesis also highlights the proper management of hypokalemia in PA patients beside antihypertensive treatment. Medicated PA patients also presented with elevated plasma sodium concentration versus the other groups. Steichen er al. reported that PA patients have increased plasma sodium concentration and the authors recommended the inclusion of plasma sodium and potassium concentration in the diagnostic algorithm of PA (Steichen et al., 2011).

Table 16. Summary of hemodynamic findings in primary aldosteronism versus essential hypertension, normotension and secondary aldosteronism; and effects of treatment in primary aldosteronism

Study	Study setting	Number in total/PA patients	Use of BP-lowering medication	BP	Functional cardiac variables	Systemic vascular resistance	Arterial stiffness	Wave reflection (AIx)
Present work Choudhary et al.	medicated PA vs. never-medicated EH, medicated EH and NT	520/130	yes, in 2 groups	↑medicated PA vs. medicated EH	cardiac output ↑ medicated PA vs. medicated EH	↑ PA vs. NT; no difference PA vs. EH	PWV ↑ medicated PA vs. medicated EH and NT	↑ PA vs. NT; no difference PA vs. EH
Bernini et al. 2008	PA vs. EH and NT	62/23	no	↑ PA vs. NT	not studied	not studied	PWV ↑ PA vs. EH and NT	↑ PA vs. NT
Mark et al. 2014	PA vs. EH and NT	64/14	yes	↑ PA vs. NT	↓ LVEDV vs. NT	not studied	PWV ↑ PA vs. EH and NT	no difference PA vs. EH
Strauch et al. 2006	PA vs. EH and NT	84/36	yes	↑ PA vs. NT	not studied	not studied	PWV ↑ PA vs. EH and NT	↑ PA vs. NT
Veglio et al. 1999	PA vs. EH and NT during rest and head-up tilt	61/23	stopped 4 week prior to study	↑ PA vs. NT, no difference PA vs. EH	cardiac output not different in PA vs. EH and NT	↑PA vs. NT during rest and tilt; ↑ PA vs. EH during tilt	arterial compliance ↓ PA vs. NT	not studied
Rosa et al. 2012	PA vs. EH	98/49	yes	no difference between PA and EH	not studied	not studied	PWV ↑ PA vs. EH	not studied
Rossi et al. 2002	PA vs. EH	27/17	off medication before ECHO	no difference between PA vs. EH	no difference between PA vs. EH	not studied	not studied	not studied
Hung et al. 2019	PA vs. EH	199/67	yes	no difference between PA vs. EH	not studied	not studied	PWV ↑ PA vs. EH	no difference between PA vs. EH
Kusunoki et al. 2018	PA vs. EH	180/60	yes	↑ PA vs. EH	cardiac output ↑ PA vs. EH	↑ PA vs. EH during daytime	no difference in PWV PA vs. EH	no difference PA vs. EH

Lin et al. 2012	PA vs. EH; pre- and post-adrenalectomy in PA	41/20	yes	↑ PA vs. EH; no change pre- and post-adrenalectomy in PA patients	not studied	not studied	PWV ↑ PA vs. EH; in PA ↓ post-adrenalectomy	not studied
Strauch et al. 2008	PA; adrenalectomy vs. spironolactone treatment	29	yes	↑ in PA before adrenalectomy or spironolactone; no difference between treatments	not studied	not studied	PWV ↓ post-adrenalectomy, no change post-spironolactone	↓ post-adrenalectomy, no change post-spironolactone
Cesari et al. 2016	PA vs. secondary aldosteronism in liver cirrhosis and NT	440/262	yes	↑ PA vs. secondary aldosteronism and NT	cardiac output ↑ PA vs. NT; difference in several ECHO variables between PA vs. secondary aldosteronism and NT	↑ PA vs. secondary aldosteronism and NT	arterial compliance ↓ PA vs. NT	not studied
Rossi et al. 2006	PA prevalence among hypertensives	1125/126	yes	↑ PA vs. non-PA	not studied	not studied	not studied	not studied
Mahmud et al. 2005	ARR in EH, spironolactone vs. bendroflumeta-zide treatment	24/0	no	↑ with higher aldo:renin ratio	not studied	not studied	No correlation between PWV and ARR; only spironolactone reduced PWV	ARR correlated with AIx (r=0.53), and spironolactone-induced reduction in AIx (r=0.64)
Gaddam et al. 2010	High vs. normal aldosterone	108/37 high aldosterone	yes	no difference	LVEDV ↑ high vs. normal aldosterone	not studied	not studied	not studied

AIx, augmentation index; ARR, aldosterone:renin ratio; BP, blood pressure; ECHO, echocardiography; EH, essential hypertension; LVEDV, left ventricular end-diastolic volume; NT, normotension; PA, primary aldosteronism; PWV, pulse wave velocity.

6.4.3.1 Extracellular volume, cardiac output, and stroke index in primary aldosteronism patients versus essential hypertension

It is noted that the body fluid and electrolyte composition of patients with PA varies from that observed in other types of hypertensive patients. As mentioned above excessive autonomous aldosterone secretion results in sodium and water retention with subsequent potassium loss. Studies have reported that patients with PA are considered to present with volume overload, largely on the basis of the elevated aldosterone:renin ratio (Acelajado et al., 2019), and elevated plasma natriuretic peptide concentration (Gaddam et al., 2010). In 1961, 11 PA patients were compared to matched normal controls, and ECW volume was found to be significantly increased by 16.8% of body weight as compared to 14.6% of body weight in the control group (Chobanian et al., 1961). Previously, patients with PA had increased plasma volume and ECW volume than patients with EH (Ichikawa et al., 1984). In comparison with the manufacturer reference values, 41 PA patients with unilateral aldosterone producing adenoma presented with ~4% overhydration as evaluated using bioimpedance spectroscopy device, a method which is widely used for the evaluation of volume status in dialysis patients (Wu et al., 2015).

In the present Study IV, ECW balance was also ~4% higher in the patients with PA, indicating fluid overload when compared with all other groups. Gaddam et al. reported higher brain natriuretic peptide and atrial natriuretic peptide levels in patients with resistant hypertension compared to controls. These vasodilatory hormones are produced in the heart in response to volume or pressure overload. This study also implicated aldosterone excess and persistent intravascular volume expansion as common underlying causes of resistant hypertension (Gaddam et al., 2008). In another study spironolactone treatment significantly decreased right and left ventricular volumes and brain natriuretic peptide concentrations, which supports the view of a relative volume overload state that may underlie treatment resistance in the high aldosterone patient group (Gaddam et al., 2010). In contrast, in patients with normal or low aldosterone levels, the reductions in heart volumes and brain natriuretic peptide were considerably smaller compared with the high-aldosterone patients. A reduction in BP in the absence of changes in cardiac output must be attributed to reductions in vascular resistance. Therefore, these data suggest that spironolactone has a large diuretic effect in patients with aldosterone excess, while in patients without demonstrable aldosterone excess, the more important factor seems to be the reduction in vascular resistance. Altogether, spironolactone has a

dual mechanism of action, the predominating effect depending upon the underlying aldosterone status: in the setting of aldosterone excess, the diuretic effect is most prominent; in the absence of aldosterone excess, reduction in vascular resistance seem to largely drive the BP reduction (Gaddam et al., 2010).

In this thesis (Study IV), high-aldosterone status was clearly associated with increased stroke volume and cardiac output. These findings are in contrast with previous studies that did not report differences in intracardiac volumes in patients with PA when compared with EH (Rossi et al., 2002) or normotensive subjects (Stowasser et al., 2005) measured using echocardiography. However, the current findings are consistent with animal experiments, particularly in the setting of high dietary salt intake (Makhanova et al., 2008) and human (Gaddam et al., 2010) studies that demonstrate an association of aldosterone excess with volume overload.

In 1973 Tarazi et al. reported higher heart rate and cardiac index in 16 PA patients compared to 30 EH patients, without differences in total peripheral resistance (Tarazi et al., 1973). Increased cardiac output using ultrasound was also found in 262 patients with PA versus 61 normotensive controls (Cesari et al., 2016). In another study based on waveform analyses form the brachial artery, Kusunoki et al. reported higher cardiac output in medicated PA than in medicated EH patients (Kusunoki et al., 2018) (Table 16). Similarly, when analyzed using magnetic resonance imaging, patients with high aldosterone had ~9% higher left ventricular end diastolic volume compared to patients with normal aldosterone, indicating intracardiac volume expansion (Gaddam et al., 2010). In study IV of this thesis, cardiac index was ~8% higher in PA patients when compared with medicated EH patients.

To conclude, study IV included rather large groups of medicated PA, medicated EH, never-medicated EH, and normotensive controls with comprehensive laboratory and hemodynamic examinations. This study showed that patients with medicated PA presented with fluid overload, and higher stroke volume and cardiac output than patients with medicated EH.

6.4.3.2 Arterial stiffness in primary aldosteronism versus essential hypertension

Aldosterone excess causes vascular, renal, and cardiac damage via mineralocorticoid receptor activation that promotes inflammation and endothelial dysfunction (Acelajado & Calhoun, 2010; Acelajado et al., 2019; Rossi et al., 2008; Stehr et al., 2010). As reviewed by McCurley and Jaffe, mineralocorticoid receptor activation

inhibits vasorelaxation, and promotes oxidative stress, fibrosis, and remodeling in the vascular wall (McCurley & Jaffe, 2012). Supporting the view that aldosterone excess contributes to arterial wall fibrosis and thickening, patients with PA had higher carotid-IMT when compared with EH (Ambrosino et al., 2016; Bernini et al., 2008; Holaj et al., 2007, 2015).

Ang II indirectly induces collagen synthesis by stimulating aldosterone secretion through stimulation of AT1, which is a potent activator of cardiac fibrosis (Navar, 2014; Valerie et al., 1994). The presence of aldosterone receptors in large arteries, especially in the aorta, suggest that this hormone plays a significant role in regulating the structure of large arteries (Lombès et al., 1992). Ang II also increases collagen synthesis by acting directly on vascular smooth muscle cells (Kato et al., 1991) and cardiac fibroblasts (Zhou et al., 1996) and promotes cardiovascular growth (Schunkert et al., 1995). Long-term treatment with ACE inhibitors can avert some of these alterations, probably independent of their antihypertensive action (Keeley et al., 1992; Rossi & Peres, 1992), suggesting that Ang II may be important in cardiovascular development and growth. The aldosterone antagonist spironolactone has been found to prevent the development of aortic fibrosis independent of BP reduction in spontaneously hypertensive rats (Benetos et al., 1997). The renin-angiotensin system is known to be the major determinant of aldosterone release and inhibition of this system can thus be anticipated to be responsible for a reduction in collagen synthesis.

In an experimental study, aldosterone was administered parenterally (1 μg/h) to uninephrectomized Sprague-Dawley rats that were fed with a high sodium diet from 8 to 12 weeks of age. This study reported increased carotid arterial stiffness in association with aortic fibronectin accumulation, and these effects were independent of the wall stress when compared with normotensive controls (Lacolley et al., 2002). Eplerenone, an aldosterone antagonist reversed these vascular changes, suggesting a direct role of mineralocorticoid receptors in mechanical and structural alteration of large vessels in hyperaldosteronism (Lacolley et al., 2002).

In patients with EH, increased aortic stiffness, measured via the recording of PWV, is an independent predictor of all-cause cardiovascular mortality (Vlachopoulos et al., 2010). Patients with PA have increased arterial stiffness compared to EH, which has been documented in multiple studies (Ambrosino et al., 2016; Bernini et al., 2008; Holaj et al., 2007, 2015; Rosa et al., 2012). Studies have suggested that high aldosterone levels and high aldosterone:renin ratio predispose to increase arterial stiffness (Mahmud & Feely, 2005). The fibroproliferative effects of aldosterone can lead to alterations in central and peripheral arteries with subsequent

increases in arterial stiffness. Aldosterone excess has also been attributed to an increase in left ventricular wall thickness when compared with demographically similar EH patients (Rossi et al., 2006), possibly by promoting the deposition of extracellular matrix and collagen (Weber & Brilla, 1991).

When compared with EH and normotensive controls, patients with PA had pronounced fibrosis of small resistance arteries with higher collagen and type III vascular collagen content, which were more evident than in BP-matched patients with EH (Rizzoni et al., 2006). The profibrotic action of aldosterone has been found to involve wall thickening and increased collagen deposition in the carotid arteries and higher central arterial stiffness when compared with EH patients and normotensive controls (Bernini et al., 2008). According to a systematic review, PA patients have higher aortic PWV than EH patients (Ambrosino et al., 2016).

The results from my investigation (Study IV) also demonstrated higher large arterial stiffness in PA patients in comparison with medicated EH patients and normotensive controls. The difference in PWV between medicated PA and medicated EH was independent of all other clinical characteristics. However, among all groups PWV was highest in the never-medicated EH patients. This indicates that these subjects had been unaware of their high BP for long period of time.

In conclusion, PA had increased arterial stiffness when compared with medicated EH patient even after the adjustment for confounding factors (study IV). This finding could be caused by the deleterious effects of aldosterone excess on the fibrosis and remodeling of the arterial wall (Acelajado & Calhoun, 2010; Ambrosino et al., 2016; McCurley & Jaffe, 2012). This observation in study IV is in agreement with results from the German Conn's Registry (Reincke et al., 2012) and a systematic review (Monticone et al., 2018), and indicates that individuals with PA are at a higher risk of cardiovascular morbidity than patients with EH.

6.4.4 Unawareness of hypertension

High BP is one of the most important modifiable risk factors for stroke, heart, and kidney diseases (WHO | Blood Pressure, 2015). In spite of the availability of effective BP treatments, less than half of the subjects with hypertension have their BPs under control even to what are currently considered conservative targets (<140 mmHg systolic and <90 mmHg diastolic BP) (Beaney et al., 2019; Chow, 2013). A large number of studies have suggested that aldosterone excess is an important underlying cause of resistant hypertension (Acelajado et al., 2019; Calhoun et al.,

2002; Clark et al., 2012; Gaddam et al., 2010; Stowasser, 2014; Stowasser & Gordon, 2016). Treatment intensification with new medication has been found to occur in only 17% of uncontrolled hypertensive patients. This common phenomenon is termed as clinical inertia, and it becomes more prevalent as the number of medications increases (Mu & Mukamal., 2016). Among 468,887 hypertensive patients, 31.5% were found to have uncontrolled hypertension. In the large group of uncontrolled hypertensive patients, inertia to the treatment of hypertension was common because only ~30% were prescribed ≥3 BP medications. Of these patients, only ~15% had been prescribed an optimal antihypertensive regimen (Egan et al., 2013). Recently published results from the May measurement month in 2018 also showed that significant numbers of patients were untreated or inadequately treated for hypertension (Beaney et al., 2019).

Three independent cross-sectional population surveys conducted in 1982, 2002 and 2007 with age stratified samples of men and women aged 25–64 years from the national population register of Finland with a sample size of 16775 reported that the prevalence of hypertension fell from 63.3 to 52.1% in men, and from 48.1 to 33.6% in women. Regardless of the obvious improvement in all aspects of hypertension care since 1982, still in 2007, only 68% of all hypertensive individuals were aware of their condition, 52% of those who were aware were treated with antihypertensive drugs, and only 37% of the drug-treated patients had normal BP (Kastarinen et al., 2009). Zhou et al. reported that Finland had the highest prevalence of hypertension (52% in female and 59% in male) among 123 national health examination surveys from 1976 to 2017 in 12 high-income countries. Finland, Ireland, Japan, and Spain had the lowest rates of awareness, treatment, and control among the high-income countries (Zhou et al., 2019). In 2017, about 46% of the patients with high BP were not receiving medications. Although the proportion of those on treatment has increased in Finland in the recent decades, only 42% of those treated for hypertension have BP values below 140/90 mmHg (Koponen et al., 2018). The Finnish national health examination survey (FinHealth 2017) data provided the prevalence of hypertension of 43 %, while only 12 % of such individuals were identified as hypertensives in the registered data in the administrative hospitals and in the primary care registers (Koponen et al., 2019).

Though there has been substantial improvement since 1982, the control rates of hypertension have plateaued in the past decade in Finland. In the present thesis (Study IV), among 130 patients from the PA and medicated EH groups, 104 (80%) and 90 (69%) patients respectively, did not have office BP optimized to the

acceptable level, may be due to inadequate adherence, under treatment, or lack of appropriate treatment intensification. These finding support the view that inadequate adherence and physician inertia to intensify the treatments contribute to the lack of adequate BP control in the clinical practice.

Increased sympathetic tone is associated with hypertension. Elevated resting heart rate, which is a surrogate of cardiac autonomic tone, is also an independent risk for the development of hypertension (Aladin et al., 2016). Particularly, there is a direct relationship between elevated resting heart rate and peripheral BP, while there is an inverse association between elevated resting heart rate and central BP (Messerli et al., 2016; Stergiou et al., 2016). However, in my studies (II-III) there was no significant association between peripheral or central BP and heart rate. In study IV, the never-medicated EH group had numerically higher heart rate than all other groups, and they also presented with the highest PWV. In studies II and III, elevated heart rate was directly associated with increased PWV. Previously, higher resting heart rate has been associated with an increased arterial stiffness (Whelton et al., 2013). In addition to untreated high BP, increased heart rate in the never-medicated EH patients in study IV provides one plausible mechanism for the higher PWV in these subjects.

Never-medicated EH patients had the highest PWV when compared with all groups in study IV. This indicates that though they were diagnosed with high BP in the current study, they must have had long-standing untreated hypertension. Previously, a significant number of subjects among 335,499 participants had systolic BP≥140 mmHg or diastolic BP≥90 mmHg, among whom 224,285 (66.9%) were not receiving antihypertensive medications (Beaney et al., 2019). Moreover, PWV has been found to be an independent predictor of incident hypertension (Koivistoinen et al., 2018), reaffirming that the never-medicated EH group in study IV must have had high BP for a number of years. In this thesis, 56% of the subjects in study I, and 59.5% of the subjects in studies II-III, were never treated hypertensive subjects. Although most of the hypertensive subjects were diagnosed with high BP for the first time in these 3 studies, they had probably had a long-standing history of hypertension and they had been unaware of their high BP.

To conclude from studies I-IV in this thesis, the prevalence of uncontrolled high BP and unawareness of hypertension is still high. National health-care systems should set large-scale targets and rigorously evaluate innovative mechanisms at the community level to improve hypertension awareness, treatment, and control. This would help to address the large burden of uncontrolled hypertension. In the future, other deleterious lifestyle factors connected closely to high BP such as obesity,

insulin resistance, and abnormal lipids levels should also be addressed to shift the whole BP distribution of the Finnish population to lower levels.

6.5 Clinical implications and future aspects

CVDs are the leading cause of premature death for both men and women globally. High BP, smoking status or abnormal lipid levels are some of the known modifiable cardiovascular risk markers (WHO | Risk prediction, 2007) and often these cardiovascular risk factors are interrelated (Figure 7). The cardiovascular disease continuum triggered by several cardiovascular risk factors should be detected and treated early in order to stop the associated increased morbidity including end-stage heart failure and reduce mortality (O'Rourke et al., 2010).

Previously, the effects of smoking on BP and large arterial stiffness were controversial, while increase in AIx has been documented in multiple studies, but none of the studies reported the underlying mechanism. In the current thesis, in agreement with previous studies we found that in the absence of changes in BP and arterial stiffness, smoking status had a substantial influence on the regulation of cardiac output and SVR. Furthermore, this study showed that the present smokers presented with enhanced wave reflection, which could be attributed to an increase in stroke volume and shortening of the aortic reflection time. These findings provide new information about cardiovascular regulation and the pathophysiological differences between smokers and non-smokers and provide for the first time this additional background to the mechanisms of the increased cardiovascular risk in present smokers.

The relationship between serum lipids and central arterial stiffness are usually investigated because serum lipids are considered to have a critical role in the pathogenesis of atherosclerosis, whereas dyslipidemias can also predict the future development of hypertension (Laaksonen et al., 2008; Oparil et al., 2003; Wang et al., 2011). Endothelial dysfunction likely plays an important role in the pathogenesis of primary hypertension (Fleming, 2017; Hadi et al., 2005; Oparil et al., 2003). The results of the current thesis showed that LDL-C was independently associated with BP and SVR. LDL-C impairs endothelium-dependent vasodilatation, the primary mechanisms being reduced nitric oxide bioavailability through increased vascular production of reactive oxygen species, and enhanced responses of arterial smooth muscle cells to vasoconstrictors like Ang II (Cominacini et al., 2001; Nickenig, 2002;

Nickenig et al., 1997). The observed impaired endothelium-mediated vasomotion in the resistance blood vessels would lead to elevated BP via increased SVR.

Due to the central role of LDL-C in atherosclerosis the influences of LDL-C often outweigh the importance of HDL-C and triglycerides in clinical practice. Previous studies have reported that AIP is a strong marker to predict the risk of atherosclerosis and CVD (Cai et al., 2017; Cure et al., 2018; Dobiášová et al., 2011; Dobiásová & Frohlich, 2001; Nam et al., 2020; Onat et al., 2010; Tan et al., 2004). The results of the study III stress the importance of the inclusion of all lipids and not only LDL-C as cardiovascular risk factors. In concordance to the majority of previously reported studies (Cecelja & Chowienczyk, 2009), the present study II showed that LDL-C was not associated with arterial stiffness. However, the logarithm of plasma triglycerides to HDL-C ratio (AIP, Study III), was independently associated with arterial stiffness. The results of study III may be of importance, as AIP can be used in daily clinical practice and it can be easily calculated from routine lipid profiles. The strong correlation of AIP with lipoprotein particle size (Dobiášová et al., 2011; Dobiásová & Frohlich, 2001) may explain its predictive value for the occurrence of CVDs. Altogether, the link between LDL-C and high BP in study II, and AIP and large arterial stiffness in study III, provide support for the more widespread use of LDL-C for the future prediction of hypertension, and AIP for prediction of large arterial stiffness in standard clinical practice and cardiovascular risk evaluation.

In the clinic, the diagnosis of PA is not straightforward (Funder et al., 2008; Mulatero et al., 2005; Steichen et al., 2011; Stowasser, 2014; Stowasser & Gordon, 2016; Young, 2019). A previous study revealed unexpected heterogeneity in the diagnostic evaluation among 555 treated PA patients (Schirpenbach et al., 2009). The early detection of PA depends on screening, but a recent study from Germany and Italy reported substantial under-diagnosis of this disease in primary care (Mulatero et al., 2016). Considering the high prevalence of hypertension in the general population (Beaney et al., 2019), and the high prevalence of PA among hypertensive individuals (Calhoun, 2007; Funder et al., 2008; Rossi et al., 2006; Schwartz & Turner, 2005), reliable, easy-to-perform, and cost-effective diagnostic tests are required for the early diagnosis of PA. It would be clinically important to identify early the existence of PA among hypertensive patients. To determine which patients should be suspected for PA and to manage them properly, understanding of the hemodynamic profiles in PA can be helpful.

Finally, from the results of this thesis it is clear that modifiable cardiovascular risk factors matter. If left untreated, the cardiovascular disease continuum triggered by

several cardiovascular risk factors inevitably culminates in excess end stage-heart failure, end-stage kidney disease, stroke, and death. Future research in this field should consider taking into account the inclusion of the whole lipid profile in the prediction of the future development of hypertension and arterial stiffness. Also, the routine determinations of ECW volume, and evaluations of cardiac output and arterial stiffness would benefit the clinical diagnostics of PA.

7 SUMMARY AND CONCLUSIONS

CVD due to hypertension, atherosclerosis and the associated complications, are the leading cause of mortality worldwide (Benjamin et al., 2017; WHO | Cardiovascular diseases, 2017). Cardiovascular risk factors such as high BP, smoking and dyslipidemias predispose to the development of atherosclerosis. The present study adds to the knowledge of the hemodynamic changes induced by cardiovascular risk factors and provides new information regarding functional cardiovascular regulation. Particularly, new knowledge is presented about the hemodynamic changes in smokers, in association with plasma lipids and in PA patients.

The principal findings of the current study are as follows:

1. Smoking status had a significant influence on the regulation of cardiac output and SVR in the absence of changes in BP and arterial stiffness. The present smokers presented with hyperdynamic circulation and enhanced wave reflection when compared with previous smokers, while the previous smokers had increased upright SVR and lower cardiac output when compared with never smokers. These findings indicate that increased AIx in present smokers is attributed to increased cardiac stroke volume and shortening of the aortic reflection time (**I**).

2. In 615 subjects, LDL-C was independently associated with BP and the effect could be attributed to elevated SVR that also resulted in enhanced wave reflection. In contrast, AIP was directly and independently associated with large arterial stiffness (**II, III**).

3. Patients with PA were characterized by treatment resistant hypertension, elevated plasma sodium concentration, reduced potassium concentrations despite potassium supplements, and increased ECW volume, cardiac output and arterial stiffness when compared with medicated EH (**IV**).

4. Never-medicated EH patients presented with highest large arterial stiffness, which stresses the importance of early diagnosis and treatment of primary hypertension (**IV**).

5. Our findings in a group of 130 untreated hypertensive patients selected form altogether 1349 normotensive and hypertensive subjects indicate that the prevalence of elevated BP and unawareness of hypertension remain high in the general population (**IV**).

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10 ORIGINAL PUBLICATIONS

PUBLICATION I

Effect of present versus previous smoking on non-invasive haemodynamics

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Effect of present versus previous smoking on non-invasive haemodynamics

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We examined cardiovascular function in 637 volunteers (19–72 years) without antihypertensive medication in never smokers (n = 365), present smokers (n = 81) and previous smokers (n = 191, median abstinence 10 years). Haemodynamics during passive head-up tilt were recorded using whole-body impedance cardiography and radial pulse wave analysis. Results were adjusted for age, sex, body mass index, LDL cholesterol and alcohol use. Systolic and diastolic blood pressure, heart rate, and pulse wave velocity were not different between the groups. Supine aortic reflection times did not differ, while upright values were shorter in present versus previous smokers ($p = 0.04$). Heart rate adjusted augmentation index was increased in the supine position in present smokers versus controls ($p = 0.045$), and in present ($p < 0.001$) and previous ($p = 0.031$) smokers versus controls in the upright position. Supine and upright cardiac output was higher ($p \leq 0.016$) and systemic vascular resistance lower ($p \leq 0.001$) in present versus previous smokers. In spite of the long abstinence, in the upright position previous smokers had lower cardiac output ($p = 0.032$) and higher systemic vascular resistance ($p = 0.014$) than never smokers. In the absence of differences in blood pressure and arterial stiffness, present smokers presented with hyperdynamic circulation and enhanced wave reflection compared with previous smokers.

Cigarette smoking is one of the most important preventable risk factors for mortality in the Western world[1,2], accounting for more than 5 million premature deaths globally per year[3]. Smoking is also the second most common cause for cardiovascular disease (CVD) after elevated blood pressure (BP)[4]. According to World Health Organisation more than one billion people smoke and the prevalence is continuously rising[5]. Cardiovascular deaths account for >54% of all deaths worldwide, and more than 10% of these deaths are attributed to smoking[6].

Smoking predisposes to the progression of atherosclerosis, shown as increased arterial intima-media thickness (IMT)[7], and higher prevalence of atherosclerotic plaques in autopsy studies[8]. In a study with 10,914 patients the progression of atherosclerosis in current smokers was increased by 50% versus non-smokers, documented using measurements of IMT in the carotid artery[7]. Smoking is also associated with adverse effects on serum lipids[9,10], insulin resistance[11], and activation of the sympathetic nervous system[12]. Carbon monoxide in the inhaled cigarette smoke increases the levels of carboxyhemoglobin, the proportion of which can exceed 7.5% in smokers, while the average level in non-smokers is 0.32%[13]. Although very high levels are uncommon, symptomatic effects may occur at carboxyhemoglobin levels of 2.5% or more[13].

Controversial reports have been published about the effect of smoking on BP[14–17]. Gropelli *et al.* reported that smoking causes an acute 15–20 mmHg rise in systolic BP, but the effect starts declining after 10 minutes and can be missed if BP is measured more than 30 minutes after smoking[15]. Some previous reports found that male smokers have increased BP[16]. In contrast, some studies reported that smokers have lower BP than non-smokers[17,18]. The putative reduction of BP in smokers may be related to lower body weight, while previous smokers often have higher body weight and increased BP versus never smokers[19]. The vasodilator effect of the nicotine metabolite cotinine may contribute to the reduction of BP in current smokers[20].

Increased arterial stiffness is an independent predictor of CVD[21]. Many studies have reported that chronic smoking is a risk factor for increased arterial stiffness, however, a number of investigations have not found differences in arterial stiffness between smokers and never smokers[22,23]. Higher augmentation index (AIx), a marker of

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wave reflections, has been found to be associated with smoking in several studies[14,24–27]. Argacha *et al*. reported that acute smoking increases AIx[14], while 1-hour exposure to passive smoking was found to increase AIx by 15.7 percentage points[25]. Polonia *et al*. found that AIx was reduced by about 9 percentage points in subjects who stopped smoking for 6 months, whereas there was an increase of 1.7 percentage points in those who continued smoking[28].

Altogether, the effects of smoking on BP and arterial stiffness remain controversial, while increased AIx has been documented in many studies but the underlying mechanisms are not well understood. Here we examined putative differences in haemodynamics between present, previous, and never smokers. To gain insight about the function of the cardiovascular system in the study groups a passive head-up tilt was included in the study protocol.

Methods

Participants. All participants were from an ongoing study, the primary aim of which is to examine haemodynamics in subjects with primary and secondary hypertension versus normotensive controls, in both supine and upright positions (DYNAMIC study; ClinicalTrails.gov identifier NCT01742702). The total number of all enrolled subjects is 1349. The exclusion criteria for the present study were: use of BP-lowering or other medication with direct cardiovascular influences, secondary hypertension, and history of coronary artery disease, stroke, heart failure, valvular heart disease, diabetes, chronic kidney disease, alcohol or substance abuse, psychiatric illnesses, or other heart rhythm than sinus.

The participants were enrolled by announcements from the personnel and patients treated at Tampere University Hospital, personnel of the University of Tampere, and clients of the Varala Sports Institute and local occupational health care providers. Those who agreed to participate were recruited in the order in which they contacted the research nurses. All subjects underwent physical examination by a medical doctor and laboratory analyses for elevated BP[29]. The medical history and lifestyle habits were documented along with smoking habits, number of cigarettes smoked per day, total smoking duration, and abstinence from smoking in years along with family history for CVD. Alcohol consumption was evaluated as standard drinks (~12 grams of absolute alcohol) per week.

A total of 637 normotensive subjects and never-treated hypertensive patients, aged 19–72 years, were included in the study. They were divided into never smokers (n = 365), present smokers (n = 81) and previous smokers (n = 191). Signed informed consent was obtained from all participants. The study complies with the declaration of Helsinki, and was approved by the ethics committee of the Tampere University Hospital (study code R06086M) and the Finnish Medicines Agency (Eudra-CT registration number 2006-002065-39).

Altogether 247 (39%) of the 637 persons used one or more medications, but the proportions of subjects taking some medication in the never smokers, present smokers and previous smokers did not differ (37.8%, 35.8% and 41.9%, respectively). Seventy-eight female subjects used systemic estrogen, progestin, or their combination (for contraception or hormone replacement therapy), and 1 subject used tibolone. Forty-one subjects were taking antidepressants, 18 antihistamines, 17 inhaled corticosteroids, 15 statins, 13 proton pump inhibitors, while 22 euthyroid subjects were on a stable dose of thyroid hormone. The other medications used by the study population were hypnotics or sedatives (8), low dose acetylsalicylic acid (6), non-steroidal anti-inflammatory drugs (4), antirheumatic agents (4), antiepileptics (3), allopurinol (3), coxibs (3), antipsychotics (2), muscle relaxants (2), varenicline (2), antiviral agents (2), paracetamol (1), carbimazole (1), isotretinoin (1), and alendronate (1). One physically well and symptomless subject was treated with warfarin due to anti-phospholipid syndrome.

Laboratory analyses. Blood and urine samples were drawn after ~12 hours of fasting. Plasma C-reactive protein, sodium, potassium, glucose, cystatin-C, creatinine, triglyceride, and total, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol concentrations were determined using Cobas Integra 700/800 (F. Hoffmann-Laroche Ltd, Basel; Switzerland) or Cobas6000, module c501 (Roche Diagnostics, Basel, Switzerland), insulin using electrochemiluminescence immunoassay (Cobas e411, Roche Diagnostics), and blood cell count by ADVIA 120 or 2120 (Bayer Health Care, Tarrytown, NY, USA). Urine dipstick analysis was made by an automated refractometer test (Siemens Clinitec Atlas or Advantus, Siemens Healthcare GmbH, Erlangen, Germany). Insulin sensitivity was evaluated by calculating the quantitative insulin sensitivity check index (QUICKI)[30], and glomerular filtration rate (GFR) was estimated using the CKD-EPI creatinine-cystatin C equation[31].

Pulse wave analysis. Radial BP and pulse wave were continuously captured from the radial pulsation using a tonometric sensor (Colin BP-508T, Colin Medical Instruments Corp., USA), which was secured on the radial pulse with a wrist band. The radial BP signal was calibrated twice during each 5 minute-period of recording by brachial BP measurements from the contralateral arm. Aortic BP was derived with the SphygmoCor system (SpygmoCor PWMx^{R}, AtCor medical, Australia) by means of the validated generalized transfer function[32]. Left ventricular ejection duration, forward wave amplitude (FWA), aortic pulse pressure and reflection time, AIx (augmented pressure/pulse pressure * 100), AIx adjusted to heart rate 75/min (AIx@75), and amplification of pulse pressure and systolic pressure (radial pressure/aortic pressure) were determined.

Whole-body impedance cardiography. Beat-to-beat heart rate, stroke volume, cardiac output, and pulse wave velocity (PWV) were recorded using whole-body impedance cardiography (CircMon^{R}, JR Medical Ltd., Tallinn, Estonia). This method records changes in body electrical impedance during cardiac cycles. Systemic vascular resistance was calculated using the BP signal from the radial tonometry and the cardiac index measured by the CircMon^{R} device. Systemic vascular resistance was calculated by subtracting normal central venous pressure (4 mmHg) from mean arterial pressure and dividing it by cardiac output. Systemic vascular resistance and

	Never smoker (n = 365)	Present smoker (n = 81)	Previous smoker (n = 191)
Male/female	164/201	43/38	107/84*
Age (years)	44.2 (0.6)	44.2 (1.3)	46.7 (0.8)
Body mass index (kg/m^2)	26.2 (0.2)	26.4 (0.5)	28.0 (0.3)*†
Office systolic BP (mmHg)	139.6 (1.1)	136.9 (2.4)	144.4 (1.6)†
Office diastolic BP (mmHg)	88.7 (0.6)	87.5 (1.4)	91.8 (0.9)*†
Cigarettes/day	0	5 [2–12]*	10 [3–19] *†
Smoking duration (years)	0	15 [7–25]*	10 [3.0–16.5]*†
Total number of cigarettes	0	21900 [7300–87600]*	21900 [4562–79387]*
Smoking abstinence (years)	n.a.	0	10 [3–20]†
Alcohol (standard drinks/week)	2.0 [0.0–4.0]	5.5 [2.0–13.0]*	3.0 [1.0–9.5]*†
Estimated GFR (ml/min/1.73 m^2)	99.3 (0.8)	98.5 (1.6)	96.4 (1.0)
Hemoglobin (g/L)	143.0 (0.7)	146.0 (1.2)	145.6 (0.8)
Fasting Plasma			
Sodium (mmol/l)	140.4 (0.1)	140.5 (0.2)	140.3 (0.1)
Potassium (mmol/l)	3.81 (0.01)	3.79 (0.02)	3.81 (0.02)
C-Reactive Protein (mg/l)	1.5 (0.1)	1.8 (0.3)	2.0 (0.3)
Triglycerides (mmol/l)	0.97 [0.68–1.34]	1.18 [0.86–1.75]*	1.18 [0.86–1.58]*
HDL cholesterol (mmol/l)	1.60 (0.02)	1.52 (0.04)	1.54 (0.03)
LDL cholesterol (mmol/l)	2.91 (0.05)	3.21 (0.11)*	3.26 (0.07)*
Glucose (mmol/l)	5.39 (0.03)	5.54 (0.08)	5.54 (0.04)*
Insulin (mU/l)	9.04 (1.15)	8.86 (0.84)	9.02 (0.47)
QUICKI	0.361 (0.002)	0.355 (0.004)	0.352 (0.003)#

Table 1. Basic Clinical Characteristics and Laboratory Results. Results shown as mean (standard error of mean) or median [25th to 75th percentile]; n.a., not applicable; GFR, glomerular filtration rate (CKD-EPI cystatin-C creatinine formula); QUICKI, quantitative insulin sensitivity check index; *$P < 0.05$ vs. never smoker; †$P < 0.05$ vs. present smoker (#$p = 0.059$ vs. never smoker).

cardiac output were related to body surface area and presented as indexes – cardiac index, and systemic vascular resistance index (SVRI), respectively. The method and electrode configuration have been previously reported in detail[33,34].

The stroke volume values measured using CircMon^R agree well with 3-dimensional ultrasound[35]. The supine and upright cardiac output values measured with CircMon^R agree well with the values measured using thermodilution[33,34]. The PWV values recorded using CircMon^R show very good correlations with values measured using ultrasound and the tonometric SphygmoCor method[33,34,36].

Experimental protocol. Hemodynamics were recorded in a quiet, temperature-controlled laboratory by trained research nurses[37,38]. Caffeine containing products, smoking or heavy meal were to be avoided for ≥4 hours, and alcohol consumption for >24 hours prior to the studies. The subjects rested supine on the tilt-table with the electrodes placed on body surface, the tonometric sensor on the left radial pulsation, and the oscillometric brachial cuff to the right upper arm. The left arm with the tonometric wrist sensor was abducted to 90 degrees in an arm support, which held the extended arm steady and kept the measurement probes at the heart level both supine and upright.

The actual measurement consisted of one 5-minute period supine and second 5-minute period upright. For the statistical analyses the mean values of each 1-minute period of recording were calculated. The analyses provided information about peripheral and central BP and heart rate[39], and evaluated large arterial stiffness by measurements of central pulse pressure, FWA, and PWV[21,26,40]. The transit of forward pressure waves in the arterial tree was evaluated by recording the amplification of the systolic pressure and pulse pressure[26,41–43], and the influence of reflected waves by the variables aortic reflection time and AIx[27,39,44]. Cardiac performance was examined by the evaluations of left ventricular ejection duration, stroke volume, and cardiac output, while resistance arterial tone was estimated by the calculation of systemic vascular resistance[37,38]. Previously, the good repeatability and reproducibility of the measurement protocol has been demonstrated[45].

Statistics. The demographic and laboratory data was analysed using analysis of variance (ANOVA), and the homogeneity of variances was tested with the Levene's test. If variable distribution was skewed, Kruskal-Wallis was applied with Mann-Whitney U-test in the post-hoc analyses (Table 1). The Bonferroni correction was applied in the post-hoc analyses. Haemodynamic differences between the individual groups were examined in supine and upright positions using ANOVA for repeated measures. The analyses were adjusted for age, and for the following variables that presented with significant differences between the groups in univariate analyses: sex, body mass index (BMI), use of alcohol as standard doses per week, LDL cholesterol; and in analyses concerning PWV also

Augmentation index	b	beta	95% confidence interval for b		P value
			Lower	Upper	
Supine, $R^2 = 0.609$, $p < 0.001$					
Constant	7.069		−16.932	31.069	0.563
Male sex	−6.936	−0.291	−8.423	−5.450	<0.001
Systemic vascular resistance index	0.005	0.245	0.003	0.007	<0.001
Lg_{10} of pulse wave velocity	36.760	0.290	28.668	44.851	<0.001
Stroke index	0.230	0.138	0.106	0.353	<0.001
Heart rate	−0.232	−0.187	−0.337	−0.127	<0.001
Ejection duration	0.093	0.154	0.048	0.138	<0.001
Aortic reflection time	−0.361	−0.474	−0.406	−0.317	<0.001
Upright, $R^2 = 0.733$, $p < 0.001$					
Constant	−10.109		−28.205	7.987	0.273
Male sex	−1.867	−0.076	−3.286	−0.448	0.010
Systemic vascular resistance index	0.002	0.086	0.001	0.003	0.005
Lg_{10} of pulse wave velocity	21.012	0.161	14.842	27.183	<0.001
Stroke index	−0.127	−0.047	−0.295	0.041	0.137
Heart rate	−0.089	−0.080	−0.167	−0.010	0.027
Ejection duration	0.328	0.600	0.292	0.365	<0.001
Aortic reflection time	−0.532	−0.454	−0.585	−0.478	<0.001

Table 2. Linear regression analysis with the enter method: hemodynamic variables and sex as explanatory variables for augmentation index. Variables used: Systemic vascular resistance index, the common logarithm of PWV, heart rate, stroke volume index. Lg_{10}, the common logarithm; n = 631 subjects.

for systolic BP. The analyses were not adjusted for triglycerides, since increased plasma triglyceride concentration may represent a true effect of smoking on plasma lipids[9,10].

Linear regression analysis with the enter method was employed to examine the effect of gender and the haemodynamic variables on the level of AIx in supine and upright positions (Table 2), while stepwise linear regression analysis was employed to examine the associations of demographic, laboratory, and haemodynamic variables with AIx (Supplementary Table). For these analyses the skewed distribution of PWV and triglycerides was corrected by lg_{10}-transformation, while alcohol consumption was treated as a series of discrete variables that were assigned a score of either 0 or 1; cut-points for women 0, 1–7, 8–14, and above 15 doses per week; for men 0, 1–14, 15–24, and above 25 doses per week, according to the prevailing Finnish Guidelines[46]. Spearman's correlations (r_s) were calculated, as appropriate. The results were presented as means and standard errors of the mean (SEM) or median [25th to 75th percentile], and $p < 0.05$ was considered statistically significant. SPSS version 22.0 (IBM SPSS Statistics, Armonk, NY, USA) was used for the statistics.

Results

Study population and laboratory values. The previous smokers had slightly lower proportion of female subjects, while mean age between the study groups did not differ (Table 1). BMI was higher in previous smokers compared to never- and present smokers. In the office systolic BP was ~6 mmHg higher in previous smokers versus present smokers, while diastolic BP was higher in previous smokers compared to never and present smokers. The median number of consumed cigarettes was 21900 in present and previous smokers, while the median abstinence from smoking in previous smokers was 10 years. The weekly intake of alcohol was higher in present and previous smokers than in never smokers, with slightly higher alcohol intake was also observed in the present versus previous smokers, but the average values were well within the limits of moderate drinking in all groups (Table 1). LDL cholesterol level was higher in present and previous smokers, while triglyceride level was higher in present and previous smokers when compared with never smokers. Fasting plasma glucose was slightly higher in the previous smokers versus the never smokers, while QUICKI values were not significantly different between the groups (Table 1).

Haemodynamic effects associated with present and previous smoking. In unadjusted analyses, radial and aortic systolic and diastolic BP was higher in previous smokers than present smokers and never smokers (Fig. 1A–D). However, when adjusted for age, sex, BMI, LDL cholesterol, and use of alcohol, the differences in BP values between the groups were not significant (Supplementary Fig. A,B). In the text below, only the results of the adjusted analyses are being referred to, while the unadjusted statistics are also shown in the figures.

Aortic pulse pressure was not different between the individual groups in either supine or upright position (Fig. 2A). Supine aortic-to-radial amplification of pulse pressure (Fig. 2B) and systolic pressure (Fig. 2C) showed differences in adjusted ANOVA (p = 0.035 and 0.022, respectively), but the differences between individual study groups were not significant. In the upright position, pulse pressure amplification did not differ between the groups (Fig. 2B), while amplification of systolic BP was reduced in the present (p = 0.002) and previous (p = 0.009) smokers versus never smokers (Fig. 2C). No significant differences were found in PWV between the individual study groups in analyses adjusted for age, sex, BMI, LDL cholesterol, use of alcohol, and systolic BP (Fig. 2D).

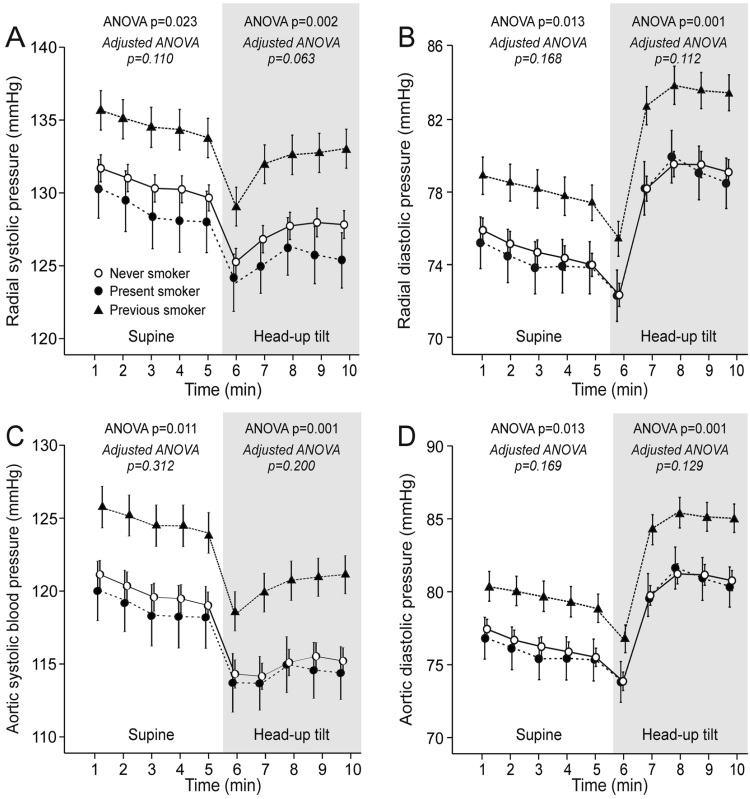

Figure 1. Supine and upright radial systolic (**A**) and diastolic (**B**) blood pressure, and aortic systolic (**C**) and diastolic (**D**) blood pressure in never smokers (n = 365), present smokers (n = 81), and previous smokers (n = 191); mean ± standard error of the mean; ANOVA results from unadjusted analyses (plain text) and from analyses adjusted for age, sex, BMI, LDL cholesterol, and alcohol use (italic) are shown (see Methods).

Neither heart rate (Fig. 3A) nor ejection duration (Fig. 3B) differed between the study groups. Both supine and upright FWA was lower in present smokers than in never smokers ($p \leq 0.042$) (Fig. 3C). Supine aortic reflection time was not different between the groups, but was shorter in present smokers than in previous smokers ($p = 0.049$) during upright position (Fig. 3D).

ANOVA of AIx in the supine position indicated differences ($p = 0.020$) but the deviations between individual groups were not significant (Fig. 3E). However, heart rate adjusted AIx@75 was increased ($p = 0.045$) in the supine position in present smokers versus never smokers (Fig. 3F). In the upright position, both AIx and AIx@75 were higher in present smokers than in never smokers ($p \leq 0.003$), while AIx@75 was also higher in present smokers than in previous smokers ($p = 0.031$, Fig. 3E,F, Supplementary Fig. C).

Supine stroke index was higher in present smokers when compared with never smokers ($p = 0.009$) and previous smokers ($p = 0.001$), while upright values were higher in present than in previous smokers ($p = 0.044$) (Fig. 4A). Cardiac index was increased in present smokers versus previous smokers both supine and upright ($p \leq 0.016$), while cardiac index was lower in previous smokers than in never smokers in the upright position ($p = 0.032$, Fig. 4B, Supplementary Fig. D). When compared with never smokers, supine but not upright SVRI was lower in present smokers ($p = 0.041$), while upright but not supine SVRI was increased in the previous smokers ($p = 0.014$). Both supine and upright SVRI was higher in previous smokers versus present smokers ($p \leq 0.001$) (Fig. 4C).

Results of analyses in subjects not taking medications. Altogether 227 never smokers, 52 present smokers, and 111 previous smokers were without any regular medications. In these subjects, supine AIx@75 was not different between never smokers and present smokers ($p = 0.133$), while the main findings showing increased AIx

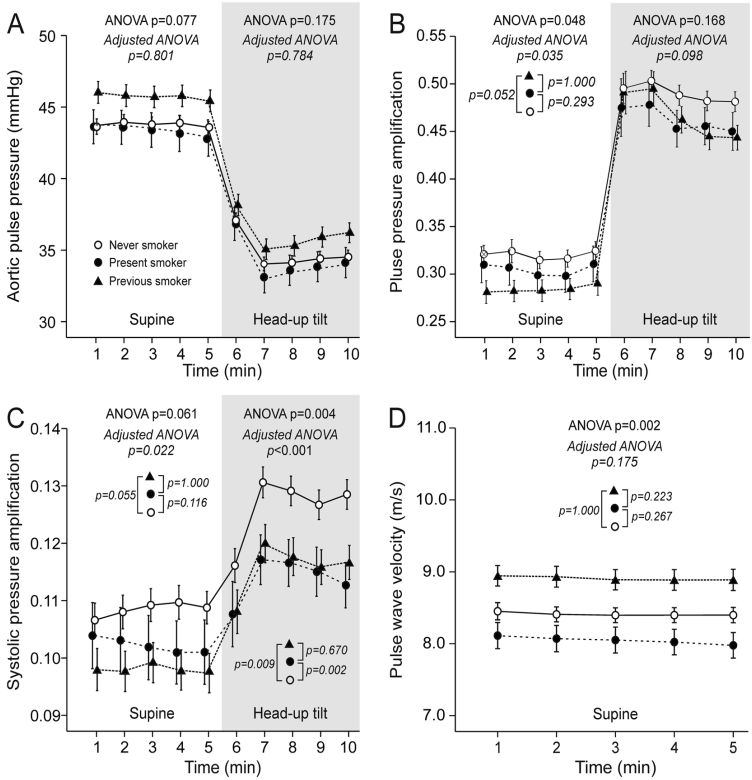

Figure 2. Supine and upright aortic pulse pressure (**A**), pulse pressure amplification (**B**), and systolic amplification (**C**), and supine pulse wave velocity (**D**) in never smokers (n = 365), present smokers (n = 81), and previous smokers (n = 191); mean ± standard error of the mean; ANOVA results from unadjusted (plain text) and adjusted (italic) analyses are shown.

(p = 0.007) and AIx@75 (p = 0.005) in the upright position in the present smokers versus never smokers were still detected. The differences in supine stroke index (p = 0.007) and cardiac index (p = 0.038), and upright aortic reflection time (p = 0.038) remained significant between present smokers and previous smokers. However, some deviations were found when compared with the whole study population: Supine SVRI was not higher in previous smokers than present smokers (p = 0.083), but was higher in previous smokers than in never smokers (p = 0.013). Supine stroke index did not differ between present smokers and never smokers (p = 0.213), while upright stroke index did not differ between present smokers and previous smokers (p = 0.063). Upright cardiac index did not show differences between the groups (previous smokers versus never smokers p = 0.074), while supine and upright SVRI were no more different between present smokers and never smokers (p = 1.000 and p = 1.000, respectively). Supine pulse pressure amplification did not differ (p = 0.072), while supine systolic amplification was lower in previous smokers than never smokers (p = 0.022).

Multivariate analysis about the factors associated with augmentation index. Present smoking increased AIx although it did not reduce heart rate, elevate PWV, or increase systemic vascular resistance, i.e. induce changes in the variables that are most often related to an increase in AIx[36,39,40,47]. Therefore, linear regression analysis was performed to examine the relations of the haemodynamic variables with AIx (Table 2). Due to its powerful confounding, sex was included in the model[36,38,39,48]. These analyses showed that sex and the hemodynamic variables SVRI, PWV, heart rate, ejection duration, and aortic reflection time were significant explanatory variables for AIx (p ≤ 0.027) in both supine and upright positions. Stroke index was a significant explanatory variable for AIx in supine (0 < 0.001) but not in the upright position (p = 0.137). The overall R^2 values for the model were 0.609 in the supine and 0.733 in the upright position.

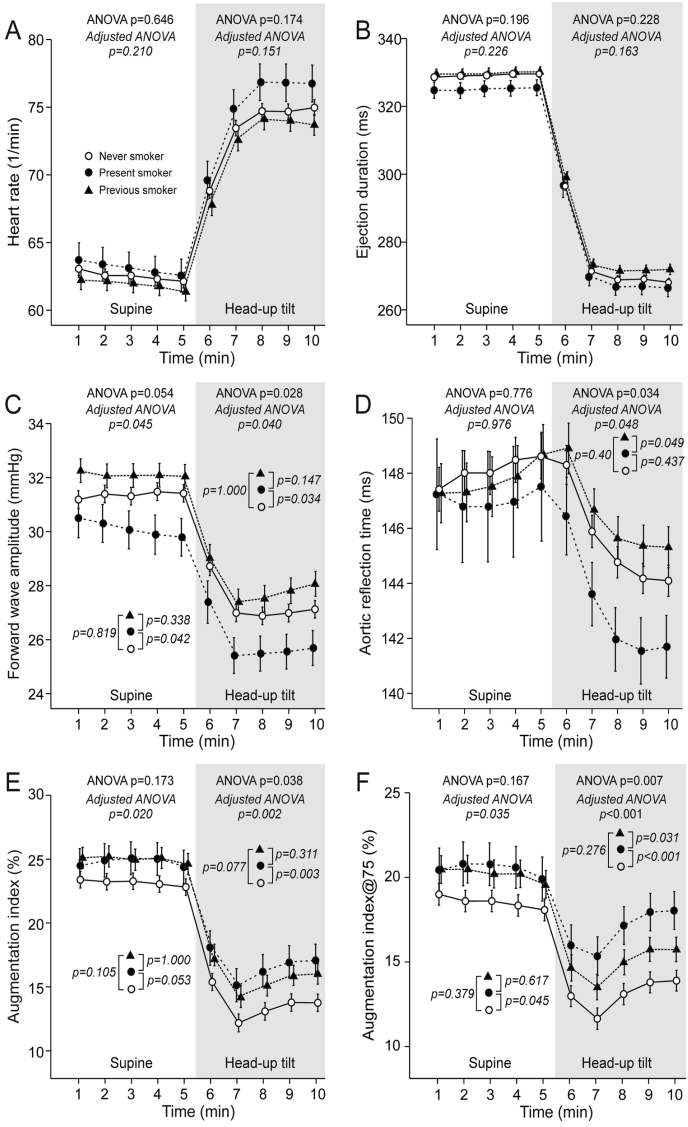

Figure 3. Heart rate (**A**), ejection duration (**B**), forward wave amplitude (**C**), aortic reflection time (**D**), augmentation index (**E**), and augmentation index adjusted to heart rate of 75 beats per minute (**F**) in never smokers (n = 365), present smokers (n = 81), and previous smokers (n = 191); mean ± standard error of the mean; ANOVA results from unadjusted (plain text) and adjusted (italic) analyses are shown.

Additionally, the relations between demographic variables, smoking status, alcohol intake, laboratory variables, haemodynamic variables, and AIx were examined by the use of regression analysis (Supplementary Table). These analyses also showed that the variables that could explain an increase in AIx in present smokers, i.e. elevated supine stroke index and shorter upright aortic reflection time, were independently associated with AIx. Moreover, present smoking was related with elevated AIx both supine and upright.

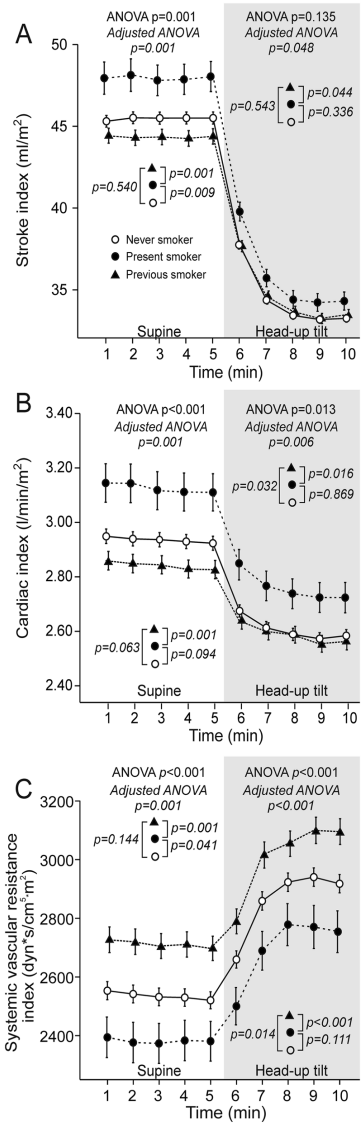

Figure 4. Stroke index (**A**), cardiac index (**B**), and systemic vascular resistance index (**C**) in never smokers (n = 365), present smokers (n = 81), and previous smokers (n = 191); mean ± standard error of the mean; ANOVA results from unadjusted (plain text) and adjusted (italic) analyses are shown.

Discussion

Previous reports about the influence of smoking on the level of BP and arterial stiffness have been contradictory[14,15,17,22,49]. Here we examined the haemodynamic effects of smoking using non-invasive recordings of central BP, arterial stiffness, cardiac performance, and systemic vascular resistance. A passive head-up tilt was included, as possible changes in haemodynamics may become more apparent during upright posture[37,38,50]. Many studies have found that smoking increases AIx, but our findings for the first time suggest that AIx may be higher in smokers due to an increase in cardiac stroke volume and shortening of the aortic reflection time.

Smoking has not been associated with consistent effects on BP[14,15,17]. In the present study, higher BP in previous smokers was attributed to the confounding effects of age, sex, BMI, use of alcohol, and LDL cholesterol. In the adjusted analyses, neither present nor previous smoking influenced BP, corresponding to some previous reports[17,51]. This emphasises that the confounding factors must be carefully taken into account in all analyses[52]. Quitting smoking predisposes to increases in weight and BP[19], while the risk of hypertension increases in previous smokers with increasing years of abstinence[53].

Smokers may have higher serum cholesterol and triglyceride levels than never smokers[54,55]. Insulin resistance in smokers can alter lipid and lipoprotein metabolism[56]. Previously, smokers had higher triglyceride levels than never smokers in the absence of differences in LDL cholesterol[10]. Smokers also exhibited higher postprandial increases in triglyceride levels than non-smokers, indicating impaired lipolytic removal capacity[9]. Altogether, smoking promotes atherosclerosis via several mechanisms including changes in blood clotting and lipids, endothelial function, insulin sensitivity, and autonomic tone[7,11–13,54,57]. In our study, the present smokers had higher plasma concentrations of LDL cholesterol and triglycerides than never smokers. In previous smokers LDL cholesterol and triglycerides were also higher than in never smokers, probably due to the increased BMI[11,58].

Increased PWV is a strong predictor of CVD mortality independent of the level of BP[21]. Early stages of atherosclerosis do not influence the stiffness of the arterial wall, while advanced calcified plaques are associated with increased arterial stiffness[59]. Previously, carotid IMT and plaques were not associated with aortic PWV when adjusted for the confounders age, gender, BP, smoking, and diabetes[60,61]. Thus, aortic stiffness does not predict the severity of carotid atherosclerosis[60,61]. The influence of smoking on PWV remains controversial, and all investigations have not found differences in arterial stiffness between smokers and non-smokers[22,23]. In the present study, PWV did not differ between present smokers and never smokers, while PWV was highest in previous smokers. However, when adjusted for the above confounders, PWV did not differ between the study groups.

The level of AIx, a marker of wave reflections, is influenced by arterial stiffness, heart rate, ventricular ejection duration, body height, BP, systemic vascular resistance, and stroke volume[36,37,39,40,47]. Previous reports have shown that acute, chronic and passive smoking are associated with increased AIx[14,24–28]. However, this has not been attributed to the variables that are known to increase the level of augmentation, like lower heart rate, increased arterial stiffness, or higher systemic vascular resistance[24,26,36,48,49,62] In our study present smokers had higher upright AIx, and higher supine and upright AIx@75, in the absence of changes in BP, heart rate, ejection duration, PWV, and systemic vascular resistance that could explain an increase in AIx. However, present smokers presented with increased stroke index in supine and upright positions, and decreased upright aortic reflection time versus previous smokers. Both of these factors are associated with higher AIx[36,39,44]. In order to elucidate the haemodynamic determinants of wave reflection, we performed regression analyses of the explanatory variables of AIx. These analyses confirmed that stroke index was an independent determinant of supine AIx, while shorter aortic reflection time was associated with higher AIx both supine and upright. Corresponding to a previous report showing that smoking cessation is associated with reductions in AIx[28], the level did not differ between previous smokers and never smokers.

Nicotine in tobacco smoke stimulates the sympathetic nervous system[12,63]. In male smokers, nicotine elevated metabolic rate at rest, and increased energy expenditure during light exercise[64]. The present results showed that smoking was associated with increased stroke index in the absence of changes in heart rate. Thus, smoking stimulated the contractile properties of the heart, probably via mechanisms that increase the metabolic rate[64] and elevate the sympathetic tone[12,63]. Increased stroke index was also translated to higher cardiac output in present smokers versus previous smokers. Previously, current smokers had higher cardiac output than never smokers in an ultrasound-based evaluation[18].

We found that systemic vascular resistance in the present smokers was lower than in never smokers in the supine position, and lower than in previous smokers in both supine and upright positions. Such haemodynamic changes may be related to the impaired oxygen transport properties of blood during smoking, as carbon monoxide in cigarette smoke increases the levels of carboxyhemoglobin in red cells[13]. Carbon monoxide has also vasodilatory properties[65]. Corresponding to our findings, male smokers presented with vasodilatation in the palmar microvasculature when compared with non-smokers[51].

Smokers have twice the death rate versus never smokers due to coronary events, while in patients with coronary heart disease the risk of mortality is reduced after 2 years of abstinence from smoking[66]. There is some immediate reduction in CVD risk after smoking cessation[41], but the period of the remaining increase in risk remains unclear[67,68]. In the present study, upright cardiac output was reduced and systemic vascular resistance was increased in previous smokers versus never smokers. These findings after 10 years of abstinence may represent persistent changes in haemodynamics after the withdrawal of the 10-year-long influence of tobacco smoke on cardiovascular regulation.

In contrast to the increase in PWV and AIx with increasing age, aortic reflection time is only moderately shortened during ageing[44]. In the present study, age correlated strongly with PWV ($r_S = 0.67$) and AIx ($r_S = 0.57$ supine, $r_S = 0.52$ upright), but only moderately with aortic reflection time ($r_S = -0.31$, $r_S = -0.23$ upright) ($p < 0.001$ for all). In concert with earlier findings[26], our results showed that upright aortic reflection time was faster in the present smokers than previous smokers. Shorter aortic reflection time provides a possible explanation for the difference in upright AIx@75 between these groups. Although upright AIx and AIx@75 were higher in the present smokers than in never smokers, neither upright aortic reflection time nor upright stroke index differed between these groups. The possibility remains that statistically insignificant changes in the above variables resulted in higher wave reflections in the present smokers. Supporting this view, the relation of stroke index to aortic reflection time was higher in present smokers than in never smokers both supine and upright (0.334 ± 0.007 versus 0.314 ± 0.03 ml/m^2/ms, $p = 0.009$; 0.248 ± 0.004 versus 0.237 ± 0.002 ml/m^2/ms, $p = 0.021$; respectively).

Aortic-to-brachial pulse pressure amplification reflects arterial compliance in the upper limb, showing reduced values with ageing[42]. Although reduced pulse pressure amplification has been suggested in smokers

versus non-smokers[41,43], the present results did not show differences in this variable between present smokers and never smokers. However, previous smokers presented with impaired amplification of supine and upright systolic pressure. This suggests prevailing differences in the circulation from the aorta to the upper limb in previous smokers, although these findings may also be attributed to the less favourable metabolic profile in this group. Furthermore, upright systolic pressure amplification was impaired in the present smokers versus never smokers. The probable explanation for this is increased augmentation that reduces systolic pressure amplification[26,43].

This study has some limitations. The results should be interpreted cautiously, as non-invasive measurements were used to evaluate cardiac output, and this requires mathematical processing and simplification of physiology[33]. However, invasive haemodynamic measurements cannot be performed without a clear clinical indication. The present methods have been validated against invasive methods, 3-dimensional ultrasound, and tonometric measurements of PWV[32,33,35,36]. The supine and upright recordings lasted in total for 10 minutes, and this gives a rather narrow window of observation for the study of haemodynamics in humans. The present cross-sectional design does not allow conclusions about causal relationship, and the present findings should be confirmed in follow-up studies. Although all subjects using antihypertensive medications and other medications with direct influences on haemodynamics were excluded, the other medications used by 39% of the study population may have influenced the results. However, the principal findings of the study remained very similar when all subjects taking regular medications were excluded from the analyses.

In conclusion, the present results showed that smoking status had a significant influence on the regulation of cardiac output and systemic vascular resistance in the absence of changes in BP and arterial stiffness. The present smokers presented with hyperdynamic circulation and enhanced wave reflection when compared with previous smokers, while the previous smokers had increased upright systemic vascular resistance and lower cardiac output when compared with never smokers. Finally, our findings suggest that increased AIx in present smokers may be attributed to an increase in stroke volume and shortening of the aortic reflection time.

Data Availability

Analyses and generated datasets during the current study are not available publicly as our clinical database contains several indirect identifiers and the informed consent obtained does not allow publication of individual patient data. The datasets are available from the corresponding author on reasonable request.

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Author Contributions

M.K.C., I.P. and E.J.H. reviewed the literature; I.P. and J.M. conceived and designed the study; A.J.T. and I.P. contributed to the collection of data, setup of the haemodynamic recording equipment, and laboratory analyses; I.P., M.K. and K.S. contributed to the technical details and methodology of the study; M.K.C., I.P., A.E., E.J.H. and H.B. analysed the data and interpreted the results; M.K.C. and I.P. drafted the first version of the manuscript. All authors provided intellectual input and contributed to the revision and final version of the manuscript.

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PUBLICATION II

LDL cholesterol is associated with systemic vascular resistance and wave reflection in subjects naive to cardiovascular drugs

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LDL cholesterol is associated with systemic vascular resistance and wave reflection in subjects naive to cardiovascular drugs

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Abstract

Background and aim: Low density lipoprotein cholesterol (LDL-C) is a primary risk factor for atherosclerosis, but it is also associated with elevated blood pressure (BP) and future development of hypertension. We examined the relationship between LDL-C and haemodynamic variables in normotensive and never-treated hypertensive subjects.

Methods: We recruited 615 volunteers (19-72 years) without lipid-lowering and BP-lowering medication. Supine haemodynamics were recorded using continuous radial pulse wave analysis, whole-body impedance cardiography, and single channel electrocardiogram. The haemodynamic relations of LDL-C were examined using linear regression analyses with age, sex, body mass index (BMI) (or height and weight as appropriate), smoking status, alcohol use, and plasma C-reactive protein, sodium, uric acid, high density lipoprotein cholesterol (HDL-C), triglycerides, estimated glomerular filtration rate, and quantitative insulin sensitivity check index as the other included variables.

Results: The mean (SD) characteristics of the subjects were: age 45 (12) years, BMI 27 (4) kg/m^2, office BP 141/89 (21/13) mmHg, creatinine 74 (14) µmol/l, total cholesterol 5.2 (1.0), LDL-C 3.1 (0.6), triglycerides 1.2 (0.8), and HDL-C 1.6 (0.4) mmol/l. LDL-C was an independent explanatory factor for aortic systolic and diastolic BP, augmentation index, pulse wave velocity (PWV), and systemic vascular resistance index ($p<0.05$ for all). When central BP was included in the model for PWV, LDL-C was no longer an explanatory factor for PWV.

Conclusions: LDL-C is independently associated with BP via systemic vascular resistance and wave reflection. These results suggest that LDL-C may play a role in the pathogenesis of primary hypertension.

Keywords: augmentation index, haemodynamics, hypertension, impedance cardiography, LDL cholesterol, pulse wave analysis, systemic vascular resistance

Introduction

Cardiovascular diseases are the leading cause of mortality worldwide representing 31% of all global deaths [1]. One fourth of all deaths in the Western countries are related to coronary heart disease [2]. Subjects with familial hypercholesterolemia are characterized by premature atherosclerosis, and the pathogenic role of low density lipoprotein cholesterol (LDL-C) in this process is well recognized [3]. LDL-C has also vasoconstrictor, pro-inflammatory and thrombogenic properties, and it functions as a mitogenic factor that can stimulate vascular hypertrophy via several growth factors [4].

Previous studies have reported that LDL-C is associated with arterial stiffness, shown as increased pulse wave velocity (PWV) [5], or reduced aortic compliance using ultrasound measurements [6,7]. A positive correlation between LDL-C and PWV was also observed in a group of 315 children aged 8–9 years [8]. However, according to a systematic review, less than 10% of studies demonstrated a positive correlation between serum lipids and arterial stiffness, and therefore the influence of risk factors other than age and blood pressure (BP) on PWV appears to be small [9].

Dyslipidaemias are not only a risk factor for cardiovascular disease, but can also predict the future development of hypertension [10–13] and impairment of endothelial function [14]. LDL-C can reduce nitric oxide bioavailability and blunt the vasodilator response to acetylcholine [4,14–17], and the resulting endothelial dysfunction could be manifested as increased BP. Following treatment with statins, several trials have shown moderate but statistically significant lowering effect on BP [18–23], reduction of arterial stiffness [19,20,24], and improved endothelial function [25,26].

Some previous studies that addressed the association of LDL-C with haemodynamic variables included subjects taking antihypertensive medications and even diabetic patients [5–7]. The objective of this cross-sectional study was to investigate the association of LDL-C with haemodynamic variables that

could potentially explain differences in BP between normotensive subjects and never-treated patients with primary hypertension.

Methods

Participants

All subjects participated in an ongoing study with the aim to examine haemodynamics in primary and secondary hypertension versus normotension (DYNAMIC study; ClinicalTrails.gov identifier NCT01742702). The participant recruitment has been described before [27,28], and the study flow-chart is presented in Supplementary Figure 1. Subjects taking lipid-lowering or BP-lowering medication or with a history of coronary artery disease, stroke, heart failure, valvular heart disease, diabetes, chronic kidney disease, secondary hypertension, alcohol or substance abuse, psychiatric illnesses, or heart rhythm other than sinus were excluded (total number of enrolled subjects 1349). All subjects underwent physical examination by a medical doctor, measurement of office BP, and routine laboratory analyses for elevated BP according to the guidelines of the European Society of Hypertension [29]. The medical history, lifestyle habits and use of medicines, dietary supplements, and other substances not registered as drugs were documented along with information about smoking and alcohol consumption as standard drinks (~12 grams of absolute alcohol) per week.

A total of 615 normotensive and never-treated subjects with primary hypertension, aged 19-72 years, were included in the study. Based on the office BP measurements on a single occasion, 249 (40.5%) of the participants were normotensive and 366 (59.5%) were hypertensive. For the graphical illustrations, they were divided into age- and sex-adjusted LDL-C quartiles (Q1, n=153; Q2, n=158; Q3, n=148; Q4, n=156). Signed informed consent was obtained from all participants. The study complies with the

declaration of Helsinki, and was approved by the ethics committee of the Tampere University Hospital (study code R06086M) and the Finnish Medicines Agency (Eudra-CT registration number 2006-002065-39).

Altogether 230 (37.4%) of the 615 persons used some medications. Seventy-six females were treated with systemic oestrogen, progestin, or their combination (contraception, hormone replacement therapy), and 1 subject with tibolone. Forty-one subjects were treated with antidepressants, 18 with antihistamines, 17 with inhaled corticosteroids, 13 with proton pump inhibitors, while 22 euthyroid subjects were on a stable dose of thyroid hormone. Other medications in use were hypnotics or sedatives (8), low dose acetylsalicylic acid (6), non-steroidal anti-inflammatory drugs (4), antirheumatic agents (4), antiepileptics (3), allopurinol (3), coxibs (3), antipsychotics (2), muscle relaxants (2), varenicline (2), antiviral agents (2), paracetamol (1), carbimazole (1), isotretinoin (1), and alendronate (1). One subject was treated with warfarin because of an anti-phospholipid syndrome, and she was physically well and symptomless during the recordings.

Laboratory analyses

Blood and urine sampling was preceded by about 12 hours of fasting. Plasma total, high density lipoprotein cholesterol (HDL-C), LDL-C, triglyceride, C-reactive protein (CRP), sodium, potassium, glucose, cystatin-C, and creatinine concentrations were determined using Cobas Integra 700/800 (F. Hoffmann-Laroche Ltd, Basel; Switzerland) or Cobas6000, module c501 (Roche Diagnostics, Basel, Switzerland), insulin using electrochemiluminescence immunoassay (Cobas e411, Roche Diagnostics), and blood cell count by ADVIA 120 or 2120 (Bayer Health Care, Tarrytown, NY, USA). Urine dipstick analysis was made by an automated refractometer test (Siemens Clinitec Atlas or Advantus,

Siemens Healthcare GmbH, Erlangen, Germany). For evaluation of insulin sensitivity the quantitative insulin sensitivity check index (QUICKI) [30] was calculated, and glomerular filtration rate (eGFR) was estimated using the CKD-EPI cystatin C formula [31].

Pulse wave analysis

Radial BP and pulse wave were continuously recorded from radial pulsation using a tonometric sensor (Colin BP-508T, Colin Medical Instruments Corp., USA), secured on the radial pulse with a wrist band. The radial BP signal was calibrated twice during each 5 minute-period by contralateral brachial BP measurements. Aortic BP was derived with the SphygmoCor system (SpygmoCor PWMx®, AtCor medical, Australia) by means of the validated generalized transfer function [32]. Left ventricular ejection duration, aortic pulse pressure and reflection time, augmentation index (AIx, augmented pressure/pulse pressure*100), AIx adjusted to heart rate 75/min (AIx@75), and amplification of pulse pressure and systolic pressure (radial pressure/aortic pressure) were determined [27,28].

Whole-body impedance cardiography

Beat-to-beat heart rate, stroke volume, cardiac output, and PWV were recorded using whole-body impedance cardiography (CircMon®, JR Medical Ltd., Tallinn, Estonia) that detects changes in body electrical impedance during cardiac cycles. The method and electrode configuration have been previously reported [33]. Systemic vascular resistance (SVR) was calculated from the tonometric BP signal and cardiac index measured by CircMon®. SVR was calculated by subtracting normal central venous pressure (4 mmHg) from mean arterial pressure and dividing it by cardiac output. SVR and cardiac output were presented as indexes related to body surface area – cardiac index, and systemic

vascular resistance index (SVRI), respectively. The stroke volume values measured using CircMon® correlate well with 3 dimensional ultrasound [34]. The supine cardiac output values measured with CircMon® correlate well with the values measured using thermodilution [33]. The whole-body impedance cardiography tends to overestimate PWV, and a validated equation was utilized to calculate values correspond to the ultrasound method (PWV = PWVimpedance * 0.696 + 0.864) [35]. By the use of this equation, the PWV values recorded using CircMon® show very good correlations with values measured using either ultrasound (r=0.91) [35] or the tonometric SphygmoCor® method (r=0.82, bias 0.02 m/s, 95% confidence interval -0.21 to 0.25) [28].

Experimental protocol

Haemodynamics were recorded in a quiet, temperature-controlled laboratory by research nurses [36]. Caffeine containing products, smoking or heavy meals were to be avoided for ≥4 hours, and alcohol consumption for >24 hours prior to the studies. The subjects rested supine, the left arm with the tonometric sensor abducted to 90 degrees in an arm support. After getting accustomed to the laboratory for about 10 minutes, supine haemodynamics were recorded for five minutes. For the statistical analyses the mean values of each 1-minute period of recording were calculated. The good repeatability and reproducibility of the measurement protocol has been demonstrated [37].

Statistics

Continuous variables were expressed as the mean and standard deviation (SD) or standard error of the mean (SEM). Baseline characteristics were depicted as age- and sex-adjusted quartiles of LDL-C (Table 1). The demographic and laboratory data was analysed using analysis of variance (ANOVA),

and the Bonferroni correction was applied in the post-hoc analyses. The homogeneity of variances was tested with the Levene's test. For the illustrations, the haemodynamic differences between the quartiles were examined using ANOVA for repeated measures adjusted for age and sex. Spearman's correlations (rS) were calculated, as appropriate.

A multiple regression analysis with stepwise elimination was applied to evaluate the associations between age, sex, body mass index (BMI) (for systemic vascular resistance index BMI was replaced by height and weight), smoking status, alcohol consumption, QUICKI, plasma CRP, sodium, uric acid, HDL-C, LDL-C, triglycerides, and eGFR (independent variables), and aortic systolic and diastolic BP, aortic pulse pressure, AIx, PWV and SVRI (dependent variables) in model 1. The variables in model 2 were model 1 + PWV (independent variables) for aortic systolic and diastolic BP, aortic pulse pressure (dependent variables); for AIx (dependent variable): model 1 + heart rate, SVRI, and PWV (independent variables); for PWV (dependent variable): model 1 + aortic systolic BP (independent variables). For these analyses the skewed distribution of PWV was corrected by lg_{10}-transformation. $P<0.05$ was considered statistically significant. SPSS version 22.0 (IBM SPSS Statistics, Armonk, NY, USA) was used for the statistics.

Results

Study population and laboratory values

In total, 615 subjects were included in the analyses, consisting of 314 male (51%) and 301 female (49%) subjects (Table 1). The age range was 19–72 years, mean (SD) age was 44.9 (11.9) years, BMI 26.8 (4.4) kg/m^2, office systolic/diastolic BP 140.5 (21.1) / 89.3 (12.5) mmHg, creatinine 73.7 (13.5) µmol/l, total cholesterol 5.2 (1.0), LDL-C 3.1 (0.6), triglycerides 1.2 (0.8) and HDL-C 1.6 (0.4) mmol/l

(Table 1). Altogether 80 (13%) of the subjects had impaired fasting plasma glucose (6.1-7.0 mmol/l), while in 6 subjects (1%) the fasting plasma glucose was in the range of 7.1-10.3 mmol/l. However, none of the study participants had glucosuria or proteinuria in the morning urine sample.

For the graphical illustrations the participants were divided into age- and sex-adjusted LDL-C quartiles. The average LDL-C in the quartiles ranged from 2.05 (0.51) (Q1) to 4.15 (0.75) (Q4) mmol/l (Table 1). After adjustments for age and sex, the quartiles presented with differences in BMI, office systolic and diastolic BP, eGFR, QUICKI, and plasma cystatin C, total cholesterol, triglycerides, HDL-C, and glucose concentrations (Table 1). Alcohol intake, smoking status, and plasma creatinine, sodium, potassium, uric acid, CRP, and insulin concentrations were not significantly different between the quartiles.

Haemodynamic variables in the quartiles of LDL-C adjusted for age and sex

Aortic and radial systolic and diastolic BP were not significantly different in adjacent quartiles, but were different in all other comparisons between the quartiles, so that the highest LDL-C quartile (Q4) presented with the highest BP (Figures 1A-1B, Supplementary Figures 2A-2B). Aortic pulse pressure differed between the highest (Q4) and lowest LDL-C quartile (Q1) (Figure 1C), while AIx (Figure 1D) and AIx@75 (Supplementary Figure 2C), and heart rate and cardiac index (Figures 2A-2B) were not significantly different between the quartiles. SVRI differed between Q4 and Q1 (Figure 2C), while PWV was higher in Q4 than in Q1 and Q2 (Figure 2D).

LDL-C and haemodynamic variables in stepwise linear regression analyses

We performed linear regression analyses to examine the relationships between the haemodynamic variables and age, sex, BMI (for systemic vascular resistance index replaced by height and weight),

smoking status, weekly alcohol consumption, insulin sensitivity (QUICKI) [30], cystatin C based eGFR [31], and plasma CRP, sodium, uric acid, HDL-C, LDL-C, and triglyceride concentrations (model 1, Table 2). These analyses showed that LDL-C was a significant independent explanatory factor for aortic systolic and diastolic BP, aortic pulse pressure, AIx, PWV, and SVRI ($p<0.05$ for all) (model 1, Table 2).

The model 2 for aortic systolic and diastolic BP, and aortic pulse pressure included PWV in addition to the above variables of model 1 (model 2, Table 2). These results showed that LDL-C was a significant independent explanatory factor for aortic systolic and diastolic BP ($p<0.05$ for all), while in this model LDL-C was no more an explanatory factor for aortic pulse pressure.

The model 2 for AIx contained the variables heart rate, SVRI and PWV in addition to the above variables of model 1. The outcome was that LDL-C, smoking status, sex, age, BMI, heart rate, and SVRI were independent significant explanatory factors for AIx (model 2, Table 2).

The model 2 for PWV contained aortic systolic BP in addition to the variables of model 1. In this model LDL-C was no more an explanatory factor for PWV. The significant explanatory factors for PWV were age, aortic systolic BP, uric acid, triglycerides, HDL-C, smoking status and sodium (model 2, Table 2). If aortic mean BP or aortic diastolic BP was used in the model 2 instead of aortic systolic BP, LDL-C was not an independent explanatory factor for PWV, either (not shown).

Discussion

LDL-C is an established risk factor for atherosclerosis and endothelial dysfunction, but it has also been linked with elevated BP [10,11,18,20]. We examined the relations of LDL-C with several cardiovascular variables using non-invasive recordings of haemodynamics. The present results indicated that LDL-C was independently associated with BP, AIx, and SVRI. LDL-C was also

associated with increased PWV, but the relation between PWV and LDL-C was no longer significant when central BP was included in the model.

Increased large artery stiffness, manifested as accelerated PWV and elevated pulse pressure, increases with aging and is an independent cardiovascular risk factor [38]. As atherosclerosis and plaque formation alter the properties of the arterial wall, the measures of arterial stiffness have been considered as surrogate markers of atherosclerosis [9,39]. Due to the central role of LDL-C in atherosclerosis, its relation with arterial stiffness would seem evident, and a positive correlation between LDL-C and PWV has been reported [5–8]. However, the relationship between LDL-C and arterial stiffness, a process characterized by increased fibrosis and collagen deposition in the arterial wall, remains controversial: according to a comprehensive review, the majority of studies did not find a positive correlation between serum lipids and arterial stiffness, as measured using determinations of PWV [9].

In addition to age, PWV is strongly dependent on the prevailing level of BP [9,39]. In our study LDL-C was associated with PWV, but when central BP was included in the regression model, LDL-C was no more an explanatory factor for arterial stiffness. Our results indicated that triglyceride concentration was directly, and HDL-C was inversely, associated with PWV in the linear regression model 2. Previously, higher triglyceride and lower HDL-C levels have been associated with increased PWV [40,41]. Of note, the characteristic components of the metabolic syndrome, i.e. lower HDL-C, higher triglycerides, and higher uric acid were all associated with higher PWV in the present statistical model 2, corresponding to previous findings [27,40]. The influence of glucose metabolism on haemodynamics was taken into consideration in the regression analyses by the inclusion of the QUICKI index in the variables. Many reports support the view that statins can improve arterial stiffness [19,20,24]. The present results raise the possibility that the lowering effect of statins on PWV could partially be mediated via the beneficial effect on central BP.

LDL-C may be a significant risk factor for the development of hypertension. Laaksonen et al. found that abnormal LDL-C and triglyceride metabolism predicted future development of hypertension in middle-aged men [11]. In a study comprising 20,074 subjects, the incidence of new onset of hypertension was lower in subjects with lower LDL-C [13]. Several studies have revealed small but significant reductions in BP after treatment with statins [18,19,22,23]. A meta-analysis comprising 828 subjects reported a decrease of BP by 1.9/0.9 mmHg following statin therapy that was unrelated to age, changes in serum cholesterol, or length of the trial [21]. Another meta-analysis including 22,602 statin-treated patients and 22,511 controls found that statins decreased BP by 2.62/0.94 mmHg, an effect that was not related to patient age, follow-up duration, or the evaluated quality of the study [22]. In the Anglo-Scandinavian Cardiac Outcomes Trial, 10,305 hypertensive patients were randomly assigned to receive atorvastatin 10 mg daily or placebo for a median follow-up of 3.3 years [42]. Office BP throughout the trial was similar in the atorvastatin and placebo groups. However, no conclusions could be drawn about the lack of BP-lowering effect of atorvastatin in this study, as antihypertensive medication was titrated upwards based on achieved BP, and this potentially masked any impact of atorvastatin on BP [42].

Endothelial dysfunction can be manifested as elevated BP [16], and factors like dyslipidaemia that impair endothelium-dependent vasomotion may play a role in the pathogenesis of primary hypertension [12,14]. Blood lipids, including LDL-C, have a number of non-atheromatous effects on blood vessels, which increase oxidative stress and inflammation, and promote elastin damage and deposition of calcium within the arterial wall [43,44]. LDL-C has been found to impair endothelial nitric oxide bioavailability through increased vascular production of reactive oxygen species and enhanced responses to vasoconstrictors like angiotensin II [15,17,45,46]. Therefore, the consequence of impaired endothelium-mediated dilatation in the resistance vessels would be the elevation of SVR. Twelve month treatment with statins improved flow-mediated dilatation in the brachial artery [25], while a

meta-analysis reported increased flow-mediated dilatation following treatment with pitavastatin [26]. As the deleterious effect of LDL-C on endothelial dysfunction can be reversed by statins, this provides a potential explanation for the beneficial effects of these agents on BP [20,24–26]. Experimental evidence suggests that LDL-C can increase the angiotensin II type 1 receptor density in the arterial wall [15,17], while statin treatment *in vivo* can reduce the vasoconstrictor responses elicited by angiotensin II in isolated human internal thoracic artery segments *in vitro* [47]. Collectively, the above findings support the view that LDL-C is a significant explanatory factor for BP via increased SVR. Supporting this view, plasma total cholesterol was recently found to be independently associated with the media:lumen ratio of small arteries obtained from humans by biopsy [48]. This variable that characterises resistance vessel structure is directly linked to the regulation of SVR [49].

The present results indicated that LDL-C was an independent determinant of AIx (models 1 and 2) and aortic pulse pressure (model 1), corresponding to previous findings [50,51]. AIx and central pulse pressure were higher in subjects with hypercholesterolemia than in controls [50]. Men with higher LDL-C level had increased AIx in all age groups, and a similar finding was observed in women under 60 years of age [51]. A significant proportion of the reflected pressure wave originates from resistance arteries [49], and the level of augmentation is equally influenced by SVR and arterial stiffness [28]. Therefore, the association between LDL-C and wave reflection can be explained via SVR, the lowering of which reduces the magnitude of AIx. We also found an inverse relationship between smoking and SVR, which could be mediated via the vasodilating influence of carbon monoxide in tobacco smoke [52]. Unexpectedly, smoking showed a small inverse association with PWV, and we can speculate that lower SVR may also favour reductions in PWV. Of note, according to a comprehensive review, smoking has not influenced PWV in the majority of studies [9].

The current study has limitations and the interpretation of the results must be done cautiously. Although the methods have been validated against invasive measurements, 3 dimensional ultrasound,

and tonometric recordings of PWV [28,32–34], the non-invasive evaluation of stroke volume and cardiac output is based on mathematical processing of the bioimpedance signal and simplification of physiology [33]. The supine recordings lasted 5 minutes, and this gives a rather narrow window of observation for the study of haemodynamics. However, when compared with single measurements of BP and heart rate, these continuous evaluations were still based on variables collected from more than 300 cardiac cycles. The cross-sectional design does not allow conclusions about causal relationship, and the present findings should be confirmed in follow-up studies. As the haemodynamic recordings were performed in subjects who were themselves willing to participate, this makes a potential source for selections bias. Although the results were adjusted for multiple covariates that may be associated with LDL-C, the possibility of residual confounding remains. Finally, LDL-C and haemodynamics were measured during one single occasion, and repeated measurements of all variables would strengthen the findings.

In conclusion, the present results showed that LDL-C was independently associated with BP and this effect could be attributed to elevated SVR that also resulted in enhanced wave reflection. Therefore, LDL-C could play a role in the pathogenesis of primary hypertension, possibly via its harmful influence on endothelium-dependent vasodilation.

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Disclosure statement

The authors declare no conflict of interest with respect to this manuscript.

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Data availability

Analyses and generated datasets that support the current study are not available publicly. The datasets are available from the corresponding author on reasonable request.

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FIGURE LEGENDS

Figure 1. Supine aortic systolic (A) and diastolic (B) blood pressure, and aortic pulse pressure (C) and augmentation index (D) in age- and sex-adjusted quartiles (Q1-Q4) of LDL-cholesterol during 5-minute recordings. Q1 (n=153), Q2 (n=158), Q3 (n=148) and Q4 (n=156); mean±standard error of the mean; *p<0.05, ANOVA for repeated measurements.

Figure 2. Heart rate (A), cardiac index (B), systemic vascular resistance index (C), and pulse wave velocity (D) in age- and sex-adjusted quartiles of LDL- cholesterol during 5-minute recordings. Quartiles as in Figure 1; mean±standard error of the mean; *p<0.05, ANOVA for repeated measurements.

Figure 1.

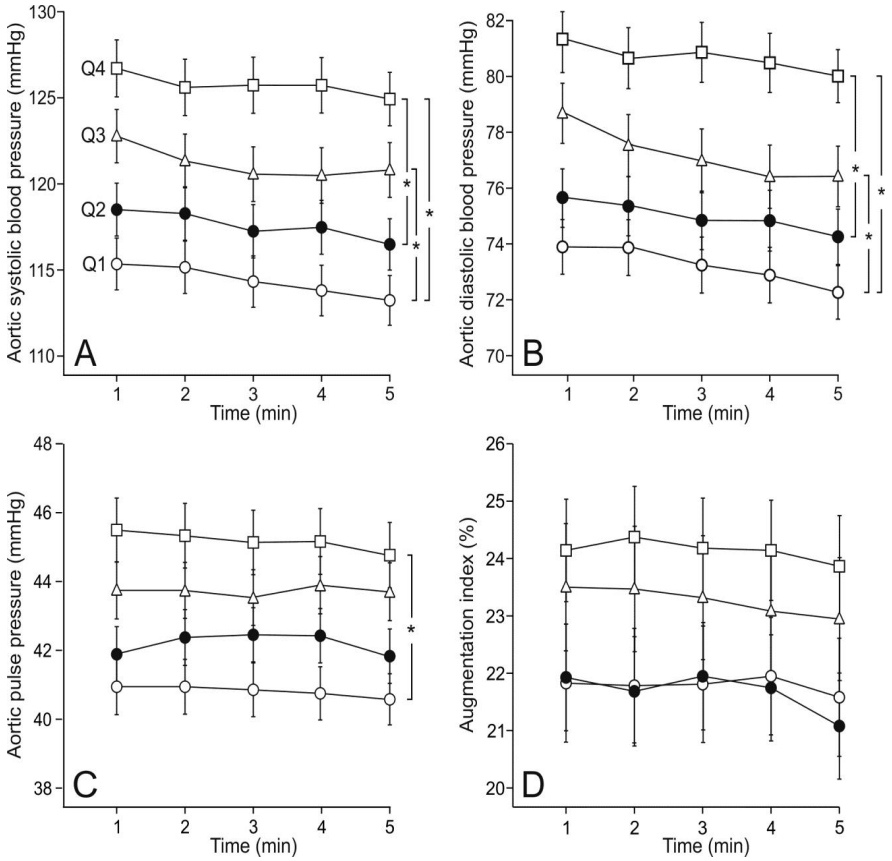
Aortic systolic blood pressure (mmHg)
130
125
120
115
110
Q4
Q3
Q2
Q1
A
1
2
3
4
5
Time (min)
*
*
*
Aortic diastolic blood pressure (mmHg)
82
80
78
76
74
72
70
B
Time (min)
*
*
*
Aortic pulse pressure (mmHg)
48
46
44
42
40
38
C
Time (min)
*
Augmentation index (%)
26
25
24
23
22
21
20
D
Time (min)

Figure 2.

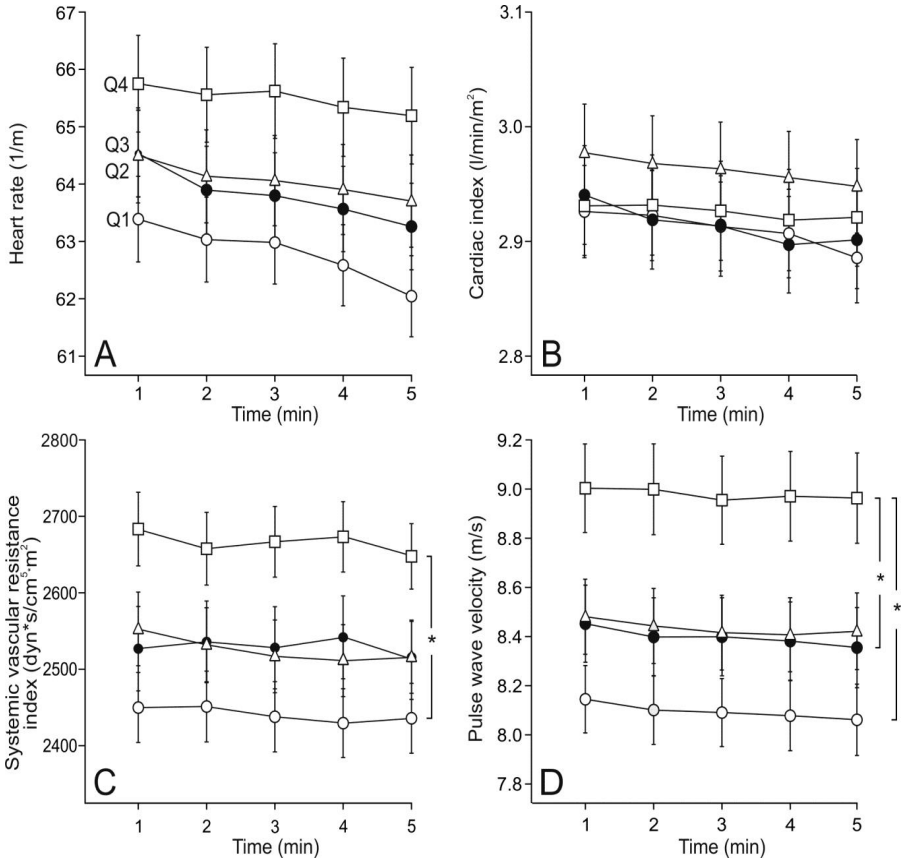
Heart rate (1/m)
Q4
Q3
Q2
Q1
A
Time (min)
Cardiac index (l/min/m²)
B
Time (min)
Systemic vascular resistance index (dyn*s/cm⁵*m²)
C
Time (min)
Pulse wave velocity (m/s)
D
Time (min)
*
*
*

TABLE 1. Age and sex adjusted characteristics of the study population in quartiles of LDL cholesterol.

	Overall	Q1	Q2	Q3	Q4
Number	615	153	158	148	156
Male / female	314 / 301	80 / 73	79 / 79	75 / 73	80 / 76
Age (years)	44.9 (11.9)	44.6 (11.8)	44.1 (12.6)	45.1 (12.6)	45.7 (10.7)
Age range (years)	19-72	20-67	19-71	20-72	21-72
BMI (kg/m^2)	26.8 (4.4)	25.6 (4.5)	26.4 (4.5)	27.2 (4.1)*	28.1 (4.2)*†
Alcohol (standard doses/week)	4.5 (5.7)	3.8 (4.9)	4.1 (5.1)	4.9 (6.5)	5.0 (6.3)
Smokers (number / percentage)	70 / 11.4%	14 / 9.1%	16 / 10.1%	21 / 14.2%	25 / 16.0%
Office blood pressure (mmHg)					
Systolic	140.5 (21.1)	134.7 (19.9)	138.8 (20.9)	139.4 (19.8)*	149.0 (20.9)*†
Diastolic	89.3 (12.5)	85.3 (12.0)	88.6 (12.9)	90.0 (11.4)*	94.0(12.1)*†
eGFR (ml/min per 1.73 m^2)	98.8 (18.1)	102.0 (18.1)	101.3 (18.3)	97.7 (17.1)	94.2 (18.0)*†
QUICKI	0.358 (0.042)	0.369 (0.041)	0.365 (0.056)	0.349 (0.032)*†	0.349 (0.032)*†
Fasting plasma					
Creatinine (µmol/l)	73.7 (13.5)	72.2 (12.3)	74.8 (14.1)	74.1 (13.5)	73.6 (13.9)
Cystatin C (mg/l)	0.85 (0.15)	0.82 (0.15)	0.83 (0.15)	0.85 (0.14)	0.89 (0.14)*†
Sodium (mmol/l)	140.3 (2.0)	140.3 (2.1)	140.4 (2.0)	140.5 (2.0)	140.3 (1.7)
Potassium (mmol/l)	3.81 (0.28)	3.82 (0.28)	3.81 (0.28)	3.84 (0.26)	3.77 (0.31)
Uric acid (µmol/l)	303 (76)	291 (78)	297 (79)	310 (76)	312 (71)
CRP (mg/l)	1.7 (2.9)	1.6 (3.0)	1.7 (4.1)	1.5 (1.5)	2.0 (2.2)
Total cholesterol (mmol/l)	5.15 (1.02)	4.23 (0.68)	4.78 (0.64)*	5.33 (0.63)*†	6.25 (0.85)*$^{\dagger\#}$
Triglycerides (mmol/l)	1.23 (0.76)	1.05 (0.96)	1.17 (0.69)	1.23 (0.62)	1.49 (0.70)*$^{\dagger\#}$
HDL-C (mmol/l)	1.58 (0.44)	1.71 (0.488)	1.54 (0.41)*	1.56 (0.41)*	1.52 (0.43)*
LDL-C cholesterol (mmol/l)	3.05 (0.95)	2.05 (0.51)	2.75 (0.48)*	3.26 (0.50)*†	4.15 (0.75)*$^{\dagger\#}$
Insulin (mU/L)	8.9 (17.0)	7.2 (5.6)	7.9 (6.1)	11.6 (33.3)	9.1 (5.4)
Glucose (mmol/l)	5.44 (0.58)	5.34 (0.69)	5.40 (0.59)	5.49 (0.48)	5.55 (0.54)*

Mean (standard deviation), *p<0.05 vs Q1; †p<0.05 vsQ2; $^{\#}$$p$<0.05 vs Q3; BMI, body mass index; eGFR, cystatin C based CDK-EPI formula for estimated glomerular filtration rate [31]; QUICKI, quantitative insulin sensitivity check index [30]; CRP, C-reactive protein; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol.

TABLE 2. Explanatory factors for haemodynamic variables in linear regression analyses with stepwise elimination.

Aortic systolic blood pressure: model 1					**Aortic systolic blood pressure: model 2**				
	B	Beta	R squared	*p*		B	Beta	R squared	*p*
(Constant)	-16.615			0.738	(Constant)	-98.362			0.044
eGFR	-0.222	-0.208	0.205	<0.001	PWV	77.269	0.377	0.306	<0.001
LDL-C	3.343	0.165	0.259	<0.001	eGFR	-0.187	-0.175	0.352	<0.001
Age	0.332	0.204	0.285	<0.001	LDL-C	2.586	0.128	0.366	0.001
BMI	0.531	0.122	0.304	0.003	Sodium	1.077	0.109	0.374	0.001
Sodium	0.945	0.096	0.312	0.006	QUICKI	-34.232	-0.076	0.380	0.036
QUICKI	-38.761	-0.086	0.317	0.026	HDL-C	4.509	0.103	0.384	0.005
					BMI	0.434	0.099	0.389	0.014

Aortic diastolic blood pressure: model 1					**Aortic diastolic blood pressure: model 2**				
	B	Beta	R squared	*p*		B	Beta	R squared	*p*
(Constant)	19.283			0.588	(Constant)	-32.811			0.316
eGFR	-0.204	-0.286	0.170	<0.001	PWV	54.974	0.401	0.244	<0.001
LDL-C	2.265	0.168	0.217	<0.001	eGFR	-0.155	-0.218	0.284	<0.001
QUICKI	-33.714	-0.112	0.238	0.005	QUICKI	-35.114	-0.116	0.306	0.001
Sex	2.564	0.099	0.252	0.008	Alcohol amount	0.198	0.088	0.317	0.011
BMI	0.283	0.097	0.257	0.021	Sodium	0.628	0.095	0.324	0.005
Sodium	0.525	0.080	0.261	0.032	Age	-0.149	-0.137	0.330	0.006
					LDL-C	1.466	0.109	0.337	0.006

Aortic pulse pressure: model 1					**Aortic pulse pressure: model 2**				
	B	Beta	R squared	*p*		B	Beta	R squared	*p*
(Constant)	-46.757			0.092	(Constant)	-50.479			0.076
Age	0.307	0.354	0.206	<0.001	Age	0.190	0.219	0.206	<0.001
BMI	0.355	0.152	0.223	<0.001	PWV	24.365	0.222	0.238	<0.001
LDL-C	1.331	0.123	0.230	0.003	eGFR	-0.047	-0.083	0.244	0.064

HDL-C	2.398	0.102	0.235	0.012	BMI	0.291	0.125	0.248	0.003
Sodium	0.417	0.079	0.240	0.032	HDL-C	2.677	0.114	0.255	0.005
					Sodium	0.393	0.074	0.259	0.041

Augmentation index: model 1					**Augmentation index: model 2**				
	B	Beta	R squared	*p*		B	Beta	R squared	*p*
(Constant)	-1.268			0.414	(Constant)	7.678			0.016
Age	0.576	0.570	0.350	<0.001	Age	0.532	0.527	0.350	<0.001
Sex	-9.060	-0.378	0.483	<0.001	Sex	-9.687	-0.404	0.483	<0.001
LDL-C	0.841	0.067	0.485	0.043	SVRI	0.005	0.247	0.549	<0.001
					Heart rate	-0.205	-0.164	0.576	<0.001
					Smoker	2.930	0.080	0.583	0.003
					BMI	-0.240	-0.088	0.587	0.004
					LDL-C	0.774	0.062	0.589	0.047

Pulse wave velocity: model 1					**Pulse wave velocity: model 2**				
	B	Beta	R squared	*p*		B	Beta	R squared	*p*
(Constant)	0.566			<0.001	(Constant)	0.916			<0.001
Age	0.004	0.544	0.419	<0.001	Age	0.004	0.501	0.419	<0.001
Uric acid	0.0002	0.172	0.493	<0.001	Aortic systolic blood pressure	0.001	0.281	0.510	<0.001
Triglycerides	0.013	0.102	0.509	0.002	Uric acid	0.0002	0.154	0.555	<0.001
LDL-C	0.011	0.116	0.518	0.001	Triglycerides	0.012	0.095	0.568	0.003
Smoker	-0.023	-0.081	0.525	0.005	Smoker	-0.019	-0.067	0.571	0.013
BMI	0.002	0.080	0.528	0.019	HDL-C	-0.019	-0.088	0.575	0.006
					Sodium	-0.003	-0.056	0.578	0.045

Systemic vascular resistance index: model 1				
	B	Beta	R squared	*p*
(Constant)	3417.956			<0.001
eGFR	-5.613	-0.172	0.106	<0.001
Weight	9.149	0.246	0.142	<0.001
Age	5.122	0.103	0.159	0.031
Smoker	-220.835	-0.123	0.171	0.001
Height	-8.445	-0.133	0.181	0.005
LDL-C	64.294	0.104	0.188	0.016

For all these analyses, the skewed distribution of pulse wave velocity (PWV) was Lg10 transformed.

Variables in **model 1**: age, sex, body mass index (BMI) (for systemic vascular resistance BMI was replaced by height and weight), smoking status, consumption of standard drinks of alcohol per week, quantitative insulin sensitivity check index (QUICKI) [30], plasma C-reactive protein (CRP), sodium, uric acid, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triglycerides, and cystatin C based CDK-EPI formula for estimated glomerular filtration rate (eGFR) [31].

Variables in **model 2**: **model 1** + PWV; for augmentation index: **model 1** + heart rate, systemic vascular resistance index (SVRI), and PWV; for PWV: **model 1** + aortic systolic blood pressure.

PUBLICATION III

Atherogenic index of plasma is related to arterial stiffness but not to blood pressure in normotensive and never-treated hypertensive subjects

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ORIGINAL ARTICLE

Atherogenic index of plasma is related to arterial stiffness but not to blood pressure in normotensive and never-treated hypertensive subjects

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ABSTRACT

Background and aims: Atherogenic index of plasma (AIP), defined as the logarithm of triglycerides to high-density lipoprotein cholesterol (HDL-C) ratio, is a strong predictor of future cardiovascular disease. Our aim was to examine the association of AIP with haemodynamic variables in normotensive and never-treated hypertensive subjects in a cross-sectional study.

Methods: Supine haemodynamics in 615 subjects without antihypertensive and lipid-lowering medications were examined using whole-body impedance cardiography and radial pulse wave analysis. Linear regression analysis was applied to investigate the association of AIP with haemodynamic variables and age, sex, body mass index (BMI), smoking status, alcohol consumption, plasma C-reactive protein, electrolytes, uric acid, low density lipoprotein cholesterol (LDL-C), estimated glomerular filtration rate, and quantitative insulin sensitivity check index.

Results: The demographics and laboratory values of the study population were (mean ± 95% confidence interval): age 44.9 ± 1.0 years, BMI 26.8 ± 0.4 kg/m^2, office blood pressure 140.6 ± 1.6/89.4 ± 1.0 mmHg, total cholesterol 5.2 ± 0.08, LDL-C 3.1 ± 0.08, triglycerides 1.2 ± 0.08, HDL-C 1.6 ± 0.04 mmol/l, and AIP −0.15 ± 0.02. Age (standardized coefficient Beta 0.508, $p < .001$) and aortic systolic blood pressure (Beta 0.239, $p < .001$) presented with the strongest associations with pulse wave velocity. However, AIP was also associated with pulse wave velocity (Beta 0.145, $p < .001$). AIP was not related with aortic or radial blood pressure, cardiac output, systemic vascular resistance, or augmentation index.

Conclusions: AIP is directly and independently associated with arterial stiffness, a variable strongly related to cardiovascular risk. This supports more widespread use of AIP in standard clinical cardiovascular disease risk evaluation.

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arterial stiffness; atherogenic index; haemodynamics; HDL cholesterol; hypertension; triglycerides

Introduction

Cardiovascular diseases due to atherosclerosis and its complications, such as myocardial infarction and stroke, are the leading cause of mortality worldwide representing 31% of all deaths [1]. Among 82.6 million U.S. adults, the prevalence of cardiovascular diseases due to high blood pressure (BP), coronary heart disease, and stroke is estimated to exceed 33%, with the majority of the cases found in subjects older than 60 years of age [2].

Dyslipidaemia is a major risk factor for cardiovascular disease, and the primary focus has been on the dominant role of low density lipoprotein cholesterol (LDL-C) in atherosclerosis. The benefits of LDL-C lowering in cardiovascular disease are well recognized [3,4]. In clinical practice, the influence of LDL-C has overridden the significance of high density lipoprotein cholesterol (HDL-C) and triglycerides [3,4]. Previous studies have reported that not only low, but also extremely high, levels of HDL-C increase the risk of cardiovascular disease and mortality [5–7]. Elevated serum triglycerides level is also a risk factor for cardiovascular disease [8,9]. A meta-analysis of 17 population-based prospective studies with 46,413 men and 10,864 women reported that plasma triglyceride level, independent of HDL-C, was a risk factor for cardiovascular disease [10].

Increased pulse wave velocity (PWV) that designates arterial stiffness is a strong predictor of cardiovascular disease and mortality, independent of the level

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of BP [11]. The role of unfavourable lipid profile in atherosclerosis is well recognized, but the associations of plasma lipids with arterial stiffness are not straightforward. In spite of the dominant role of LDL-C in atherosclerosis, the relationship of LDL-C with PWV is rather weak [12]. Recently, we found that LDL-C was not associated with PWV when the level of BP was taken into account [13], and this finding is concordant with the majority of published papers [12]. High triglycerides concentration in 11,640 and 1,447 subjects, and low HDL-C levels in 15,302 subjects, were associated with increased PWV [14–16]. However, Wang et al. found that HDL-C was inversely associated with PWV in 2,375 Chinese subjects, while total cholesterol or triglycerides were not associated with PWV [17].

The atherogenic index of plasma (AIP) is defined as the logarithm of plasma triglycerides to HDL-C ratio [18–22]. In contrast to plasma triglycerides concentration, AIP shows normal distribution [23], and is therefore well suited for the mathematical modelling of cardiovascular variables. AIP is particularly useful in predicting plasma atherogenicity [18,20–22]. AIP is also a strong marker for the future risk of atherosclerosis and cardiovascular disease [18–21,24–27], and the routine calculation of AIP in clinical cardiovascular disease risk evaluation would seem warranted.

To our knowledge, the association of AIP with haemodynamic variables has not been previously examined. Due to the weak association of LDL-C with arterial stiffness in our previous report [13], our objective in this cross-sectional study was to examine the associations of AIP with functional haemodynamic variables, and especially to test the hypothesis whether AIP is related to arterial stiffness.

Methods

Participants

All subjects were from an ongoing study with the primary aim to examine haemodynamics in primary and secondary hypertension versus normotensive controls (DYNAMIC study; ClinicalTrails.gov identifier NCT01742702). The participant recruitment was recently published in the form of a study flow-chart [13], and altogether 615 from 1349 subjects were included. The exclusion criteria for the study were volunteers taking (1) statin or other lipid-lowering or BP-lowering medication, or with a history of (2) coronary artery disease, (3) stroke, (4) heart failure, (5) valvular heart disease, (6) diabetes, (7) chronic kidney disease, (8) secondary hypertension, (9) alcohol or substance abuse, (10) psychiatric illness other than mild depression or anxiety, or (11) abnormal heart rhythm other than sinus.

Physical examination and office BP measurements were performed by a medical doctor, and routine laboratory analyses for elevated BP according to the guidelines of the European Society of Hypertension were performed to all enrolled subjects [28]. Beside the medical history, lifestyle habits and use of dietary supplements, medicines, and other substances not registered as drugs were also documented along with information about smoking and alcohol consumption as standard drinks (~12 grams of absolute alcohol) per week.

The study included 314 men and 301 women, altogether 615 normotensive and never-treated subjects with primary hypertension, aged 19–72 years. Based on the office BP measurements on a single occasion, 249 (40.5%) of the participants were normotensive and 366 (59.5%) were hypertensive. The subjects were divided into age- and sex-adjusted AIP tertiles (Tertile 1, $n = 202$; Tertile 2, $n = 208$; Tertile 3, $n = 205$). The study complies with the declaration of Helsinki, and was approved by the ethics committee of the Tampere University Hospital (study code R06086M) and the Finnish Medicines Agency (EudraCT registration number 2006-002065-39). Signed informed consent was obtained from all participants.

Altogether 230 (37.4%) of the 615 persons used some medications. Full description about medicine consumption has been described in our previous study [13].

Laboratory analyses

Blood and urine sampling was performed after ~12 hours of fasting. Plasma total, HDL-C, LDL-C, triglycerides, C-reactive protein (CRP), sodium, potassium, glucose, cystatin-C, and creatinine concentrations were determined using Cobas Integra 700/800 (F. Hoffmann-Laroche Ltd, Basel; Switzerland) or Cobas6000, module c501 (Roche Diagnostics, Basel, Switzerland), insulin using electrochemiluminescence immunoassay (Cobas e411, Roche Diagnostics), and blood cell count by ADVIA 120 or 2120 (Bayer Health Care, Tarrytown, NY, USA). To exclude patients with renal disease, urine dipstick analysis was made by an automated refractometer test (Siemens Clinitec Atlas or Advantus, Siemens Healthcare GmbH, Erlangen, Germany). AIP was defined as Lg_{10}(plasma triglycerides/plasma HDL-C) [18–21]. Quantitative insulin sensitivity check index (QUICKI) was calculated for evaluation of insulin sensitivity [29], and glomerular filtration rate (eGFR) was estimated using the CKD-EPI cystatin C formula [30].

Pulse wave analysis

Continuous pulse wave and radial BP were recorded using a tonometric sensor (Colin BP-508T, Colin Medical Instruments Corp., USA) that was attached on the left radial artery pulsation pulse with a wrist band. The radial BP signal was calibrated twice during each 5 minute-period by right brachial BP measurements. Aortic BP was derived with the SphygmoCor system (SpygmoCor PWMx®, AtCor medical, Australia) [31], and augmentation index (AIx, augmented pressure/pulse pressure*100), and AIx adjusted to heart rate 75/min (AIx@75) were determined [32].

Whole-body impedance cardiography

Beat-to-beat heart rate, stroke volume, cardiac output, and PWV were recorded using whole-body impedance cardiography (CircMon®, JR Medical Ltd., Tallinn, Estonia). This method detects changes in body electrical impedance during cardiac cycles, and the electrode configuration has been previously reported [33]. Systemic vascular resistance was calculated from the tonometric BP and cardiac index measured by CircMon® so that normal central venous pressure (4 mmHg) was subtracted from mean arterial pressure and the value was divided by cardiac output. Systemic vascular resistance and cardiac output were related to body surface area and presented as indexes (cardiac index, and systemic vascular resistance index (SVRI), respectively). The stroke volume values measured using CircMon® correlate well with 3 dimensional ultrasound [34]. The supine cardiac output values measured with CircMon® correlate well with the values measured using thermodilution [33].

To measure the PWV, the CircMon® software records the time difference between the onset of the decrease in the impedance of the whole-body signal and the signal from the popliteal artery region, and PWV is then determined from the time difference and the distance between the electrodes [35]. Thus, the values measured using this method reflect cardio-popliteal PWV. The whole-body impedance cardiography tends to overestimate PWV, and a validated equation was utilized to calculate values correspond to the ultrasound method (PWV = PWVimpedance * 0.696 + 0.864) [35]. By the use of this equation, the PWV values recorded using CircMon® show very good correlations with values measured using either the tonometric SphygmoCor® method ($r = 0.82$, bias 0.02 m/s, 95% confidence interval −0.21 to 0.25) [32] or ultrasound ($r = 0.91$) [35].

Experimental protocol

Haemodynamics were recorded by research nurses in a quiet, temperature-controlled laboratory. Smoking, caffeine containing products or heavy meals were to be avoided for ≥4 hours, and alcohol consumption for >24 hours prior to the participation in the studies. The subjects rested supine, the left arm with the tonometric sensor abducted to 90 degrees in an arm support. After getting accustomed to the laboratory for about 10 minutes, supine haemodynamics were recorded for five minutes. For the statistical analyses the mean values of each 1-minute period of recording were calculated. The good repeatability and reproducibility of the measurement protocol has been demonstrated [36].

Statistics

Continuous variables were expressed as the mean, standard deviation (SD) or 95% confidence interval (CI) of the mean. Baseline characteristics were depicted as age- and sex-adjusted tertiles of AIP (Table 1). The demographic and laboratory data was analysed using analysis of variance (ANOVA), and the Bonferroni correction was applied in the post-hoc analyses. For the illustrations, the haemodynamic differences between the tertiles were examined using one-way ANOVA with the Bonferroni correction in the post-hoc analyses. The homogeneity of variances was tested with the Levene's test.

Spearman's correlations (rS) were calculated, and the variables that correlated with the variable of interest with $p < .1$ were included in the regression analyses, as appropriate. The skewed distributions of CRP and PWV were corrected by Lg_{10}-transformation for these analyses, while alcohol intake was treated as a series of discrete variables that were assigned a score of either 0 or 1; cut-points for women 0, 1–7, 8–14, and above 15 doses per week; for men 0, 1–14, 15–24, and above 25 doses per week, according to the Finnish Guidelines [37]. Multiple regression analysis with stepwise elimination was applied to evaluate the associations between age, sex, body mass index (BMI), smoking status, alcohol consumption, insulin sensitivity evaluated by QUICKI [29], plasma CRP, sodium, uric acid, LDL-C, AIP, and cystatin C based eGFR [30] (independent variables), and radial systolic and diastolic BP, heart rate, and PWV (dependent variables). In the case of PWV, heart rate was also included as an independent variable. The above variables comprised the model 1. The variables in the model 2 were model 1 + PWV (independent

Table 1. Age and sex adjusted characteristics of the study population in tertiles of atherogenic index of plasma.

	Overall	Tertile 1	Tertile 2	Tertile 3
Male / female (n/n)	314 /301	104 / 98	106 / 102	104 / 101
Age (years)	44.9 (11.9)	44.7 (12.3)	44.1 (11.9)	44.9 (11.6)
BMI (kg/m^2)	26.8 (4.4)	25.1 (3.7)	26.4 (4.0)*	28.9 (4.7)*†
Alcohol (standard doses/week)	4.5 (5.7)	3.9 (5.4)	4.2 (5.3)	5.2 (6.4)
Smokers (number / percentage)	76 / 12.4%	19 / 9.4%	27 / 13%	30 / 14.6%
Office blood pressure (mmHg)				
Systolic	140.6 (20.6)	135.7 (19.8)	140.2 (20.3)	145.7 (20.7)*†
Diastolic	89.5 (12.3)	86.1 (12.1)	89.5 (12.7)*	93.0 (11.4)*†
eGFR (ml/min per 1.73 m^2)	98.8 (18.1)	102.6 (16.8)	97.9 (18.2)*	95.1 (18.7)*
QUICKI	0.360 (0.042)	0.375 (0.048)	0.360 (0.037)*	0.341 (0.040)*†
Creatinine (μmol/l)	74.0 (13.5)	72.4 (13.2)	75.0 (13.0)	73.8 (14.3)
Cystatin C (mg/l)	0.85 (0.15)	0.81 (0.14)	0.85 (0.14)*	0.87 (0.15)*
Sodium (mmol/l)	140.4 (2.0)	141.0 (2.0)	140.3 (1.8)	140.3 (2.1)
Potassium (mmol/l)	3.81 (0.28)	3.80 (0.29)	3.78 (0.27)	3.83 (0.28)
Uric acid (μmol/l)	303 (76)	280 (71)	300 (70)*	327 (81)*†
CRP (mg/l)	1.7 (2.9)	1.3 (2.3)	1.4 (1.7)	2.4 (4.0)*†
Total cholesterol (mmol/l)	5.2 (1.0)	4.83 (1.0)	5.17 (1.0)*	5.44 (1.0)*†
Triglycerides (mmol/l)	1.23 (0.77)	0.70 (0.21)	1.09 (0.33)*	1.92 (0.92)*†
HDL-C (mmol/l)	1.58 (0.44)	1.90 (0.40)	1.58 (0.37)*	1.30 (0.34)*†
LDL-C (mmol/l)	3.1 (1.0)	2.70 (0.90)	3.12 (0.90)*	3.40 (1.0)*†
Atherogenic index	−0.15 (0.31)	−0.44 (0.17)	−0.17 (0.16)*	0.15 (0.23)*†
Insulin (mU/L)	8.89 (17.0)	8.1 (28.4)	7.6 (4.7)	10.9 (7.10)
Glucose (mmol/l)	5.44 (0.59)	5.33 (0.62)	5.40 (0.52)	5.60 (0.58)*†

Mean (standard deviation),*$p < .05$ vs Tertile 1; †$p < .05$ vs Tertile 2.
BMI: body mass index; eGFR: cystatin C based CDK-EPI formula for estimated glomerular filtration rate [30]; QUICKI: quantitative insulin sensitivity check index [29]; CRP: C-reactive protein; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol.

variables) for radial systolic and diastolic BP and heart rate (dependent variables), and model 1 + aortic systolic BP (independent variables) for PWV (dependent variable). The coefficient B, standardized coefficient Beta, and R squared values were presented in the Table 2, and $p < .05$ was considered statistically significant. SPSS version 25.0 (IBM SPSS Statistics, Armonk, NY, USA) was used for the statistics.

Results

Study population and laboratory values

Altogether, 314 (51%) male and 301 (49%) female subjects were included in the analyses (Table 1). The age range was 19–72 years. The demographics and laboratory values of the study population were (mean ± SD): age 45 ± 12 years, BMI 27 ± 4 kg/m^2, office systolic/diastolic BP 141 ± 21/90 ± 12 mmHg, eGFR 98.8 ± 18.1 ml/min/1.73 m^2, total cholesterol 5.2 ± 1.0, LDL-C 3.1 ± 0.6, triglycerides 1.2 ± 0.8, HDL-C 1.6 ± 0.4 mmol/l, and AIP −0.15 ± 0.3 (Table 1). In the morning urine sample, none of the study participants had glucosuria or proteinuria. The fasting plasma glucose was in the range of 7.1–10.3 mmol/l in 6 (1%) subjects, while impaired fasting plasma glucose (6.1–7.0 mmol/l) was detected in 80 (13%) of the subjects.

The participants were divided into age- and sex-adjusted AIP tertiles. The average AIP in the tertiles ranged from −0.44 ± 0.17 (Tertile 1) to 0.15 ± 0.23 (Tertile 3) (Table 1). The age and sex adjusted AIP tertiles presented with differences in BMI, office systolic and diastolic BP, eGFR, QUICKI, and plasma cystatin C, uric acid, CRP, total cholesterol, triglycerides, HDL-C, LDL-C and glucose concentrations. Age, alcohol intake, smoking status, and plasma creatinine, sodium, potassium, and insulin concentrations were not different between the tertiles (Table 1).

Haemodynamic variables in the tertiles of AIP adjusted for age and sex

Radial and aortic systolic and diastolic BP and heart rate were higher in the highest than in the lowest AIP tertile, while radial systolic BP and heart rate were also higher in the highest versus the middle AIP tertile (Figures 1(A–D), Figure 2(A)). Cardiac index, SVRI and AIx@75 (Figures 2(B,C), Figure 3(A)) were not different between the tertiles. PWV was higher in the highest and the middle tertile than in the lowest AIP tertile (Figure 3(B)).

AIP and haemodynamic variables in stepwise linear regression analyses

To examine the relationships of AIP with BP, heart rate and PWV, we performed linear regression analyses with two applied models (see methods) (Table 2). The regression analyses were not performed for

Table 2. Explanatory factors for haemodynamic variables in linear regression analyses with stepwise elimination.

Radial systolic BP: model 1 (R squared 0.278)				Radial systolic BP: model 2 (R squared 0.338)			
	B	Beta	*p*		B	Beta	*p*
(Constant)	5.667		.915	(Constant)	−54.968		.281
eGFR	−0.265	−0.251	<.001	PWV	81.530	0.396	<.001
BMI	0.613	0.142	.001	QUICKI	−53.779	−0.120	.001
Male sex	5.257	0.137	<.001	eGFR	−0.207	−0.196	<.001
LDL-C	3.242	0.162	<.001	Male sex	3.270	0.086	.021
Sodium	1.000	0.102	.006	Sodium	1.089	0.111	.002
QUICKI	−45.295	−0.101	.010	LDL-C	2.220	0.111	.006
Present smoker	−4.690	−0.081	.025	Age	−0.209	−0.130	.011
Radial diastolic BP: model 1 (R squared 0.274)				**Radial diastolic BP: model 2 (R squared 0.333)**			
	B	Beta	*p*		B	Beta	*p*
(Constant)	21.741		.540	(Constant)	−37.667		.254
eGFR	−0.199	−0.282	<.001	PWV	54.136	0.393	<.001
LDL-C	2.227	0.166	<.001	eGFR	−0.157	−0.222	<.001
QUICKI	−31.547	−0.105	.008	QUICKI	−33.656	−0.112	.002
Male sex	2.851	0.112	.003	LDL-C	1.622	0.121	.003
High alcohol intake	8.612	0.096	.008	Sodium	0.661	0.101	.004
Present smoker	−3.173	−0.082	.024	Age	−0.150	−0.140	.006
BMI	0.276	0.096	.024	High alcohol intake	6.602	0.074	.033
Sodium	0.495	0.076	.042				
Heart rate: model 1 (R squared 0.108)				**Heart rate: model 2 (R squared 0.171)**			
	B	Beta	*p*		B	Beta	*p*
(Constant)	80.017		<.001	(Constant)	51.106		<.001
QUICKI	−38.761	−0.172	<.001	PWV	43.525	0.421	<.001
CRP	2.310	0.096	.023	QUICKI	−42.282	−0.188	<.001
Male sex	−3.532	−0.184	<.001	Male sex	−4.161	−0.217	<.001
Atherogenic index	4.485	0.143	.003	Age	−0.215	−0.266	<.001
Moderate alcohol intake	3.798	0.111	.006	Moderate alcohol intake	4.584	0.134	.001
				Previous smoker	−1.709	−0.082	.037
Pulse wave velocity: model 1 (R squared 0.582)				**Pulse wave velocity: model 2 (R squared 0.616)**			
	B	Beta	*p*		B	Beta	*p*
(Constant)	0.482		<.001	(Constant)	0.444		<.001
Age	0.004	0.567	<.001	Age	0.004	0.508	<.001
Atherogenic index	0.030	0.100	.007	Aortic systolic BP	0.001	0.239	<.001
Heart rate	0.002	0.197	<.001	Atherogenic index	0.044	0.145	<.001
Uric acid	0.0002	0.129	.001	Heart rate	0.002	0.174	<.001
Present smoker	−0.024	−0.086	.002	Uric acid	0.0002	0.161	<.001
LDL-C	0.007	0.076	.021	Present smoker	−0.021	−0.076	.004
BMI	0.002	0.079	.020				
Male sex	0.015	0.080	.023				

Variables in model 1: age, sex, BMI, smoking status, categorised alcohol consumption, QUICKI, Lg_{10} of plasma CRP, sodium, uric acid, LDL-C, atherogenic index, and eGFR [30], and for PWV also heart rate. Model 2: model 1 + PWV; for PWV model 2: model 1 + aortic systolic BP.
Blood pressure (BP), coefficient of regression (B), standardized coefficient of regression (Beta), cystatin C based CDK-EPI formula for estimated glomerular filtration rate (eGFR) [30], body mass index (BMI), low density lipoprotein cholesterol (LDL-C), quantitative insulin sensitivity check index (QUICKI) [29]; the skewed distributions of C-reactive protein (CRP) and pulse wave velocity (PWV) were Lg_{10} transformed.

cardiac index and SVRI, as these variables were not different between the AIP tertiles (Figures 2(B,C)). The univariate correlations (rS) between AIP and radial systolic and diastolic BP, heart rate, and PWV were 0.296, 0.252, 0.169, and 0.401 ($p < .001$ for all), respectively.

In the regressions analyses AIP was not an explanatory factor for radial systolic and diastolic BP in either model, in contrast to PWV, QUICKI, eGFR, age, sex, BMI, present smoker, high alcohol consumption, and plasma sodium and LDL-C concentrations (Table 2). AIP was a moderate explanatory factor for heart rate in model 1, in addition to QUICKI, CRP, sex, and moderate alcohol consumption, however when PWV was included in the model, AIP was no longer an explanatory factor for heart rate (Table 2).

In both of the applied models, AIP was a significant independent explanatory factor for PWV (Table 2). The other significant explanatory factors for PWV were age, aortic systolic BP, heart rate, plasma uric acid and present smoking (Table 2, model 2). If aortic systolic BP was replaced by aortic mean BP or aortic diastolic BP in the model 2, AIP still remained as an independent explanatory factor for PWV (data not shown).

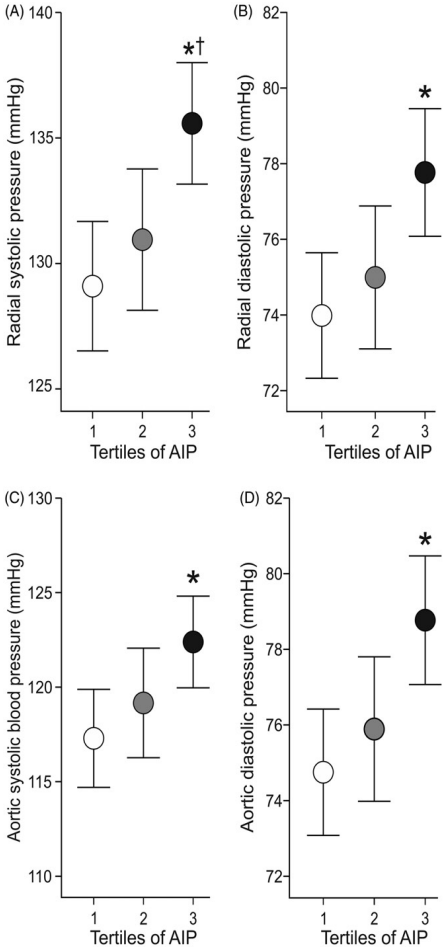

Figure 1. Averages of radial systolic (A) and diastolic (B) blood pressure, and aortic systolic (C) and diastolic (D) blood pressure in age- and sex-adjusted tertiles of atherogenic index of plasma (AIP) during 5-minute recordings in the supine position. Tertile 1 ($n = 202$), Tertile 2 ($n = 208$), and Tertile 3 ($n = 205$); mean and 95% confidence interval; $^{*}p < .05$ vs Tertile 1; $^{\dagger}p < .05$ vs Tertile 2, one-way ANOVA.

Discussion

There is paucity of studies on the associations of AIP with haemodynamic variables. Although some reports have associated triglycerides and HDL-C with arterial stiffness [14–16], all studies do not support this finding [38–40]. Moreover, the association of LDL-C with arterial stiffness has been surprisingly weak in the published literature [12,13]. Therefore, our goal was to assess the association between AIP and functional cardiovascular variables using non-invasive recordings of haemodynamics. The present results showed that AIP was independently associated with arterial stiffness, while it was not related to aortic or radial BP, cardiac output, systemic vascular resistance, or AIx.

The present evaluation of arterial stiffness was performed by the measurement of cardio-popliteal PWV, the pressure wave thus travelling along the thoracic and abdominal aorta, iliac artery, and the femoral artery. The elastic properties of the thoracic and abdominal aorta are higher than those of the more muscular iliac and femoral arteries [41]. According to an expert consensus, PWV is normally 4–5 m/s in the

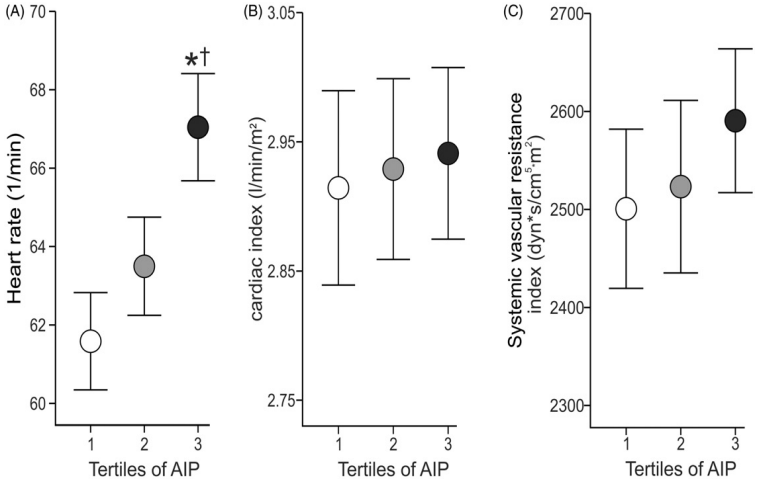

Figure 2. Averages of heart rate (A), cardiac index (B), and systemic vascular resistance index (C) in age- and sex-adjusted atherogenic index of plasma (AIP) during 5-minute recordings in the supine position; mean and 95% confidence interval; $^{*}p < .05$ vs Tertile 1; $^{\dagger}p < .05$ vs Tertile 2, one-way ANOVA.

ascending aorta, 5–6 m/s in the abdominal aorta, and 8–9 m/s in the iliac and femoral arteries [41,42]. This explains why cardio-popliteal PWV is higher than carotid-femoral PWV. Moreover, PWV in the aorta increases progressively with age due to the loss of elasticity, while PWV in the femoral artery is only moderately increased in the course of aging [43]. On the other hand, both the aorta and the femoro-popliteal arteries are frequently affected by atherosclerosis, making both of these regions relevant in the study of large arterial pathophysiology [44].

Meta-analyses have demonstrated that statin-induced reduction in LDL-C reduces cardiovascular morbidity and mortality, while several statin trials have also revealed an associated reduction in BP [3,4]. We previously found that LDL-C showed an independent inverse relation with BP and systemic vascular resistance in subjects naive to cardiovascular drugs, but was not associated with arterial stiffness. Thus, LDL-C is not only a major risk factor for atherosclerosis, but it can also be considered as a predisposing factor for elevated BP [13]. However, even when LDL-C is reduced to the recommended levels, some residual cardiovascular risk remains that has been related e.g. to inflammation, and this has encouraged the search for new cardiovascular disease predictors [45,46].

High LDL-C level, smoking, and hypertension have been identified as causes for atherosclerosis that is an intimal disease, while ageing, diabetes, and chronic kidney disease have been associated with arteriosclerosis, which is a medial disease and especially related to arterial stiffening [47]. Though LDL-C has been the major focus on the link between lipids and cardiovascular disease, the combination of reduced HDL-C and elevated triglycerides has been identified as atherogenic dyslipidaemia [48]. This combination has been associated with more unfavourable cardiovascular risk profile, higher heart rate and systolic BP than hypertriglyceridemia or low HDL-C levels alone [48]. Furthermore, reduced HDL-C together with elevated triglycerides, and also elevated AIP, have been associated with decreased insulin sensitivity [19,48,49]. This view corresponds to the present findings whereby insulin sensitivity, as evaluated by means of QUICKI, was different in every AIP tertile with the lowest values in the highest AIP tertile. In the present regression analyses, insulin sensitivity was also inversely related with BP and heart rate (Table 2).

The ratio of triglycerides to HDL-C is related to the processes involved in LDL size pathophysiology [50]. An increased proportion of small, dense LDL particles is characteristic of patients with diabetes and the metabolic syndrome, and both of these groups have increased risk for cardiovascular disease [51,52]. The mean LDL particle size was also found to be smaller in patients with stroke than in control subjects, despite similar total LDL-C concentrations [53]. When compared with age-matched men with normal

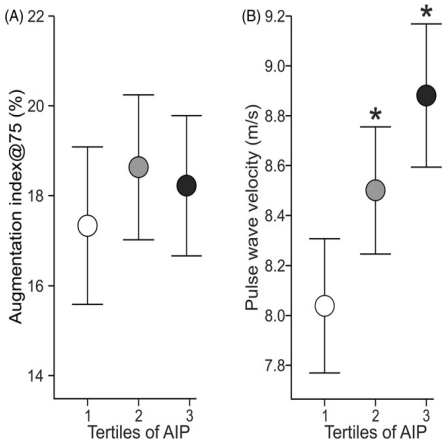

Figure 3. Averages of augmentation index adjusted to heart rate of 75 beats per minute (A), and pulse wave velocity (B) in age- and sex-adjusted atherogenic index of plasma (AIP) during 5-minute recordings in the supine position; mean and 95% confidence interval; $^{*}p < .05$ vs Tertile 1; $^{\dagger}p < .05$ vs Tertile 2, one-way ANOVA.

lipid levels, young men with hypertriglyceridemia presented with small dense LDL particles that were associated increased serum levels of adhesion molecules and impaired flow mediated vasodilation [54]. Some studies have suggested that the definite way to assess potential atherogenicity of LDL particles would be a direct measurement of the LDL particle size [55,56].

AIP reflects the lipoprotein composition in plasma, and it has been postulated as a surrogate marker for small dense LDL particles, and also as a predictor of atherosclerosis, cardiovascular risk, and even effectiveness of therapy [18–21,25,27]. AIP was found to be higher in 2936 patients with coronary artery disease versus 2451 controls [25], while AIP has also been suggested as an independent risk factor for coronary artery disease [24,25]. An AIP value below 0.11 has been associated with low, values from 0.11 to 0.24 with intermediate, and values exceeding 0.24 with high cardiovascular disease risk [20–22,24,57]. Dobiášová et al. examined 1433 subjects with various risks of atherosclerosis and reported that AIP directly correlated with the fractional esterification rate of HDL ($r = 0.803$), and inversely correlated with LDL particle size ($r = -0.776$). The fractional esterification rate of HDL strongly predicted particle size in LDL ($r = -818$), and the use of lg_{10}(triglycerides/HDL-C) ratio was considered as a useful predictor of plasma atherogenicity, as it reflected the metabolic interactions within the whole lipoprotein complex [18,20,22].

Plasma triglycerides levels have been previously associated with arterial stiffness [14,16], but contradictory findings have been published. A study in young type 1 diabetic patients found that an increase in triglycerides level of 48 mg/dl (0.54 mmol/L) resulted in a 1.0% higher PWV during a 4.8-year follow-up, but this increase was no longer significant after adjustment for baseline waist circumference, LDL-C, and HbA1c [39]. In 917 middle-aged French men and women, neither plasma triglycerides nor HDL-C were independently related with carotid-femoral PWV [38]. Although some reports have inversely associated HDL-C with arterial stiffness [15,17], HDL-C was not correlated with brachial-ankle PWV in 12,900 Chinese adults aged 20–79 years [40].

In the present study, the highest tertile with a mean AIP of 0.15 presented with the highest PWV. In the regression analyses, age and the prevailing level of BP showed the strongest associations with PWV, corresponding to previous studies [12,13]. However, AIP was also significantly and independently related with arterial stiffness (Beta values in the two models 0.100–0.145, $p \leq .007$ for both). As discussed above, controversies remain about the associations of triglycerides and HDL-C with arterial stiffness, while our findings for the first time suggest that AIP is directly and independently associated with PWV, an acknowledged marker of large arterial stiffness that is also strongly related to cardiovascular risk [11]. The present results do not provide an explanation why AIP is better correlated with arterial stiffness than LDL-C. The process leading to increased large arterial stiffness is complex and comprises influences mediated via

mechanical pulsatile stress, inflammatory cells, growth factors, and alterations in endothelial function, enzymes that degrade elastin, changes in smooth muscle cells from the contractile to the synthetic phenotype, and increased extracellular matrix production by fibroblasts [47]. Plasma triglycerides and HDL-C are known to have opposite influences on oxidative stress, inflammation, extracellular matrix formation, and on the change in vascular smooth muscle from the contractile to the synthetic phenotype, and the index AIP summarizes these influences [43].

The current study has limitations and the interpretation of the results should be done cautiously. The present methods have been validated against invasive measurements, 3 dimensional ultrasound, and tonometric recordings of PWV [31–34]. Nevertheless, the non-invasive evaluation of stroke volume and cardiac output is based on mathematical analysis of the bioimpedance signal that simplifies physiology [33]. The present recordings lasted for 5 minutes, which gives a rather narrow window of observation for the examination of haemodynamics. Yet, when compared with single measurements of BP and heart rate, the present analyses were based on recordings collected from more than 300 cardiac cycles. The haemodynamic recordings were performed in subjects who themselves were willing to participate, and this makes a potential source for selection bias. The inclusion of PWV in the regression model 2 resulted in an inverse relationship between age and systolic and diastolic BP in the present population, probably due to the strong interrelationship between PWV and age (rS = 0.67, $p < .001$). A small but significant inverse association between present smoking and PWV was perceived. According to our previous report that was focused on the haemodynamic effects of smoking, and also to a comprehensive review, smoking does not usually influence PWV [12,58]. In our previous study, present smokers had a clear reduction in systemic vascular resistance [58], and such a haemodynamic change may favour reductions in PWV. Finally, the cross-sectional design does not allow conclusions about causality, and the present findings should be confirmed in follow-up studies.

In conclusion, the present results showed that AIP was directly and independently associated with arterial stiffness. AIP is known to inversely correlate with LDL particle size [18,21], and it can be readily calculated from the routine lipid profiles. The link between AIP and large arterial stiffness further supports the view that calculation of AIP should be included in the normal clinical cardiovascular disease risk evaluation.

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Disclosure statement

The authors declare no conflicts of interest with respect to this manuscript.

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Data availability

Analyses and generated datasets that support the current study are not available publicly. The datasets are available from the corresponding author on reasonable request.

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PUBLICATION IV

Primary aldosteronism: Higher volume load, cardiac output and arterial stiffness than in essential hypertension

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Primary aldosteronism: Higher volume load, cardiac output and arterial stiffness than in essential hypertension

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Abstract. Choudhary MK, Värri E, Matikainen N, Koskela J, Tikkakoski AJ, Kähönen M, Niemelä O, Mustonen J, Nevalainen PI, Pörsti I (Tampere University, Tampere; University of Helsinki, Helsinki; Tampere University Hospital, Tampere; and Seinäjoki Central Hospital, Seinäjoki, Finland). Primary aldosteronism: Higher volume load, cardiac output and arterial stiffness than in essential hypertension. *J Intern Med* 2020; https://doi.org/10.1111/joim.13115

Background. The diagnostics of primary aldosteronism (PA) are usually carried out in patients taking antihypertensive medications. We compared haemodynamics between medicated PA, medicated essential hypertension (EH), never-medicated EH and normotensive controls (n = 130 in all groups).

Methods. The hypertensive groups were matched for age (53 years), sex (84 male/46 female) and body mass index (BMI) (30 kg m^{-2}); normotensive controls had similar sex distribution (age 48 years, BMI 27 kg m^{-2}). Haemodynamics were recorded using whole-body impedance cardiography and radial pulse wave analysis, and the results were adjusted as appropriate. Radial blood pressure recordings were calibrated by brachial blood pressure measurements from the contralateral arm.

Results. Radial and aortic systolic and diastolic blood pressure was similar in PA and never-medicated EH, and higher than in medicated EH and normotensive controls ($P \leq 0.001$ for all comparisons). Extracellular water balance was ~ 4% higher in PA than in all other groups ($P < 0.05$ for all), whilst cardiac output was ~ 8% higher in PA than in medicated EH ($P = 0.012$). Systemic vascular resistance and augmentation index were similarly increased in PA and both EH groups when compared with controls. Pulse wave velocity was higher in PA and never-medicated EH than in medicated EH and normotensive controls ($P \leq 0.033$ for all comparisons).

Conclusions. Medicated PA patients presented with corresponding systemic vascular resistance and wave reflection, but higher extracellular water volume, cardiac output and arterial stiffness than medicated EH patients. Whether the systematic evaluation of these features would benefit the clinical diagnostics of PA remains to be studied in future.

Keywords: arterial stiffness, cardiac output, extracellular water, hypertension, primary aldosteronism.

Introduction

In 2015, the prevalence of elevated blood pressure (BP) in adult females was around 20% and in males around 24%, affecting ~ 1.13 billion people worldwide [1]. Several studies have indicated that the prevalence of primary aldosteronism (PA) exceeds 5% amongst hypertensive patients [2,3]. Aldosterone excess predisposes to sodium retention, increased extracellular water (ECW) volume, hypokalemia, alkalosis and hypertension [4]. Accordingly, increased ECW volume was reported in patients with PA versus controls in small previous studies (≤16 participants per group) [5,6].

Aldosterone excess promotes oxidative stress, inflammation, endothelial dysfunction, impairs vasorelaxation, promotes fibrosis, and causes vascular, renal and cardiac damage [7,8]. Supporting these views, carotid intima–media thickness was

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higher in patients with PA than in essential hypertension (EH) [9,10]. In patients with EH, increased aortic stiffness, measured via the recording of pulse wave velocity (PWV), is an independent predictor of cardiovascular mortality [11]. According to a review, PA patients (n = 272) had higher aortic PWV than EH patients (n = 240), whereas no significant difference was found in the variables of wave reflection, augmentation index (AIx) and AIx adjusted to heart rate 75 beats per minute (AIx@75) [10]. Recently, the forward and backward wave amplitudes were reported to be higher in medicated PA than in medicated EH, possibly reflecting vascular damage in PA patients [12,13] (see Supplementary Table S1).

Typically, patients with PA have higher BP and they need more antihypertensive medications than patients with EH [14]. Independent of the level of BP, increased incidence of myocardial infarction and stroke, and increased prevalence of atrial fibrillation have been reported in patients with PA [15]. According to the German Conn's Registry, patients with PA are at a higher risk of cardiovascular mortality than patients with EH [16]. However, higher BP does not seem to entirely explain the increase in cardiovascular morbidity and mortality in PA patients.

The suspicion of PA most often arises because of a poor response to antihypertensive medications [14]. Subsequently, in general the clinicians carry out the diagnostics of PA in patients who are ingesting BP-lowering agents [14]. At present, PA is probably less severe and better treated than previously [3,15], whilst the range of the haemodynamic changes in contemporary PA is still not entirely known [12]. Also, limited information exists about how parallel changes in systemic vascular resistance and ECW volume could influence wave reflections in PA [17]. To gain insight about the principal haemodynamic features of PA, our objective in this cross-sectional study was to examine cardiovascular function in patients with medicated PA, medicated EH, never-medicated EH and normotensive controls.

Methods.

Participants

All subjects participated in an ongoing study with the primary aim to examine haemodynamics in primary and secondary hypertension (Eudra-CT 2006-002065-39, ClinicalTrials.gov NCT01742702). Patients with confirmed aldosteronism from all five university clinics in Finland are referred to Tampere University Hospital for adrenal vein sampling. These patients were invited to participate in noninvasive haemodynamic recordings. The other participants were enrolled by announcements from the employees of, and patients treated at, Tampere University Hospital, and from staff of Tampere University, and clients of Varala Sports Institute and local occupational healthcare providers. Participants were recruited in the order in which their contact information reached the research nurses.

The 520 subjects of the present study were chosen from 1260 hypertensive and normotensive subjects recruited during 2006-2019. The groups were normotensive controls, never-medicated EH, medicated EH and medicated PA (Table 1). The study included 336 men and 184 women aged 21-80 years. The three hypertensive groups were matched for age (53 years), sex (84 male/46 female) and body mass index (BMI) (30 kg m^{-2}). Additionally, the medicated PA and medicated EH groups were matched for the use of beta blockers, or beta + alpha blockers (Table S2). The normotensive controls were matched for sex (n = 130, age 48 years, BMI 27 kg m^{-2}).

The exclusion criteria were the following: history of (1) coronary artery disease, (2) stroke, (3) heart failure, (4) valvular heart disease, (5) chronic kidney disease, (6) secondary hypertension other than PA, (7) alcohol or substance abuse, (8) psychiatric illnesses other than mild depression or anxiety and (9) heart rhythm other than sinus rhythm. The study complies with the Declaration of Helsinki and was approved by the Ethics Committee of the Tampere University Hospital (study code R06086M). Signed informed consent was obtained from all participants.

The never-medicated EH patients had elevated office BP (≥140/90 mmHg) [18]. The diagnosis of PA was based on screening and confirmatory testing [3]. Screening of aldosteronism (n = 130) was defined as serum aldosterone (pmol L^{-1}) to plasma renin activity (ng mL^{-1} h^{-1}) ratio > 750, with serum aldosterone concentration ≥ 280 pmol L^{-1} [3,19]; or serum aldosterone (pmol L^{-1}) to plasma renin concentration (mU L^{-1}) ratio > 30, with serum aldosterone concentration ≥ 280 pmol L^{-1} [3,20,21]. Most of the patients (n = 82) had

Table 1. *Basic clinical characteristics and laboratory results*

	Normotensive controls (n = 130)	Never-medicated essential hypertension (n = 130)	Medicated essential hypertension (n = 130)	Medicated primary aldosteronism (n = 130)
Male/female	84/46	84/46	84/46	84/46
Age (years)	47.9 (0.9)	53.5 (0.8)*	52.9 (1.1)*	53.0 (1.0)*
Height (cm)	175.6 (0.8)	174.7 (0.8)	173.7 (0.8)	173.8 (0.8)
Weight (kg)	82.5 (1.1)	91.8 (1.4) *	89.0 (1.4) *	92.1 (1.7) *
Body mass index (kg m^{-2})	26.7 (0.3)	30.1 (0.5)*	29.5 (0.4)*	30.3 (0.5)*
Number of type 1 diabetics	0	0	1	1
Number of type 2 diabetics	1	3	21*†	31*†
Office systolic BP (mmHg)	126.3 (0.8)	161.7 (1.6)*	145.2 (1.8)*†	154.1 (1.5)*†‡
Office diastolic BP (mmHg)	81.9 (0.5)	99.3 (0.8)*	90.9 (1.2)*†	91.6 (1.0)*†
Hypertension duration (years)	0.0	1.6 (0.5)	11.2 (0.9)*†	14.9 (0.9)*†‡
Office heart rate	65 (1)	70 (1)*	66 (1)	69 (1)*
Smoking status (never/present/previous)	67/23/40	64/15/51	70/15/45	68/22/40
Alcohol (standard drinks/week)	4.3 (0.5)	5.7 (0.7)	5.6 (0.6)	4.0 (0.4)
eGFR (ml min^{-1} 1.73 m^{-2})	96.2 (1.3)	89.5 (1.1)*	88.5 (1.6)*	86.8 (1.8)*
Sodium (mmol L^{-1})	140.5 (0.2)	140.7 (0.2)	140.0 (0.2)†	142.8 (0.2)*†‡
Potassium (mmol L^{-1})	3.80 (0.02)	3.82 (0.03)	3.75 (0.03)	3.48 (0.03)*†‡
C-reactive protein (mg L^{-1})	1.5 (0.2)	2.5 (0.4)	2.5 (0.3)	3.1 (0.6)*
Creatinine (μmol L^{-1})	76.6 (1.2)	75.7 (1.2)	77.1 (1.3)	80.7 (2.9)
Cystatin C (mg L^{-1})	0.87 (0.01)	0.93 (0.01)*	0.95 (0.02)*	1.0 (0.02)*
Uric acid (μmol L^{-1})	303 (6)	336 (6)*	349 (8)*	327 (7)
Total cholesterol (mmol L^{-1})	5.1 (0.1)	5.6 (0.1)*	5.1 (0.1)†	4.7 (0.1)*†‡
Triglycerides (mmol L^{-1})	1.07 (0.05)	1.53 (0.08)*	1.49 (0.07)*	1.48 (0.09)*
HDL cholesterol (mmol L^{-1})	1.60 (0.04)	1.45 (0.04)*	1.41 (0.04)*	1.36 (0.04)*
LDL cholesterol (mmol L^{-1})	3.04 (0.09)	3.54 (0.08)*	3.20 (0.09)†	2.96 (0.08)†
Glucose (mmol L^{-1})	5.42 (0.05)	5.81 (0.06)*	6.33 (0.14)*†	6.55 (0.13)*†
Cornell voltage-duration product (mm*ms)	1569 (50)	1917 (47)*	2017 (98)*	2163 (75)*

Results shown as mean (standard error of mean); eGFR, estimated glomerular filtration rate (CKD-EPI cystatin C creatinine formula).
*$P < 0.05$ versus normotensive.
†$P < 0.05$ versus never-medicated essential hypertension.
‡$P < 0.05$ versus medicated essential hypertension.

presented with hypokalemia (Table 2), and confirmatory testing was performed in the majority (n = 113), showing urine aldosterone excretion > 33 nmol day^{-1} during oral sodium loading [3,19]. Seven subjects who had borderline screening tests for PA were included, as they were hypokalemic (plasma potassium < 3.3 mmol L^{-1}), presented with elevated serum aldosterone (range 513-1290 pmol L^{-1}) in control samples and showed elevated 24-hour urine aldosterone excretion (range 44-132 nmol day^{-1}) during oral sodium loading (Table 2) [3].

Office BP measurements and laboratory analyses for elevated BP were performed according to the guidelines of the European Society of Hypertension

[18]. The participants were examined by a physician, and medical history, lifestyle habits, dietary supplements, medicines, smoking status and alcohol consumption as standard drinks (~12 grams of absolute alcohol) per week were documented. Leg oedema was classified clinically: no oedema, cuff part of the socks made impressions in the ankle region (mild), pitting in the feet and ankles (moderate), and oedema extending to the proximal parts of the calves (severe).

Altogether 362 (69.7%) participants used medications, and the BP and lipid-lowering medications are shown in Table 3. Amongst 130 PA patients, spironolactone was previously used by 51 subjects, but was discontinued in 47 of them 6 weeks before the recordings. Prazosin and calcium channel blockers were prescribed by the treating physicians when needed. Four PA patients continued spironolactone for safety reasons (Table 3). Other regular medications are listed in Supplementary Table S3.

Laboratory analyses

Blood and urine sampling were performed after ~ 12 hours of fasting. Plasma sodium, potassium, glucose, cystatin C, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, C-reactive protein, uric acid, and creatinine concentrations, and urine sodium and potassium concentrations, were determined using Cobas Integra 700/800 (F. Hoffmann-Laroche Ltd., Basel, Switzerland) or Cobas 6000, module c501 (Roche Diagnostics, Basel, Switzerland), and blood cell count by ADVIA 120 or 2120 (Bayer Health Care, Tarrytown, NY, USA). Plasma renin activity (PRA) was initially determined using radioimmunoassay (DiaSorin, Saluggia, Italy), but this method was replaced by the analysis of plasma direct renin concentration (LIAISON immunoanalyzer, DiaSorin, Saluggia, Italy) [20]. In patients with very low renin values, the concentrations were given as the following low detection limits: 0.2 ng mL^{-1} h^{-1} for PRA and 2 mU L^{-1} for direct renin concentration. Plasma and urine aldosterone was quantified using liquid chromatography–mass spectrometry (LC–MS/MS) on API 4000 (Sciex) as described earlier [22]. To exclude patients with renal diseases, automated urine dipstick refractometer analysis was performed (Siemens Clinitec Atlas or Advantus, Siemens Healthcare GmbH, Erlangen, Germany). Glomerular filtration rate (eGFR) was estimated using the CKD-EPI creatinine–cystatin C formula [23].

Pulse wave analysis

Continuous pulse wave and radial BP were captured using an automated tonometric sensor (Colin BP-508T, Colin Medical Instruments Corp., USA) recordings from the left radial artery. The radial BP signal was calibrated twice during the 5-minute period by contralateral brachial BP measurements. Aortic BP, AIx (augmented pressure/pulse pressure*100) and AIx@75 were determined using the SphygmoCor software (SpygmoCor PWMx®, AtCor medical, Australia) [17,24].

Whole-body impedance cardiography

Beat-to-beat heart rate, stroke volume, cardiac output, ECW and PWV were recorded using

Table 2. *Laboratory characteristics of 130 patients with primary aldosteronism*

		95% confidence interval			
	Mean	Lower bound	Upper bound	Number[a]	Normal range
Lowest plasma potassium (mmol L^{-1})	3.14	3.06	3.21	130	3.3–4.8
Serum aldosterone (pmol L^{-1})	822	720	925	130	<520
Plasma renin activity (ng of Ang I mL^{-1} h^{-1})	0.32	0.24	0.42	79	1.5–5.7
Plasma renin concentration (mU L^{-1})	13.3	9.2	17.4	51	4.4–46
Ratio of aldosterone to renin activity	3234	2727	3740	79	<750
Ratio of aldosterone to renin concentration	119	79	158	51	<30
Urinary aldosterone (nmol/24h)	100.5	55.3	145.6	113	<40
Urinary sodium (mmol/24h)	223	205	242	89	130–240
Urinary potassium (mmol/24h)	113	104	122	78	60–90

[a]Number of subjects with available result of the laboratory determination.

whole-body impedance cardiography (CircMon®, JR Medical Ltd., Tallinn, Estonia). The electrode configuration has been previously reported [25]. Systemic vascular resistance was calculated from the tonometric BP and cardiac index measured by CircMon®: normal central venous pressure (4 mmHg) was subtracted from mean arterial pressure, and the value was divided by cardiac output. Systemic vascular resistance, stroke volume and cardiac output were related to body surface area (cardiac index, stroke index and systemic vascular resistance index (SVRI), respectively). The stroke volume values measured using CircMon® correlate well with 3 dimensional ultrasound [26], and the cardiac output values correlate well with values measured using thermodilution (bias 0.00 L min^{-1}, 95% confidence interval (CI) −0.26 to 0.26) and direct oxygen Fick method (bias −0.32 L min^{-1}, 95% CI −0.69 to 0.05) [25].

The CircMon® evaluates ECW volume by the formula ECW = k*(H^2/Z), coefficient k (Ω*cm) derived from blood resistivity and the relation between the distance of voltage electrodes, H is body height (cm), and Z is the recorded impedance of the body. The bioimpedance-derived ECW volume correlates well with ^{51}Cr-EDTA dilution-based ECW measurement (n = 15, r = 0.74, bias 0.2 ± 1.1 L, mean ± SD) [27]. The ECW balance is calculated as $ECW/ECW_{predicted}$. The formula for predicted ECW is 2.4 * (0.0236 * $H^{0.725}$ * $W^{0.423}$ – 1.229) in males and 2.6 * (0.0248 * $H^{0.725}$ * $W^{0.423}$ – 1.9549) in females [28–30]. In the current results, the ECW balance of the normotensive group was adjusted to 1.0.

To measure PWV, the CircMon® software records the time difference between the onset of the decrease in the impedance of the whole-body signal and the signal from the popliteal artery region [31].

Table 3. *Number of subjects using antihypertensive or lipid-lowering medications*

	Normotensive controls (n = 130)	Never-medicated essential hypertension (n = 130)	Medicated essential hypertension (n = 130)	Medicated primary aldosteronism (n = 130)
Number of antihypertensive medications (median)	0	0	2	3
ACE inhibitor	0	0	42	22*
Angiotensin II receptor blocker	0	0	52	65
Beta blocker	0	0	65	64
Beta and alpha blocker	0	0	4	6
Calcium channel blocker	0	0	60	115*
Thiazide	0	0	53	15*
Furosemide	0	0	6	5
Spironolactone	0	0	7	4
Amiloride	0	0	8[a]	0
Nitrate	0	0	2	0
Moxonidine	0	0	5	18*
Minoxidil	0	0	0	1
Potassium supplement	0	1	4	82*
Prazosin	0	0	6	23*
Statin	5	3	46	36
Ezetimib	1	0	1	0
Fibrate	0	0	0	1

Statistic is only about the differences between primary aldosteronism versus medicated essential hypertension; spironolactone was previously used by 51 patients with PA, and this medication was discontinued in 47 of them 6 weeks before the recordings.
*$P < 0.05$.
[a]in 7/8 combination with hydrochlorothiazide.

PWV is calculated from the time difference and the distance between the electrodes. As the whole-body impedance cardiography slightly overestimates PWV, a validated equation was utilized to calculate values that correspond to the ultrasound method (PWV = PWVimpedance * 0.696 + 0.864) [31]. With this equation, the PWV values recorded using CircMon® show good correlations with values measured using SphygmoCor® (r = 0.82, bias 0.02 m s^{-1}, 95% CI −0.21 to 0.25) [17] or ultrasound (r = 0.91) [31].

Experimental protocol

Research nurses recorded haemodynamics in a noiseless temperature-controlled laboratory. Prior to the recordings smoking, caffeine-containing products and heavy meals were to be avoided for ≥ 4 hours, and alcohol consumption for > 24 hours. The subjects rested supine, and the left arm with the tonometric sensor was abducted to 90 degrees in a support. After getting accustomed for about 10 minutes, supine haemodynamics were recorded for 5 minutes. For the statistical analyses, the mean values of each 1-minute period of recording were calculated. The good repeatability and reproducibility of the measurement protocol has been demonstrated previously [32].

Statistics

The demographic and laboratory data were analysed using analysis of variance (ANOVA). The homogeneity of variances was tested with the Levene's test. Haemodynamic differences between the groups were examined using generalized estimating equation (GEE) analyses. This method enabled the analyses of repeated measurements over the 5-minute recording period to compare differences between the study groups in the haemodynamic variables. Linear scale response was applied, and the autoregressive option was chosen for the correlation matrix, as successive serial measures of haemodynamic variables in individual participants are autocorrelated. The Bonferroni correction was applied in all post hoc analyses. The groups presented with differences in age, BMI, proportions of diabetic subjects, eGFR; plasma uric acid, triglycerides, HDL cholesterol, LDL cholesterol and glucose (Table 1). If any of the above variables correlated with the haemodynamic variable of interest with $P < 0.1$ (Pearson), they were included in the GEE analyses as covariates. The PWV analyses were additionally adjusted for mean aortic pressure [33]. As changes in plasma sodium, potassium and C-reactive protein probably reflect true effects of aldosterone [3,15], no adjustments were performed for these variables. Lean body mass was used instead of BMI in analyses concerning ECW volume and balance, as lean body mass is more suitable for normalization of body fluid volumes [34]. The results were presented as mean and standard error of the mean (SEM), or as mean and 95% CI of the mean, and $P < 0.05$ was considered statistically significant. SPSS version 26.0 (IBM SPSS Statistics, Armonk, NY, USA) was used.

Results

Study population

Altogether, 336 (65 %) male and 184 (35%) female subjects participated in the analyses (age range 21–80 years) (Table 1). Sex distribution was equal in all groups, whilst the normotensive subjects were ~ 5 years younger with ~ 3 kg m^{-2} lower BMI than in the other groups. The number of type 2 diabetic subjects was higher in the medicated EH and PA groups than amongst the never-medicated EH and normotensive groups. Office systolic BP was ~ 10 mmHg higher in PA versus medicated EH, whilst office systolic and diastolic BP was highest in the never-medicated EH group (Table 1). There were no differences in clinically evaluated lower extremity oedema between the groups: even in the PA group 91% were without oedema, 6% had mild oedema, whilst 3% had moderate oedema.

Average alcohol intake and smoking habits were not different between the groups. Patients with PA had the longest known hypertension history (Table 1), whilst the median number of antihypertensive medications was not different between PA and medicated EH ($P = 0.135$, Table 3). Although 82 PA patients were taking potassium supplements, plasma potassium concentration was lowest, whilst plasma sodium concentration was highest, in the PA group (Tables 1 and 3). Amongst the PA patients 36 subjects and amongst the medicated EH patients 46 subjects, were taking statins (Table 3). Plasma total cholesterol was lowest in the PA group, and LDL cholesterol was highest in the never-medicated EH group (Table 1). Plasma triglycerides and glucose were higher, and

HDL cholesterol was lower, in the 3 hypertensive groups than in normotensive controls. Fasting plasma glucose was slightly higher in the PA group and the medicated EH group than in the never-medicated EH group. Cornell voltage product did not differ between the 3 hypertensive groups and was higher than in normotensive controls (Table 1).

Noninvasive haemodynamics in the laboratory

Radial systolic and diastolic BP, calibrated from contralateral brachial BP signal, and aortic systolic and diastolic BP were not different in medicated PA and never-medicated EH, and were higher than in medicated EH and normotensive controls ($P \leq 0.001$ for all comparisons). BP was also higher in medicated EH than in normotensive controls ($P < 0.001$ for all comparisons) (Figures 1a-d).

When adjusted for confounding variables, ECW volume was higher in PA patients than in medicated EH and normotensive subjects ($P < 0.05$ for both) (Figure 2a). Also, the bioimpedance-derived ECW balance was ~ 4% higher in PA than in all other groups ($P \leq 0.009$ for all) (Figure 2b).

Aortic-to-popliteal PWV, adjusted for mean aortic pressure in addition to demographic and metabolic factors, was higher in medicated PA than in medicated EH and normotensive subjects ($P \leq 0.033$) (Figure 2c). However, PWV was highest in never-medicated EH ($P \leq 0.004$ for all comparisons) (Figure 2c). Aortic-to-popliteal PWV without conversion to values that correspond to the ultrasound method is presented in Figure S1. Aortic pulse pressure was higher in all hypertensive groups than in normotensives ($P < 0.001$ for all), and it was also higher in medicated PA than in medicated EH ($P = 0.008$) (Figure 2d).

Never-medicated EH patients had higher heart rate when compared with PA, medicated EH and normotensive subjects ($P < 0.05$ for all) (Figure 3a). Stroke volume related to body surface area (stroke index) did not differ between PA patients and normotensive controls and was higher than in medicated and never-medicated EH ($P \leq 0.033$ for all) (Figure 3b). Despite similar beta-blocker use, cardiac index was ~ 8% higher in medicated PA than in medicated EH ($P = 0.012$) (Figure 3c). SVRI was similar in the PA and both EH groups, and higher than in normotensive controls ($P < 0.001$ for all) (Figure 3d).

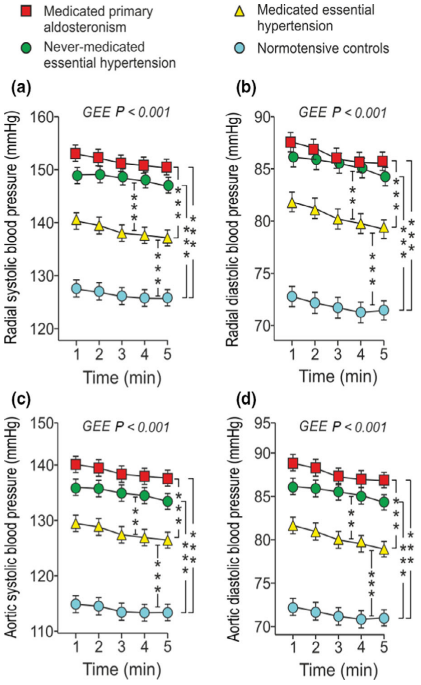

Figure 1 *Radial systolic (a) and diastolic (b) blood pressure calibrated from brachial blood pressure measurements, and aortic systolic (c) and diastolic (d) blood pressure in medicated primary aldosteronism (n = 130), never-medicated essential hypertension (n = 130), medicated essential hypertension (n = 130) and normotensive controls (n = 130) during 5-minute recordings in supine position; mean ± SEM; statistics by generalized estimating equations (GEE) adjusted for age, BMI, presence of diabetes, eGFR; and plasma triglycerides, HDL cholesterol, LDL cholesterol, uric acid and glucose (see Methods); **P < 0.01, ***P < 0.001*

The forward wave amplitude (FWA) did not differ between PA and never-medicated EH, and was higher in PA than in medicated EH and normotensive controls ($P \leq 0.002$) (Figure 4a). AIx@75 was corresponding in all hypertensive groups, and higher than in normotensive controls ($P < 0.001$ for all) (Figures 4b). A summary of the main haemodynamic findings of this study is presented in Table 4.

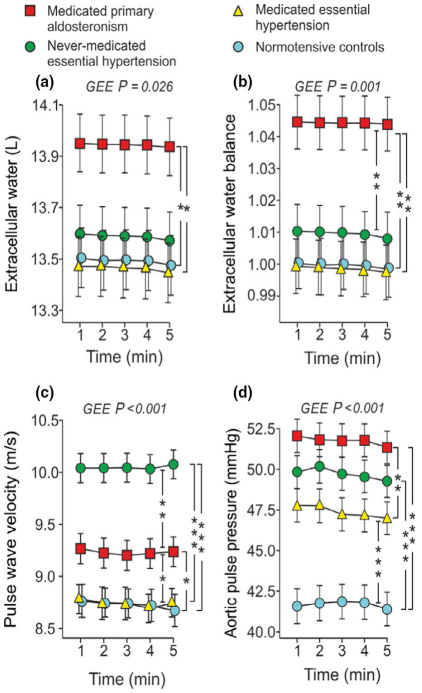

Figure 2 *Extracellular water volume (a), extracellular water balance (b), aortic-to-popliteal pulse wave velocity (PWV) (c) and aortic pulse pressure (d). Groups and statistics as in Figure 1, except that in extracellular water analyses, BMI was replaced with lean body mass, and in PWV analyses, the results were also adjusted for mean aortic blood pressure;* *P < *0.05,* **P < *0.01,* ***P < *0.001*

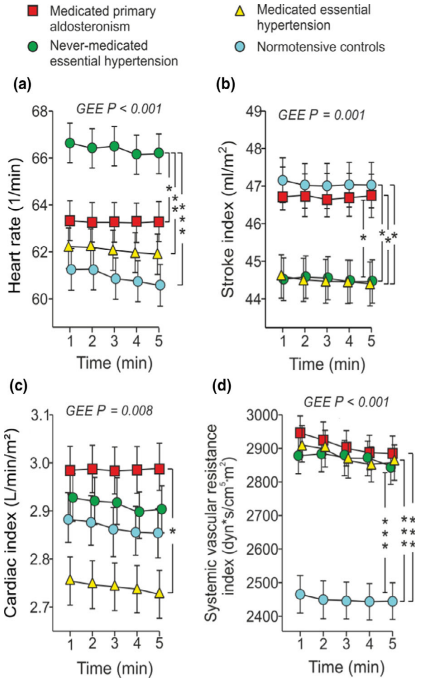

Figure 3 *Heart rate (a), stroke index (b), cardiac index (c) and systemic vascular resistance index (d). Groups and statistics as in Figure 1;* *P < *0.05,* **P < *0.01,* ***P < *0.001*

Adrenal vein sampling was successful in 114/130 subjects with PA. Lateralization to either adrenal was detected in 63/114 patients. However, no significant haemodynamic differences were detected between subjects with bilateral versus unilateral aldosterone excess. Amongst the PA patients, serum aldosterone to plasma renin activity ratio correlated with radial and aortic diastolic BP ($r_S = 0.23$, $P = 0.04$ for both), and 24-hour urine aldosterone excretion correlated with radial and aortic diastolic BP ($r_S = 0.20$, $P = 0.04$ for both), but not with other haemodynamic variables.

Discussion

Rather few studies have examined haemodynamic differences between PA and EH patients carefully matched for confounding factors. Here, we compared haemodynamics between patients with medicated PA, medicated EH, never-medicated EH and normotensive controls. The PA group presented with the typical characteristics of aldosterone excess [3], and only four PA patients and seven EH patients were taking spironolactone during the recordings. In addition to age, BMI, sex, plasma lipids and glucose, the medicated groups were matched for the use of beta adrenoceptor blockers, as this class drugs interferes with both cardiac function and regulation of systemic vascular resistance [35].

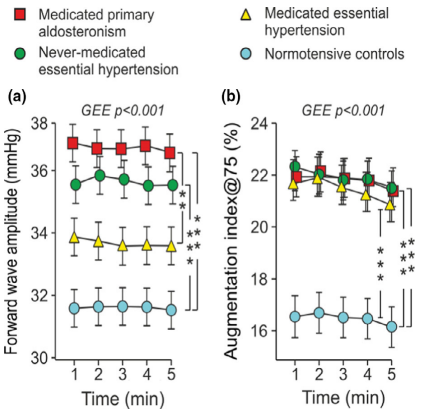

Figure 4 *Forward wave amplitude (a) and augmentation index adjusted to heart rate of 75 beats per minute (b). Groups and statistics as in Figure 1;* **P < *0.01,* ***P < *0.001*

In separate previous reports, PA has been associated with increased ECW volume [5,6], increased cardiac output [36] and increased large arterial stiffness [9,10,37] (see Supplementary Table S1 for a summary of haemodynamic findings in PA). Our results showed that SVRI and AIx@75 were corresponding in all hypertensive groups, whilst medicated PA patients had higher ECW balance, cardiac index and PWV than medicated EH patients.

Largely due to elevated aldosterone-to-renin ratio [14] and elevated plasma natriuretic peptide concentration [38], the PA patients are considered to have volume overload. In 279 patients with resistant hypertension, plasma natriuretic peptide and aldosterone concentrations, and aldosterone-to-renin ratio, were higher than in 53 subjects with normotension or controlled hypertension [39]. ECW volume, estimated by means of radioactive sodium sulphate injections, was higher in 11 PA patients than in 11 normal controls (16.8% vs. 14.6% of body weight, respectively) [5], and in 10 PA patients than in 7 EH patients [6]. When compared with the manufacturer reference values, Wu et al. reported ~ 4% overhydration in 41 patients with PA by bioimpedance spectroscopy, a method widely used for the evaluation of volume status in dialysis patients [40]. In the present study, ECW balance was also ~ 4% higher in the PA group than in all other groups (Figure 2b), indicating fluid overload.

In the current study, stroke index was higher, and cardiac index was ~ 8% higher, in medicated PA patients than in medicated EH patients (Figure 3c). Already in 1973, 16 patients with PA were found to have higher heart rate and cardiac index than 30 patients with EH, without differences in total peripheral resistance [36]. Cesari et al. found increased cardiac output in PA versus normotensive controls using ultrasound [41], whilst Kusunoki et al. reported higher cardiac output in medicated PA than in medicated EH based on waveform analyses form the brachial artery [42]. When examined using magnetic resonance imaging, 37 patients with high aldosterone had ~ 9% higher left ventricular end diastolic volume than 71 patients with normal aldosterone, indicating intracardiac volume expansion [38]. In contrast, in an echocardiography analysis, no differences were detected in stroke volume or cardiac output of 17 PA patients versus 10 EH patients [43] (see Supplementary Table S1). Aldosterone excess has also been associated with thicker left ventricular walls when compared with EH patients [44,45], which was attributed to deposition of extracellular matrix and collagen in the heart [44,45]. However, in the present study the hypertensive groups demonstrated corresponding increases in Cornell voltage product when compared with normotensive controls.

High aldosterone levels and high aldosterone:renin ratio predispose to increased arterial stiffness [46]. Increased intima–media thickness and fibrous tissue content were reported in the carotid artery of 23 PA patients versus 24 EH patients [9]. In small arteries, PA patients had higher type III collagen content than EH patients, indicating increased fibrosis [47]. Accordingly, several studies have reported increased large arterial PWV in patients with PA, although in all investigations the analyses were not adjusted for BP and other confounders [9,10,37]. In the present study, PWV was higher in patients with medicated PA than in medicated EH. However, PWV in the never-medicated EH patients was highest amongst all groups. This indicates that although they had not been diagnosed with hypertension previously, they must have had long-standing untreated high BP. Our results agree with the view that unawareness of hypertension is a major problem [48], and stress the importance of early diagnosis and treatment of hypertension. Of

Table 4. *Summary of the noninvasive haemodynamic results in the laboratory*

	Normotensive controls (n = 130)	Never-medicated essential hypertension (n = 130)	Medicated essential hypertension (n = 130)	Medicated primary aldosteronism (n = 130)
Radial systolic blood pressure (mmHg)[a]	126.7 (1.1)	148.0 (1.6)*	138.7 (1.4)*	151.8 (1.7)*
Radial diastolic blood pressure (mmHg)[a]	71.9 (0.8)	85.2 (1.0)*	80.4 (0.8)*	86.8 (0.9)*
Extracellular water balance (%)	0.0 (0.7)	0.9 (0.7)	0.0 (1.0)	4.6 (0.9)*
Pulse wave velocity (m s^{-1})[b]	8.7 (0.1)	10.1 (0.2)*	8.8 (0.1)†	9.3 (0.1)*
Cardiac index (L min^{-1} m^{-2})	2.89 (0.05)	2.93 (0.05)	2.76 (0.04)	2.97 (0.05)‡
Systemic vascular resistance index (dyn*s/cm^5·m^2)	2467 (45)	2850 (51)*	2861 (53)*	2922 (58)*
Augmentation index adjusted to heart rate 75 beats per minute (%)	16.3 (0.8)	21.8 (0.7)*	21.1 (0.8)*	21.8 (0.7)*

Results shown as mean (standard error of the mean).
[a]Calibrated from contralateral brachial blood pressure.
[b]Adjusted for age, BMI, presence of diabetes, triglycerides, HDL cholesterol, LDL cholesterol, uric acid, glucose, eGFR and mean aortic pressure.
*$P < 0.05$ versus normotensive.
†$P < 0.05$ versus never-medicated essential hypertension.
‡$P < 0.05$ versus medicated essential hypertension.

note, PWV measured by the present method is an independent predictor of incident hypertension [49].

In many studies, patients with PA had higher AIx and AIx@75 than normotensive subjects, but these variables of wave reflection were found to be similar in patients with PA and EH [10,37]. These results correspond to our findings showing similar AIx@75 in all hypertensive groups. Recently, the amplitude of the forward and backward waves were found to be higher, although the AIx values were not different, in medicated PA patients than in medicated EH patients [12]. This was suggested to reflect dysfunction of the arterial system, but information about central BP, or the effect of volume load on pressure waves were lacking. The proportion spironolactone users was also higher in the PA group than in the EH group (15% vs. 2%), and office BP and wave reflection were recorded sequentially [12], not simultaneously like in the present study. The FWA depends critically on the level of BP [50], and we found no differences in FWA between never-medicated EH and medicated PA patients with comparable laboratory BP values. We evaluated peripheral arterial function by the recording of SVRI that was not different between the EH and PA groups.

In our study, 82 of the PA patients were hypokalemic at screening and they were treated with potassium supplements. However, mean plasma potassium concentration was still lower amongst PA patients than in the other groups, whilst the PA group also presented with elevated plasma sodium concentration. Previously, increased plasma sodium concentration was reported in PA [51], and Steichen et al. have discussed the inclusion of plasma sodium and potassium concentrations in the diagnostic algorithm of PA [51]. The 24-hour urine collections are unreliable and depend largely on the intake of electrolytes, whilst urine sodium excretion is also variable due to water-free sodium storage in the body [52].

Without treatment with mineralocorticoid receptor antagonists or adrenalectomy, PA increases the risk of cardiovascular events and death [15]. However, the diagnosis of PA is not straightforward [3,4,51,53,54]. Early PA detection depends on screening, but under-diagnosis is characteristic in primary care [54]. High heterogeneity in the diagnosis of PA was even revealed in specialized care in Germany [53]. Considering the high prevalence of hypertension in the population [48], and the high prevalence of PA amongst hypertensive individuals [2,3], reliable, easy-to-perform, and

cost-effective diagnostic tests for PA are welcome. Whether screening and diagnosis of PA would benefit from information about the haemodynamic characteristics and ECW volume makes an interesting research topic in the future.

The current study has limitations. Screening for aldosteronism by serum aldosterone and plasma renin analyses was not performed in the EH groups. However, we can assume that ≥ 90% of the participants in these groups had primary hypertension [2,3], and the inclusion of subjects with unrecognized PA would have reduced the haemodynamic differences between PA and EH. The present methods have been validated against invasive measurements, three-dimensional ultrasound and tonometric PWV recordings [17,24–26]. Yet, noninvasive evaluation of stroke volume is based on mathematical analyses of bioimpedance with a formula containing body height, and a coefficient including BMI [25]. Similar heights in all study groups ($P = 0.314$) and similar weights and BMIs ($P = 0.285$ and 0.377, respectively) in the hypertensive groups should increase the analysis reliability. The haemodynamic recordings were performed in voluntary subjects, which make a source for selection bias, and lasted for five minutes, which gives a rather short window of observation. Still, the analyses were based on average from ≥ 300 cardiac cycles in each subject. For patient safety, the antihypertensive medications in the PA group were not being discontinued. For this reason, we included both never-medicated and medicated EH groups in the study. Previous spironolactone treatment may also have influenced the haemodynamic results in 47 subjects of the PA group. Finally, the cross-sectional design does not allow conclusions about causality. However, a strength of this study is the large number PA patients.

Conclusions

In this study, we examined noninvasive haemodynamics in patients with PA and EH versus normotensive controls. The inclusion of never-medicated and medicated EH groups allowed us to conclude that aldosterone excess *per se*, not only the aldosterone-induced elevation of BP, invoked changes in haemodynamic variables that may contribute to the reported excess cardiovascular risk in PA [16]. Whether the routine determinations of extracellular water volume, cardiac output and arterial stiffness would benefit the clinical diagnostics of PA remains to be studied in future.

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Conflict of interest

The authors declare no conflicts of interest with respect to this manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 Summary of haemodynamic findings in primary aldosteronism versus essential hypertension, normotension and secondary aldosteronism; and effects of treatment in primary aldosteronism.

Table S2 Number of subjects using beta blockers or beta+alpha blockers.

Table S3 Number of subjects using other than anti-hypertensive or lipid-lowering medications.

Figure S1 Aortic-to-popliteal pulse wave velocity (PWV) presented as unprocessed raw data without conversion to values that correspond to the ultrasound method (see Methods) in medicated primary aldosteronism (n = 130), never-medicated essential hypertension (n = 130), medicated essential hypertension (n = 130), and normotensive controls (n = 130) during 5-minute recordings in supine position; mean±standard error of the mean; statistics by generalized estimating equations (GEE) adjusted for age, BMI, proportions of diabetic subjects, eGFR; and plasma triglycerides, HDL cholesterol, LDL cholesterol, uric acid, glucose, and mean aortic pressure (see Methods); $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

