Tampere University

PAULIINA KANGAS

Influence of Metabolic Syndrome and Sex on Cardiovascular Function

Non-Invasive Recordings of Supine and Upright Hemodynamics

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Influence of Metabolic Syndrome and Sex on Cardiovascular Function

Non-Invasive Recordings of Supine and Upright Hemodynamics

ACADEMIC DISSERTATION
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Tampere University, Faculty of Medicine and Health Technology
Tampere University Hospital, Department of Internal Medicine
Tampere University Hospital, Department of Clinical Physiology and Nuclear Medicine
Finland

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To my ever-supportive parents

ABSTRACT

Cardiovascular diseases (CVDs) are the major cause of death and disability globally. Metabolic syndrome (MetS), a cluster of abnormalities including abdominal obesity, dyslipidemias, glucose intolerance, and hypertension, is significantly increasing the risk of CVDs. The background mechanisms in this risk increase have been under active investigation, but the underlying pathogenesis is still unclear. Previous studies have reported that the MetS-related risk of CVDs is higher in women than in men. Also in the general population, men and women show divergence in CVDs. However, only very few previous studies have systematically examined the hemodynamic differences between the sexes.

The aim of this thesis was to examine the hemodynamic changes associated with MetS. Therefore, hemodynamics in men and women, with and without MetS, were evaluated. In detail, the aims were to assess: I) supine hemodynamics in MetS, with and without hypertension, II) sex-related hemodynamic differences during head-up tilt test, III) supine and upright hemodynamic changes associated with MetS in men and women, and IV) MetS-related cardiac autonomic regulation during head-up tilt in men and women.

The study populations consisted of subjects without previously diagnosed CVD (other than hypertension), diabetes, or medication having direct effects on cardiovascular function. Hemodynamics were recorded non-invasively using whole body impedance cardiography, pulse wave analysis, and tonometric radial blood pressure measurements. Cardiac autonomic tone was evaluated using power spectral analyses of heart rate variability (HRV). MetS was defined using the criteria of Alberti et al. from 2009.

In Study I, 166 subjects (88 men and 78 women) were allocated to four groups (mean age 44-46 years): healthy controls, hypertension only, MetS without hypertension, and MetS with hypertension. Cut-point for hypertension was blood pressure ≥ 140/90mmHg. The population of Study II consisted of 167 men and 167 women of matching age (mean 45 years) and body mass index (mean 26.5 kg/m^2). In Study III, 502 subjects (252 men and 250 women, mean age 48 years) were divided to four groups: control men, men with MetS, control women, and women with MetS. In Study IV, 501 subjects (252 men and 249 women, mean age 48 years) were similarly divided to groups based on their sex and MetS status.

In good concordance with previous studies, MetS was associated with increased arterial stiffness, defined as higher large arterial pulse wave velocity (PWV). Importantly, PWV was also higher in MetS subjects without hypertension. Additionally, MetS was associated with other changes in hemodynamics: I) Elevated blood pressure that was predominantly explained by increased systemic vascular resistance in the supine position and increased cardiac output in the upright position, II) increased aortic pulse pressure and left cardiac work, and lower subendocardial viability ratio (index of myocardial oxygen supply and demand) in both supine and upright positions.

Several hemodynamic changes associated with MetS seemed to be more pronounced in women than in men. While the MetS-related increase in PWV was similar in men and women, the increase in aortic characteristic impedance (which contributes to left ventricle pulsatile load) was higher in women. In addition, upright aortic reflection time, a variable reflecting the

propagation of the pressure wave in large arteries, was shortened in women, but not in men with MetS. In the evaluations of cardiac autonomic tone by the use of HRV, MetS was associated with lower total power, lower high-frequency power, and lower low-frequency power of HRV when compared with controls. However, when the results were adjusted for the confounding factors, the differences between the MetS-subjects and controls diminished, and lower total and high frequency power of HRV were still found in women but not in men with MetS.

When hemodynamics were compared between 167 men and 167 women, supine hemodynamic differences were minor, but in the upright position systemic vascular resistance was lower, and stroke index, cardiac index, and left cardiac work were significantly higher in men than in women. Corresponding results were also found in a subgroup consisting of postmenopausal women and men of matching age.

In conclusion, MetS was associated with clear hemodynamic changes. The changes in HRV indices and hemodynamic variables that burden the heart were found to be more pronounced in women than in men. These findings may partly explain the greater MetS-related increase in cardiovascular risk in women, and emphasize the importance of prevention and treatment of MetS. Generally, upright workload for the heart was clearly higher in men than in women, and this finding was not explained by hormonal differences before menopause or typical cardiovascular risk factors. This deviation in cardiovascular regulation may play a role in the different cardiovascular risk between the sexes.

TIIVISTELMÄ

Sydän- ja verisuonitaudit ovat yleisin kuolinsyy maailmanlaajuisesti. Miesten ja naisten sairastuvuus näihin tauteihin on erilaista. Tutkimuksia verenkiertoelimistön säätelyn eroista sukupuolten välillä on julkaistu vain vähän. Metabolinen oireyhtymä (MBO) on riskikasauma, jolle on tunnusomaista keskivartalolihavuus, rasva- ja sokeriaineenvaihdunnan häiriö sekä kohonnut verenpaine, ja oireyhtymä lisää merkittävästi riskiä sairastua sydän- ja verisuonitauteihin. Mekanismeja tämän riskin lisääntymisen taustalla on tutkittu paljon, mutta patogeneesi on silti osin epäselvä. Aiemmissa tutkimuksissa on todettu MBO:ään liittyvän sydän- ja verisuonitautiriskin olevan naisilla miehiä suurempi.

Tämän väitöskirjatutkimuksen tavoitteena oli tutkia verenkiertoelimistön säätelyä eli hemodynamiikkaa MBO:ssä. Lisäksi selvitettiin miesten ja naisten hemodynaamisia eroja. Eriteltyinä tavoitteina oli arvioida I) makuuasennon hemodynamiikkaa sekä hypertensiivisillä että normotensiivisillä MBO-henkilöillä, II) sukupuolten välisiä hemodynamiikan eroja kallistuskokeen aikana, III) MBO:ään liittyviä hemodynamiikan muutoksia makuu- ja pystyasennossa, sekä tutkia onko näissä muutoksissa sukupuolten välillä eroa, ja IV) MBO:ssä esiintyviä sydämen autonomisen säätelyn muutoksia kallistuskokeen aikana, sekä niihin mahdollisesti liittyviä sukupuolieroja.

Tutkimusaineisto koostui henkilöistä, joilla ei ollut diagnosoitua sydän- ja verisuonitautia (hypertensiota lukuun ottamatta) tai diabetesta. Tutkimushenkilöillä ei myöskään ollut käytössä potentiaalisesti verenkiertoelimistön toimintaan vaikuttavia lääkkeitä kuten verenpainelääkkeitä. Hemodynaamiset mittaukset tehtiin kajoamattomasti koko kehon impedanssikardiografiaa, jatkuvaa pulssiaaltoanalyysiä ja tonometrista ranneverenpaineen mittausta käyttäen. Autonomisen hermoston toimintaa arvioitiin rekisteröimällä sydämen sykevälivaihtelua. MBO:n määrittämiseen käytettiin Albertin kriteeristöä vuodelta 2009. I tutkimuksessa 166 henkilöä (88 miestä ja 78 naista) jaettiin neljään ryhmään (keski-ikä 44-46 vuotta): kontrolli, pelkkä hypertensio, normotensiivinen MBO, hypertensiivinen MBO. II tutkimuksessa oli mukana iältään (keski-ikä 45 vuotta) ja painoindeksiltään (keskimäärin 26,5 kg/m^2) yhteneväiset 167 miestä ja 167 naista. III tutkimus käsitti 502 henkilöä (252 miestä ja 250 naista, keski-ikä 48 vuotta), jotka jaettiin neljään ryhmään: kontrolli-miehet, MBO-miehet, kontrolli-naiset, ja MBO-naiset. IV tutkimuksessa oli mukana 501 henkilöä (252 miestä, 249 naista,keski-ikä 48 vuotta), ja kuten tutkimuksessa III, heidät oli jaettu ryhmiin sukupuolen ja MBO:n suhteen.

Yhteneväisesti aiempien tutkimusten kanssa, MBO:ään todettiin liittyvän lisääntynyt pulssiaallon etenemisnopeus, suure, jota yleisesti pidetään luotettavimpana menetelmänä evaluoimaan valtimojäykkyyttä. Merkittävää on, että pulssiaallon etenemisnopeus oli lisääntynyt myös niillä MBO-henkilöillä, joilla ei ollut hypertensiota. MBO:ään todettiin liittyvän myös muita hemodynaamisia muutoksia: korkeampi verenpaine näytti liittyvän kohonneeseen ääreisverenkieroon vastukseen makuulla ja kohonneeseen sydämen minuuttitilavuuteen pystyasennossa. Lisäksi MBO:ään liittyi korkeampi sentraalinen pulssipaine ja vasemman kammion työ, sekä matalampi "subendocardial viability ratio", joka kuvaa sydänlihaksen

hapensaantia ja -tarvetta. Nämä muutokset todettiin MBO:ssä sekä makuulla että pystyasennossa.

Useat MBO:ään liittyvät muutokset olivat selvempiä naisilla kuin miehillä. Vaikka pulssiaallon etenemisnopeus oli samalla tavalla lisääntynyt sekä MBO-naisilla että -miehillä, aortan ominaisimpedanssi (joka vaikuttaa vasemman kammion pulsoivaan painekuormaan) oli suurentunut enemmän MBO-naisilla verrattuna MBO-miehiin. Lisäksi pulssiaallon takaisinheijastuma-aika oli MBO-naisilla lyhentynyt pystyasennossa, mutta MBO-miehillä tässä ei todettu eroa kontrolliryhmään verrattuna. Sykevälivaihtelumittauksissa kokonaissykevälivaihtelu, korkeataajuuksinen sykevälivaihtelu sekä matalataajuuksinen sykevälivaihtelu olivat kaikki vähentyneet MBO:ssä. Kun tulokset vakioitiin sekoittavilla tekijöillä, MBO:ään liittyvät muutokset selvästi vähenivät. Kuitenkin vakioinnin jälkeenkin muutoksia sykevälivaihtelussa voitiin edelleen todeta MBO-naisilla, mutta ei MBO-miehillä.

Kun hemodynamiikan eroja tutkittiin 167 miehen ja 167 naisen välillä, makuuasennossa ei huomattavia sukupuolten välisiä eroja ollut. Sen sijaan pystyasennossa miehillä todettiin naisia suurempi sydämen isku- ja minuuttitilavuus sekä vasemman kammion työindeksi, kun taas ääreisverenkierron vastus oli naisia pienempi. Vastaavat tulokset todettiin myös alaryhmällä, jossa oli mukana postmenopausaalisia naisia ja heitä iältään vastaavia miehiä.

Tutkimustulokset osoittivat, että MBO:ään liittyy selkeitä hemodynamiikan muutoksia. Useat hemodynamiikan muutokset sekä vähentynyt sykevälivaihtelu korostuivat naisilla miehiä enemmän. Tulokset saattavat osin selittää MBO:ään naisilla miehiä voimakkaammin liittyvää sydän- ja verisuonitautiriskiä, ja korostavat MBO:n ehkäisyn ja hoidon tärkeyttä. Sukupuolieroihin keskittyvässä tutkimuksessa todettiin miehillä selkeästi suurentunut sydämen kuormitus pystyasennossa. Tämä löydös ei selittynyt menopaussia edeltävillä hormonaalisilla eroilla, eikä myöskään yleisesti tunnetuilla sydän- ja verisuonitautien riskitekijöillä. Tutkimuksessa löydetty ilmiö voi osaltaan selittää miesten ja naisten eroja sydän- ja verisuonitaudeissa.

CONTENTS

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:

I Kangas P, Tikkakoski AJ, Tahvanainen AM, Leskinen MH, Viitala JM, Kähönen M, Kööbi T, Niemelä OJ, Mustonen JT and Pörsti IH. Metabolic syndrome may be associated with increased arterial stiffness even in the absence of hypertension: A study in 84 cases and 82 controls. Metabolism 2013; 62:1114-22.

II Kangas P, Tahvanainen A, Tikkakoski A, Koskela J, Uitto M, Viik J, Kähönen M, Kööbi T, Mustonen J and Pörsti I. Increased Cardiac Workload in the Upright Posture in Men: Noninvasive Hemodynamics in Men Versus Women. Journal of the American Heart Association 2016; 5: e002883.

III Kangas P, Tikkakoski A, Kettunen J, Eräranta A, Huhtala H, Kähönen M, Sipilä K, Mustonen J and Pörsti I. Changes in hemodynamics associated with metabolic syndrome may be more pronounced in women than in men. Submitted.

IV Kangas P, Tikkakoski A, Uitto M, Viik J, Bouquin H, Niemelä O, Mustonen J, Pörsti I. Metabolic syndrome is associated with decreased heart rate variability in a sex-dependent manner: a comparison between 252 men and 249 women. Clinical Physiology and Functional Imaging 2018; doi: 10.1111/cpf.12551.

LIST OF ABBREVIATIONS

AHA	American Heart Association
AIx	Augmentation index
AIx@75	Augmentation index related to heart rate (75/min)
ANCOVA	Analysis of covariance
ANOVA	One-way analysis of variance
ANS	Autonomic nervous system
BMI	Body mass index
CAD	Coronary artery disease
CHD	Coronary heart disease
CRP	C-reactive protein
CV	Cardiovascular
CVD	Cardiovascular disease
ECG	Electrocardiogram
FFA	Free fatty acids
GFR	Glomerular filtration rate
HbA1c	Glycated hemoglobin
HDL-C	High-density lipoprotein cholesterol
HF power	Power in high-frequency range
HIIT	High intensive interval training
HRV	Heart rate variability
IAS	International Atherosclerosis Society
IASO	International Association for the Study of Obesity
ICG_{WB}	Whole body impedance cardiography
IHD	Ischemic heart disease
IDF	International Diabetes Federation
IL-6	Interleukin-6
LCWI	Left cardiac work index

LDL-C	Low-density lipoprotein cholesterol
LF power	Power in low-frequency range
LVH	Left ventricular hypertrophy
N	Number of subjects
MetS	Metabolic syndrome
NAFLD	Non-alcoholic fatty liver disease
NHLBI	National Heart, Lung, and Blood Institute
PAI-1	Plasminogen activator inhibitor-1
PP	Pulse pressure
PWV	Pulse wave velocity
RAAS	Renin-angiotensin-aldosterone system
ROS	Reactive oxygen species
SD	Standard deviation
SEVR	Subendocardial viability ratio
SHBG	Sex hormone-binding globulin
SI	Stroke index
SV	Stroke volume
SVRI	Systemic vascular resistance index
T2DM	Type 2 diabetes mellitus
TNF-α	Tumor necrosis factor alpha
VLDL	Very low-density lipoprotein
WHF	World Heart Federation
WHO	World Health Organization

1 INTRODUCTION

Metabolic syndrome (MetS) is a disorder characterized by a cluster of cardiovascular (CV) risk factors. The typical features in MetS are abdominal obesity, dyslipidemias, hypertension, hyperglycemia, and insulin resistance. Nowadays overweight and physical inactivity are common problems associated with the Western lifestyle, and as a result the prevalence of MetS is rapidly growing. MetS is related to a remarkable increase in the risk of CVDs and type 2 diabetes (T2DM) (Alberti et al. 2009).

CVDs are the most significant cause of death and disability worldwide. According to the report of World Health Organization from 2017 (WHO 2017), CVDs caused globally an estimated number of 17.7 million deaths in 2015, representing 31% of all deaths in the world. Consequently, different features, risk factors and background mechanisms of CVDs have been under active investigation during the last decades.

One clear feature of CVDs is the fact that men have a greater risk of CVDs compared to women. This is true especially before the middle age (Kannel & Wilson 1995). For example, research based on the Framingham Heart Study reported that at 45 years of age, the lifetime risk of sudden cardiac death was 19.9% for men, while in women the risk was 2.8% (Bogle et al. 2016). When it comes to Mets and the risk of CVDs, men and women show also differences: in women MetS is associated with greater relative risk of CV mortality when compared with men (Hunt et al. 2004, Schillaci et al. 2006). Furthermore, early atherosclerosis related to MetS has been found to be more prevalent in women than in men (Iglseder et al. 2005, Tabatabaei-Malazy et al. 2012).

Autonomic nervous system (ANS) plays an important role in CV regulation. Many studies have shown that impaired ANS function is related to CVDs and early mortality (Mäkikallio et al. 2001, Thayer et al. 2010, Wulsin et al. 2015). An imbalance in ANS is a common feature related to MetS (Grassi 2006). Heart rate variability (HRV) is a generally used, non-invasive method to evaluate cardiac autonomic function. A recent review article published by Stuckey et al. (Stuckey et al. 2014) reported that many

studies have found clear evidence for the relationship between MetS and impaired HRV. Like in the risk of CVDs, clear sex-related differences have also been found in the function of ANS (Koenig & Thayer 2016, Pothineni et al. 2016), while the MetS-related changes in HRV seem to be more pronounced in women than in men (Koskinen et al. 2009b, Stuckey et al. 2014, Stuckey et al. 2015). However, there are some discrepancies in the results concerning the relationship between MetS and HRV. Many factors like heart rate (Billman 2013a, Sacha 2014) and antihypertensive medication (Vaile et al. 1999, Karas et al. 2005, Okano et al. 2009) have strong influence on ANS, but in most of the studies on MetS and HRV, these confounding factors have not been taken into account.

Albeit the differences in CVDs are well known between the sexes, the treatment recommendations for CVDs, such as hypertension are the same for both men and women (excluding the pregnancy time). Surprisingly, only few studies have examined the sex-related differences in hemodynamic regulation. Furthermore, the upright position is characteristic for the human race, but nevertheless almost all of the hemodynamic studies have been performed in the supine position. Most of the studies concerning hemodynamic changes associated with MetS have also been focused on the supine measurements.

The aim of this thesis was to provide new information about the hemodynamic changes associated with MetS, and to evaluate the sex-related physiological and pathophysiological differences in the hemodynamic regulation. In the current study the hemodynamic measurements were performed in both supine and upright positions to subjects not using medications with direct CV actions. In the study protocol, the beat-to-beat information of heart rate, peripheral and central blood pressures, aortic compliance, cardiac function, central wave reflection, arterial stiffness, and HRV were obtained. CVDs and MetS are major problems on both individual and public health levels. Understanding their pathophysiological and hemodynamic background may lead to better and more accurate approach in the prevention and treatment of these conditions.

2 REVIEW OF THE LITERATURE

2.1 Regulation of blood pressure - the physiological basis

2.1.1 Cardiac function and peripheral vascular resistance

The function of the CV system is based on the heart, which operates as an electromechanical pump, and on the vasculature, which has different functions depending on the level and the structure of the vessel. The volume of blood pumped from the left ventricle per beat is called stroke volume, and cardiac output represents the blood volume pumped into the aorta by the heart during one minute (Guyton & Hall 2011). According to the Frank-Starling mechanism, the left ventricle is able to increase its force of contraction and therefore stroke volume in response to increases in venous return and hence preload. In other words, up to a physiologic limit, preload and contractility are positively associated (Guyton & Hall 2011).

Systemic vascular resistance (SVR) (sometimes called as total peripheral resistance) depicts the tendency of the vessels to resist blood flow. It can be calculated as the difference between mean aortic pressure and the mean right atrial pressure divided by cardiac output. The pressure of right atrium is normally 2-6 mmHg, but often in the formula it is assumed to be zero as the pressure in the great veins is very small compared to the aortic pressure (Wilmer & Vlachopoulos 2011). Since the main pressure difference occurs during the blood flow in the arterioles, SVR is dominated by the caliber of the arterioles. However, there are also other components of the vascular bed having some effects on it (Wilmer & Vlachopoulos 2011). SVR is controlled by several mechanisms including humoral, structural, neural, and renal regulation. The control is both regional and systemic (Guyton & Hall 2011). It seems that specific structural changes during aging are not so clear in the microcirculation (i.e. in small arteries, arterioles and capillaries) than in larger arteries (O'Rourke & Hashimoto 2007). but, SVR increases during aging as a result of vascular rarefaction and for the reason that the cross-

sectional area of the arterioles decreases (Wilmer & Vlachopoulos 2011). Furthermore, for example hypertension (Schiffrin 2012), sympathetic overdrive (Grassi et al. 2015) and obesity (Bogaert & Linas 2009) can increase SVR.

Mean arterial pressure depends on SVR and cardiac output, and it represents the average value of the arterial pressures measured over a period of time. Since the diastolic phase is longer than the systolic phase during the cardiac cycle mean arterial pressure is determined approximately 60% by the diastolic pressure and 40% by the systolic pressure (Guyton & Hall 2011). Pulse pressure represents the difference between systolic and diastolic blood pressure.

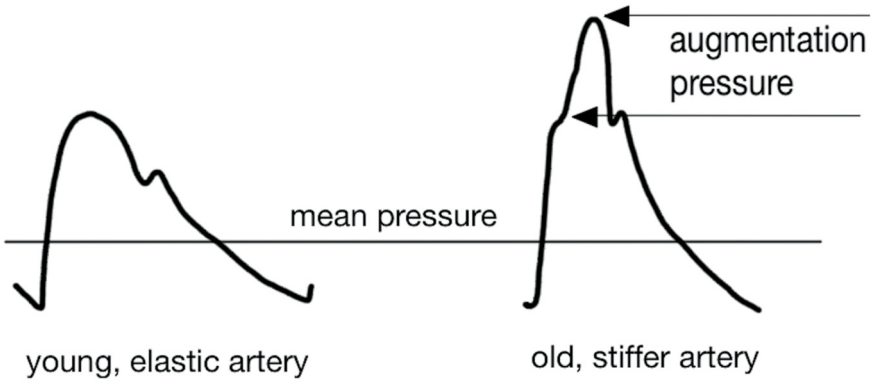
2.1.2 Central wave reflection and arterial stiffness

In a simple mechanical way, the circulation of blood can be defined as a closed system consisting of a pump (the heart) and tubes (the blood vessels). In real life, the circulatory system is a complex entity having multiple components regulating it. Importantly, blood flow is that it is pulsatile, not constant. When evaluating circulation of blood in the arteries, both flow wave and pressure wave have to be taken into account. The arterial tree has basically two functions: 1. delivering blood from the heart to the capillaries of the organs and tissues, and 2. cushioning the central pulsation so that the blood flow in capillaries is as continuous as possible (O'Rourke et al. 2007).

When the pressure wave travels forward from the left ventricle and reaches the branching points in the arterial tree, the wave reflects backward. This phenomenon occurs mainly at the changing point from the low resistance arteries into the high-resistance arterioles (Laurent et al. 2006, O'Rourke et al. 2007). Thus, the pressure wave measured from an artery is a composite of forward pressure wave and reflecting backward pressure wave. In young, elastic arteries, the velocity of the pulse wave is low, and the reflected wave has a tendency to return to the aortic root during diastole. When the arteries stiffen, the reflected wave arrives back earlier, augmenting the systolic pressure and enlarging the forward wave. The addition of the reflected wave to the systolic pressure is termed as augmentation pressure, and augmentation index (AIx) represents the ratio

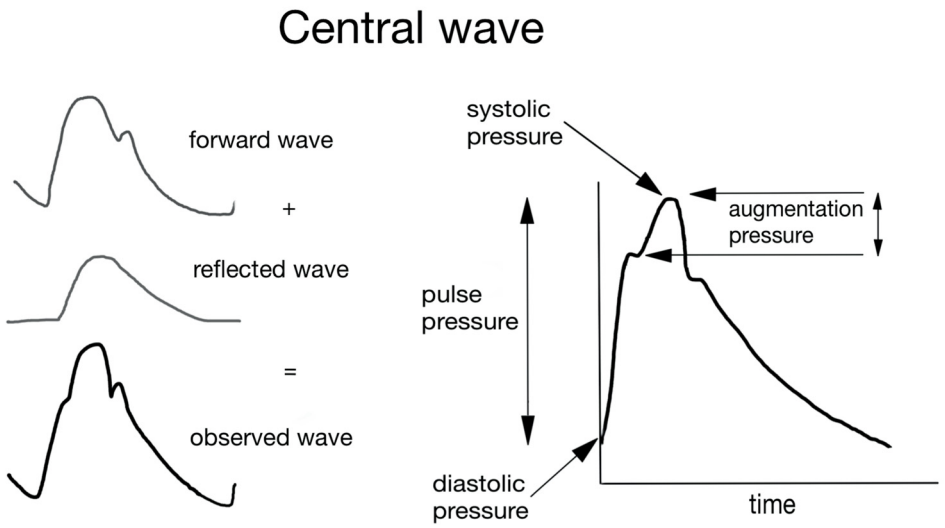
between augmentation pressure and pulse pressure (Laurent et al. 2006, Wilmer & Vlachopoulos 2011). The difference in the shape of the central pressure wave between elastic and stiffened artery is represented in Figure 1.

Figure 1. Central wave pressure curve in elastic and in stiffened artery (modified from Safar et al. 2013).



Since AIx depends on the PWV, the arterial stiffness is an important determinant of it (Safar 2008, Wilmer & Vlachopoulos 2011). However, several other factors like height and sex (London et al. 1995), age (McEniery et al. 2005), and SVR (Wilenius et al. 2016) have a clear effect on AIx. In addition, AIx is influenced by heart rate (Wilkinson et al. 2000), and therefore AIx is often depicted in relation to heart rate 75/min (AIx@75). The composition of the central wave and pulse pressure is represented in Figure 2.

Figure 2. Schematic presentation of the central blood pressure curve (modified from Safar et al. 2013).



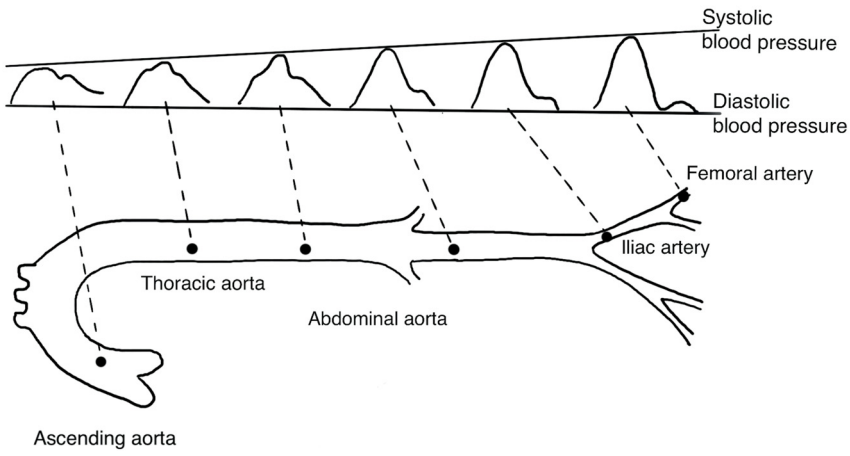
When the reflected wave returns to the aortic root earlier, during systole instead of diastole, it has several harmful effects. Central systolic blood pressure increases, and this intensifies left ventricle load, thus stressing the heart. This predisposes to the development of left ventricle hypertrophy (LVH) and has also an increasing effect on left ventricle oxygen demand. Furthermore, the contraction time of the hypertrophic heart muscle is prolonged, so that the duration of systole is increased and diastole is shortened, and this phenomenon causes even more augmentation of late systolic pressure by the reflected wave. Since the coronary blood supply occurs during diastole, the decreased diastolic pressure and shortened diastolic time add to an adverse effect on the heart. (O'Rourke et al. 2007)

Amplification

The elasticity of the arteries varies along the arterial tree. While the central, large arteries have elastin as a dominant component in the vessel wall, in the distal arteries extracellular matrix is less stretchy. Consequently, the

arterial stiffness increases physiologically from central to the peripheral arteries (Wilmer & Vlachopoulos 2011). In addition, the shape of the pressure wave changes as it travels from the left ventricle to the periphery. The reflection sites of the pulse wave are closer in peripheral arteries than in large arteries, and this amplifies the peripheral pressure wave (Latham et al. 1985). For these reasons systolic blood pressure and pulse pressure increase in peripheral arteries to a higher level than what occurs in the central arteries. This physiological phenomenon is called amplification, and it helps to maintain the central systolic blood pressure and pulse pressure low, thus protecting the heart from an increase in post-load. However, during aging the amplification reduces while augmentation increases (Safar et al. 2013). The amplification phenomenon is represented in the Figure 3.

Figure 3. Amplification phenomenon along the arterial tree (modified from Wilmer & Vlachopoulos 2011).



Stiffening of arteries

During the life course, elastic arteries are under repetitive pulsations (about 30 million/year), and this stress causes some structural changes in the artery wall. Dilatation and stiffening are the most prominent physical alterations with age (O'Rourke et al. 2007). Compared to the muscular arteries, the aging effects on the central large arteries are more pronounced, thus the large arteries stiffen more than the distal arteries (Boutouyrie et al. 1992, Bortolotto et al. 1999). One reason for this phenomenon is the fact that each beat of the heart causes a wider proportional dilatation in the aorta and in the proximal elastic arteries than in the peripheral muscular arteries. Consequently, the central arteries are more stressed, and on the basis of material fatigue, the structural changes are more pronounced in these arteries during life course (O'Rourke et al. 2007).

When the central arteries stiffen, the cushioning, i.e. the other main function of the arterial tree, becomes disturbed. As a result, the arterial pulsation flow is transferred further to the periphery. This leads to unfavorable influences in the microcirculation. This is especially harmful for the brain and kidneys, organs with naturally high resting flow (O'Rourke & Safar 2005, O'Rourke et al. 2007). Moreover, as described earlier, arterial stiffness causes increased PWV leading to earlier wave reflection, and consequently increased systolic blood pressure and pulse pressure. In addition, arterial stiffness stresses the vessel wall, and as a result increases the risk of atherogenesis, and also the risk of plaque rupture (Van Bortel 2002), albeit it also has been postulated that atherosclerotic changes can precede the increase of arterial stiffness (Hansen & Taylor 2016). Thus, arterial stiffness has several harmful effects not only on the heart, but also locally in the artery wall, and due to disturbed microcirculation it causes organ damage as well. Accordingly, in several studies and reviews, increased arterial stiffness has been stated as an independent risk factor of fatal and non-fatal CV end-points in both healthy subjects and patients with medical disorders like hypertension (Laurent et al. 2001, Safar et al. 2002, Laurent et al. 2003, Willum Hansen 2006, Mitchell et al. 2010a, Veerasamy et al. 2014). It has been evaluated that when aortic PWV increases by 1m/s, the corresponding risk increase in total CV events, CV mortality, and all-cause mortality is 14%, 15%, and 15%, respectively. When aortic PWV

increases by 1 standard deviation (SD), it is associated with respective risk increases of 47%, 47%, and 42%. (Vlachopoulos et al. 2010).

Determination of arterial stiffness

When an arterial segment is examined, compliance represents a change in volume for a given change in pressure, while distensibility is defined as compliance divided by the initial volume. Arterial stiffness or elastance (sometime called as elastic modulus) are the inverse of distensibility and compliance (Wilmer & Vlachopoulos 2011). A good, non-invasive tool for evaluating arterial stiffness is to measure PWV. PWV is defined as a distance of two measurement sites divided by the travelling time of the pressure wave, and higher PWV indicates stiffer arteries. Large arteries play a major role in the pathophysiology of CV problems related to arterial stiffness, and the prognostic value of carotid-femoral PWV for CV events and mortality has been shown in several studies (Laurent et al. 2006). Consequently, carotid-femoral PWV, as a marker of aortic stiffness, is considered the gold standard measurement of arterial stiffness (Laurent et al. 2006). However, especially in the Asian population, evidence of the prognostic value of brachial-ankle PWV in CVDs has also been reported (Vlachopoulos et al. 2012).

Aortic stiffness can also be evaluated by aortic characteristic impedance. Vascular impedance represents the relationship between arterial pressure and flow at the same site in an artery, in the absence of wave reflections. A major determinant of vascular impedance is arterial stiffness (Wilmer & Vlachopoulos 2011, Townsend et al. 2015). Aortic characteristic impedance depicts the impedance in the proximal part of aorta, and can be used as a marker of aortic stiffness (Chemla et al. 2008, Townsend et al. 2015). However, it is important to notice that not only the aortic wall stiffness, but also the diameter of the aortic lumen has a clear effect on aortic characteristic impedance (Mitchell 2015).

Also pulse pressure can be considered a crude estimate of large artery stiffness (Cohn et al. 2004). However, several other physiological factors such as wave reflection have an effect on pulse pressure, and for instance the influence of aging is different on pulse pressure and on carotid-femoral PWV (Mitchell 2015). In addition, in young persons, pulse pressure can

elevate due to an increase of stroke volume, while in older persons the relation seems not to be clear (Alfie et al. 1999).

Cardiovascular risk factors and arterial stiffness

According to a review of Benetos et al. (2002b), aortic and carotid stiffness increases approximately 10% to 15% during a 10 year period of aging. In addition to aging, several CV risk factors have important effects on arterial stiffness. Hypertension is strongly associated with arterial stiffness, and hypertension has been observed to cause similar structural changes in the arterial wall as aging, thus accelerating the aging process (Greenwald 2007). In a longitudinal study with comparison to normotensive subjects, hypertensive subjects had greater annual rates of progression in PWV (Benetos et al. 2002). However, some studies have reported that arterial stiffness is associated with blood pressure progression and future development of hypertension, thus suggesting that arterial stiffness can also be a cause of hypertension (Dernellis & Panaretou 2005, Takase et al. 2011, Kaess et al. 2012).

Diabetes (both type 1 and 2) has frequently been reported to associate with increased arterial stiffness (Theilade et al. 2013, Prenner & Chirinos 2015, Fu et al. 2017a). Furthermore, the stiffness is increased in subjects with impaired glucose tolerance (Xu et al. 2010, Li et al. 2012), and with increased glycated hemoglobin (HbA1c) (Lee et al. 2016), even without diabetes. Just as in diabetes, in MetS arterial stiffness has also been noticed to be increased (Stehouwer et al. 2008, Scuteri et al. 2014, Vilmi-Kerala et al. 2017). Also insulin resistance (Westerbacka et al. 2001, Yki-Järvinen & Westerbacka 2007, Fu et al. 2017b) and hyperinsulinemia (Hansen et al. 2004) seem to associate with large arterial stiffness. Of note, the relation of insulin resistance and arterial stiffness may occur independently of glucose tolerance status (Sengstock et al. 2005).

Even if high low-density lipoprotein cholesterol (LDL-C) and low high-density lipoprotein cholesterol (HDL-C) are both strong independent predictors of CVDs (NCEP 2001), the study results of the association of lipid disorders with arterial stiffness are contradictory. Some studies have reported a clear relation with lipid disorders and artery stiffness (or elasticity) indices (Juonala et al. 2005, Wang et al. 2011b), while some

others have not found the association (Saba et al. 1999, Nguyen et al. 2008).

Also chronic kidney disease has repeatedly been reported to associate with increased arterial stiffness (Ma et al. 2015). Importantly, Schillaci et al. (2006a) showed that in hypertensive patients with normal renal function, decreased glomerular filtration rate (GFR) was related with arterial stiffness, even if age, sex and blood pressure values were taken into account.

2.1.3 Cardiac autonomic tone, heart rate variability

Autonomic nervous system (ANS), consisting of sympathetic and parasympathetic parts, is involved in numerous physiological functions in the human body. Disturbances in ANS have been reported to associate with CVDs and early mortality (Mäkikallio et al. 2001, Thayer et al. 2010, Grassi et al. 2015, Wulsin et al. 2015). Cardiac autonomic tone can be evaluated non-invasively by the use of HRV (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996). The interval between consecutive heart beats physiologically oscillates, and this phenomenon is called HRV. HRV can be evaluated using different methods: time domain method, frequency domain method (i.e. spectral analysis), rhythm pattern analysis, and nonlinear methods are most commonly used. The frequency domain method is typically used when data is captured from short-term (approximately 5-minutes) recordings, and the time domain method when long-term (approximately 24-hours) data is available (Xhyheri et al. 2012). In the frequency domain method, power spectral density analysis is used to obtain information about how power (i.e. variance) is distributed as a function of frequency (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996). Then, by the use of a mathematical algorithm, total power, power in the low frequency (LF) range, and power in the high frequency (HF) range are typically represented. In addition, LF/HF ratio is often calculated, and occasionally also very low frequency range is analyzed and presented. The HF component represents cardiac parasympathetic activity (Eckberg 1997, Cooke et al. 1999, Xhyheri et al. 2012), while the LF

component predominantly reflects sympathetic activity, although parasympathetic contributions also influence the LF component (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996, Eckberg 1997, Xhyheri et al. 2012). The meaning of the LF/HF ratio remains somewhat controversial. It is typically considered to represent the sympathovagal balance (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996, Xhyheri et al. 2012), but this interpretation has repeatedly been criticized, and conclusions should be drawn with caution (Eckberg 1997, Cooke et al. 1999, Billman 2013b). Consequently, HRV is a valuable method for describing especially the parasympathetic cardiac tone (Stuckey et al. 2015).

Several studies have shown the clinical and prognostic value of HRV in the evaluation of the risk of CVDs. However, some discrepancy exists between the study results, and large prospective longitudinal studies are needed to better understand the value of HRV in the evaluation of future morbidity and mortality. The strongest evidence indicates decreased HRV as a predictor of risk after acute myocardial infarction and as an initial warning signal of diabetic neuropathy (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996). Many factors like age (Reardon & Malik 1996), sex (Koenig et al. 2016), heart rate (Billman 2013a, Sacha 2014) and antihypertensive medication (Vaile et al. 1999, Karas et al. 2005, Okano et al. 2009) have been reported to clearly influence HRV. Furthermore, the circadian rhythm (Li et al. 2011) and breathing (Billman 2011) also influence HRV.

2.2 The meaning of the posture, tilt table test

Even if the upright posture is characteristic for the human race, and we spend a lot of time standing or sitting instead of lying down, most of the studies evaluating hemodynamics have been performed in the supine position. Consequently, the knowledge of upright hemodynamics is very limited, and the meaning of the upright position may have been neglected in the evaluation of the CV risk.

Changing the posture from supine to upright causes many remarkable alterations in hemodynamics, and this can be claimed to create a challenge to the CV system. In the laboratory conditions, these hemodynamic alterations can be evaluated by means of a head-up tilt table test. During the postural change, body fluid shifts and the gravity influence on fluid columns leads to reactions in the autonomic nervous functions such as in baroreflexes (Avolio & Parati 2011). Thus, disruptions in the autonomic nervous regulation are often revealed during the head up tilt test (Teodorovich & Swissa 2016).

2.3 Cardiovascular differences between the sexes

2.3.1 Sex-related differences in cardiovascular diseases

Men and women differ in the prevalence and incidence of CVDs, also in mortality due to CVDs. Men have a greater risk of CVDs, and the sex-related difference in that risk is clear especially before the middle age (Kannel et al. 1995, Mozaffarian et al. 2016). In men, the risk of sudden cardiac death is much higher compared to women (Bogle et al. 2016). Prevalence of hypertension is greater in young men than in young women, but after the middle age the prevalence increases rapidly in women. Consequently, the prevalence in older women is even higher than in men of the same age (Wenner & Stachenfeld 2012). Especially, the prevalence of isolated systolic hypertension is higher in elderly women than in men (Martins et al. 2001).

The age-adjusted prevalence and incidence of ischemic heart disease (IHD) is greater in men (Crea et al. 2015). However, in the recent decades, data from the National Health and Nutrition Examination Surveys shows that the prevalence of myocardial infarction has declined in midlife (35-56 years) men, but not in women (Towfighi et al. 2009). Moreover, many studies have reported that women have a worse prognosis during acute coronary syndrome (Davis et al. 2017). This may be due to the fact that women with acute coronary syndrome are often older compared to men, and also have more comorbidities (Bucholz et al. 2014). However, it also seems that the CV risk is sometimes underestimated in women, and medical undertreatment is more common in women than in men with IHD

(Bugiardini et al. 2011, Johnston et al. 2011). This phenomenon is called the Yentl syndrome, and the term was first used by Dr Healy in 1991 with a purpose to pay attention to the unfavorable outcome in women with IHD, and the underdiagnosis and undertreatment of women (Healy 1991).

Furthermore, the characteristics of CVDs differ between men and women. In women, non–ST-segment elevation myocardial infarction occurs with non-obstructed coronary arteries more often than in men (Gehrie et al. 2009). Spontaneous coronary artery dissection (Tweet et al. 2012), coronary microvascular dysfunction (Reis et al. 2001) and stress-induced cardiomyopathy (Takotsubo syndrome) (Templin et al. 2015) are also more common in female subjects. There is also evidence showing that in cases of sudden coronary deaths, the prevalence of acute thrombosis is higher in men than in women. As a cause for thrombosis, plaque erosion is more frequent in women, while plaque rupture is more frequent in men (Yahagi et al. 2015). Moreover, CV-ageing is different between the sexes: when compared to women, men have greater endothelial dysfunction and arterial stiffness before the middle age (Mitchell et al. 2004, Merz & Cheng 2016). Young and middle-aged men have higher abundance of atherosclerotic plaques than women, and this phenomenon can also be found in asymptomatic individuals (Kelley et al. 2011). The myocardial remodeling in response to aging seems to be more favorable in women than in men (Piro et al. 2010), but diastolic heart failure is more common in women (Merz et al. 2016). However, the prognosis of heart failure has been reported to be better in women than in men (Parashar et al. 2009, Martinez-Selles et al. 2012).

The most important atherosclerotic CVD risk factors are the same for both sexes: high blood pressure, dyslipidemias, diabetes, obesity, physical inactivity, and smoking. A prospective study in middle-aged Finnish subjects showed that many of these risk factors are more favorable for women, albeit the sex-related differences diminish with increasing age. These sex-related differences in risk factors, especially in HDL-C/total cholesterol ratio and in smoking explain almost half of the difference in coronary artery disease (CAD) risk between the sexes (Jousilahti et al. 1999). However, some differences in the relationship of the most common risk factors and CVDs have been reported. A meta-analysis of over 400000 individuals showed that the relative risk of fatal CAD was even 50% higher in women with diabetes compared to men with diabetes (Huxley et al. 2006). Also

smoking seems to increase the risk of CVDs more in women than in men. According to a large meta-analysis comparing the CAD risk between smokers and non-smokers, female smokers had a 25% greater relative risk of CAD than did male smokers (Huxley & Woodward 2011).

Thus, plenty of evidence exists of the sex-related differences in CVDs: in the prevalence, incidence, prognosis, and characteristics of CVDs. However, the background mechanisms for these sex-related differences are poorly known. Furthermore, traditional treatment of CVDs remains the same for both sexes.

2.3.2 Hemodynamics in male and female subjects

In hemodynamic regulation, many sex-related differences have been noticed. Since the body size is different between men and women, it is obvious that also the size of the heart and major blood vessels are greater in men compared with women of the same race and age (Vasan et al. 1997). This leads to greater stroke volume and also greater cardiac output in men (Huxley 2007). By contrast, women have higher heart rate (HR) than men (Umetani et al. 1998). Pulse pressure is higher in young men than in young women, but at older ages, pulse pressure becomes higher in women (Skurnick et al. 2010). Studies have consistently found that men have higher blood pressure than women. According to a large, recently published report, global age-standardized mean systolic blood pressure in 2015 was 127.0 mmHg in men and 122.3 mmHg in women; age-standardized mean diastolic blood pressure was 78.7 mmHg for men and 76.7 mmHg for women (Collaboration 2017). Furthermore, already in adolescents aged 12-18 years, sex-related differences in hemodynamics can be found: heart rate is higher in females while systolic blood pressure is higher in males (Rabbia et al. 2002). Arterial stiffness has been observed to be higher in men than in women (Kim et al. 2014b, Wen et al. 2015). However, also with arterial stiffness, age has an influence on the sex-related differences. Tomiyama et al. (2003) showed that in non-medicated subjects, men had higher arterial stiffness compared to women before the age of 60, but after that the results became similar in both sexes. Thus, it seems that in the elderly subjects, vascular ageing is faster in women than in men.

When it comes to sex-related hemodynamic differences in the upright position, the knowledge of it is quite scarce. Only a few studies have been

published, and most of these with a small number of participants. In previous reports, it has been found that women have lower orthostatic tolerance than men (Convertino 1998, Waters et al. 2002). However, the study results concerning the physiological mechanisms beneath the sex-related hemodynamic differences in the upright position vary from one report to another. During head up tilt or posture change from lying to sitting position, heart rate has been reported to increase less in men when compared with women (Shoemaker et al. 2001), more in men than in women (Huikuri et al. 1996), or the increase of heart rate has been reported to be similar in both sexes (Barnett et al. 1999, Jarvis et al. 2010). Regarding cardiac output and stroke volume, some previous studies did not find sex-related differences in the response to head-up tilt (Shoemaker et al. 2001, Jarvis et al. 2010).

Sex-related differences have also been reported regarding HRV. According to a large meta-analysis, women are characterized by lower total power, greater HF power, and lower LF power of HRV than men, indicating greater parasympathetic activity in women when compared with men (Koenig et al. 2016). Despite the finding that women have greater vagal activity, higher heart rate is also characteristic for them (Koenig et al. 2016). During the tilt-test or active standing men have been reported to have higher LF/HF ratio when compared with women (Barantke et al. 2008, Reulecke et al. 2018). Orthostatic intolerance is more common in women *(Anjum et al. 2018),* and the sex-related differences in cardiac autonomic regulation during the change from the supine to the standing position may be one explanation for this finding (Barantke et al. 2008).

2.4 Metabolic syndrome

2.4.1 Definitions of metabolic syndrome

In 1923, a Swedish physician Kylin noticed the association of hypertension, high blood glucose, and gout (Kylin 1923). Already before that, attention had been given to a few main factors having a great influence on CVDs. The clustering of some CVD risk factors was named as syndrome X by Reaven in 1988 (Reaven 1988). Later this phenomenon was more specifically defined,

and the term "metabolic syndrome" (MetS) was developed. MetS is a cluster of CVD risk factors including impaired glucose tolerance, elevated triglycerides, decreased HDL-C, elevated blood pressure, and abdominal obesity. Since these factors occur together more often than would be expected by chance alone, MetS can be defined as a distinct disorder, a syndrome. In addition to the risk factors mentioned above, also prothrombotic and proinflammatory states are common disorders associated with MetS (Alberti et al. 2009). An important component in MetS is insulin resistance, and consequently also the term “insulin resistance syndrome” has been used (Bloomgarden 2003, Kashyap & Defronzo 2007).

During the last decades, there have been several definitions of MetS. Some of the definitions have been applied mainly for research purposes, while others are more suitable for clinical use. At first, the existence of insulin resistance was the core of the definition (of syndrome X by Reaven). The other criteria were hyperinsulinemia, high level of very low-density lipoprotein (VLDL), low level of HDL-C, impaired glucose tolerance, and hypertension. Later, also obesity (general and abdominal), triglycerides, and microalbuminuria have been noticed as parts of the syndrome (Sarafidis & Nilsson 2006). The latest definitions of MetS also take the ethnic differences in waist circumference into account. The definition from 2009 is a joint statement by the International Diabetes Federation (IDF), the National Heart, Lung, and Blood Institute (NHLBI), the American Heart Association (AHA), World Heart Federation (WHF), the International Atherosclerosis Society (IAS), and International Association for the Study of Obesity (IASO) (Alberti et al. 2009). According to this definition, at least three of the following five risk factors should be met in order to make the diagnosis of MetS: increased waist circumference, elevated fasting blood glucose, elevated triglycerides, elevated blood pressure, and decreased HDL-C. The specific features of the most used definitions of MetS are presented in Table 1.

Table 1. Different definitions of metabolic syndrome.

WHO, 1998 (Alberti & Zimmet 1998, Organization 1999)	**EGIR, 1999** (Balkau & Charles 1999)	**NCEP, 2001**	**IDF, 2005** (Alberti et al. 2006)	**IDF, NHLBI, AHA, WHF, IAS, IASO, 2009** (Alberti et al. 2009)
Insulin resistance[a] and/or impaired glucose regulation[b] or diabetes, together with ≥ 2 of following	Insulin resistance[c] or fasting hyperinsulinemia[c] in non-diabetic population, together with ≥ 2 of following	≥ 3 of following	Abdominal obesity defined by ethnicity specific waist circumference (European men ≥ 94cm, women ≥ 80 cm) or BMI >30 kg/m^2 together with ≥ 2 of following	≥ 3 of following
Waist-to-hip ratio > 0.9 in men and/or > 0.85 in women or BMI > 30 kg/m^2	Waist circumference > 94 cm in men and > 80 cm in women	Waist circumference > 102 cm in men and > 88 cm in women		Ethnicity specific waist circumference (European men ≥ 94cm, women ≥ 80 cm)
Systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90mmHg	Systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90mmHg	Systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 85 mmHg	Systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 85 mmHg	Systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 85 mmHg
Triglycerides > 1.7 mmol/l and/or HDL-C < 0.9 mmol/l in men, < 1.0 mmol/l in women	Triglycerides > 2.0 mmol/l and/or HDL-C < 1.0 mmol/l	Triglycerides ≥ 1.7 mmol/l	Triglycerides ≥ 1.7 mmol/l	Triglycerides ≥ 1.7 mmol/l
		HDL-C < 1.0 mmol/l in men, < 1.3 mmol/l in women	HDL-C < 1.0 mmol/l in men, < 1.3 mmol/l in women	HDL-C < 1.0 mmol/l in men, < 1.3 mmol/l in women
Urinary albumin excretion rate > 20 µg/min or albumin:creatinine ratio > 30 mg/g	Fasting plasma glucose ≥ 6.1 mmol/l (but not diabetic)	Fasting plasma glucose ≥ 6.1 mmol/l	Fasting plasma glucose ≥ 5.6 mmol/l or previously diagnosed diabetes	Fasting plasma glucose ≥ 5.6 mmol/l or medication for elevated glucose

[a]Under hyperinsulinemic euglycemic conditions, glucose uptake below lowest quartile for background population under investigation.
[b]Fasting plasma glucose < 7.0 mmol/l and 2 hours post glucose load ≥ 7.8 mmol/l < 11.1 mmol/l.
[c]The highest quartile of the non-diabetic population.
If specific medication is used for lowering triglycerides or blood pressure, or for raising HDL-C, the cut points in question are not needed.
WHO, World Health Organization; EGIR, European Group for the Study of Insulin Resistance; NCEP, National Cholesterol Education Program; IDF, International Diabetes Federation; NHLBI, National Heart, Lung, and Blood Institute; AHA, American Heart Association; WHF, World Heart Federation; IAS, International Atherosclerosis Society; IASO, International Association for the Study of Obesity.

2.4.2 Pathophysiology of metabolic syndrome

Especially in the Western countries, lifestyle has become more sedentary in the last decades. Obesity is an epidemic, and it is obvious that it has a great influence on the prevalence of MetS. It seems clear that the more specific pathophysiology of MetS is multifaceted, and still incompletely understood.

According to the latest definition of MetS from 2009 (Alberti et al. 2009) the abdominal obesity is just one and equal criterion of the five factors. However, obesity has been noticed to have a remarkable effect on the pathogenesis of MetS (Furukawa et al. 2004, Alam et al. 2007). It has been proposed that when adipocytes are getting larger, the blood supply to the adipocytes is reduced, which may cause hypoxia. That hypoxia can lead to many kinds of changes in molecular metabolism, such as overproduction of free fatty acids (FFA) and glycerol, and biologically active metabolites named adipocytokines (Kaur 2014). These adipocytokines include for example proinflammatory mediators [interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α)], C-reactive protein (CRP), leptin, and plasminogen activator inhibitor-1 (PAI-1). Adipocytokines have influences on the endocrine, autocrine and paracrine signaling mediating many disorders characteristic in MetS (Kaur 2014). There is some evidence that the enlarging of the adipose tissue and the increasing amount of FFA accelerate the production of reactive oxygen species (ROS), leading to elevated oxidative stress. Oxidative stress causes dysregulated production of adipocytokines (Furukawa et al. 2004). One adipocytokine having a great influence in the pathogenesis of MetS is adiponectin. In contrast to the other adipocytokines, the amount of adiponectin is inversely correlated to obesity (Yamauchi et al. 2001, Furukawa et al. 2004). Adiponectin has an effect on lipid and glucose metabolism, and it increases insulin sensitivity, regulates the energy homoeostasis, and protects against chronic inflammation (Liu & Liu 2009). It has also a multifactorial antiatherogenic action (Matsuzawa et al. 2004). When the influence of the adipocytes on the metabolic disorders is evaluated, visceral adipose tissue is more pathogenic than subcutaneous adipose tissue (Fox et al. 2007).

Insulin resistance, i.e. the impaired ability of insulin to stimulate glucose uptake in peripheral tissues, plays a key role in MetS. In the pathogenesis of

insulin resistance related to MetS, multiple components are involved. Many adipocytokines mediate insulin resistance and its adverse effects in MetS (Kaur 2014). FFAs have a significant effect on insulin resistance (Boden 2011). Moreover, when insulin resistance affects the adipose tissue, lipolysis is disturbed, and it further releases FFAs into the circulation (Delarue & Magnan 2007). When the amount of FFAs increases, the capability of adipose tissue to take up and store them is exceeded. Consequently, FFAs accumulate in other tissues, such as the liver and skeletal muscle. This phenomenon is called ectopic fat accumulation, and it has been noticed to be strongly associated with insulin resistance (Seppala-Lindroos et al. 2002, Yki-Järvinen 2002). Insulin resistance leads to compensatory oversecretion of insulin from the pancreatic beta cells. Consequently, insulin resistance and hyperinsulinemia exist at the same time. They both have effects on multiple metabolic functions, and this leads to the clinical manifestations of MetS (Gill et al. 2005).

Nutrition has an influence on the development of MetS. It is obvious that positive energy balance increases the amount of adipose tissue, and thereby contributes the development of MetS. However, the quality of food also plays an important role (Paniagua 2016). For example, low whole-grain intake and high dietary glycemic index are positively associated with the prevalence of MetS (McKeown et al. 2004). Also a high intake of saturated fat (Nupponen et al. 2015) and sodium (Baudrand et al. 2014), and a low intake of potassium (Cai et al. 2016) have been reported to relate with the development of MetS. In addition, some evidence has been found about the association of exposure to synthetic chemicals (such as chemicals used in food production and in household goods) with MetS. It seems that especially undesirable exposures early in life may have a significant impact (De Long & Holloway 2017).

Sleeping disorders like obstructive sleep apnea and short sleeping period have been noticed to associate with MetS (Xi et al. 2014, Xu et al. 2015). Interestingly, the association with MetS and obstructive sleep apnea seems to exist also independently of obesity (Qian et al. 2016). Also stressful life events and chronic stress seem to be associated with the prevalence of MetS (Pyykkonen et al. 2010)

Even if lifestyle and environmental factors play remarkable roles in the pathophysiology of MetS, also the genetic predisposition is important, and some genetic information about MetS has been obtained recently (Brown &

Walker 2016). However, the clinical significance regarding the reported genetic predispositions to the development of MetS at the individual level remains to be determined.

In conclusion, MetS is a multifaceted disorder with several factors in its pathophysiology. Even though the area has been under active investigation, the background mechanisms are still incompletely understood. Insulin resistance, metabolically active adipose tissue, oxidative stress, and inflammatory state are the key players in the development of the syndrome. Importantly, one factor may strenghten the influence of another factor.

2.4.3 Clinical importance of metabolic syndrome

Since obesity is an epidemic in a large part of the world, also the prevalence of MetS is rapidly growing (van Vliet-Ostaptchouk et al. 2014). The worldwide prevalence of MetS varies from below 10% to as high as 84%, depending on the region, environmental aspect (rural or urban), sex, age, race, ethnicity, and concurrent existence of other diseases (such as diabetes) of the population studied. The prevalence of MetS also depends on the used definition (Kolovou et al. 2007). According to the estimation of IDF, about a quarter of the world's adult population has MetS (IDF 2006).

When comparing the subjects with and without MetS, MetS confers a 5-fold increase in the risk of developing type 2 diabetes, and a 2-fold risk of developing CVD over the next 5 to 10 years (Alberti et al. 2009). For instance, the risk of stroke (Li et al. 2017) and the risk of myocardial infarction are clearly increased (Mottillo et al. 2010). Even if CVDs and diabetes may be the most renowned consequences of MetS, MetS has an influence on other organs too. Non-alcoholic fatty liver disease (NAFLD) is a disease consisting of liver disorders from hepatic steatosis to non-alcoholic steatohepatitis, cirrhosis, end-stage liver failure, and hepatocellular carcinoma (Wainwright & Byrne 2016). It is clearly associated with MetS (Cortez-Pinto et al. 1999, Marchesini et al. 2003, Hamaguchi et al. 2005, Yki-Järvinen 2014), and it seems that the relationship is bidirectional (Vanni et al. 2010, Wainwright et al. 2016). NAFLD is the most common cause of chronic liver disease in the developed countries, and liver failure induced by NAFLD is one of the most important reasons for liver transplantation

(Targher & Byrne 2013). In addition, the risk of some cancers, such as breast cancer has been noticed to associate with MetS (Bhandari et al. 2014). Furthermore, MetS is associated with a 1.5-fold increase in risk of all-cause mortality (Mottillo et al. 2010).

Accordingly, MetS causes significant clinical problems, and furthermore, MetS has a distinct public health aspect. CVDs have huge financial consequences (only in the United States, the annual cost of CVDs and stroke in 2011-2012 was an estimated $316.6 billion (Mozaffarian et al. 2016)). Thus, in addition to MetS being a remarkable risk factor of CVDs, it has also significant economical consequences.

Some studies and meta-analyses have proposed that the CV risk associated with MetS is above and beyond its specific components (Gami et al. 2007, Gupta et al. 2010). However, most of the recent studies have shown that the CV risk in MetS is clearly increased, but the correlation between the number of MetS components and CV diseases is linear. Thus, the MetS does not predict the CVDs more than would be expected from the sum of its risk components (Koskinen et al. 2009a, Rachas et al. 2012, Hanchaiphiboolkul et al. 2013).

2.4.4 Hemodynamic features associated with metabolic syndrome

One characteristic hemodynamic feature related to MetS is elevated systolic and diastolic blood pressure. This is obvious, since blood pressure is one of the five criteria of MetS (Alberti et al. 2009). Salt-sensitivity (Uzu et al. 2006) and increased activity of renin-angiotensin-aldosterone system (RAAS) (Owen & Reisin 2015) play important roles in hypertension associated with MetS. Higher resting heart rate has repeatedly been noticed to associate with MetS (Rogowski et al. 2009, Perlini et al. 2013, Jiang et al. 2015). Furthermore, high resting heart rate seems to have a predictive value for the development of MetS (Jiang et al. 2015, Liu et al. 2017). Sympathetic overdrive related to MetS has been proposed to have a significant influence on the elevation of heart rate in MetS (Mancia et al. 2007b, Rogowski et al. 2009).

Increased arterial stiffness has been reported to associate with MetS in many studies (Safar et al. 2006b, Stehouwer et al. 2008, Scuteri et al. 2014, Vilmi-Kerala et al. 2017). Since arterial stiffness is an important,

independent predictor of fatal and non-fatal CV end-points (Willum Hansen 2006, Mitchell et al. 2010a), it has been proposed that increased arterial stiffness could be a prominent link between MetS and CVDs (Stehouwer et al. 2008). Elevated blood pressure is an important component of MetS, and the association with hypertension and arterial stiffness is indisputable (Laurent & Boutouyrie 2007, Mitchell 2014). Thus, it has been suggested that also in MetS elevated blood pressure is the most significant factor determining arterial stiffness (Czernichow et al. 2005, Sipilä et al. 2007). However, insulin resistance has also a clear impact on arterial stiffness via many mechanisms (Stehouwer et al. 2008). Insulin has been shown to act as a vasodilator and also can decrease arterial stiffness. However, insulin resistance also affects the vasculature, and during this process the arterial tree becomes resistant to the lowering influence of insulin on arterial stiffness (Westerbacka et al. 1999, Yki-Järvinen et al. 2007).

In addition, elevated blood glucose (Aronson 2003), low-grade inflammation and endothelial dysfunction (Stehouwer et al. 2008) may also have important roles in the pathophysiology of arterial stiffening in MetS.

MetS has a clear influence on the function of the heart. A large, population-based study demonstrated a direct association between left ventricular remodeling and body mass index (BMI) (Turkbey et al. 2010). In MetS, the systolic function of the left ventricle has been reported to be impaired (Gong et al. 2009). Decreased left ventricular stroke index, i.e. stroke volume value normalized to body surface area (Koivistoinen et al. 2010), is also associated with MetS. In addition, subclinical left ventricle diastolic dysfunction is common in MetS, and insulin resistance is suggested to be an important factor in the pathophysiology of that association (Fontes-Carvalho et al. 2015). Subsequently, the risk of heart failure is clearly increased in MetS patients (Ingelsson et al. 2006, Bozkurt et al. 2016). Importantly, the risk of heart failure in MetS patients is occurring even without concurrent obesity (Voulgari et al. 2011). Besides systolic heart failure, also diastolic heart failure, i.e. heart failure with preserved ejection fraction, is common in MetS (Tang et al. 2014). Of note, when compared to healthy controls, MetS patients have been reported to have increased left ventricular mass and left ventricular wall thickness even in the absence of hypertension (Al-Daydamony & El-Tahlawi 2016).

Increased pulse pressure in both brachial (Perlini et al. 2013, Kwon et al. 2017) and aortic level (Emre et al. 2009) has been noticed to associate with

MetS. Pulse pressure typically increases with age, and this age-related increase is stronger in MetS (Safar et al. 2011). Pulse pressure amplification seems also to be higher in MetS patients (Protogerou et al. 2007, Safar et al. 2013). Increased systemic vascular resistance is also associated with MetS (Koivistoinen et al. 2010, Edgell et al. 2012).

The current concept is that MetS is associated with dysfunction of the ANS with sympathetic predominance (Grassi 2006). As stated above, upright position challenges the ANS and influences the regulation hemodynamics in many ways (Teodorovich et al. 2016). However, previous studies have not evaluated upright hemodynamics in MetS.

2.4.5 Sex-related differences in metabolic syndrome

MetS is a common disorder in both sexes. However, it seems that men and women have somewhat different features related to MetS. When it comes to a CV risk associated with MetS, many studies (both original studies and meta-analyses) have reported greater MetS-related risk in women compared to men (McNeill et al. 2005, Mottillo et al. 2010, Suh et al. 2014, Vishram et al. 2014, Li et al. 2017). On the other hand, it has been suggested that this greater MetS-related CV risk in women is mainly attributed to the existence of diabetes (Hunt et al. 2004). However, in another study, this sex-related difference in the CV risk associated with MetS, was reported to persist even in the absence of diabetes (McNeill et al. 2005). A large population-based study (the MOnica, Risk, Genetics, Archiving and Monograph - MORGAM project) revealed that the increase in the prevalence of MetS from the age group 19-39 years to 60-78 years was clearly greater in women than in men: a 5-fold increase was found in women, while in men the increase was 2-fold. Compared to men, this caused a higher prevalence of MetS in women after the age of 50 years (Vishram et al. 2014).

The background factors influencing the possible sex-related difference in the CV risk associated with MetS is poorly known. However, some mechanisms have been suggested. Elevated triglycerides, one of the five components in the criteria of MetS, have been noticed to associate with CAD more strongly in women than in men (Hokanson & Austin 1996). In addition, hormonal differences may play an important role in these sex-

related differences in CV risk. In men, MetS is associated with reduced level of testosterone (Wang et al. 2011a), but in women the situation is contrariwise as the testosterone levels are increased in MetS (Brand et al. 2011, Moulana et al. 2011). Thus, it is possible that androgens affect differently the CV system in men and women, and this divergence may have a significant influence on the different prognosis of MetS between the sexes.

With regard to the sex-related hemodynamic differences in MetS, the association of MetS with arterial stiffness has been reported to be stronger in women when compared to men (Weng et al. 2013, Kim et al. 2015, Wen et al. 2015). However, in an Italian study, the effect of MetS on central arterial function was similar in men and women (Scuteri et al. 2010). When plaques and intima-media thickness in carotid arteries were measured with ultrasound, women with MetS were found to have a higher predisposition to early atherosclerosis when compared with men (Iglseder et al. 2005).

Among patients at high risk of CAD, the impact of MetS on left ventricular diastolic dysfunction has been reported to be stronger in women compared with men (Kim et al. 2016). Among untreated, hypertensive, non-diabetic patients, MetS was associated with LVH in women, but not in men. Also left ventricular function was disturbed in women with MetS, but not in men, suggesting that MetS has greater deteriorating influence on left ventricle in women when compared with men (Schillaci et al. 2006).

2.4.6 Metabolic syndrome and autonomic nervous system

Sympathetic overdrive and imbalance in ANS have been reported to associate with MetS. Therefore, disturbances in the ANS may be an important link between MetS and CVDs (Grassi 2006). Changes in cardiac autonomic tone associated with MetS have been under active investigation during the last decades. A systematic review from 2014 reported that numerous cross-sectional studies have shown impaired HRV associated with MetS (Stuckey et al. 2014). In addition, a longitudinal study showed that low HRV increased the odds of developing MetS during 12 years of follow-up (Wulsin et al. 2016). The changes in HRV related to MetS seem to be more pronounced in women when compared with men (Koskinen et al.

2009b, Stuckey et al. 2014, Stuckey et al. 2015). Thus, the sex-related difference in autonomic tone, as deducted from studies on HRV, may partly explain the stronger association of MetS with CVDs found in women (Hunt et al. 2004, Schillaci et al. 2006).

The underlying pathogenesis for the association of MetS with impaired HRV is not clear, but some possible explanations have been suggested. Insulin resistance is an important feature of MetS, and several studies have revealed an association between insulin resistance and impaired HRV (Rodriguez-Colon et al. 2010, Hillebrand et al. 2015, Saito et al. 2015). However, a study in 220 participants, containing subjects with MetS and controls, showed that insulin resistance was associated with heart rate, but not with HRV (Stuckey et al. 2015). Inflammatory markers like IL-6 have been reported to inversely associate with HRV (Brunner et al. 2002, Haensel et al. 2008). Also plasma leptin level has been found to relate with HRV indices (Paolisso et al. 2000), and it seems that this relationship is stronger in women than in men (Flanagan et al. 2007). Both IL-6 and leptin may strongly influence the metabolic background of MetS (Kaur 2014). When the effects of the different MetS components (increased waist circumference, low HDL-C, high triglycerides, impaired fasting glucose, and elevated blood pressure) on HRV were evaluated in a large cohort of men, all of these components had strong, linear association (Hemingway et al. 2005). However, the strongest association was found between waist circumference and impaired HRV (Hemingway et al. 2005, Stuckey et al. 2015). Yet, a study in 2441 participants emphasized the role of impaired glycemic status above all other MetS components as the background for impaired HRV (Jarczok et al. 2013). Furthermore, metabolic disturbances may also cause axonal degeneration and demyelination, and such mechanisms may well be a cause for an impairment in autonomic tone (Xhyheri et al. 2012).

Although the association of MetS with impaired HRV has been shown in several studies, some discrepancy has been observed among the results (Stuckey et al. 2014). In particular, a wealth of other factors can influence HRV: age (Bonnemeier et al. 2003), sex (Koenig et al. 2016), heart rate (HR) (Billman 2013a, Sacha 2014), circadian rhythm (Bonnemeier et al. 2003), and breathing frequency (Stolarz et al. 2003, Billman 2011) all have an effect on HRV. According to a review article, these confounding factors have been often neglected in studies evaluating the association of MetS with

HRV (Stuckey et al. 2014), and this may well be the cause for the discrepancy in the published results.

3 AIMS OF THE STUDY

The aim of the present study was to comprehensively examine the hemodynamic changes associated with MetS, in both supine and upright positions. Furthermore, hemodynamic features specific for each sex were investigated.

The specific objectives were:

1. To examine supine hemodynamics in MetS, and more specifically evaluate if the MetS-related changes can be found in normotensive subjects with MetS (Study 1).

2. To investigate physiological differences in the regulation of supine and upright hemodynamics between men and women without atherosclerosis or medication with direct effect on cardiovascular function (Study II).

3. To evaluate MetS-related hemodynamic changes in both supine and upright positions, and to examine if sex-related differences in these changes can be found (Study III).

4. To investigate whether MetS is associated with impaired cardiac autonomic regulation during head-up tilt test, and whether sex has an influence on the autonomic regulation (Study IV).

4 SUBJECTS AND METHODS

4.1 Study subjects

All participants were volunteers in an ongoing study with the primary target of investigating the hemodynamic changes in hypertensive subjects and normotensive controls in both supine and upright positions (DYNAMIC study; ClinicalTrials.gov identifier NCT01742702). To enroll participants, announcements of recruitment were distributed at the University of Tampere, Tampere University Hospital, local occupational health care centers, and Varala Sports Institute. Additionally, 2 announcements were published in a local newspaper.

By the time of Study IV analyses, hemodynamic measurements were performed altogether to 1091 participants. From that population, suitable subjects were selected for the Studies I-IV using the following exclusion criteria. The most important exclusion criteria were usage of any medication with a direct influence on CV function (such as antihypertensive drugs, α_1-adrenoceptor blockers for prostate problems, β_2-adrenoceptor agonists, β-blocker eye drops for glaucoma, and digoxin). Furthermore, diagnosed atherosclerosis, diabetes, cardiac insufficiency or cerebrovascular diseases were not allowed in the subjects chosen to the Studies I-IV. Moreover, missing measurement variables or missing laboratory values, and construction of the study groups of corresponding ages, influenced the composition of the populations in the Studies I-IV.

Some medications were regularly used among the study participants. The percentage of subjects using some medication varied from 34 to 37 in Studies I-IV. The most commonly used medications were systemic female hormones, statins, antidepressants (selective serotonin reuptake Inhibitors or serotonin-norepinephrine reuptake inhibitors), thyroxin, intranasal or inhaled corticosteroids for asthma or allergy, and proton pump inhibitors. In Study II, the medications used among the participants were specifically listed, and these are represented in Table 2.

Table 2. Medications used regularly by the subjects of Study II (number of subjects with each type of medication).

	Men n = 167	Women n = 167
Acetylsalicylic acid	3	0
Acyclovir	0	1
Allopurinol	0	1
Amitriptyline	0	1
Amoxicillin	0	1
Antidepressant (SSRI or SNRI)	3	12
Antihistamine	2	5
Doxycycline (low dose)	1	0
Ezetimibe	1	0
Female hormones		
Systemic (including tibolone)	0	41
Topical	0	3
Glucosamine	3	3
Intranasal or inhaled corticosteroid	2	6
Isotretinoin	0	1
Letrozole	0	1
Levomepromazine	0	1
Levonorgestrel via intrauterine device	0	16
Liothyronine	0	1
Lymecycline	1	0
Mefloquine	0	1
Melatonin	1	1
Mepacrine	1	0
Non-steroidal anti-inflammatory drug	1	3
Oxybutynine	0	1
Pramipexole	0	1
Pregabalin	1	1
Proton pump inhibitor	3	4
Quetiapine	0	1
Statin	7	1
Thyroxine	0	10
Valproate	0	1
Vitamin D supplementation	6	11
Warfarin	1	0

MetS (in Studies I, III and IV) was defined using the criteria of Alberti et al. (Alberti et al. 2009), so that at least three of the following criteria were met: waist circumference ≥ 94 cm (men) and ≥ 80 cm (women); triglycerides ≥ 1.7 mmol/l; HDL-C < 1.0 mmol/l (men) and < 1.3 mmol/l (women); fasting plasma glucose ≥ 5.6 mmol/l; systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 85 mmHg. However, since in Study I the aim was to examine the influence of hypertension in MetS, the criterion for blood pressure was set to 140/90 mmHg. Blood pressure was measured continuously during the hemodynamic recordings using a radial tonometric device, and the average values of blood pressure readings during the last 3 minutes of the first 5-minute-period in supine position were used for the definition of MetS.

All study subjects were interviewed by a physician to record lifestyle habits, medical history, and family history. Clinical examination of the CV status and routine laboratory tests were performed.

In Study I altogether 166 subjects (89 men and 78 women) were allocated to four groups: 1) healthy controls (all five MetS components in normal level), n = 58, 2) hypertension only (normal plasma glucose, HDL-C, triglycerides, and waist circumference), n = 24, 3) MetS without hypertension, n = 27, and 4) MetS with hypertension, n = 57. The abbreviations of the groups were: control, HT (hypertensive), NT-MetS (normotensive MetS), and HT-MetS (hypertensive MetS).

In Study II 167 men and 167 women were chosen to participate in the main analysis. It has been shown that age and body weight have a clear influence on hemodynamics (Safar et al. 2006a, Tahvanainen et al. 2009b, Russo et al. 2011, Recio-Rodriguez et al. 2012), and to avoid their confounding effect on the study results, the following protocol was used in the inclusion: A male participant was chosen, followed by selection of a female subject with corresponding age (difference ≤ 3 years) and BMI (difference ≤ 1.5 units). The analyses were also performed in groups in which all subjects using statins or systemic female hormones were excluded, and in which all participants were at least 55 years old and without hormone replacement therapy. For having a wider view, additional analyses were performed to a population of 878 subjects, many of whom were with CVDs and antihypertensive drugs.

In Study III, 502 subjects were allocated to four groups: 1) men without MetS (M-control, n = 133), 2) men with MetS (M-MetS, n = 119), 3) women without MetS (W-control, n = 196), and 4) women with MetS (W-MetS, n = 54). The statistical analyses were performed separately in men and in women (MetS vs. controls).

In Study IV, the population only slightly differed from Study III. In Study IV, HRV was evaluated altogether in 501 subjects, and the groups were allocated as followes: 1) men without MetS (M-control, n = 131), 2) men with MetS (M-MetS, n = 121), 3) women without MetS (W-control, n = 191), and 4) women with MetS (W-MetS, n = 58).

4.2 Hemodynamic measurements

4.2.1 Measurement protocol

Hemodynamic measurements were performed by trained nurses in temperature controlled laboratory. The participants were instructed to refrain from usage of caffeine-containing products, eating heavy meals, and smoking for at least 4 hours, and from alcohol drinking for at least 24 hours prior to the investigation. Participants were resting supine on a tilt table, and the electrodes for ICG_{WB} were placed on the body surface, a tonometric sensor for radial artery blood pressure on the left wrist (Colin BP-508T; Colin Medical Instruments Corp), and an oscillometric brachial cuff for blood pressure calibration on the right upper arm. For ensuring that the left arm with the tonometric sensor was at the level of the heart both in supine and upright positions, the arm was supported to 90 degrees abduction.

To familiarize the participants with tilting, an introductory head-up tilt was performed before the actual measurements. The measurement protocol consisted of 3 consecutive 5-minute periods with continuous data capturing: 5 minutes supine, 5 minutes of passive head-up tilt to 60 degrees, and 5 minutes supine. The mean values of the last 3 minutes in the first supine period, and of the tilt period were used in the analyses, as the signal was the most stabile then. Previously, a good repeatability and

reproducibility of the supine and upright measurements have been shown (Tahvanainen et al. 2009a).

4.2.2 Pulse wave analysis

Blood pressure and pulse wave form were captured continuously from the radial pulsation by the tonometric sensor (Colin BP-508T; Colin Medical Instruments Corp). Radial blood pressure signal was calibrated about every 2.5 minutes by contralateral brachial blood pressure measurements. Aortic blood pressure and variables of wave reflection were derived with the SphygmoCor pulse wave monitoring system (SphycmoCor PWMx, AtCor medical, Australia) using a generalized transfer function that has previously been validated (Chen et al. 1997).

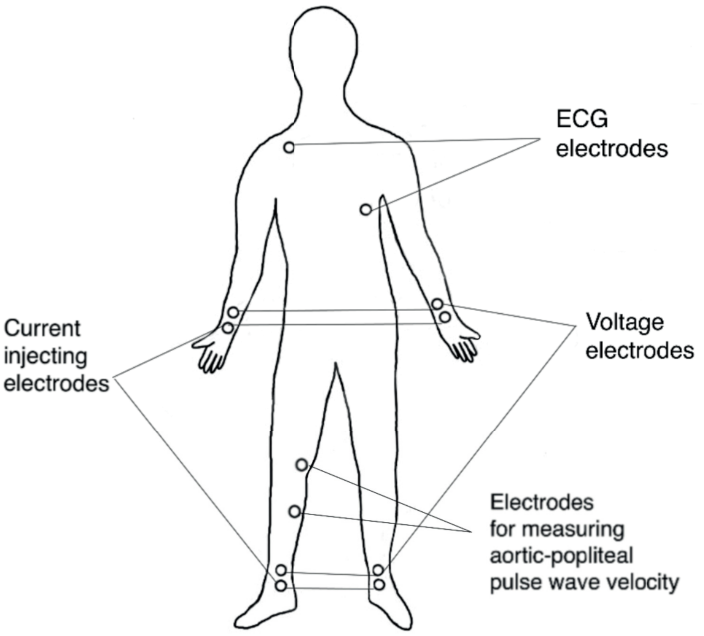
4.2.3 Whole-body impedance cardiography

A whole-body impedance cardiography (ICG_{WB}) device (CircMon^{R}; JR Medical Ltd) records changes in body electrical impedance during cardiac cycles. To determine beat-to-beat heart rate, stroke volume, and cardiac output, this device was used as described previously (Kööbi et al. 1997a, Kööbi et al. 2003, Tahvanainen et al. 2012, Tikkakoski et al. 2013). When appropriate, the values were normalized to body surface area, and expressed as stroke index, cardiac index, systemic vascular resistance index (SVRI), and left cardiac work index (LCWI). SVRI was calculated from tonometric blood pressure and cardiac index measured by the CircMon^{R} device. LCWI (in kg x m/min per m^2) was calculated as 0.0143 x (mean arterial pressure - pulmonary artery occlusion pressure) x cardiac index, where pulmonary artery occlusion pressure was assumed to be 6 mmHg (normal), and 0.0143 was the conversion factor of pressure from millimeter of mercury to centimeters of water, volume to density of blood (in kg/L), and centimeters to meters (Gorlin et al. 1955, Koskela et al. 2013). PWV

was measured between aortic and popliteal level. The placements of the electrodes are presented in the Figure 4.

The cardiac output values of CircMon^{R} device have been reported to be in a good agreement with values measured using the thermodilution method (Kööbi et al. 1997a), and stroke volume values have shown good correlation with 3-dimensional ultrasound (Koskela et al. 2013). The latter correlation has been shown in both supine and upright positions. Furthermore, the PWV values measured by CircMon^{R} device well agree with the values of the widely used tonometric method (Wilenius et al. 2016).

Figure 4. Placements of the electrodes in the ICG_{WB} measurement (modified from CircMon^{R} - manual).



4.3 Evaluating of cardiac autonomic tone

To assess cardiac autonomic tone, HRV analysis from electrocardiogram (ECG) was used. ECG was recorded by the CircMon^{R} device (sampling rate 200 Hz), and the data were analyzed using the Matlab software (MathWorks Inc., Natick, Massachusetts, USA). Normal R-R intervals were recognized, and a beat was considered ectopic if the interval differed more than 20% from the previous values. The cubic spline interpolation method was used in the artifacts processing (Peltola 2012). Since the data were collected from short-term recordings, the frequency domain method was used (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996). Using the fast Fourier transformation method, the following variables were calculated from the recordings in supine and in upright positions: (1) total power, (2) low-frequency (LF) power (0.04-0.15 Hz), (3) high-frequency (HF) power (0.15-0.40 Hz), and (4) LF/HF ratio.

4.4 Laboratory tests

Blood samples were obtained from the antecubital vein after about 12 hours of fasting. Plasma glucose, triglycerides, total cholesterol, LDL-C, HDL-C, creatinine, and cystatin-C were determined using Cobas Integra 700/800 or Cobas 6000, module c501 (Roche Diagnostics, Basel; Switzerland). In case of some participants, LDL-C was calculated using the Friedewald formula (Friedewald et al. 1972). Insulin was determined using electrochemiluminescence immunoassay (Cobas e 411, Roche Diagnostics). Estimated GFR was calculated using either RULE formula (Study I) (Rule et al. 2004) or the creatinine- and cystatin-C-based Chronic Kidney Disease Epidemiology Collaboration formula (Studies II-IV) (Inker et al. 2012). Left ventricular mass was estimated using the Cornell voltage QRS duration product in a standard 12-lead ECG, and the cut-point for LVH was set in 2440 mm x ms (Mancia et al. 2013). Insulin resistance was evaluated from fasting insulin and glucose values using Homeostasis model assessment of insulin resistance (HOMA-IR) (Matthews et al. 1985) (Study I) and

Quantitative insulin sensitivity check index (QUICKI) (Katz et al. 2000) (Studies III and IV).

4.5 Statistical analyses

All statistical analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, Ill., USA). The testing was 2-sided, and the P-values < 0.05 were considered statistically significant. In the tables, the results were reported as means and SDs for normally distributed variables, and medians and lower and upper quartiles for variables with skewed distribution, and numbers of cases and percentages with categorical variables. In the figures, mean values and 95 % confidence interval for the mean, or box plots were depicted.

To compare the differences in the characteristics between two study groups, independent samples t-test was used. The skewed distributions (i.e. of triglycerides and of HOMA-IR) were corrected using logarithmic transformation before statistical analyses, or a non-parametric method (Mann-Whitney U-test) was used in the analyses. Categorical variables were analyzed using Pearson chi-square test or the Fisher's exact test.

In Study I, hemodynamics were compared between four study groups, and the statistical analyses were performed using the one-way analysis of variance (ANOVA), and the adjusted analyses using the analysis of covariance (ANCOVA) with age, sex, height, and smoking habits as confounding factors. To investigate the associations of different components of MetS with arterial stiffness, the linear regression analysis was used. To compare the influence of SVRI and PWV on aortic pulse pressure, the scatter plots graphs were made. These graphs are presented as an additional data in this thesis, and were not included in the original article.

In the Studies II and III, the differences in the hemodynamic variables between two groups (between men and women in Study II, and between MetS and control groups in Study III) were compared using ANOVA for repeated measures, and the analyses were adjusted for possible confounding factors. Mean values of the hemodynamic variables during each minute (values from the last 3 minutes of the 5-minute recording in

both supine and upright positions) were used in the analyses. When the magnitude of the changes in hemodynamic variables in response to upright posture was evaluated, the difference between the study groups was analyzed using ANCOVA. In Study III, the MetS-related increase in PWV and aortic characteristic impedance was evaluated performing additional analyses with 108 age- and sex-matched pairs (the differences in PWV and aortic characteristic impedances between 54 male controls and male MetS-subjects were compared with the differences between 54 female controls and female MetS-subjects).

In the analyses of HRV (Studies II and IV), the skewed distribution of total power, LF power, HF power, and LF/HF ratio were logarithmically transformed before statistical analyses. The differences between two study groups (between men and women in Study II, and between MetS and control groups in Study IV) were analyzed using independent samples t-test, and the adjusted analyses were performed using ANCOVA. In the figures, the original power values (without logarithmic transformation) were shown as box plot representations.

4.6 Ethical aspects

The participants gave written informed consent, and the study was approved by the ethics committee of Tampere University Hospital, Tampere, Finland (study code R06086M). The study was performed following the principles of good clinical practice.

5 RESULTS

The results of this thesis are presented in two entities not following the order of the original publications: The first entity shows sex-related differences in hemodynamics, and the second entity concerns the hemodynamic changes associated with MetS.

5.1 Sex-related differences in hemodynamics (Study II)

Characteristics in the study population

In Study II, hemodynamic features were evaluated in 167 men and 167 women with corresponding age and BMI. Both supine and upright recordings were performed. The characteristics of the study population are represented in Table 3.

Hemodynamics in men and women

Based on the statistical analyses described in the Methods chapter, the following differences were detected between men and women.

In the supine position, men had higher mean arterial pressure, stroke index and LCWI than women, while heart rate was higher in women ($P < 0.05$ in unadjusted analyses) (Figure 5A - 5C and 5E). No differences between the sexes were found in supine cardiac index or SVRI (Figure 5D

and 5F). After adjusting for smoking habits, alcohol intake, LDL-C, HDL-C, triglycerides, glucose, height, and mean arterial pressure (as appropriate), the differences only in heart rate and stroke index between men and women remained statistically significant (Figure 5B and 5C).

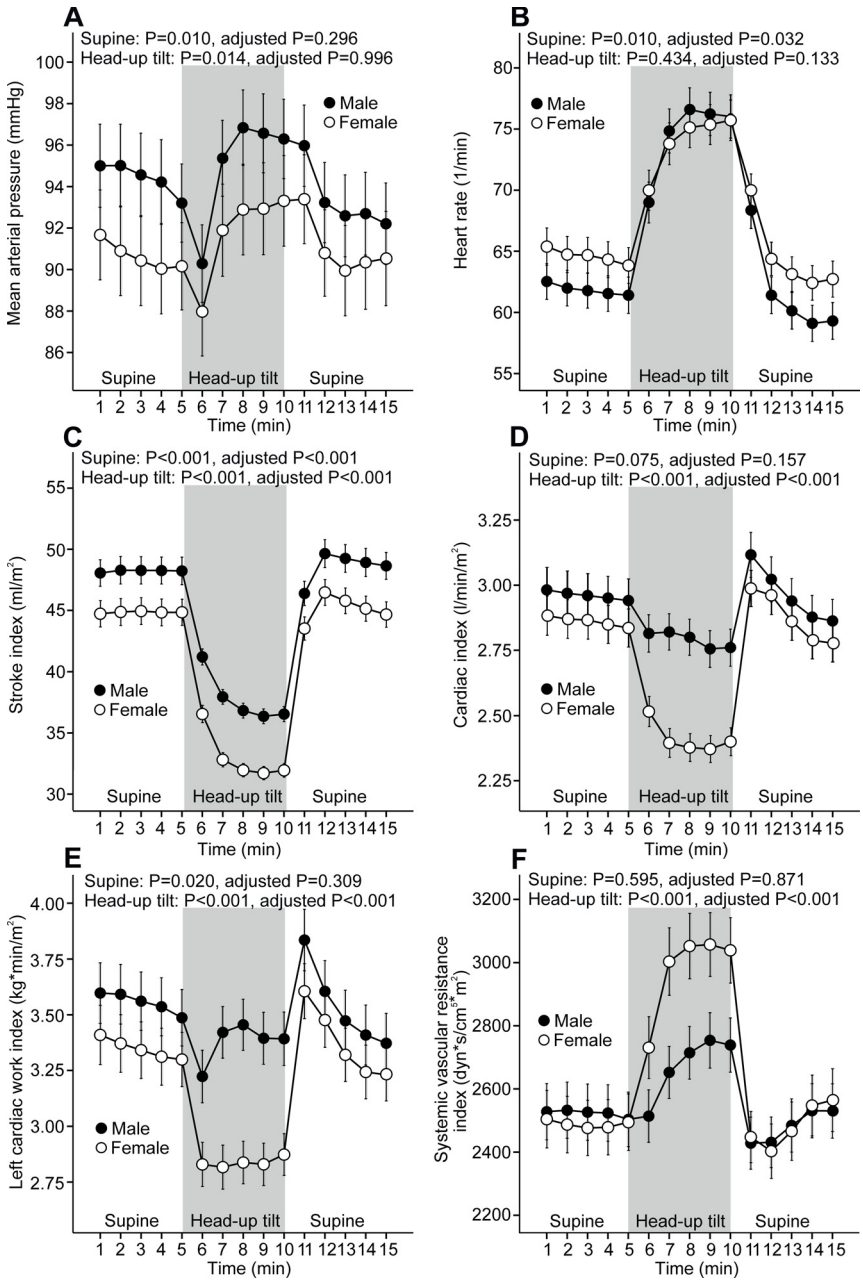
In the upright position (change of the body position during passive head-up tilt to 60°), mean arterial pressure, stroke index, cardiac index, and LCWI (Figure 5A, 5C, 5D, and 5E) were higher in men than in women ($P < 0.05$ in unadjusted analyses), while SVRI was higher in women ($P < 0.001$ in unadjusted analysis) (Figure 5F). Upright heart rate did not differ between men and women ($P = 0.434$ in unadjusted analysis) (Figure 5B). After the adjusting process, with the exception of mean arterial pressure, all of the above mentioned hemodynamic differences between the sexes remained statistically significant $P < 0.001$) (Figure 5A and 5C-5F).

Table 3. Characteristics in the groups of Study II.

	Men n = 167	Women n = 167	P-value
Age (years)	45 ± 12	45 ± 11	0.967
Body mass index (kg/m^2)	26.5 ± 3.7	26.6 ± 3.8	0.898
Height (cm)	180 ± 6	166 ± 6	<0.001
Weight (kg)	88 ± 12	73 ± 11	<0.001
Systolic blood pressure (mmHg)	136 ± 17	131 ± 20	0.006
Diastolic blood pressure (mmHg)	79 ± 13	76 ± 13	0.024
Smoking			
Current (n / %)	22 / 13.2 %	17 / 10.2 %	0.394
Previous (n / %)	51 / 30.5 %	46 / 27.5 %	0.547
Never (n / %)	94 / 56.3 %	104 / 62.3 %	0.265
Alcohol (standard drinks/week)	4 (1-10)	2 (0-4)	<0.001
Creatinine (μmol/l)	82 ± 12	65 ± 9	<0.001
Cystatin-C (mg/l)	0.87 ± 0.14	0.80 ± 0.14	<0.001
eGFR (ml/min/1.73 m^2)	99.2 ± 14.5	99.3 ± 14.0	0.956
Total cholesterol (mmol/l)	5.1 ± 1.0	5.1 ± 1.0	0.804
LDL-C (mmol/l)	3.1 ± 1.0	2.8 ± 0.9	0.021
HDL-C (mmol/l)	1.4 ± 0.3	1.8 ± 0.4	<0.001
Triglycerides (mmol/l)	1.3 (0.7-1.5)	1.1 (0.7-1.3)	0.007
Fasting plasma glucose (mmol/l)	5.5 ± 0.5	5.3 ± 0.5	<0.001
Cornell voltage product in ECG (ms x mm)	1638 ± 615	1621 ± 509	0.779

Values are means ± SD except the values for smoking, which are the numbers of cases and percentages, and the values for alcohol intake and triglycerides, which are shown as medians (lower and upper quartiles) due to skewed distribution; eGFR, estimated glomerulus filtration rate; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ECG, electrocardiogram.

Figure 5. Line graphs show mean arterial pressure (A), heart rate (B), stroke index (C), cardiac index (D), left cardiac work index (E), and systemic vascular resistance index (F) in 167 men and 167 women during supine position and passive head-up tilt; means and 95% confidence intervals of the mean; P values denote differences between the sexes in unadjusted analysis, and in analyses adjusted for low-density and high-density lipoprotein cholesterol, triglycerides, glucose, mean arterial pressure, smoking habits, alcohol intake, and height.



In addition to the original study in 334 men and women, three supplementary analyses were performed: 1) analyses in 285 subjects who did not use systemic female hormones or statins; 2) analyses in 76 elderly (≥ 55 years) subjects without systemic female hormones in use; 3) all 878 subjects who had participated in the hemodynamic recordings (no exclusions, these subjects could have had several medications or CVDs, diabetes, or renal diseases). The sex-related differences in the upright hemodynamics were similar in these additional analyses than in the original study group. The more detailed results are presented in the original publication (Kangas et al. 2016).

5.2 Hemodynamics in metabolic syndrome

5.2.1 Supine hemodynamics in metabolic syndrome with and without hypertension (Study I)

Characteristics in the study population

In Study I, supine hemodynamics were evaluated in the following groups: healthy controls, HT group, NT-MetS group, and HT-MetS group.

The differences in average age (44-46 years), plasma creatinine concentration and eGFR were not statistically significant between the four study groups ($P > 0.1$). Cornell voltage product did not differ either ($P = 0.076$). When the proportion of smokers (current or previous smoking) was examined, the percentages ranged from 25% to 52% with the highest prevalence in NT-MetS group. However, the difference in the smoking habits between the study groups was not quite statistically significant ($P = 0.05$).

Due to the allocation of the study groups, the metabolic features like BMI, waist circumference, total cholesterol, triglycerides, LDL-C, fasting glucose concentration, and HOMA-IR were all higher, and HDL-C lower in the two MetS groups when compared with the control and HT groups ($P <$

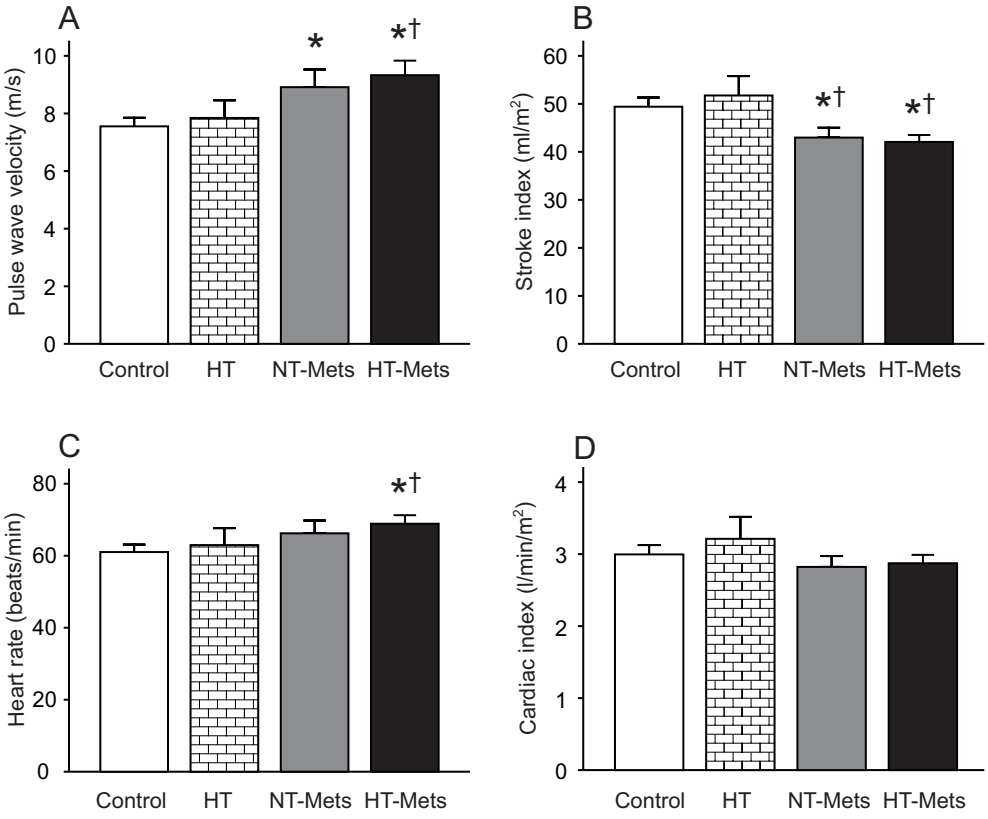
0.05 for all). Mean blood pressure was higher in the HT and HT-MetS groups than in the control and NT-MetS groups ($P < 0.05$, blood pressure values 153/87 mmHg, 151/89 mmHg, 119/69 mmHg, and 126/75 mmHg, respectively). Even though the numerical difference in systolic blood pressure between the NT-MetS and control groups was rather small, it was statistically significant ($P = 0.036$), while the difference in diastolic blood pressure was not significant.

Hemodynamics in the study groups

PWV was higher in the HT-MetS group as well as in the NT-MetS group when compared with the control group ($P < 0.05$). In a comparison between the NT-MetS and HT groups, the mean PWV was numerically higher in NT-MetS group (8.9 m/s vs. 7.8 m/s), but the difference was not statistically significant ($P = 0.073$) (Figure 6A). After adjusting for age, smoking, height and sex, the observed PWV differences still were significant. PWV did not significantly differ between control and HT groups, or between NT-MetS and HT-MetS groups.

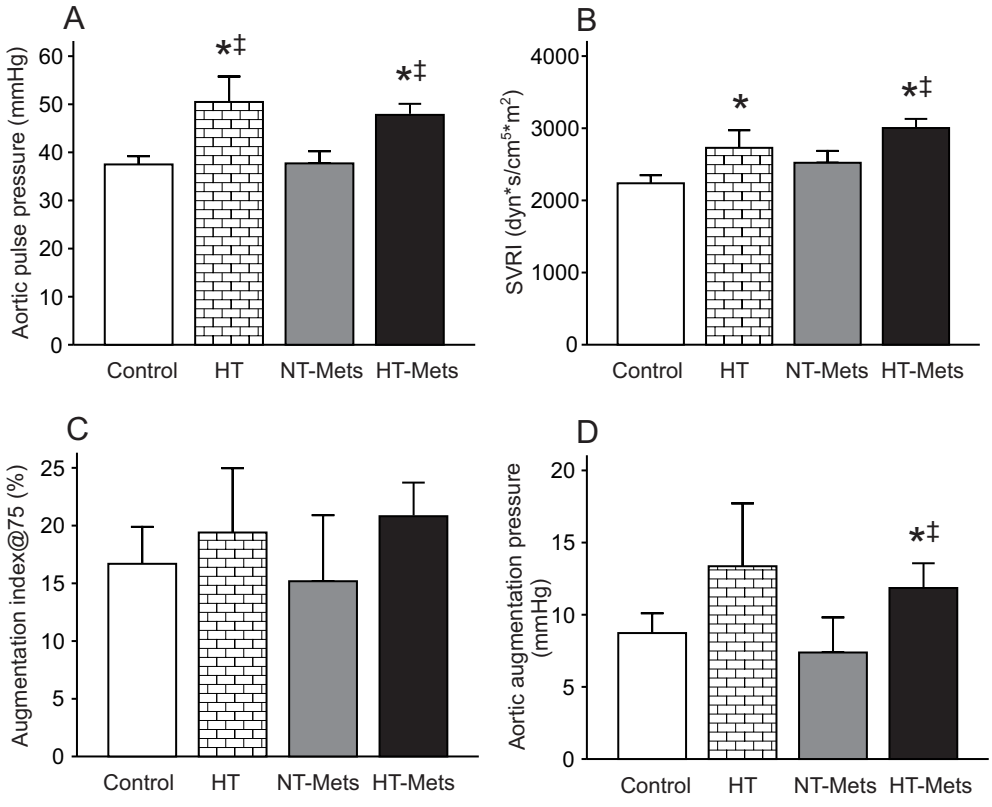
Stroke index was lower in both NT-MetS and HT-MetS groups than in the control and HT groups ($P < 0.05$)(Figure 6B), while heart rate was higher only in HT-MetS group in comparison with the control and HT groups ($P < 0.05$)(Figure 6C).

Figure 6. Pulse wave velocity (A), stroke index (B), heart rate (C), and cardiac index (D) in the study groups. HT, hypertensive subjects without any other components of MetS; NT-MetS, normotensive subjects with MetS; HT-MetS, hypertensive subjects with MetS; values as mean ± CI; *P < 0.05 vs. controls, †P < 0.05 vs. HT.



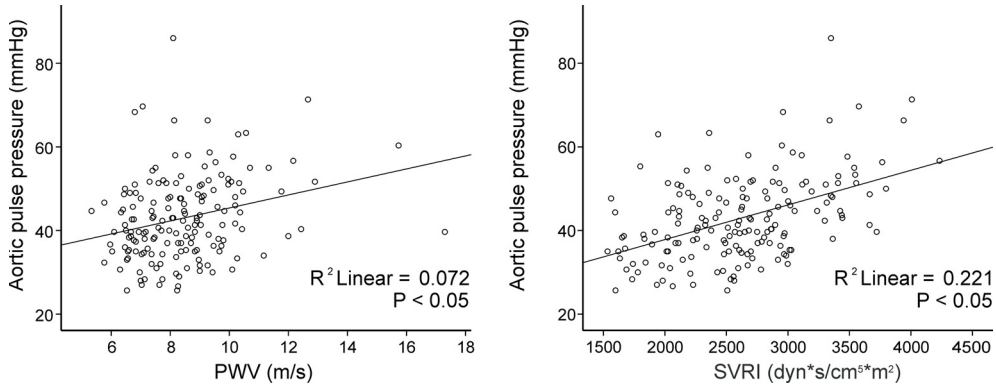
Aortic pulse pressure was higher in both hypertensive groups (HT and HT-MetS) than in the control and NT-MetS groups (P < 0.05) (Figure 7A), while SVRI was higher in the HT and HT-MetS groups when compared with the control group (P < 0.05). In addition, SVRI was also higher in the HT-MetS group than in the NT-MetS group (P < 0.05) (Figure 7B). No statistically significant differences between the study groups were found in cardiac index (Figure 6D) or in AIx@75 (Figure 7C), but augmentation pressure was higher in the HT-MetS group than in the control and NT-MetS groups (P < 0.05) (Figure 7D).

Figure 7. Aortic pulse pressure (A), systemic vascular resistance index (SVRI) (B), augmentation index related to heart rate 75/min (C), and aortic augmentation pressure (D) in the study groups. HT, hypertensive subjects without any other components of MetS; NT-MetS, normotensive subjects with MetS; HT-MetS, hypertensive subjects with MetS; values as mean ± CI; *P < 0.05 vs. controls, ‡P < 0.05 vs. NT-MetS.



Aortic pulse pressure was influenced by both arterial stiffness (evaluated using measurements of PWV) and peripheral arterial resistance (SVRI). Figure 8 shows that the correlation of SVRI with central pulse pressure was stronger than the correlation of PWV to central pulse prerssure.

Figure 8. The relations of pulse wave velocity and systemic vascular resistance index to aortic pulse pressure. This is an additional figure and was not included in the original publication.



The association of different risk factors with PWV (linear regression analysis)

To evaluate the effect of different factors on PWV, a linear regression analysis was performed (R^2 of the model = 0.461, $P < 0.001$) (Table 4). The results showed that higher age, higher triglycerides, and lower HDL-C were significantly associated with increased PWV ($P < 0.05$ for all). The P-value for the association of systolic blood pressure with PWV was statistically not quite significant ($P = 0.057$).

Table 4. Linear regression analysis of variables associated with pulse wave velocity.

			95% Confidence Interval for B		
	B	Beta	Lower Bound	Upper Bound	P - value
Age	0.075	0.432	0.052	0.098	<0.001
Sex	0.273	0.079	-0.464	1.009	0.466
Height	0.016	0.095	-0.019	0.052	0.366
Waist circumference	-0.001	-0.011	-0.024	0.021	0.913
Current or previous smoking	0.108	0.030	-0.323	0.539	0.622
Systolic blood pressure	0.019	0.205	0.000	0.038	0.057
Diastolic blood pressure	0.004	0.031	-0.024	0.032	0.770
HDL-C	-0.837	-0.240	-1.470	-0.203	0.010
Triglycerides	0.349	0.172	0.014	0.683	0.041
Fasting plasma glucose	0.324	0.101	-0.164	0.812	0.191

The enter method was used in the linear regression analysis. R squared of the model was 0.461. Male = 0, female = 1; non-smoking = 0, current or previous smoking = 1.

5.2.2 Supine and upright hemodynamics in men and women with metabolic syndrome (Study III)

Characteristics in the study population

In Study III, supine and upright hemodynamics were evaluated in 252 men and in 250 women. According to the criteria of Alberti et al. (Alberti et al. 2009), the subjects were divided to M-control, M-MetS, W-control, W-MetS groups (men and women without and with MetS).

The characteristics of the study population are presented in Table 5. In women, mean age was slightly higher in the MetS group than in the control

group (50 vs. 47 years, P = 0.035). No differences in smoking or alcohol usage were found between the MetS and control groups (P > 0.1 for all). BMI, waist circumference, systolic and diastolic blood pressure, fasting plasma glucose, total cholesterol, triglycerides, and LDL-C were all higher, while HDL-C and QUICKI were lower in the MetS groups than in the controls (P < 0.001 for all). These differences between the MetS and the control groups were found in both men and women. In women, the MetS group had higher plasma creatinine than controls (P = 0.005), but eGFR was corresponding between the groups (P = 0.216). W-MetS had higher Cornell voltage product than W-control (P = 0.007), while in men the difference between the groups was not significant (P = 0.092). Both men and women with MetS had higher PWV compared with controls (P < 0.001).

Table 5. Characteristics in the groups of Study III.

	M-control n=133	M-MetS n=119	W-control n=196	W-MetS n=54
Age (years)	48 ± 10	49 ± 9	47 ± 9	50 ± 10*
BMI (kg/m^2)	26 ± 3	30 ± 3*	25 ± 4	30 ± 5*
Waist circumference (cm)	95 ± 9	105 ± 8*	85 ± 12	96 ± 12*
Systolic blood pressure (mmHg)	129 ± 15	144 ± 16*	124 ± 18	141 ± 18*
Diastolic blood pressure (mmHg)	74 ± 10	84 ± 10*	72 ± 12	80 ± 12*
Pulse wave velocity (m/s)	8.50 ± 1.8	9.88 ± 2.0*	7.87 ± 1.4	9.21 ± 1.9*
Current smoker (N/%)	21 (16%)	14 (12%)	24 (12%)	6 (11%)
Alcohol intake (standard doses/week)	4 (1-9)	4 (1-10)	2 (0-3)	2 (0-4)
Creatinine (µmol/l)	83 ± 12	81 ± 11	66 ± 9	62 ± 9*
eGFR (ml/min/1.73 m^2)	95 ± 13	95 ± 12	95 ± 13	98 ± 13
Fasting plasma glucose (mmol/l)	5.4 ± 0.4	5.9 ± 0.4*	5.2 ± 0.4	5.8 ± 0.5*
Total cholesterol (mmol/l)	5.1 ± 0.9	5.7 ± 1.1*	5.1 ± 1.0	5.7 ± 0.8*
Triglycerides (mmol/l)	1.0 (0.7 - 1.4)	1.8 (1.1 - 2.4)*	0.9 (0.6 - 1.1)	1.5 (1.1 - 2.1)*
HDL-C (mmol/l)	1.5 ± 0.3	1.2 ± 0.3*	1.9 ± 0.4	1.5 ± 0.4*
LDL-C (mmol/l)	3.1 ± 0.9	3.7 ± 0.9*	2.8 ± 0.9	3.5 ± 0.7*
QUICKI	0.365 ± 0.045	0.342 ± 0.040*	0.373 ± 0.041	0.338 ± 0.031*
Cornell voltage product in ECG (ms x mm)	1621 ± 817	1772 ± 557	1546 ± 516	1764 ± 518*

Values are means ± SD except the values for smoking, which are the numbers of cases and percentages, and the values for triglycerides and alcohol doses per week, which are shown as medians (lower and upper quartiles) due to skewed distribution. M-control, men without MetS; M-MetS, men with MetS; W-control, women without MetS; W-MetS, women with MetS; * P<0.05 MetS vs control group; BMI, body mass index; eGFR, estimated glomerulus filtration rate; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; QUICKI, quantitative insulin sensitivity check index.

Hemodynamics during the head-up tilt test

In the supine position, both men and women with MetS had higher systolic and diastolic blood pressure (Figure 9A, 9B), heart rate (Figure 9C), SVR (Figure 9D), LCW (Figure 10B), aortic pulse pressure (Figure 10C), aortic characteristic impedance (Figure 10D), and lower SEVR (Figure 10F) than controls ($P < 0.05$ for all). The differences between the MetS and control groups in supine cardiac output and aortic reflection time were not statistically significant.

In the upright position, the MetS groups had higher systolic and diastolic blood pressure (Figure 9A, 9B), cardiac output (Figure 10A), LCW (Figure 10B), aortic pulse pressure (Figure 10C), and lower SEVR (Figure 10F) than the control groups. These differences were found in both men and women ($P < 0.05$). However, statistically significant differences between the MetS groups and control groups in upright heart rate (Figure 9C), aortic characteristic impedance (Figure 10D) and aortic reflection time (Figure 10E) were only found in women ($P < 0.05$). Upright SVR did not differ between the study groups in either men or women ($P > 0.1$)(Figure 9D). The differences between the study groups were adjusted for age.

Figure 9. Radial systolic blood pressure (A), diastolic blood pressure (B), heart rate (C), and systemic vascular resistance (SVR) in the study groups; means and 95% confidence intervals for the mean of each minute of recording; supine and upright P-values calculated using ANOVA for repeated measures, analyses adjusted for age; n=133 in men without metabolic syndrome (MetS), n=119 in men with MetS; n=196 in women without MetS, n=54 in women with MetS. The figure is from submitted, not yet published article.

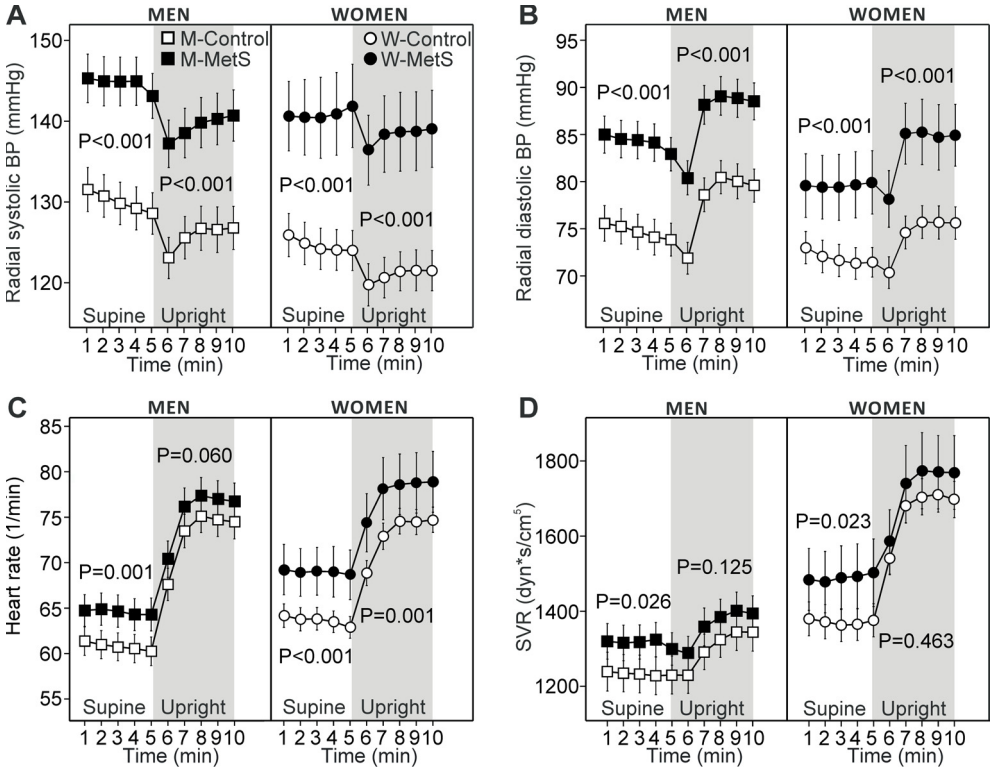
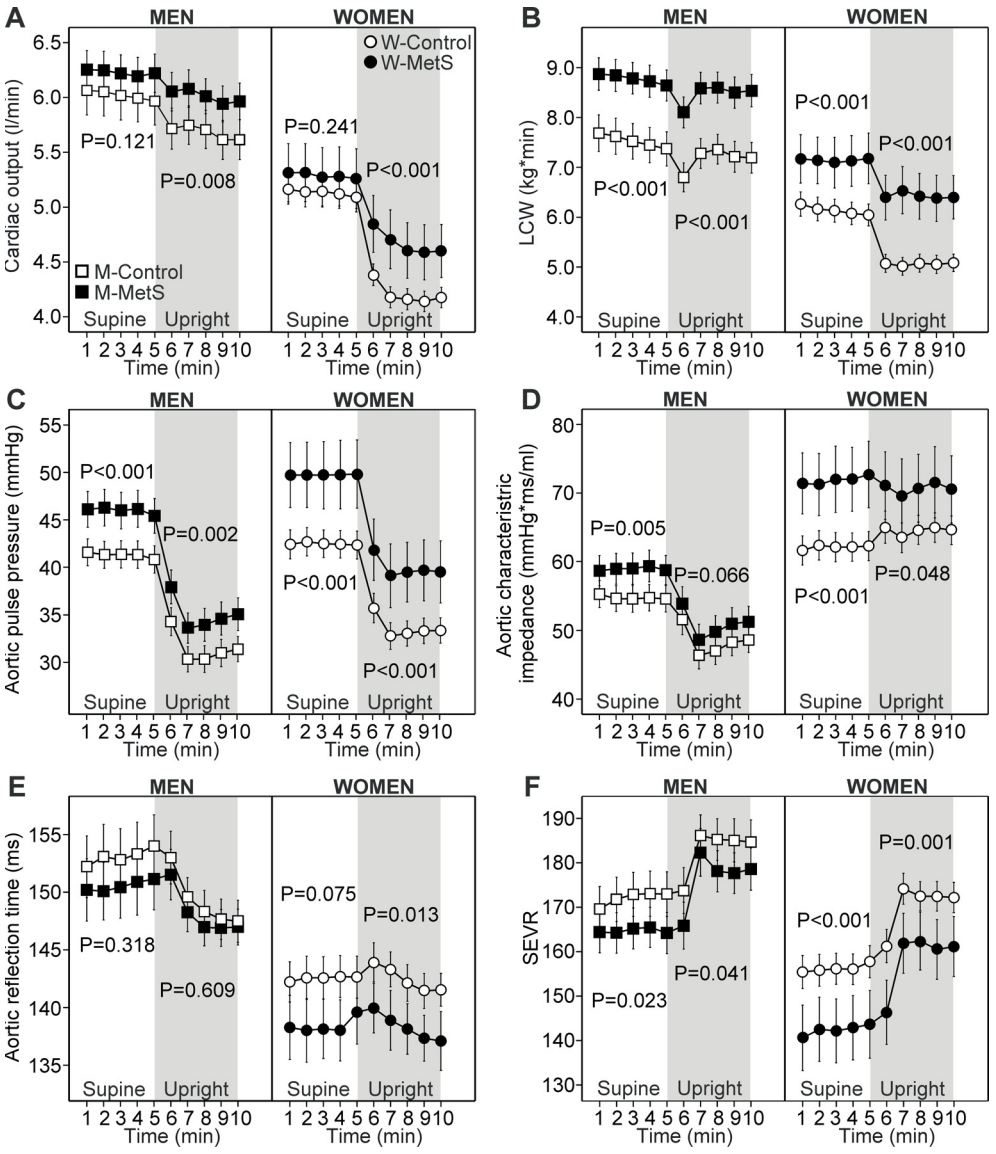
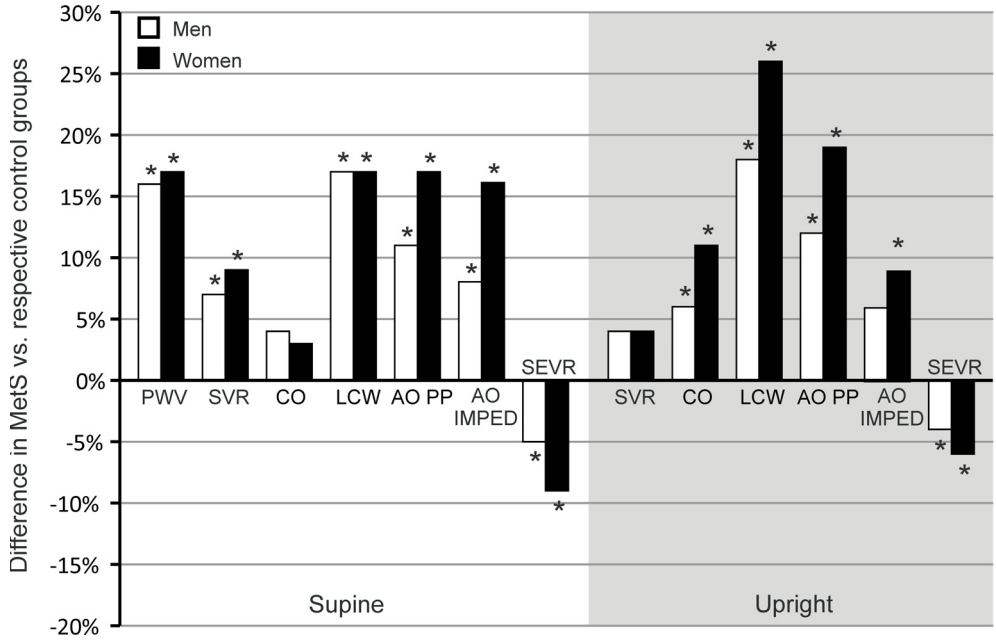


Figure 10. Cardiac output (A), left cardiac work (LCW) (B), aortic pulse pressure (C), aortic characteristic impedance (D), time to return of the reflected wave (E), and subendocardial viability ratio (SEVR) (F) in the study groups; means and 95% confidence intervals of the mean of each minute of recording; supine and upright P values calculated using ANOVA for repeated measures, analyses adjusted for age; n=132-133 in men without metabolic syndrome (MetS), n=118-119 in men with MetS; n=196 in women without MetS, n=51-54 in women with MetS. The figure is from submitted, not yet published article.



The MetS-related increases in systolic and diastolic blood pressure were quite similar in men and women, in both supine and upright positions (Table 5, Figure 9A, 9B). Also the MetS-related increases in PWV, supine SVR, and supine LCW were comparable in men and women (Table 5, Figure 9D and Figure 10B). However, several hemodynamic changes related to MetS seemed to be more pronounced in women. In a comparison with men, women had higher MetS-related increase in aortic pulse pressure (supine 17% vs. 11%, upright 19% vs. 12%), aortic characteristic impedance (supine 16% vs. 8%, upright 9% vs. 6%), upright cardiac output (11% vs. 6%), and in upright LCW (26% vs. 18%). The MetS-related decrease in subendocardial viability ratio (SEVR) was greater in women than in men (supine -9% vs. -5%, upright -6% vs. -4%) (Figure 11).

Figure 11. Bar graphs show percent differences in pulse wave velocity (PWV), systemic vascular resistance (SVR), cardiac output (CO), left cardiac work (LCW), aortic pulse pressure (AO PP), aortic characteristic impedance (AO IMPED), and subendocardial viability ratio (SEVR) in the MetS groups versus respective controls. The analyses were adjusted for age, and outcomes of MetS groups vs. controls indicated as *P<0.05. The differences in percents were calculated from the mean values. The figure is from submitted, not yet published article.



Since the dispersions for the above MetS-related changes were not possible to calculate, the statistical significances for the sex-related differences could not be analyzed. However, in order to further evaluate the sex-related differences in large artery stiffness, the MetS-related changes in PWV and aortic characteristic impedance were additionally analyzed between 54 MetS-men and MetS-women and 54 age- and sex-matched controls. This allowed the statistical comparisons for the sex-related differences. In these analyses, PWV was 1.1 ± 0.28 vs. 1.2 ± 0.29 m/s (mean ± standard error) higher in MetS subjects than in controls in men and women, respectively (P = 0.82). The MetS-related supine increase in aortic characteristic impedance was 2.8 ± 2.1 vs. 12.1 ± 2.9 mmHg x ms/ml in men and women, respectively (P = 0.011), while the upright increase did not statistically differ between the sexes (3.42 ± 2.70 vs. 6.04 ± 3.35 mmHg x ms/ml, respectively, P = 0.54).

5.2.3 Metabolic syndrome and heart rate variability (Study IV)

Characteristics in the study population

In Study IV, supine and upright HRV were evaluated in 252 men and in 249 women. According to the criteria of Alberti et al. (Alberti et al. 2009), the subjects were divided to M-control, M-MetS, W-control, W-MetS groups (men and women without and with MetS).

The differences in the mean age (ranged 47-49) or alcohol usage were not statistically significant between MetS and control groups, either in women or in men (P > 0.1 for all). In women, the proportion of previous smokers was higher in MetS group than in control group (P = 0.047), but the proportions of current smokers were similar in MetS and control groups, in both women and men (P > 0.1). Creatinine was higher in W-control group than in W-MetS group (P = 0.004), but in eGFR W-MetS and W-control groups or M-MetS and M-control groups did not differ (P > 0.1 for both). Both women and men with MetS had higher Cornell voltage product in ECG compared with controls (P < 0.05). As expected, subjects with MetS had

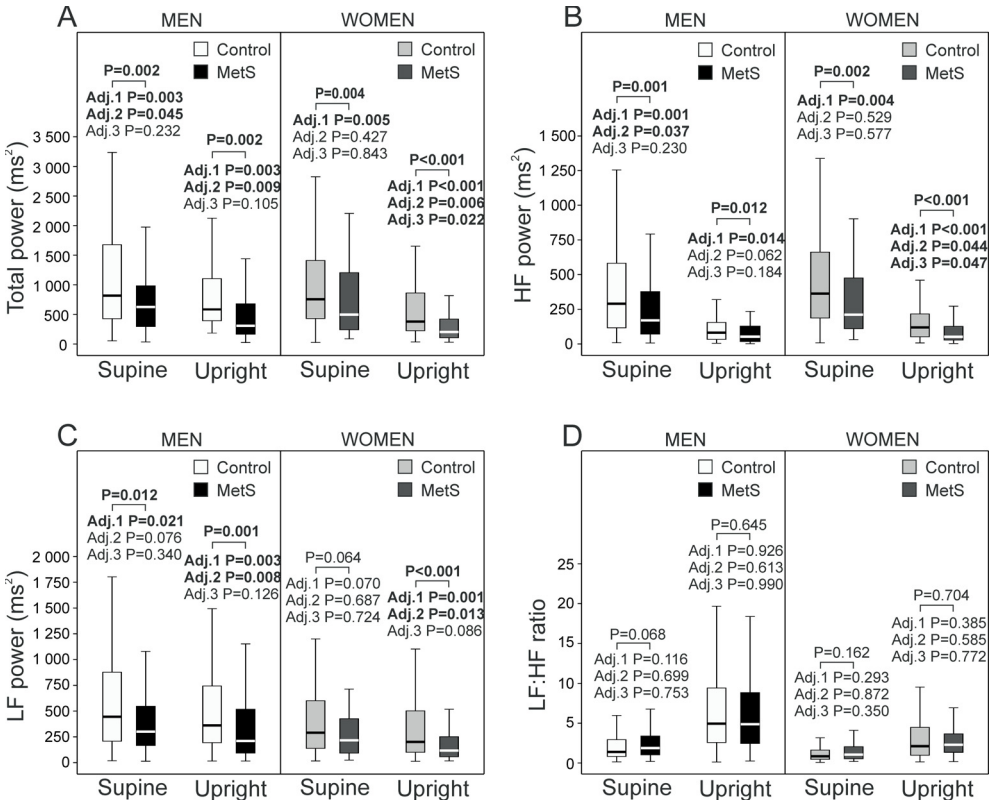
higher systolic and diastolic blood pressure, BMI, waist circumference, fasting plasma glucose, total cholesterol, triglycerides, LDL-C ($P < 0.001$ for all), and lower HDL-C and QUICKI ($P < 0.001$ for all) compared with controls.

Heart rate variability in supine and upright positions

In unadjusted analyses, supine total power (Figure 12A) and HF power (Figure 12B) were lower in the MetS groups than in the control groups ($P < 0.05$ for both analyses in women and in men). The M-MetS group had lower supine LF power (Figure 12C) than the M-control group ($P = 0.012$), while in women supine LF power did not differ statistically significantly between the study groups ($P = 0.064$). In the upright position total power (Figure 12A), HF power (Figure 12B), and LF power (figure 12C) all differed in the comparisons between the M-MetS and M-control groups and between the W-MetS and W-control groups ($P < 0.05$ for all). The LF/HF ratio (Figure 12D) did not differ between the MetS and the control groups in either supine or upright positions ($P > 0.1$ for all).

To evaluate the effect of different confounding factors on HRV results, three separate models of adjustment were created: the results were adjusted for 1) age, smoking (current smoking amount), alcohol intake, and height; 2) all of the variables in model 1 plus heart rate; 3) all of the variables in model 2 plus breathing frequency. When the results were adjusted for these confounding factors, the differences between the MetS and control groups were not as clear as in the unadjusted analyses. The detailed comparisons with exact p-values are presented in Figure 12. With the adjustments of model 3, only the differences in the upright total power (Figure 12A) and the upright HF power (Figure 12B) between the MetS and the control groups were statistically significant, and these differences were only found in women ($P < 0.05$ for both).

Figure 12. Box plots of total power (A) and high frequency (HF) power (B) of heart rate variability in the study groups (median [line inside box], 25th to 75th percentile [box], and range [whiskers]; outliers were excluded from the figure, but were included in the statistics). Supine and upright P values in unadjusted analyses and in analyses adjusted for 1) age, smoking (current smoking amount), alcohol intake, and height; 2) model 1 plus heart rate; 3) model 2 plus breathing frequency. Numbers of subjects in the groups of men without metabolic syndrome (MetS), men with MetS, women without MetS, and women with MetS, respectively: unadjusted n=131, n=121, n=191, and n=58; adjusted models 1 and 2 n=125, n=120, n=180, and n=57; adjusted model 3 n=110, n=90; n=160, and n=47-48.



6 DISCUSSION

6.1 Methodological aspects

6.1.1 Study population

The studies in this thesis were all part of a continuing clinical study on hemodynamics in the University of Tampere (DYNAMIC-study, clinical trial registration NCT01742702). Based on the exclusion criteria, the timing and the setting of the study, the population varied slightly between the four studies. In contrast to many previous hemodynamic investigations, the subjects included in the present studies were all without medicines with direct CV influences, even though many of them were never-treated hypertensive subjects. This allowed the evaluation of the primary hemodynamic changes in hypertension and MetS without the confounding effects of anti-hypertensive medications on the hemodynamic measurements.

In the DYNAMIC-study, many organizations took part in recruiting of the participants to the study. For example, some subjects were patients at Tampere University Hospital, and some were from the local occupational health care units. Furthermore, announcements were published in a local newspaper. Naturally, participating was voluntary, and we can assume that these subjects were more interested in their own health than the average population. In addition, the primary aim of the DYNAMIC-study is to evaluate the hemodynamic changes in primary and secondary hypertension and normotensive controls, and therefore in comparison with normal population, the hypertensive subjects may be overrepresented. Thus, it seems likely that the selection bias cannot be completely avoided in this study. However, none of the participants had diabetes mellitus, CAD, or any other diagnosed atherosclerotic vascular disease, cardiac insufficiency, or renal disease. Due to these exclusion criteria, confounding factors were

minimized, and the study groups were as comparable to each other as possible.

The ages of the participants varied from 19 - 72 years, thus there were a preponderance of working-age subjects (1%, 0,5%, 3%, and 3% of the study population were over the age of 65 years in Studies I, II, III, and IV, respectively). Consequently, the results should be applied to elderly subjects with caution.

Even if the participants in the main analyses of the Studies I-IV were chosen as described above (i.e. subjects did not had antihypertensive medications or diagnosed CVDs), in Study II, an additional analysis with 878 subjects was performed. In this additional analysis some subjects had diabetes, chronic renal disease, atherosclerotic vascular disease, and several medications. However, very similar sex-related hemodynamic differences in the upright position were found when compared with the main study groups consisting of 167 men and 167 women with corresponding age and BMI. Among these 878 subjects, 63 (7%) were over the age of 65 years.

6.1.2 Definition of the metabolic syndrome

As described in the chapter 2.4.1, several definitions of MetS can be found. Despite an intensive investigation of the field, a concerted decision of the best definition of MetS does not exist, and the definition varies between different studies. Furthermore, some criticism of the relevance of MetS as a clinical tool has been postulated. Simmons et al. (2010) recommended that MetS should not be used as a clinical diagnosis. Moreover, to function as a complete risk indicator of CVD, MetS has some defects, since CV risk factors like age, sex, smoking, and LDL-C are not included in the definition of the syndrome (Alberti et al. 2009). However, there is a clear consensus that the risk factor combination in MetS exhibits an important increase in the risk of CVD and diabetes (Alberti et al. 2009, Mottillo et al. 2010). Sundström et al. (2006) showed that MetS has an independent long-term value in predicting total and CV mortality, even if age, smoking, diabetes, hypertension and cholesterol were taken into account. In addition, the diagnosis of MetS is very simple to assign without the requirement for invasive or expensive

investigations. Thus, despite some deficiencies concerning the diagnosis of MetS, it can be considered as a valuable tool when CV risk is estimated. For patients, it may be clearer to having a diagnosis of MetS, rather than recognizing just increased fasting glucose level, blood pressure, waist circumference etc. Thus, the diagnosing of MetS creates an educational aspect, too.

In this thesis, MetS was defined by the criteria of Alberti et al. (Alberti et al. 2009). When compared with the widely used NCEP criteria, in this criteria clearly lower waist circumference and lower fasting blood glucose concentration are considered as abnormal. Thus, according to the criteria of Alberti et al (2009), healthier subjects are defined as individuals with MetS. It is notable that despite the above background, and regardless of the fact that all of the present subjects were without antihypertensive medications, clear hemodynamic differences between the Mets and the control groups were observed in Studies I, III and IV.

6.1.3 Hemodynamic measurements

In this thesis, the hemodynamic variables were evaluated using ICG_{WB}, tonometric blood pressure recordings, single channel ECG recordings, and continuous pulse wave analysis. The ICG_{WB} is rooted in the identification of the changes in the electrical conduction properties during cardiac cycles, and stroke volume is specified on the basis of these changes using a mathematical model (Kööbi et al. 1997a). The other hemodynamic variables like LCWI and SVR are derivatives of blood pressure and cardiac output. Some criticism has been presented against ICG. The method is not commonly used in large epidemiological studies, and ICG has been blamed to over-simplify the human physiology by a mathematical model, and especially in the case of thoracic bioimpedance measurements, to ignore the individual variety of thoracic anatomy (Geerts et al. 2011). In this thesis, ICG_{WB} was used instead of thoracic application, and the electrodes were placed on the distal parts of extremities (Figure 4). The latest ICG technologies have shown improved reproducibility and less variability when compared with the older ones (Van De Water et al. 2003). Stroke volume and cardiac output measured using CircMon ICG_{WB} have been observed to

be in good agreement with values measured using the thermodilution method (Kööbi et al. 1997a, Kööbi et al. 1997b, Kööbi et al. 1999), and also with 3-dimensional ultrasound (Koskela et al. 2013). Furthermore, good repeatability and reproducibility of the measurements during head-up tilt have been shown (Tahvanainen et al. 2009a).

When PWV is measured, the tonometric carotid-femoral PWV is considered as the gold standard, and most of the epidemiological studies have been done using that method (Laurent et al. 2006). However, a good reliability for the ICG_{WB} in measuring PWV was shown when the tonometric carotic-femoral PWV method was compared with ICG_{WB} in 80 volunteers. The correlation between these two methods was very good (r = 0.82) (Wilenius et al. 2016). Furthermore, the ICG_{WB} method has also been shown to agree well with Doppler ultrasound in assessing aortic-popliteal PWV (Kööbi et al. 2003).

The advance of the method used in this thesis is the simultaneous continuous recording of the hemodynamic variables. In an expert consensus on arterial stiffness measurements, a recommendation was given that when PWV is evaluated, an average of 10 consecutive tonometric pulse waves should be analyzed (Laurent et al. 2006). In the DYNAMIC study, hemodynamic variables were measured continuously for 3-5 minutes in each recording phase. Thus, for example during a heart rate of 60/min, 60 x 5 = 300 readings were captured during the first 5-minute supine phase recordings. The large number of the readings significantly increases the reliability of the recordings. As an expert consensus from 2012 (Van Bortel et al. 2012) recommended, the measurements of arterial stiffnes in this thesis were performed in in a quiet room with stable room temperature, and study subjects were instructed to refrain from usage of caffeine-containing products, eating heavy meals, and smoking for at least 4 hours prior to measurements, and speaking and sleeping were not allowed during measurements.

When MetS was defined in this thesis, the radial tonometric measurements were applied for the criterion of blood pressure. This differs from the conventional brachial measurements. In general, radial blood pressure measurements are considered less reliable than brachial measurements. Furthermore, for the definition of MetS, blood pressure was measured only during the hemodynamic measurements, not in home or by ambulatory blood pressure monitoring. Thus, the measurement

method for blood pressure may be considered as a limitation of the study. However, also blood pressure was captured beat-to-beat from the radial pulsation, and the large number of the readings significantly increases the reliability of the recordings. In addition, the tonometric blood pressure signal was calibrated approximately every 2.5 minutes by contralateral brachial measurements, and the stable recordings were ensured by a support, which held the wrist steady at the level of the heart in both supine and upright positions.

6.2 Major findings of the study

6.2.1 Arterial stiffness and metabolic syndrome

The association of MetS and arterial stiffness has frequently been shown in previous studies (Safar et al. 2006b, Stehouwer et al. 2008, Scuteri et al. 2014, Vilmi-Kerala et al. 2017). Some reports have suggested the elevated blood pressure as the strongest factor determining arterial stiffness in MetS (Czernichow et al. 2005, Sipilä et al. 2007). These conclusions were based on statistical multivariate regression models. In this thesis (Study I), the role of hypertension was evaluated by allocating the participants to the different groups according to their blood pressure level and MetS status (controls, HT, NT-MetS, and HT-MetS). The most important result in this study was the finding that arterial stiffness was increased in MetS even without the presence of hypertension. To our knowledge, this was the first study that compared the hemodynamic features between hypertensive and normotensive subjects with MetS, and the study provides new information about the large artery stiffening in MetS.

The background mechanisms for the association of arterial stiffness and MetS are not completely clear, but some pathophysiological pathways have been postulated. As described in the chapter 2.4.4, there is no doubt that insulin resistance has an important role in the pathophysiology of arterial stiffening (Stehouwer et al. 2008). In addition, elevated blood glucose (Aronson 2003), low HDL-C level (Wang et al. 2011b), and low-grade inflammation and endothelial dysfunction (Stehouwer et al. 2008) all have

an impact on arterial stiffness in MetS. The evidence of the independent role of central obesity in arterial stiffness is not clear. However, especially in hypertensive subjects, low adiponectin level (which is known to occur in obesity) seems to associate with increased arterial stiffness (Chen et al. 2017). Moreover, it seems clear that intra-abdominal fat has both metabolic and anatomical predisposing effects on the development of atherosclerosis (Czernichow et al. 2005).

Also in the study population of 502 subjects (Study III), MetS was shown to associate with arterial stiffness. PWV was significantly higher in the MetS groups compared with controls, suggesting increased large artery stiffness. Furthermore, aortic characteristic impedance, a variable related to the stiffening of the proximal aorta, was increased in the MetS groups. Also aortic pulse pressure was clearly higher in the MetS groups, although in addition to arterial stiffness, several other factors have an effect on pulse pressure.

The findings of Studies I and III further strengthen the previously reported significance of arterial stiffness in MetS. Importantly, it seems that large arteries are stiffened already in the early stage of MetS; i.e. under circumstances when MetS is defined by the relatively tight criteria of Alberti et al. (2009), in subjects without medication for hypertension, and even in the absence of hypertension.

6.2.2 Hemodynamic differences between men and women

It is clear that men and women differ from each other in many physiological ways. As described in chapter 2.3.1, for example the incidence and the prevalence of CVDs are different in men and in women. However, surprisingly little is known about the hemodynamic differences between the sexes, and even less in the upright position. Only a few previous studies reported a comparison of the upright hemodynamics between men and women (White et al. 1996, Barnett et al. 1999, Shoemaker et al. 2001, Jarvis et al. 2010, Smetana & Malik 2013, Ndayisaba et al. 2015). In many of these studies, the sample size was small. In addition, some incoherence of the results can be found.

In Study II of this thesis, clear sex-related differences were found in the regulation of upright hemodynamics. As it is well known, mean arterial pressure is defined by the product of cardiac output and SVR. The findings of this thesis (Study II) showed that men and women have very different ways for maintaining blood pressure in the upright position: In men, the upright position seemed to stress the heart more (men had higher cardiac index), while in women the hemodynamic balance was preserved more by peripheral arterial changes (women had higher SVRI).

The sex-related findings were clear also in Study III. (The following results are presented as an additional data in this thesis, and were not published in the article.) In a comparison of 252 men and 250 women, quite similar results were found as in Study II: In an age-adjusted analysis, upright SVRI was lower in men, while upright cardiac index was higher in men when compared with women (in Study III, non-indexed variables cardiac output and SVR were used, but the upright results were similar with these values normalized to body surface area). Furthermore, the sex-related differences in aortic pulse pressure, aortic characteristic impedance, the time to the return of the reflected wave, and AIx@75 were examined in Study III. In both supine and upright positions, time to the return of the reflected wave was higher, while aortic characteristic impedance and AIx@75 were lower in men than in women. These findings are in concordance with previous studies (Hayward & Kelly 1997, Coutinho et al. 2013). Men had also lower aortic pulse pressure in the upright position, while in this study the sex-related difference in the supine position in this variable was not statistically significant. Previously, central pulse pressure (in supine position) has been reported to be lower in men than in women (Dart et al. 2008, Shim et al. 2011, Coutinho et al. 2013). However, no sex-related difference in central pulse pressure was found in another study (Cauwenberghs et al. 2017), and it seems that the sex-related variation of central pulse pressure is strongly dependent on age: In the young ages central pulse pressure seems to be lower in females than in males, while in the elderly population the tendency is opposite (Hayward et al. 1997, Segers et al. 2007).

The underlying mechanisms of the sex-related hemodynamic differences are not fully understood, and it is clear that these mechanisms are multifaceted. However, some physiological backgrounds can be postulated. Since the body size in women is smaller than in men, also the female diameter of the aorta is smaller. In the proximal aorta, the relationship

between pressure and flow until the arrival of the pressure wave reflection is regulated by aortic characteristic impedance (Chemla et al. 2008, Phan et al. 2016). When the aorta stiffens, the characteristic impedance increases. However, in a comparison between PWV and characteristic impedance, the latter is far more dependent on the diameter of aorta lumen, and also on the remodeling process of the proximal part of the aorta (Chemla et al. 2008, Mitchell 2015). Furthermore, central pulse pressure is highly attributable to the forward pressure wave amplitude, an important part of the formula defining aortic characteristic impedance (Chemla et al. 2008, Mitchell et al. 2010b). Thus, aortic diameter can have a great influence on pulse pressure too. However, in a study in elderly hypertensive subjects, sex had an independent effect on central pulse pressure, even if aortic cross sectional area, aortic length, and aortic stiffness were taken into account (Dart et al. 2008).

Since the structural properties of the CV system can be assumed to remain quite similar in both supine and upright positions, the finding that the upright position seems to stress the heart in men and to increase SVRI in women (Study II) is not rationally explained by the different size of the vascularity and the heart in men and women. Thus, it seems that the functional hemodynamic regulation clearly differs between men and women.

Autonomic tone during the head up tilt (Study II) was evaluated by the use of the frequency domain analyses of HRV, and the LF/HF ratio was noticed to be higher in men. The LF/HF ratio is considered to be a marker of sympathovagal balance (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996, Xhyheri et al. 2012). However, the interpretation of LF/HF ratio remains controversial, thus caution should be exercised in the conclusion of this finding (Eckberg 1997, Cooke et al. 1999). In Study II, the sex-related differences in cardiac autonomic tone were mainly caused by decreased HF power, that is to say lower parasympathetic tone in men when compared with women.

An obvious difference between the sexes is hormonal function. The sex-related differences in CVD:s are well known to narrow with ageing (Jousilahti et al. 1999). Sex hormones, both endogenous and exogenous, affect the vasculature (Orshal & Khalil 2004, Wenner et al. 2012), but the effects are not straightforward. Progesterone, for instance, has vasodilatory

or vasoconstrictive influences depending on the site of the vessel and the rate of exposure (Wenner et al. 2012). Furthermore, exogenous progestins have more androgenic properties than endogenous progesterone. Also baroreflex sensitivity during oral contraceptive pill usage can be different than in the normal menstrual cycle (Wenner et al. 2012). In addition, estradiol and testosterone may have an opposite effects on the RAAS (Orshal et al. 2004). Even though it seems clear that the withdrawal of estrogen in menopause increases the age-related CV risk, chronological aging likely plays a more significant role in the increase of the incidence of CVD:s (Merz et al. 2016). In Study II, the role of female hormones in the hemodynamic findings was evaluated by performing analyses in a subgroup consisting of postmenopausal subjects not using hormonal replacement therapy. Similar sex-related differences in the upright hemodynamics were found in this subgroup, too. Consequently, the divergence in sex hormones cannot solely explain the higher upright cardiac stress found in men. It seems that hemodynamics in women may be more cardioprotective, and in this matter the female hormones could have a lower impact than what has been previously thought. However, several factors influence the incidence and prevalence of CVD:s, and it is probable that female hormones have significant effects on many of these factors (like structural properties of the vasculature (Usselman et al. 2016) and plaque accumulation (Naftolin et al. 2016)).

Another physiological distinction between the sexes is body height, and it is reasonable to presume that the difference in height could explain some of the deviations in hemodynamics between men and women. In many studies, an inverse relation between height and the risk of CVD:s has been reported (Rich-Edwards et al. 1995, Batty et al. 2009, Nelson et al. 2015, Korhonen et al. 2017, Yeboah et al. 2017). However, criticism has also been presented about the causality of this relationship. For example, social background factors could largely explain the phenomenon (Allebeck & Bergh 1992, Samaras 2013). From the hemodynamic aspect, height seems to correlate with lower heart rate, lower AIx, and longer travelling time of the reflected wave (Smulyan et al. 1998). Height is also associated with higher systolic pressure amplification from central to peripheral arteries (London et al. 1995). Based to these previous studies, higher body stature seems to associate with putatively positive, heart protective findings. In Study II, the main discovery was that the upright position stresses the heart

more in men than in women, that is to say the taller study group performed higher cardiac work in the upright posture. Thus, it is unlikely that the higher body stature in men can explain all of these study results. Furthermore, in the formulas of SVRI, LCWI and cardiac index, the variables are normalized to body surface area, and for calculating it, the body height has been taken into account.

Of note, the higher upright cardiac workload in men (Study II) was not explained by the generally known CV risk factors, since the results were adjusted for smoking, alcohol use, lipid and glucose values, and blood pressure level.

The strengths of Study II were the relatively large sample size (334), and the similarity in age and BMI in men and women. Furthermore, the study subjects were without antihypertensive medicines, a fact that improves the reliability of the results. However, the same results were found in the larger study group (n=878) that consisted of more heterogenic population, and also included subjects with CVDs and antihypertensive medications.

6.2.3 Metabolic syndrome with cardiac workload - the significance of sex

MetS is known to increase the CV risk, and the increase seems to be more pronounced in women than in men (McNeill et al. 2005, Mottillo et al. 2010, Suh et al. 2014, Vishram et al. 2014, Li et al. 2017). In this thesis (Studies III and IV) hemodynamic background to this sex-related difference was evaluated. MetS was observed to associate with many hemodynamic changes in both sexes. However, while corresponding increases in PWV, and supine and upright blood pressure were found in both men and women with MetS, several MetS-related changes were more pronounced in women. In a comparison of MetS subjects and controls, differences in aortic pulse pressure, aortic characteristic impedance, and SEVR were all numerically greater in women than in men, in both supine and upright positions. Additionally, the differences between MetS and control subjects in upright left cardiac work and upright cardiac output were numerically higher in women when compared with men. In the upright position, women with MetS had shorter time to the return of the reflected wave than controls, while in men a statistically significant difference was not found.

As described above, in Study III, comparable MetS-related increases in large artery stiffness and blood pressure were associated with hemodynamic changes potentially stressing the heart more in women than in men. Accordingly, women with MetS had significantly higher Cornell voltage product (14 % higher than controls), but in men, the difference between MetS and control groups was not statistically significant. In previous studies, MetS has been reported to associate with increased left ventricular mass and impaired cardiac function (Gong et al. 2009, Crendal et al. 2014), and this relationship seems to be stronger in women than in men (Schillaci et al. 2006, Nicolini et al. 2013). The findings of Study III are in good concordance with these previous reports, and it is probable that the hemodynamic, sex-related differences found in Study III can at least partly explain the more pronounced structural changes of the heart, and higher CV risk reported in women with MetS.

When sex-related differences are evaluated, it is natural that sex hormones are assumed to have an important role. In men, obesity and MetS are known to associate with lower testosterone levels (Wang et al. 2011a). In women, the situation is reversed, and the levels of testosterone are higher in MetS (Brand et al. 2011, Moulana et al. 2011). This MetS-associated difference between men and women is not only found in the quantity of total testosterone, but also in the levels of free testosterone (Brand et al. 2011). According to a review, no sex-related association was observed in sex hormone-binding globulin (SHBG), but in both sexes MetS was related to lower SHBG levels (Brand et al. 2011). It has been suggested that the sexes respond differently to androgens, and that insulin resistance has a different impact on androgens in men and in women (Moulana et al. 2011). The associations of sex hormones with insulin resistance also seem to be different in premenopausal versus postmenopausal women (Matsui et al. 2013). Estrogen has an important cardioprotective role in premenopausal women (Dworatzek & Mahmoodzadeh 2017), and it has been shown to attenuate the development of LVH (van Eickels et al. 2001, Donaldson et al. 2009). Schillaci et al. (2006b) suggested that insulin resistance could counterbalance the positive CV impact of estrogen in women, and withdraw some of the favorable effects of estrogens on LV mass. Altogether, the sex-specific differences in sex hormone metabolism potentially influence the hemodynamic changes in men and women with MetS.

In a large cohort study in almost 15.000 Finnish subjects coronary heart disease (CHD) was clearly more common in men than in women, and the difference in the HDL-C/total cholesterol ratio, one of the characteristic factors in MetS, was the most important determinant of the sex-related difference in CHD risk (Jousilahti et al. 1999). A large population-based study in 10 European countries found that when the different components of MetS were evaluated in men and in women, men had a higher prevalence of increased blood pressure and triglyceride levels, while in women increased waist circumference and decreased HDL-C levels were more commonly observed (Vishram et al. 2014). Additionally, another study showed that elevated body weight, waist circumference and low HDL-C level were more significant contributors to MetS in women than in men, while elevated blood pressure was more commonly found in men than in women with MetS (Dallongeville et al. 2004). Thus, men and women seem to differ in the metabolic disorders found in MetS, and these differences probably have an impact on the worse CV prognosis in women with MetS. Moreover, it seems very probable that the sex-specific hemodynamic differences in subjects with MetS, which were discovered in the present study, also play a role. Altogether, premenopausal women seem to have several features that are naturally cardioprotective. However, in women with MetS especially HDL-C level and waist circumference change towards values typically seen in men, and subsequently these women begin to resemble men in their hemodynamic features and in the incidence of CVDs.

6.2.4 Metabolic syndrome and cardiac autonomic tone

Many studies have reported impaired cardiac autonomic tone associated with higher CV risk (Tsuji et al. 1996, Schuster et al. 2016, Patel et al. 2017) and mortality (Thayer et al. 2010, Wulsin et al. 2015). In addition, previous studies have shown decreased HRV indices in MetS (Stuckey et al. 2014), and it has been suggested that disturbances in the autonomic nervous tone may be an important link between MetS and CVDs (Grassi 2006). However, the study results concerning the association of MetS with impaired HRV are not consistent. Even though it is well known that HRV indices are influenced by several confounding factors like heart rate, sex, and breathing

frequency, these factors have not been usually taken into account in the studies evaluating HRV in MetS (Stuckey et al. 2014).

In Study IV MetS was associated with lower total power, HF power, and LF power of HRV in both sexes. However, when the results were adjusted for age, alcohol intake, smoking habits, height, heart rate, and breathing frequency, only upright total power and HF power differed between the female MetS group and the control groups. Importantly, after the above adjustments, no difference was found between men with and without MetS. Also in previous studies the changes in HRV associated with MetS seemed to be more pronounced in women than in men (Koskinen et al. 2009b, Stuckey et al. 2014, Stuckey et al. 2015). The tilt test challenges the ANS (Avolio et al. 2011, Teodorovich et al. 2016), thus it is logical that the differences between the female MetS and control groups were highlighted in the upright position. To our knowledge, before Study IV only one study with small numbers of participants (33 MetS subjects and 31 controls) evaluated HRV in MetS during a tilt test (Kubickova et al. 2016). In that study, clear differences were found between the MetS and the control groups. Worth of note is that in Study IV, all participants were without antihypertensive medication. Many hypertensive drugs have an influence on HRV (Vaile et al. 1999, Karas et al. 2005, Okano et al. 2009), and in the present study these influences were eliminated by the exclusion criterion.

HRV is significantly influenced by heart rate. Higher HRV represents higher parasympathetic nervous system activity, which leads to slower heart rate (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996). Thus, there is a clear physiological association between heart rate and indices of HRV. In addition, a nonlinear relationship exists between the heart period (RR interval) and heart rate, causing a mathematical dependence between HRV and heart rate (Sacha & Pluta 2008, Sacha 2014). When evaluating HRV, it has been highly recommended to take heart rate into account (Billman 2013a, Sacha 2013, Monfredi et al. 2014). Higher heart rate is characteristic of MetS (Mancia et al. 2007a), and therefore heart rate should be taken into consideration in the evaluation of HRV especially when comparing subject with MetS and healthy controls. However, according to a review from 2014 (Stuckey et al. 2014), among the studies reporting an association between MetS and impaired HRV, only in one study the results were adjusted for heart rate (Koskinen et al. 2009b). In Study IV of this thesis, the

heart rate was taken into account in the adjusting process, and it influenced the results.

Also breathing frequency influences HRV (Stolarz et al. 2003, Billman 2011). HRV increases when respiratory frequency decreases, and HRV decreases when tidal volume decreases (Brown et al. 1993). Furthermore, mechanical factors (stretch of the atria that results from both changes in thoracic pressure and in cardiac filling) influence HRV independent of changes in cardiac autonomic nerve activity (Billman 2013b). Several studies have shown that slow, paced breathing can strengthen vagal activity and modify HRV (Howorka et al. 2013, Prinsloo et al. 2013, Kromenacker et al. 2018, Li et al. 2018). Since in Study IV we did not want to attenuate the possible impairment in the vagal activity associated with MetS, paced breathing was not applied before the HRV measurements. However, the effect of breathing on HRV indices was taken into account by adjusting the results for the prevailing breathing frequency. Information about tidal volume could not be obtained by the present techniques, and this may be regarded as a limitation of the study. Furthermore, the collection of breathing frequency data was not optimal in Study IV, because due to technical problems, this information was missing from 77 subjects of the total number of 501. In some subjects the time window of the breathing frequency data was also slightly shorter than the time window for the recordings of HRV.

In Study IV, the LF/HF ratio did not differ between the MetS and the control groups in either unadjusted or adjusted analyses. In the previous studies, the changes in the LF/HF ratio associated with MetS have varied very much (Stuckey et al. 2014). Even though the LF/HF ratio has been suggested to represent the sympathovagal balance (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996, Xhyheri et al. 2012), serious criticism has been presented against this interpretation (Eckberg 1997, Cooke et al. 1999, Billman 2013b), and therefore all conclusions from the LF/HF ratio should be drawn with caution. An important factor that influences the significance of the LF/HF ratio is the fact that the LF power is also influenced by vagal contributions (Eckberg 1997, Billman 2013b).

In comparisons between the sexes, women have been shown to have greater vagal activity as indexed by the HF power (Koenig et al. 2016). Estrogen and oxytocin have been suggested to influence this phenomenon

(Koenig et al. 2016). Since MetS is associated with changes in sex hormones (although mainly in testosterone and SHBG levels) (Kim & Halter 2014a), it is possible that these hormonal changes participate in the appearance of the decreased vagal tone in female subjects with MetS. However, further research of this field is needed.

To conclude, Study IV showed that MetS was associated with impaired HRV in both supine and upright positions, but when the confounding factors were taken into account, the differences in HRV between the MetS and the control groups diminished. Nevertheless, in the upright position, women but not men with MetS showed lower total power and HF power of HRV when compared with controls, even in the adjusted analyses. Impaired cardiac autonomic tone in women with MetS may partially explain why MetS seems to increase CV risk more in women than in men (Hunt et al. 2004, Iglseder et al. 2005, Schillaci et al. 2006).

6.3 Clinical implications and future aspects

Cardiovascular diseases (CVD) are the leading cause of death for both men and women globally (Laslett et al. 2012). However, especially before middle age, men and women clearly differ in the risk and outcome of CVD - that is to say, the morbidity and mortality caused by CVDs are higher in men (Kannel et al. 1995). Furthermore, the typical features of CVDs differ between the sexes (Garcia et al. 2016, Merz et al. 2016). Nevertheless, the mechanisms of these sex-related differences are not completely understood, and the treatment of CVDs has typically been similar for both sexes. Also in MetS and the risk of CVDs men and women have different features. Previous studies have reported that in women MetS is associated with greater relative risk of CVDs compared with men (Hunt et al. 2004, Schillaci et al. 2006).

In the current thesis, remarkable differences were found in the upright hemodynamics between men and women. Furthermore, this study showed hemodynamic changes associated with MetS that were more pronounced in women. These findings provide new information about CV regulation and

the physiological differences between men and women, and provide possible background to the mechanisms of the CV risk in MetS.

The global prevalence of hypertension has increased over the past 25 years (Forouzanfar et al. 2017), and hypertension is estimated to be the most important single risk factor decreasing individuals' healthy living years (Lim et al. 2012). The FINRISK study, performed in a large Finnish population, showed that among the medicated hypertension patients, only 30% of men and 36% of women had reached a good response to the treatment in year 2012 (Laatikainen T 2013). The results of the current thesis show differences in CV regulation between the sexes, and between subjects with and without MetS. These results emphasize the need for more individualized treatment strategies for hypertension, and this makes an important area of future research.

The results of this thesis further stressed the importance of MetS as a CV risk factor, as hemodynamics changes were observed in subjects having the diagnosis of MetS as based on the relatively tight criteria of Alberti et al.(2009). Importantly, the changes were revealed in subjects who were all without anti-hypertensive medication, and who were in the early stages of hypertension. These results highlight the importance of the prevention and treatment of MetS.

Training, particularly intensive exercises like high intensive interval training (HIIT), has been reported to quite rapidly improve insulin resistance (Sjoros et al. 2018), lower blood pressure (Ramirez-Jimenez et al. 2017), and influence indices of HRV (Boudet et al. 2017) in subjects with MetS or T2DM. Furthermore, dietary changes, especially modulation/restriction of carbohydrate intake, have been shown to improve manifestations of MetS (von Bibra et al. 2017). In the future, more investigation in the area is needed, and prospective studies about the impact of lifestyle changes on hemodynamics in MetS seem particularly interesting.

Finally, it is clear that sex matters. This is also true in the CV system, and sex should be carefully taken into account in all future research of this field.

7 SUMMARY AND CONCLUSIONS

Metabolic syndrome (MetS) is a common disorder worldwide, and it is related to an increase in the risk of CVDs and T2DM. Previous studies have revealed that in women MetS is associated with greater relative risk of CVDs when compared to men. Clear sex-related differences in the CVDs have also been shown in the general population. The present study adds the knowledge about hemodynamic changes in MetS, and provides new information ragarding the sex-related differences in CV regulation. Particularly, new knowledge is presented about the hemodynamics in the upright position.

The principal findings of the current study are as follows:

1. Increased arterial stiffness, evaluated by supine measurements of PWV, is associated with MetS. Importantly, increased arterial stiffness is present even in subjects with MetS in the absence hypertension. Since arterial stiffness is a strong predictor of CV events, the findings of this study highlight the importance of MetS as a CV risk factor.

2. Men and women differ in CV regulation. In the upright position, men have increased cardiac workload represented by higher cardiac index and LCWI when compared with women. In women, upright systemic vascular resistance is higher than in men. Of note, the above findings are not explained by the generally known CV risk factors or hormonal differences between men and women before menopause. The differences in the upright hemodynamics may play a role in the deviations of CV risk between men and women.

3. While MetS is associated with increased blood pressure and arterial stiffness in both sexes, several hemodynamic changes

associated with MetS seem to be more pronounced in women. Women have numerically higher MetS-related increase in aortic pulse pressure, aortic characteristic impedance, upright cardiac output, and upright left cardiac work. Also the MetS-related decrease in SEVR was greater in women than in men. These results show that MetS associates with hemodynamic changes that potentially burden the heart more in women than in men, and this may partially explain the higher increase in CV risk related to MetS in women.

4. MetS is associated with decreased total power, HF power, and LF power of HRV in both men and women. However, when age, smoking habits, alcohol intake, height, heart rate, and breathing frequency are taken into account, only upright total power and HF power are lower in subjects with MetS than in controls. The finding is found only in women, and this may influence the sex-dependent difference in the CV risk related to MetS.

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9 REFERENCES

Al-Daydamony MM, El-Tahlawi M (2016). What Is the Effect of Metabolic Syndrome without Hypertension on Left Ventricular Hypertrophy? Echocardiography:33, 1284-9.

Alam I, Lewis K, Stephens JW, Baxter JN (2007). Obesity, metabolic syndrome and sleep apnoea: all pro-inflammatory states. Obes Rev:8, 119-27.

Alberti KG, Zimmet P, Shaw J (2006). Metabolic syndrome--a new world-wide definition. A Consensus Statement from the International Diabetes Federation. Diabet Med:23, 469-80.

Alberti KG, Zimmet PZ (1998). Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med:15, 539-53.

Alberti KGMM, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, Fruchart JC, James WPT, Loria CM, Smith SC (2009). Harmonizing the Metabolic Syndrome: A Joint Interim Statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation:120, 1640-45.

Alfie J, Waisman GD, Galarza CR, Camera MI (1999). Contribution of stroke volume to the change in pulse pressure pattern with age. Hypertension:34, 808-12.

Allebeck P, Bergh C (1992). Height, body mass index and mortality: do social factors explain the association? Public Health:106, 375-82.

Anjum I, Sohail W, Hatipoglu B, Wilson R (2018). Postural Orthostatic Tachycardia Syndrome and Its Unusual Presenting Complaints in Women: A Literature Minireview. Cureus:10, e2435.

Aronson D (2003). Cross-linking of glycated collagen in the pathogenesis of arterial and myocardial stiffening of aging and diabetes. J Hypertens:21, 3-12.

Avolio A, Parati G (2011). Reflecting on posture. J Hypertens:29, 655-7.

Balkau B, Charles MA (1999). Comment on the provisional report from the WHO consultation. European Group for the Study of Insulin Resistance (EGIR). Diabet Med:16, 442-3.

Barantke M, Krauss T, Ortak J, Lieb W, Reppel M, Burgdorf C, Pramstaller PP, Schunkert H, Bonnemeier H (2008). Effects of gender and aging on differential autonomic responses to orthostatic maneuvers. J Cardiovasc Electrophysiol:19, 1296-303.

Barnett SR, Morin RJ, Kiely DK, Gagnon M, Azhar G, Knight EL, Nelson JC, Lipsitz LA (1999). Effects of age and gender on autonomic control of blood pressure dynamics. Hypertension:33, 1195-200.

Batty GD, Shipley MJ, Gunnell D, Huxley R, Kivimäki M, Woodward M, Lee CM, Smith GD (2009). Height, wealth, and health: an overview with new data from three longitudinal studies. Econ Hum Biol:7, 137-52.
Baudrand R, Campino C, Carvajal CA, Olivieri O, Guidi G, Faccini G, Vohringer PA, Cerda J, Owen G, Kalergis AM, Fardella CE (2014). High sodium intake is associated with increased glucocorticoid production, insulin resistance and metabolic syndrome. Clin Endocrinol (Oxf):80, 677-84.
Benetos A, Adamopoulos C, Bureau JM, Temmar M, Labat C, Bean K, Thomas F, Pannier B, Asmar R, Zureik M, Safar M, Guize L (2002). Determinants of accelerated progression of arterial stiffness in normotensive subjects and in treated hypertensive subjects over a 6-year period. Circulation:105, 1202-7.
Bhandari R, Kelley GA, Hartley TA, Rockett IR (2014). Metabolic syndrome is associated with increased breast cancer risk: a systematic review with meta-analysis. Int J Breast Cancer:2014, 189384.
Billman GE (2011). Heart rate variability - a historical perspective. Front Physiol:2, 86.
Billman GE (2013a). The effect of heart rate on the heart rate variability response to autonomic interventions. Front Physiol:4, 222.
Billman GE (2013b). The LF/HF ratio does not accurately measure cardiac sympatho-vagal balance. Front Physiol:4, 26.
Bloomgarden ZT (2003). American Association of Clinical Endocrinologists (AACE) consensus conference on the insulin resistance syndrome: 25-26 August 2002, Washington, DC. Diabetes Care:26, 1297-303.
Boden G (2011). Obesity, insulin resistance and free fatty acids. Curr Opin Endocrinol Diabetes Obes:18, 139-43.
Bogaert YE, Linas S (2009). The role of obesity in the pathogenesis of hypertension. Nat Clin Pract Nephrol:5, 101-11.
Bogle BM, Ning H, Mehrotra S, Goldberger JJ, Lloyd-Jones DM (2016). Lifetime Risk for Sudden Cardiac Death in the Community. J Am Heart Assoc:5.
Bonnemeier H, Richardt G, Potratz J, Wiegand UK, Brandes A, Kluge N, Katus HA (2003). Circadian profile of cardiac autonomic nervous modulation in healthy subjects: differing effects of aging and gender on heart rate variability. J Cardiovasc Electrophysiol:14, 791-9.
Bortolotto LA, Hanon O, Franconi G, Boutouyrie P, Legrain S, Girerd X (1999). The aging process modifies the distensibility of elastic but not muscular arteries. Hypertension:34, 889-92.
Boudet G, Walther G, Courteix D, Obert P, Lesourd B, Pereira B, Chapier R, Vinet A, Chamoux A, Naughton G, Poirier P, Dutheil F (2017). Paradoxical dissociation between heart rate and heart rate variability following different modalities of exercise in individuals with metabolic syndrome: The RESOLVE study. Eur J Prev Cardiol:24, 281-96.
Boutouyrie P, Laurent S, Benetos A, Girerd XJ, Hoeks AP, Safar ME (1992). Opposing effects of ageing on distal and proximal large arteries in hypertensives. J Hypertens Suppl:10, S87-91.
Bozkurt B, Aguilar D, Deswal A, Dunbar SB, Francis GS, Horwich T, Jessup M, Kosiborod M, Pritchett AM, Ramasubbu K, Rosendorff C, Yancy C (2016). Contributory Risk and Management of Comorbidities of Hypertension, Obesity, Diabetes Mellitus,

Hyperlipidemia, and Metabolic Syndrome in Chronic Heart Failure: A Scientific Statement From the American Heart Association. Circulation:134, e535-e78.
Brand JS, van der Tweel I, Grobbee DE, Emmelot-Vonk MH, van der Schouw YT (2011). Testosterone, sex hormone-binding globulin and the metabolic syndrome: a systematic review and meta-analysis of observational studies. Int J Epidemiol:40, 189-207.
Brown AE, Walker M (2016). Genetics of Insulin Resistance and the Metabolic Syndrome. Curr Cardiol Rep:18, 75.
Brown TE, Beightol LA, Koh J, Eckberg DL (1993). Important influence of respiration on human R-R interval power spectra is largely ignored. J Appl Physiol (1985):75, 2310-7.
Brunner EJ, Hemingway H, Walker BR, Page M, Clarke P, Juneja M, Shipley MJ, Kumari M, Andrew R, Seckl JR, Papadopoulos A, Checkley S, Rumley A, Lowe GD, Stansfeld SA, Marmot MG (2002). Adrenocortical, autonomic, and inflammatory causes of the metabolic syndrome: nested case-control study. Circulation:106, 2659-65.
Bucholz EM, Butala NM, Rathore SS, Dreyer RP, Lansky AJ, Krumholz HM (2014). Sex differences in long-term mortality after myocardial infarction: a systematic review. Circulation:130, 757-67.
Bugiardini R, Yan AT, Yan RT, Fitchett D, Langer A, Manfrini O, Goodman SG (2011). Factors influencing underutilization of evidence-based therapies in women. Eur Heart J:32, 1337-44.
Cai X, Li X, Fan W, Yu W, Wang S, Li Z, Scott EM, Li X (2016). Potassium and Obesity/Metabolic Syndrome: A Systematic Review and Meta-Analysis of the Epidemiological Evidence. Nutrients:8, 183.
Cauwenberghs N, Knez J, Boggia J, D'Hooge J, Yang WY, Wei FF, Thijs L, Staessen JA, Kuznetsova T (2017). Doppler indexes of left ventricular systolic and diastolic function in relation to haemodynamic load components in a general population. J Hypertens.
Chemla D, Plamann K, Nitenberg A (2008). Towards new indices of arterial stiffness using systolic pulse contour analysis: a theoretical point of view. J Cardiovasc Pharmacol:51, 111-7.
Chen CH, Nevo E, Fetics B, Pak PH, Yin FC, Maughan WL, Kass DA (1997). Estimation of central aortic pressure waveform by mathematical transformation of radial tonometry pressure. Validation of generalized transfer function. Circulation:95, 1827-36.
Chen MC, Lee CJ, Yang CF, Chen YC, Wang JH, Hsu BG (2017). Low serum adiponectin level is associated with metabolic syndrome and is an independent marker of peripheral arterial stiffness in hypertensive patients. Diabetol Metab Syndr:9, 49.
Cohn JN, Quyyumi AA, Hollenberg NK, Jamerson KA (2004). Surrogate markers for cardiovascular disease: functional markers. Circulation:109, Iv31-46.
Collaboration NCDRF (2017). Worldwide trends in blood pressure from 1975 to 2015: a pooled analysis of 1479 population-based measurement studies with 19.1 million participants. Lancet:389, 37-55.
Convertino VA (1998). Gender differences in autonomic functions associated with blood pressure regulation. Am J Physiol:275, R1909-20.

Cooke WH, Hoag JB, Crossman AA, Kuusela TA, Tahvanainen KU, Eckberg DL (1999). Human responses to upright tilt: a window on central autonomic integration. J Physiol:517 (Pt 2), 617-28.
Cortez-Pinto H, Camilo ME, Baptista A, De Oliveira AG, De Moura MC (1999). Non-alcoholic fatty liver: another feature of the metabolic syndrome? Clin Nutr:18, 353-8.
Coutinho T, Borlaug BA, Pellikka PA, Turner ST, Kullo IJ (2013). Sex differences in arterial stiffness and ventricular-arterial interactions. J Am Coll Cardiol:61, 96-103.
Crea F, Battipaglia I, Andreotti F (2015). Sex differences in mechanisms, presentation and management of ischaemic heart disease. Atherosclerosis:241, 157-68.
Crendal E, Walther G, Dutheil F, Courteix D, Lesourd B, Chapier R, Naughton G, Vinet A, Obert P (2014). Left ventricular myocardial dyssynchrony is already present in nondiabetic patients with metabolic syndrome. Can J Cardiol:30, 320-4.
Czernichow S, Bertrais S, Blacher J, Oppert J, Galan P, Ducimetiere P, Hercberg S, Safar M, Zureik M (2005). Metabolic Syndrome in Relation to Structure and Function of Large Arteries: A Predominant Effect of Blood PressureA Report From the SU.VI.MAX. Vascular Study. American Journal of Hypertension:18, 1154-60.
Dallongeville J, Cottel D, Arveiler D, Tauber JP, Bingham A, Wagner A, Fauvel J, Ferrieres J, Ducimetiere P, Amouyel P (2004). The association of metabolic disorders with the metabolic syndrome is different in men and women. Ann Nutr Metab:48, 43-50.
Dart AM, Kingwell BA, Gatzka CD, Willson K, Liang YL, Berry KL, Wing LM, Reid CM, Ryan P, Beilin LJ, Jennings GL, Johnston CI, McNeil JJ, MacDonald GJ, Morgan TO, West MJ, Cameron JD (2008). Smaller aortic dimensions do not fully account for the greater pulse pressure in elderly female hypertensives. Hypertension:51, 1129-34.
Davis E, Gorog DA, Rihal C, Prasad A, Srinivasan M (2017). "Mind the gap" acute coronary syndrome in women: A contemporary review of current clinical evidence. Int J Cardiol:227, 840-49.
De Long NE, Holloway AC (2017). Early-life chemical exposures and risk of metabolic syndrome. Diabetes Metab Syndr Obes:10, 101-09.
Delarue J, Magnan C (2007). Free fatty acids and insulin resistance. Curr Opin Clin Nutr Metab Care:10, 142-8.
Dernellis J, Panaretou M (2005). Aortic stiffness is an independent predictor of progression to hypertension in nonhypertensive subjects. Hypertension:45, 426-31.
Donaldson C, Eder S, Baker C, Aronovitz MJ, Weiss AD, Hall-Porter M, Wang F, Ackerman A, Karas RH, Molkentin JD, Patten RD (2009). Estrogen attenuates left ventricular and cardiomyocyte hypertrophy by an estrogen receptor-dependent pathway that increases calcineurin degradation. Circ Res:104, 265-75, 11p following 75.
Dworatzek E, Mahmoodzadeh S (2017). Targeted basic research to highlight the role of estrogen and estrogen receptors in the cardiovascular system. Pharmacol Res:119, 27-35.
Eckberg DL (1997). Sympathovagal balance: a critical appraisal. Circulation:96, 3224-32.
Edgell H, Petrella RJ, Hodges GJ, Shoemaker JK (2012). Central versus peripheral cardiovascular risk in metabolic syndrome. Front Physiol:3, 38.
Emre A, Oz D, Yesilcimen K, Sayar N, Ergun D (2009). Impact of the metabolic syndrome on aortic pulse pressure and ascending aortic pulsatility in patients with angiographically normal coronary arteries. Canadian Journal of Cardiology:25, 411-14.

Flanagan DE, Vaile JC, Petley GW, Phillips DI, Godsland IF, Owens P, Moore VM, Cockington RA, Robinson JS (2007). Gender differences in the relationship between leptin, insulin resistance and the autonomic nervous system. Regul Pept:140, 37-42.
Fontes-Carvalho R, Ladeiras-Lopes R, Bettencourt P, Leite-Moreira A, Azevedo A (2015). Diastolic dysfunction in the diabetic continuum: association with insulin resistance, metabolic syndrome and type 2 diabetes. Cardiovasc Diabetol:14, 4.
Forouzanfar MH, Liu P, Roth GA, Ng M, Biryukov S, Marczak L, Alexander L, Estep K, Hassen Abate K, Akinyemiju TF, Ali R, Alvis-Guzman N, Azzopardi P, Banerjee A, Barnighausen T, Basu A, Bekele T, Bennett DA, Biadgilign S, Catala-Lopez F, Feigin VL, Fernandes JC, Fischer F, Gebru AA, Gona P, Gupta R, Hankey GJ, Jonas JB, Judd SE, Khang YH, Khosravi A, Kim YJ, Kimokoti RW, Kokubo Y, Kolte D, Lopez A, Lotufo PA, Malekzadeh R, Melaku YA, Mensah GA, Misganaw A, Mokdad AH, Moran AE, Nawaz H, Neal B, Ngalesoni FN, Ohkubo T, Pourmalek F, Rafay A, Rai RK, Rojas-Rueda D, Sampson UK, Santos IS, Sawhney M, Schutte AE, Sepanlou SG, Shifa GT, Shiue I, Tedla BA, Thrift AG, Tonelli M, Truelsen T, Tsilimparis N, Ukwaja KN, Uthman OA, Vasankari T, Venketasubramanian N, Vlassov VV, Vos T, Westerman R, Yan LL, Yano Y, Yonemoto N, Zaki ME, Murray CJ (2017). Global Burden of Hypertension and Systolic Blood Pressure of at Least 110 to 115 mm Hg, 1990-2015. JAMA:317, 165-82.
Fox CS, Massaro JM, Hoffmann U, Pou KM, Maurovich-Horvat P, Liu CY, Vasan RS, Murabito JM, Meigs JB, Cupples LA, D'Agostino RB, Sr., O'Donnell CJ (2007). Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. Circulation:116, 39-48.
Friedewald WT, Levy RI, Fredrickson DS (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem:18, 499-502.
Fu S, Chen W, Luo L, Ye P (2017a). Roles of fasting and postprandial blood glucose in the effect of type 2 diabetes on central arterial stiffness: a 5-year prospective community-based analysis. Diabetol Metab Syndr:9, 33.
Fu S, Lin Y, Luo L, Ye P (2017b). Relationship between Central Arterial Stiffness and Insulin Resistance in Chinese Community-Dwelling Population without Diabetes Mellitus. Int J Endocrinol:2017, 1073919.
Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, Makishima M, Matsuda M, Shimomura I (2004). Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest:114, 1752-61.
Gami AS, Witt BJ, Howard DE, Erwin PJ, Gami LA, Somers VK, Montori VM (2007). Metabolic syndrome and risk of incident cardiovascular events and death: a systematic review and meta-analysis of longitudinal studies. J Am Coll Cardiol:49, 403-14.
Garcia M, Mulvagh SL, Merz CN, Buring JE, Manson JE (2016). Cardiovascular Disease in Women: Clinical Perspectives. Circ Res:118, 1273-93.
Geerts BF, Aarts LP, Jansen JR (2011). Methods in pharmacology: measurement of cardiac output. Br J Clin Pharmacol:71, 316-30.
Gehrie ER, Reynolds HR, Chen AY, Neelon BH, Roe MT, Gibler WB, Ohman EM, Newby LK, Peterson ED, Hochman JS (2009). Characterization and outcomes of women and men with non-ST-segment elevation myocardial infarction and nonobstructive

coronary artery disease: results from the Can Rapid Risk Stratification of Unstable Angina Patients Suppress Adverse Outcomes with Early Implementation of the ACC/AHA Guidelines (CRUSADE) quality improvement initiative. Am Heart J:158, 688-94.
Gill H, Mugo M, Whaley-Connell A, Stump C, Sowers JR (2005). The key role of insulin resistance in the cardiometabolic syndrome. Am J Med Sci:330, 290-4.
Gong HP, Tan HW, Fang NN, Song T, Li SH, Zhong M, Zhang W, Zhang Y (2009). Impaired left ventricular systolic and diastolic function in patients with metabolic syndrome as assessed by strain and strain rate imaging. Diabetes Res Clin Pract:83, 300-7.
Gorlin R, Mc MI, Medd WE, Matthews MB, Daley R (1955). Dynamics of the circulation in aortic valvular disease. Am J Med:18, 855-70.
Grassi G (2006). Sympathetic overdrive and cardiovascular risk in the metabolic syndrome. Hypertens Res:29, 839-47.
Grassi G, Seravalle G, Mancia G (2015). Sympathetic activation in cardiovascular disease: evidence, clinical impact and therapeutic implications. Eur J Clin Invest:45, 1367-75.
Greenwald SE (2007). Ageing of the conduit arteries. Journal of Pathology:211, 157-72.
Gupta AK, Dahlof B, Sever PS, Poulter NR (2010). Metabolic syndrome, independent of its components, is a risk factor for stroke and death but not for coronary heart disease among hypertensive patients in the ASCOT-BPLA. Diabetes Care:33, 1647-51.
Guyton AC, Hall JE (2011). Textbook of Medical Physiology.
Haensel A, Mills PJ, Nelesen RA, Ziegler MG, Dimsdale JE (2008). The relationship between heart rate variability and inflammatory markers in cardiovascular diseases. Psychoneuroendocrinology:33, 1305-12.
Hamaguchi M, Kojima T, Takeda N, Nakagawa T, Taniguchi H, Fujii K, Omatsu T, Nakajima T, Sarui H, Shimazaki M, Kato T, Okuda J, Ida K (2005). The metabolic syndrome as a predictor of nonalcoholic fatty liver disease. Ann Intern Med:143, 722-8.
Hanchaiphiboolkul S, Suwanwela NC, Poungvarin N, Nidhinandana S, Puthkhao P, Towanabut S, Tantirittisak T, Suwantamee J, Samsen M (2013). Risk of metabolic syndrome for stroke is not greater than the sum of its components: Thai Epidemiologic Stroke (TES) study. J Stroke Cerebrovasc Dis:22, e264-70.
Hansen L, Taylor WR (2016). Is increased arterial stiffness a cause or consequence of atherosclerosis? Atherosclerosis:249, 226-7.
Hansen TW, Jeppesen J, Rasmussen S, Ibsen H, Torp-Pedersen C (2004). Relation between insulin and aortic stiffness: a population-based study. J Hum Hypertens:18, 1-7.
Hayward CS, Kelly RP (1997). Gender-related differences in the central arterial pressure waveform. J Am Coll Cardiol:30, 1863-71.
Healy B (1991). The Yentl syndrome. N Engl J Med:325, 274-6.
Hemingway H, Shipley M, Brunner E, Britton A, Malik M, Marmot M (2005). Does autonomic function link social position to coronary risk? The Whitehall II study. Circulation:111, 3071-7.
Hillebrand S, Swenne CA, Gast KB, Maan AC, le Cessie S, Jukema JW, Rosendaal FR, den Heijer M, de Mutsert R (2015). The role of insulin resistance in the association between body fat and autonomic function. Nutr Metab Cardiovasc Dis:25, 93-9.

Hokanson JE, Austin MA (1996). Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta-analysis of population-based prospective studies. J Cardiovasc Risk:3, 213-9.
Howorka K, Pumprla J, Tamm J, Schabmann A, Klomfar S, Kostineak E, Howorka N, Sovova E (2013). Effects of guided breathing on blood pressure and heart rate variability in hypertensive diabetic patients. Auton Neurosci:179, 131-7.
Huikuri HV, Pikkujämsä SM, Airaksinen KE, Ikäheimo MJ, Rantala AO, Kauma H, Lilja M, Kesäniemi YA (1996). Sex-related differences in autonomic modulation of heart rate in middle-aged subjects. Circulation:94, 122-5.
Hunt KJ, Resendez RG, Williams K, Haffner SM, Stern MP (2004). National Cholesterol Education Program versus World Health Organization metabolic syndrome in relation to all-cause and cardiovascular mortality in the San Antonio Heart Study. Circulation:110, 1251-7.
Huxley R, Barzi F, Woodward M (2006). Excess risk of fatal coronary heart disease associated with diabetes in men and women: meta-analysis of 37 prospective cohort studies. Bmj:332, 73-8.
Huxley RR, Woodward M (2011). Cigarette smoking as a risk factor for coronary heart disease in women compared with men: a systematic review and meta-analysis of prospective cohort studies. Lancet:378, 1297-305.
Huxley VH (2007). Sex and the cardiovascular system: the intriguing tale of how women and men regulate cardiovascular function differently. Adv Physiol Educ:31, 17-22.
IDF (2006). The IDF consensus worldwide definition of the metabolic syndrome. http://www.idf.org/metabolic-syndrome (12/2018).
Iglseder BMD, Cip PMD, Malaimare LMD, Ladurner GMD, Paulweber BMD (2005). The Metabolic Syndrome Is a Stronger Risk Factor for Early Carotid Atherosclerosis in Women Than in Men. Stroke:36, 1212-17.
Ingelsson E, Arnlov J, Lind L, Sundstrom J (2006). Metabolic syndrome and risk for heart failure in middle-aged men. Heart:92, 1409-13.
Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, Kusek JW, Manzi J, Van Lente F, Zhang YL, Coresh J, Levey AS (2012). Estimating glomerular filtration rate from serum creatinine and cystatin C. N Engl J Med:367, 20-9.
Jarczok MN, Li J, Mauss D, Fischer JE, Thayer JF (2013). Heart rate variability is associated with glycemic status after controlling for components of the metabolic syndrome. Int J Cardiol:167, 855-61.
Jarvis SS, Florian JP, Curren MJ, Pawelczyk JA (2010). Sex differences in vasoconstrictor reserve during 70 deg head-up tilt. Exp Physiol:95, 184-93.
Jiang X, Liu X, Wu S, Zhang GQ, Peng M, Wu Y, Zheng X, Ruan C, Zhang W (2015). Metabolic syndrome is associated with and predicted by resting heart rate: a cross-sectional and longitudinal study. Heart:101, 44-9.
Johnston N, Schenck-Gustafsson K, Lagerqvist B (2011). Are we using cardiovascular medications and coronary angiography appropriately in men and women with chest pain? Eur Heart J:32, 1331-6.
Jousilahti P, Vartiainen E, Tuomilehto J, Puska P (1999). Sex, age, cardiovascular risk factors, and coronary heart disease: a prospective follow-up study of 14 786 middle-aged men and women in Finland. Circulation:99, 1165-72.

Juonala M, Järvisalo MJ, Mäki-Torkko N, Kähönen M, Viikari JS, Raitakari OT (2005). Risk factors identified in childhood and decreased carotid artery elasticity in adulthood: the Cardiovascular Risk in Young Finns Study. Circulation:112, 1486-93.
Kaess BM, Rong J, Larson MG, Hamburg NM, Vita JA, Levy D, Benjamin EJ, Vasan RS, Mitchell GF (2012). Aortic stiffness, blood pressure progression, and incident hypertension. JAMA:308, 875-81.
Kangas P, Tahvanainen A, Tikkakoski A, Koskela J, Uitto M, Viik J, Kähönen M, Kööbi T, Mustonen J, Pörsti I (2016). Increased Cardiac Workload in the Upright Posture in Men: Noninvasive Hemodynamics in Men Versus Women. J Am Heart Assoc:5, e002883.
Kannel WB, Wilson PW (1995). Risk factors that attenuate the female coronary disease advantage. Arch Intern Med:155, 57-61.
Karas M, Lacourciere Y, LeBlanc AR, Nadeau R, Dube B, Florescu M, Lamarre-Cliche M, Poirier L, Larochelle P, de Champlain J (2005). Effect of the renin-angiotensin system or calcium channel blockade on the circadian variation of heart rate variability, blood pressure and circulating catecholamines in hypertensive patients. J Hypertens:23, 1251-60.
Kashyap SR, Defronzo RA (2007). The insulin resistance syndrome: physiological considerations. Diab Vasc Dis Res:4, 13-9.
Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, Quon MJ (2000). Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab:85, 2402-10.
Kaur J (2014). A comprehensive review on metabolic syndrome. Cardiol Res Pract:2014, 943162.
Kelley RE, Dasmahapatra P, Wang J, Chen W, Srinivasan SR, Fernandez C, Xu J, Martin-Schild S, Berenson GS (2011). Prevalence of atherosclerotic plaque in young and middle-aged asymptomatic individuals: the Bogalusa heart study. South Med J:104, 803-8.
Kim C, Halter JB (2014a). Endogenous sex hormones, metabolic syndrome, and diabetes in men and women. Curr Cardiol Rep:16, 467.
Kim HL, Kim MA, Oh S, Kim M, Park SM, Yoon HJ, Shin MS, Hong KS, Shin GJ, Shim WJ (2016). Sex Difference in the Association Between Metabolic Syndrome and Left Ventricular Diastolic Dysfunction. Metab Syndr Relat Disord:14, 507-12.
Kim HL, Lee JM, Seo JB, Chung WY, Kim SH, Zo JH, Kim MA (2015). The effects of metabolic syndrome and its components on arterial stiffness in relation to gender. J Cardiol:65, 243-9.
Kim JY, Park JB, Kim DS, Kim KS, Jeong JW, Park JC, Oh BH, Chung N (2014b). Gender Difference in Arterial Stiffness in a Multicenter Cross-Sectional Study: The Korean Arterial Aging Study (KAAS). Pulse (Basel):2, 11-7.
Koenig J, Thayer JF (2016). Sex differences in healthy human heart rate variability: A meta-analysis. Neuroscience & Biobehavioral Reviews:64, 288-310.
Koivistoinen T, Aatola H, Hutri-Kähönen N, Juonala M, Viikari JS, Laitinen T, Taittonen L, Lehtimäki T, Kööbi T, Raitakari OT, Kähönen M (2010). Systemic hemodynamics in young adults with the metabolic syndrome: the Cardiovascular Risk in Young Finns Study. Ann Med:42, 612-21.

Kolovou GD, Anagnostopoulou KK, Salpea KD, Mikhailidis DP (2007). The prevalence of metabolic syndrome in various populations. Am J Med Sci:333, 362-71.
Korhonen PE, Kautiainen H, Eriksson JG (2017). The shorter the person, the higher the blood pressure: a birth cohort study. J Hypertens:35, 1170-77.
Koskela JK, Tahvanainen A, Haring A, Tikkakoski AJ, Ilveskoski E, Viitala J, Leskinen MH, Lehtimaki T, Kähönen MA, Kööbi T, Niemelä O, Mustonen JT, Pörsti IH (2013). Association of resting heart rate with cardiovascular function: a cross-sectional study in 522 Finnish subjects. BMC Cardiovasc Disord:13, 102.
Koskinen J, Kähönen M, Viikari JS, Taittonen L, Laitinen T, Ronnemaa T, Lehtimäki T, Hutri-Kähönen N, Pietikäinen M, Jokinen E, Helenius H, Mattsson N, Raitakari OT, Juonala M (2009a). Conventional cardiovascular risk factors and metabolic syndrome in predicting carotid intima-media thickness progression in young adults: the cardiovascular risk in young Finns study. Circulation:120, 229-36.
Koskinen T, Kähönen M, Jula A, Mattsson N, Laitinen T, Keltikangas-Järvinen L, Viikari J, Välimäki I, Ronnemaa T, Raitakari OT (2009b). Metabolic syndrome and short-term heart rate variability in young adults. The cardiovascular risk in young Finns study. Diabet Med:26, 354-61.
Kromenacker BW, Sanova AA, Marcus FI, Allen JJB, Lane RD (2018). Vagal Mediation of Low Frequency Heart Rate Variability During Slow Yogic Breathing. Psychosom Med.
Kubickova A, Kozumplik J, Novakova Z, Plachy M, Jurak P, Lipoldova J (2016). Heart rate variability analysed by Poincare plot in patients with metabolic syndrome. J Electrocardiol:49, 23-8.
Kwon YJ, Chung TH, Shim JY, Lee YJ (2017). The association of pulse pressure with metabolic syndrome in Korean elderly: A nationwide population-based study. Diabetes Res Clin Pract:123, 75-81.
Kylin E (1923). Studien ueber das Hypertonie-Hyperglyca “mie- Hyperurika” miesyndrom. Zentralblatt fuer Innere Medizin:44, 105–27.
Kööbi T, Kaukinen S, Turjanmaa VM (1999). Cardiac output can be reliably measured noninvasively after coronary artery bypass grafting operation. Crit Care Med:27, 2206-11.
Kööbi T, Kaukinen S, Ahola T, Turjanmaa VM (1997a). Non-invasive measurement of cardiac output: whole-body impedance cardiography in simultaneous comparison with thermodilution and direct oxygen Fick methods. Intensive Care Med:23, 1132-7.
Kööbi T, Kaukinen S, Turjanmaa VM, Uusitalo AJ (1997b). Whole-body impedance cardiography in the measurement of cardiac output. Crit Care Med:25, 779-85.
Kööbi T, Kähönen M, Iivainen T, Turjanmaa V (2003). Simultaneous non-invasive assessment of arterial stiffness and haemodynamics - a validation study. Clin Physiol Funct Imaging:23, 31-6.
Laatikainen T JA, Kastarinen M, Salomaa V, Borodulin K, Harlad K, Peltonen M, Jousilahti P, Vartiainen E (2013). Verenpainetasot ja hoitotasapaino FINRISKI-tutkimusalueilla 1982-2012. Suomen Lääkärilehti:24, 1803-09.
Laslett LJ, Alagona P, Jr., Clark BA, 3rd, Drozda JP, Jr., Saldivar F, Wilson SR, Poe C, Hart M (2012). The worldwide environment of cardiovascular disease: prevalence, diagnosis, therapy, and policy issues: a report from the American College of Cardiology. J Am Coll Cardiol:60, S1-49.

Latham RD, Westerhof N, Sipkema P, Rubal BJ, Reuderink P, Murgo JP (1985). Regional wave travel and reflections along the human aorta: a study with six simultaneous micromanometric pressures. Circulation:72, 1257-69.

Laurent S, Boutouyrie P (2007). Recent advances in arterial stiffness and wave reflection in human hypertension. Hypertension:49, 1202-6.

Laurent S, Boutouyrie P, Asmar R, Gautier I, Laloux B, Guize L, Ducimetiere P, Benetos A (2001). Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. Hypertension:37, 1236-41.

Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, Pannier B, Vlachopoulos C, Wilkinson I, Struijker-Boudier H (2006). Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur Heart J:27, 2588-605.

Laurent S, Katsahian S, Fassot C, Tropeano AI, Gautier I, Laloux B, Boutouyrie P (2003). Aortic stiffness is an independent predictor of fatal stroke in essential hypertension. Stroke:34, 1203-6.

Lee YH, Shin MH, Choi JS, Rhee JA, Nam HS, Jeong SK, Park KS, Ryu SY, Choi SW, Kim BH, Oh GJ, Kweon SS (2016). HbA1c is significantly associated with arterial stiffness but not with carotid atherosclerosis in a community-based population without type 2 diabetes: The Dong-gu study. Atherosclerosis:247, 1-6.

Li C, Chang Q, Zhang J, Chai W (2018). Effects of slow breathing rate on heart rate variability and arterial baroreflex sensitivity in essential hypertension. Medicine (Baltimore):97, e0639.

Li CH, Wu JS, Yang YC, Shih CC, Lu FH, Chang CJ (2012). Increased arterial stiffness in subjects with impaired glucose tolerance and newly diagnosed diabetes but not isolated impaired fasting glucose. J Clin Endocrinol Metab:97, E658-62.

Li X, Li X, Lin H, Fu X, Lin W, Li M, Zeng X, Gao Q (2017). Metabolic syndrome and stroke: A meta-analysis of prospective cohort studies. J Clin Neurosci:40, 34-38.

Li X, Shaffer ML, Rodriguez-Colon S, He F, Wolbrette DL, Alagona P, Jr., Wu C, Liao D (2011). The circadian pattern of cardiac autonomic modulation in a middle-aged population. Clin Auton Res:21, 143-50.

Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K, Adair-Rohani H, Amann M, Anderson HR, Andrews KG, Aryee M, Atkinson C, Bacchus LJ, Bahalim AN, Balakrishnan K, Balmes J, Barker-Collo S, Baxter A, Bell ML, Blore JD, Blyth F, Bonner C, Borges G, Bourne R, Boussinesq M, Brauer M, Brooks P, Bruce NG, Brunekreef B, Bryan-Hancock C, Bucello C, Buchbinder R, Bull F, Burnett RT, Byers TE, Calabria B, Carapetis J, Carnahan E, Chafe Z, Charlson F, Chen H, Chen JS, Cheng AT, Child JC, Cohen A, Colson KE, Cowie BC, Darby S, Darling S, Davis A, Degenhardt L, Dentener F, Des Jarlais DC, Devries K, Dherani M, Ding EL, Dorsey ER, Driscoll T, Edmond K, Ali SE, Engell RE, Erwin PJ, Fahimi S, Falder G, Farzadfar F, Ferrari A, Finucane MM, Flaxman S, Fowkes FG, Freedman G, Freeman MK, Gakidou E, Ghosh S, Giovannucci E, Gmel G, Graham K, Grainger R, Grant B, Gunnell D, Gutierrez HR, Hall W, Hoek HW, Hogan A, Hosgood HD, 3rd, Hoy D, Hu H, Hubbell BJ, Hutchings SJ, Ibeanusi SE, Jacklyn GL, Jasrasaria R, Jonas JB, Kan H, Kanis JA, Kassebaum N, Kawakami N, Khang YH, Khatibzadeh S, Khoo JP, Kok C, Laden F, Lalloo R, Lan Q, Lathlean T, Leasher JL, Leigh J, Li Y, Lin JK, Lipshultz SE, London S, Lozano R, Lu Y, Mak J, Malekzadeh R, Mallinger L, Marcenes W, March L, Marks R, Martin R,

McGale P, McGrath J, Mehta S, Mensah GA, Merriman TR, Micha R, Michaud C, Mishra V, Mohd Hanafiah K, Mokdad AA, Morawska L, Mozaffarian D, Murphy T, Naghavi M, Neal B, Nelson PK, Nolla JM, Norman R, Olives C, Omer SB, Orchard J, Osborne R, Ostro B, Page A, Pandey KD, Parry CD, Passmore E, Patra J, Pearce N, Pelizzari PM, Petzold M, Phillips MR, Pope D, Pope CA, 3rd, Powles J, Rao M, Razavi H, Rehfuess EA, Rehm JT, Ritz B, Rivara FP, Roberts T, Robinson C, Rodriguez-Portales JA, Romieu I, Room R, Rosenfeld LC, Roy A, Rushton L, Salomon JA, Sampson U, Sanchez-Riera L, Sanman E, Sapkota A, Seedat S, Shi P, Shield K, Shivakoti R, Singh GM, Sleet DA, Smith E, Smith KR, Stapelberg NJ, Steenland K, Stockl H, Stovner LJ, Straif K, Straney L, Thurston GD, Tran JH, Van Dingenen R, van Donkelaar A, Veerman JL, Vijayakumar L, Weintraub R, Weissman MM, White RA, Whiteford H, Wiersma ST, Wilkinson JD, Williams HC, Williams W, Wilson N, Woolf AD, Yip P, Zielinski JM, Lopez AD, Murray CJ, Ezzati M, AlMazroa MA, Memish ZA (2012). A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet:380, 2224-60.

Liu M, Liu F (2009). Transcriptional and post-translational regulation of adiponectin. Biochem J:425, 41-52.

Liu X, Luo X, Liu Y, Sun X, Han C, Zhang L, Wang B, Ren Y, Zhao Y, Zhang D, Hu D, Zhang M (2017). Resting heart rate and risk of metabolic syndrome in adults: a dose-response meta-analysis of observational studies. Acta Diabetol:54, 223-35.

London GM, Guerin AP, Pannier B, Marchais SJ, Stimpel M (1995). Influence of sex on arterial hemodynamics and blood pressure. Role of body height. Hypertension:26, 514-9.

Ma Y, Zhou L, Dong J, Zhang X, Yan S (2015). Arterial stiffness and increased cardiovascular risk in chronic kidney disease. Int Urol Nephrol:47, 1157-64.

Mancia G, Bombelli M, Corrao G, Facchetti R, Madotto F, Giannattasio C, Trevano FQ, Grassi G, Zanchetti A, Sega R (2007a). Metabolic syndrome in the Pressioni Arteriose Monitorate E Loro Associazioni (PAMELA) study: daily life blood pressure, cardiac damage, and prognosis. Hypertension:49, 40-7.

Mancia G, Bousquet P, Elghozi JL, Esler M, Grassi G, Julius S, Reid J, Van Zwieten PA (2007b). The sympathetic nervous system and the metabolic syndrome. J Hypertens:25, 909-20.

Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Bohm M, Christiaens T, Cifkova R, De Backer G, Dominiczak A, Galderisi M, Grobbee DE, Jaarsma T, Kirchhof P, Kjeldsen SE, Laurent S, Manolis AJ, Nilsson PM, Ruilope LM, Schmieder RE, Sirnes PA, Sleight P, Viigimaa M, Waeber B, Zannad F, Task Force M (2013). 2013 ESH/ESC Guidelines for the management of arterial hypertension: the Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). J Hypertens:31, 1281-357.

Marchesini G, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, Natale S, Vanni E, Villanova N, Melchionda N, Rizzetto M (2003). Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. Hepatology:37, 917-23.

Martinez-Selles M, Doughty RN, Poppe K, Whalley GA, Earle N, Tribouilloy C, McMurray JJ, Swedberg K, Kober L, Berry C, Squire I (2012). Gender and survival in patients with heart failure: interactions with diabetes and aetiology. Results from the MAGGIC individual patient meta-analysis. Eur J Heart Fail:14, 473-9.

Martins D, Nelson K, Pan D, Tareen N, Norris K (2001). The effect of gender on age-related blood pressure changes and the prevalence of isolated systolic hypertension among older adults: data from NHANES III. J Gend Specif Med:4, 10-3, 20.

Matsui S, Yasui T, Tani A, Kunimi K, Uemura H, Yamamoto S, Kuwahara A, Matsuzaki T, Irahara M (2013). Associations of estrogen and testosterone with insulin resistance in pre- and postmenopausal women with and without hormone therapy. Int J Endocrinol Metab:11, 65-70.

Matsuzawa Y, Funahashi T, Kihara S, Shimomura I (2004). Adiponectin and metabolic syndrome. Arterioscler Thromb Vasc Biol:24, 29-33.

Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC (1985). Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia:28, 412-9.

McEniery CM, Yasmin, Hall IR, Qasem A, Wilkinson IB, Cockcroft JR (2005). Normal vascular aging: differential effects on wave reflection and aortic pulse wave velocity: the Anglo-Cardiff Collaborative Trial (ACCT). J Am Coll Cardiol:46, 1753-60.

McKeown NM, Meigs JB, Liu S, Saltzman E, Wilson PW, Jacques PF (2004). Carbohydrate nutrition, insulin resistance, and the prevalence of the metabolic syndrome in the Framingham Offspring Cohort. Diabetes Care:27, 538-46.

McNeill AM, Rosamond WD, Girman CJ, Golden SH, Schmidt MI, East HE, Ballantyne CM, Heiss G (2005). The metabolic syndrome and 11-year risk of incident cardiovascular disease in the atherosclerosis risk in communities study. Diabetes Care:28, 385-90.

Merz AA, Cheng S (2016). Sex differences in cardiovascular ageing. Heart.

Mitchell GF (2014). Arterial stiffness and hypertension: chicken or egg? Hypertension:64, 210-4.

Mitchell GF (2015). Arterial stiffness: insights from Framingham and Iceland. Curr Opin Nephrol Hypertens:24, 1-7.

Mitchell GF, Hwang SJ, Vasan RS, Larson MG, Pencina MJ, Hamburg NM, Vita JA, Levy D, Benjamin EJ (2010a). Arterial stiffness and cardiovascular events: the Framingham Heart Study. Circulation:121, 505-11.

Mitchell GF, Parise H, Benjamin EJ, Larson MG, Keyes MJ, Vita JA, Vasan RS, Levy D (2004). Changes in arterial stiffness and wave reflection with advancing age in healthy men and women: the Framingham Heart Study. Hypertension:43, 1239-45.

Mitchell GF, Wang N, Palmisano JN, Larson MG, Hamburg NM, Vita JA, Levy D, Benjamin EJ, Vasan RS (2010b). Hemodynamic correlates of blood pressure across the adult age spectrum: noninvasive evaluation in the Framingham Heart Study. Circulation:122, 1379-86.

Monfredi O, Lyashkov AE, Johnsen AB, Inada S, Schneider H, Wang R, Nirmalan M, Wisloff U, Maltsev VA, Lakatta EG, Zhang H, Boyett MR (2014). Biophysical Characterization of the Underappreciated and Important Relationship Between Heart Rate Variability and Heart Rate. Hypertension.

Mottillo S, Filion KB, Genest J, Joseph L, Pilote L, Poirier P, Rinfret S, Schiffrin EL, Eisenberg MJ (2010). The metabolic syndrome and cardiovascular risk a systematic review and meta-analysis. J Am Coll Cardiol:56, 1113-32.

Moulana M, Lima R, Reckelhoff J (2011). Metabolic Syndrome, Androgens, and Hypertension. Curr Hypertens Rep:13, 158-62.

Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, Das SR, de Ferranti S, Despres JP, Fullerton HJ, Howard VJ, Huffman MD, Isasi CR, Jimenez MC, Judd SE, Kissela BM, Lichtman JH, Lisabeth LD, Liu S, Mackey RH, Magid DJ, McGuire DK, Mohler ER, 3rd, Moy CS, Muntner P, Mussolino ME, Nasir K, Neumar RW, Nichol G, Palaniappan L, Pandey DK, Reeves MJ, Rodriguez CJ, Rosamond W, Sorlie PD, Stein J, Towfighi A, Turan TN, Virani SS, Woo D, Yeh RW, Turner MB (2016). Heart Disease and Stroke Statistics-2016 Update: A Report From the American Heart Association. Circulation:133, e38-360.

Mäkikallio TH, Huikuri HV, Mäkikallio A, Sourander LB, Mitrani RD, Castellanos A, Myerburg RJ (2001). Prediction of sudden cardiac death by fractal analysis of heart rate variability in elderly subjects. J Am Coll Cardiol:37, 1395-402.

Naftolin F, Mehr H, Fadiel A (2016). Sex Steroids Block the Initiation of Atherosclerosis. Reprod Sci:23, 1620-25.

NCEP (2001). Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA:285, 2486-97.

Ndayisaba JP, Fanciulli A, Granata R, Duerr S, Hintringer F, Goebel G, Krismer F, Wenning GK (2015). Sex and age effects on cardiovascular autonomic function in healthy adults. Clin Auton Res:25, 317-26.

Nelson CP, Hamby SE, Saleheen D, Hopewell JC, Zeng L, Assimes TL, Kanoni S, Willenborg C, Burgess S, Amouyel P, Anand S, Blankenberg S, Boehm BO, Clarke RJ, Collins R, Dedoussis G, Farrall M, Franks PW, Groop L, Hall AS, Hamsten A, Hengstenberg C, Hovingh GK, Ingelsson E, Kathiresan S, Kee F, Konig IR, Kooner J, Lehtimaki T, Marz W, McPherson R, Metspalu A, Nieminen MS, O'Donnell CJ, Palmer CN, Peters A, Perola M, Reilly MP, Ripatti S, Roberts R, Salomaa V, Shah SH, Schreiber S, Siegbahn A, Thorsteinsdottir U, Veronesi G, Wareham N, Willer CJ, Zalloua PA, Erdmann J, Deloukas P, Watkins H, Schunkert H, Danesh J, Thompson JR, Samani NJ, Consortium CACD (2015). Genetically determined height and coronary artery disease. N Engl J Med:372, 1608-18.

Nguyen QM, Srinivasan SR, Xu JH, Chen W, Berenson GS (2008). Racial (black-white) divergence in the association between adiponectin and arterial stiffness in asymptomatic young adults: the Bogalusa heart study. Am J Hypertens:21, 553-7.

Nicolini E, Martegani G, Maresca AM, Marchesi C, Dentali F, Lazzarini A, Speroni S, Guasti L, Bertolini A, Venco A, Grandi AM (2013). Left ventricular remodeling in patients with metabolic syndrome: influence of gender. Nutr Metab Cardiovasc Dis:23, 771-5.

Nupponen M, Pahkala K, Juonala M, Magnussen CG, Niinikoski H, Ronnemaa T, Viikari JS, Saarinen M, Lagstrom H, Jula A, Simell O, Raitakari OT (2015). Metabolic syndrome from adolescence to early adulthood: effect of infancy-onset dietary counseling of

low saturated fat: the Special Turku Coronary Risk Factor Intervention Project (STRIP). Circulation:131, 605-13.
O'Rourke MF, Hashimoto J (2007). Mechanical factors in arterial aging: a clinical perspective. J Am Coll Cardiol:50, 1-13.
O'Rourke MF, Safar ME (2005). Relationship between aortic stiffening and microvascular disease in brain and kidney: cause and logic of therapy. Hypertension:46, 200-4.
Okano Y, Tamura K, Masuda S, Ozawa M, Tochikubo O, Umemura S (2009). Effects of angiotensin II receptor blockers on the relationships between ambulatory blood pressure and anti-hypertensive effects, autonomic function, and health-related quality of life. Clin Exp Hypertens:31, 680-9.
Organization WH (1999). Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: Diagnosis and classification of diabetes mellitus. WHO, Geneva.
Orshal JM, Khalil RA (2004). Gender, sex hormones, and vascular tone. Am J Physiol Regul Integr Comp Physiol:286, R233-49.
Owen JG, Reisin E (2015). Anti-hypertensive drug treatment of patients with and the metabolic syndrome and obesity: a review of evidence, meta-analysis, post hoc and guidelines publications. Curr Hypertens Rep:17, 558.
Paniagua JA (2016). Nutrition, insulin resistance and dysfunctional adipose tissue determine the different components of metabolic syndrome. World J Diabetes:7, 483-514.
Paolisso G, Manzella D, Montano N, Gambardella A, Varricchio M (2000). Plasma leptin concentrations and cardiac autonomic nervous system in healthy subjects with different body weights. J Clin Endocrinol Metab:85, 1810-4.
Parashar S, Katz R, Smith NL, Arnold AM, Vaccarino V, Wenger NK, Gottdiener JS (2009). Race, gender, and mortality in adults > or =65 years of age with incident heart failure (from the Cardiovascular Health Study). Am J Cardiol:103, 1120-7.
Patel VN, Pierce BR, Bodapati RK, Brown DL, Ives DG, Stein PK (2017). Association of Holter-Derived Heart Rate Variability Parameters With the Development of Congestive Heart Failure in the Cardiovascular Health Study. JACC Heart Fail:5, 423-31.
Peltola MA (2012). Role of editing of R-R intervals in the analysis of heart rate variability. Front Physiol:3, 148.
Perlini S, Naditch-Brule L, Farsang C, Zidek W, Kjeldsen SE (2013). Pulse pressure and heart rate in patients with metabolic syndrome across Europe: insights from the GOOD survey. J Hum Hypertens:27, 412-6.
Phan TS, Li JK, Segers P, Chirinos JA (2016). Misinterpretation of the Determinants of Elevated Forward Wave Amplitude Inflates the Role of the Proximal Aorta. J Am Heart Assoc:5.
Piro M, Della Bona R, Abbate A, Biasucci LM, Crea F (2010). Sex-related differences in myocardial remodeling. J Am Coll Cardiol:55, 1057-65.
Pothineni NV, Shirazi LF, Mehta JL (2016). Gender Differences in Autonomic Control of the Cardiovascular System. Curr Pharm Des:22, 3829-34.
Prenner SB, Chirinos JA (2015). Arterial stiffness in diabetes mellitus. Atherosclerosis:238, 370-9.
Prinsloo GE, Derman WE, Lambert MI, Laurie Rauch HG (2013). The effect of a single session of short duration biofeedback-induced deep breathing on measures of

heart rate variability during laboratory-induced cognitive stress: a pilot study. Appl Psychophysiol Biofeedback:38, 81-90.
Protogerou AD, Blacher J, Mavrikakis M, Lekakis J, Safar ME (2007). Increased pulse pressure amplification in treated hypertensive subjects with metabolic syndrome. Am J Hypertens:20, 127-33.
Pyykkönen AJ, Räikkonen K, Tuomi T, Eriksson JG, Groop L, Isomaa B (2010). Stressful life events and the metabolic syndrome: the prevalence, prediction and prevention of diabetes (PPP)-Botnia Study. Diabetes Care:33, 378-84.
Qian Y, Xu H, Wang Y, Yi H, Guan J, Yin S (2016). Obstructive sleep apnea predicts risk of metabolic syndrome independently of obesity: a meta-analysis. Arch Med Sci:12, 1077-87.
Rabbia F, Grosso T, Cat Genova G, Conterno A, De Vito B, Mulatero P, Chiandussi L, Veglio F (2002). Assessing resting heart rate in adolescents: determinants and correlates. J Hum Hypertens:16, 327-32.
Rachas A, Raffaitin C, Barberger-Gateau P, Helmer C, Ritchie K, Tzourio C, Amouyel P, Ducimetiere P, Empana JP (2012). Clinical usefulness of the metabolic syndrome for the risk of coronary heart disease does not exceed the sum of its individual components in older men and women. The Three-City (3C) Study. Heart:98, 650-5.
Ramirez-Jimenez M, Morales-Palomo F, Pallares JG, Mora-Rodriguez R, Ortega JF (2017). Ambulatory blood pressure response to a bout of HIIT in metabolic syndrome patients. Eur J Appl Physiol:117, 1403-11.
Reardon M, Malik M (1996). Changes in heart rate variability with age. Pacing Clin Electrophysiol:19, 1863-6.
Reaven GM (1988). Banting lecture 1988. Role of insulin resistance in human disease. Diabetes:37, 1595-607.
Recio-Rodriguez JI, Gomez-Marcos MA, Patino-Alonso MC, Agudo-Conde C, Rodriguez-Sanchez E, Garcia-Ortiz L, Vg VG (2012). Abdominal obesity vs general obesity for identifying arterial stiffness, subclinical atherosclerosis and wave reflection in healthy, diabetics and hypertensive. BMC Cardiovasc Disord:12, 3.
Reis SE, Holubkov R, Conrad Smith AJ, Kelsey SF, Sharaf BL, Reichek N, Rogers WJ, Merz CN, Sopko G, Pepine CJ (2001). Coronary microvascular dysfunction is highly prevalent in women with chest pain in the absence of coronary artery disease: results from the NHLBI WISE study. Am Heart J:141, 735-41.
Reulecke S, Charleston-Villalobos S, Voss A, Gonzalez-Camarena R, Gonzalez-Hermosillo J, Gaitan-Gonzalez M, Hernandez-Pacheco G, Schroeder R, Aljama-Corrales T (2018). Dynamics of the cardiovascular autonomic regulation during orthostatic challenge is more relaxed in women. Biomed Tech (Berl):63, 139-50.
Rich-Edwards JW, Manson JE, Stampfer MJ, Colditz GA, Willett WC, Rosner B, Speizer FE, Hennekens CH (1995). Height and the risk of cardiovascular disease in women. Am J Epidemiol:142, 909-17.
Rodriguez-Colon SM, Li X, Shaffer ML, He F, Bixler EO, Vgontzas AN, Cai J, Liao D (2010). Insulin resistance and circadian rhythm of cardiac autonomic modulation. Cardiovasc Diabetol:9, 85.
Rogowski O, Steinvil A, Berliner S, Cohen M, Saar N, Ben-Bassat OK, Shapira I (2009). Elevated resting heart rate is associated with the metabolic syndrome. Cardiovasc Diabetol:8, 55.

Rule AD, Larson TS, Bergstralh EJ, Slezak JM, Jacobsen SJ, Cosio FG (2004). Using serum creatinine to estimate glomerular filtration rate: accuracy in good health and in chronic kidney disease. Ann Intern Med:141, 929-37.

Russo C, Jin Z, Homma S, Rundek T, Elkind MSV, Sacco RL, Di Tullio MR (2011). Effect of Obesity and Overweight on Left Ventricular Diastolic Function: A Community-Based Study in an Elderly Cohort. Journal of the American College of Cardiology:57, 1368-74.

Saba PS, Roman MJ, Longhini C, Scorzoni D, Pini R, Devereux RB, Ganau A (1999). Carotid intimal-medial thickness and stiffness are not affected by hypercholesterolemia in uncomplicated essential hypertension. Arterioscler Thromb Vasc Biol:19, 2788-94.

Sacha J (2013). Why should one normalize heart rate variability with respect to average heart rate. Front Physiol:4, 306.

Sacha J (2014). Interaction between heart rate and heart rate variability. Ann Noninvasive Electrocardiol:19, 207-16.

Sacha J, Pluta W (2008). Alterations of an average heart rate change heart rate variability due to mathematical reasons. Int J Cardiol:128, 444-7.

Safar H, Mourad JJ, Safar M, Blacher J (2002). Aortic pulse wave velocity, an independent marker of cardiovascular risk. Arch Mal Coeur Vaiss:95, 1215-8.

Safar ME (2008). Pulse pressure, arterial stiffness and wave reflections (augmentation index) as cardiovascular risk factors in hypertension. Ther Adv Cardiovasc Dis:2, 13-24.

Safar ME, Balkau B, Lange C, Protogerou AD, Czernichow S, Blacher J, Levy BI, Smulyan H (2013). Hypertension and Vascular Dynamics in Men and Women With Metabolic Syndrome. Journal of the American College of Cardiology:61, 12-19.

Safar ME, Czernichow S, Blacher J (2006a). Obesity, arterial stiffness, and cardiovascular risk. J Am Soc Nephrol:17, S109-11.

Safar ME, Lange C, Blacher J, Eschwege E, Tichet J, Balkau B (2011). Mean and yearly changes in blood pressure with age in the metabolic syndrome: the DESIR study. Hypertens Res:34, 91-7.

Safar ME, Thomas F, Blacher J, Nzietchueng R, Bureau JM, Pannier B, Benetos A (2006b). Metabolic syndrome and age-related progression of aortic stiffness. J Am Coll Cardiol:47, 72-5.

Saito I, Hitsumoto S, Maruyama K, Nishida W, Eguchi E, Kato T, Kawamura R, Takata Y, Onuma H, Osawa H, Tanigawa T (2015). Heart Rate Variability, Insulin Resistance, and Insulin Sensitivity in Japanese Adults: The Toon Health Study. J Epidemiol:25, 583-91.

Samaras TT (2013). Shorter height is related to lower cardiovascular disease risk - a narrative review. Indian Heart J:65, 66-71.

Sarafidis PA, Nilsson PM (2006). The metabolic syndrome: a glance at its history. J Hypertens:24, 621-6.

Schiffrin EL (2012). Vascular remodeling in hypertension: mechanisms and treatment. Hypertension:59, 367-74.

Schillaci G, Pirro M, Pucci G, Mannarino MR, Gemelli F, Siepi D, Vaudo G, Mannarino E (2006). Different impact of the metabolic syndrome on left ventricular structure and function in hypertensive men and women. Hypertension:47, 881-6.

Schuster AK, Fischer JE, Thayer JF, Mauss D, Jarczok MN (2016). Decreased heart rate variability correlates to increased cardiovascular risk. Int J Cardiol:203, 728-30.
Scuteri A, Cunha PG, Rosei EA, Badariere J, Bekaert S, Cockcroft JR, Cotter J, Cucca F, De Buyzere ML, De Meyer T, Ferrucci L, Franco O, Gale N, Gillebert TC, Hofman A, Langlois M, Laucevicius A, Laurent S, Mattace Raso FU, Morrell CH, Muiesan ML, Munnery MM, Navickas R, Oliveira P, Orru M, Pilia MG, Rietzschel ER, Ryliskyte L, Salvetti M, Schlessinger D, Sousa N, Stefanadis C, Strait J, Van daele C, Villa I, Vlachopoulos C, Witteman J, Xaplanteris P, Nilsson P, Lakatta EG (2014). Arterial stiffness and influences of the metabolic syndrome: a cross-countries study. Atherosclerosis:233, 654-60.
Scuteri A, Najjar SS, Orru M, Usala G, Piras MG, Ferrucci L, Cao A, Schlessinger D, Uda M, Lakatta EG (2010). The central arterial burden of the metabolic syndrome is similar in men and women: the SardiNIA Study. Eur Heart J:31, 602-13.
Segers P, Rietzschel ER, De Buyzere ML, Vermeersch SJ, De Bacquer D, Van Bortel LM, De Backer G, Gillebert TC, Verdonck PR (2007). Noninvasive (input) impedance, pulse wave velocity, and wave reflection in healthy middle-aged men and women. Hypertension:49, 1248-55.
Sengstock DM, Vaitkevicius PV, Supiano MA (2005). Arterial stiffness is related to insulin resistance in nondiabetic hypertensive older adults. J Clin Endocrinol Metab:90, 2823-7.
Seppälä-Lindroos A, Vehkavaara S, Häkkinen AM, Goto T, Westerbacka J, Sovijarvi A, Halavaara J, Yki-Järvinen H (2002). Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. J Clin Endocrinol Metab:87, 3023-8.
Shim CY, Park S, Choi D, Yang WI, Cho IJ, Choi EY, Chung N, Ha JW (2011). Sex differences in central hemodynamics and their relationship to left ventricular diastolic function. J Am Coll Cardiol:57, 1226-33.
Shoemaker JK, Hogeman CS, Khan M, Kimmerly DS, Sinoway LI (2001). Gender affects sympathetic and hemodynamic response to postural stress. Am J Physiol Heart Circ Physiol:281, H2028-35.
Sipilä K, Koivistoinen T, Moilanen L, Nieminen T, Reunanen A, Jula A, Salomaa V, Kaaja R, Kööbi T, Kukkonen-Harjula K, Majahalme S, Kähönen M (2007). Metabolic syndrome and arterial stiffness: The Health 2000 Survey. Metabolism:56, 320-26.
Sjoros TJ, Heiskanen MA, Motiani KK, Löyttyniemi E, Eskelinen JJ, Virtanen KA, Savisto NJ, Solin O, Hannukainen JC, Kalliokoski KK (2018). Increased insulin-stimulated glucose uptake in both leg and arm muscles after sprint interval and moderate-intensity training in subjects with type 2 diabetes or prediabetes. Scand J Med Sci Sports:28, 77-87.
Skurnick JH, Aladjem M, Aviv A (2010). Sex differences in pulse pressure trends with age are cross-cultural. Hypertension:55, 40-7.
Smetana P, Malik M (2013). Sex differences in cardiac autonomic regulation and in repolarisation electrocardiography. Pflugers Arch:465, 699-717.
Smulyan H, Marchais SJ, Pannier B, Guerin AP, Safar ME, London GM (1998). Influence of body height on pulsatile arterial hemodynamic data. J Am Coll Cardiol:31, 1103-9.

Stehouwer CDA, Henry RMA, Ferreira I (2008). Arterial stiffness in diabetes and the metabolic syndrome: a pathway to cardiovascular disease. Diabetologia:51, 527-39.

Stolarz K, Staessen JA, Kuznetsova T, Tikhonoff V, State D, Babeanu S, Casiglia E, Fagard RH, Kawecka-Jaszcz K, Nikitin Y (2003). Host and environmental determinants of heart rate and heart rate variability in four European populations. J Hypertens:21, 525-35.

Stuckey MI, Kiviniemi A, Gill DP, Shoemaker JK, Petrella RJ (2015). Associations between heart rate variability, metabolic syndrome risk factors, and insulin resistance. Appl Physiol Nutr Metab:40, 734-40.

Stuckey MI, Tulppo MP, Kiviniemi AM, Petrella RJ (2014). Heart rate variability and the metabolic syndrome: a systematic review of the literature. Diabetes/Metabolism Research and Reviews:30, 784-93.

Suh S, Baek J, Bae JC, Kim KN, Park MK, Kim DK, Cho NH, Lee MK (2014). Sex factors in the metabolic syndrome as a predictor of cardiovascular disease. Endocrinol Metab (Seoul):29, 522-9.

Tabatabaei-Malazy O, Fakhrzadeh H, Sharifi F, Mirarefin M, Badamchizadeh Z, Larijani B (2012). Gender differences in association between metabolic syndrome and carotid intima media thickness. J Diabetes Metab Disord:11, 13.

Tahvanainen A, Koskela J, Tikkakoski A, Lahtela J, Leskinen M, Kähönen M, Nieminen T, Kööbi T, Mustonen J, Pörsti I (2009a). Analysis of cardiovascular responses to passive head-up tilt using continuous pulse wave analysis and impedance cardiography. Scand J Clin Lab Invest:69, 128-37.

Tahvanainen A, Leskinen M, Koskela J, Ilveskoski E, Nordhausen K, Oja H, Kähönen M, Kööbi T, Mustonen J, Pörsti I (2009b). Ageing and cardiovascular responses to head-up tilt in healthy subjects. Atherosclerosis:207, 445-51.

Tahvanainen AM, Tikkakoski AJ, Leskinen MH, Nordhausen K, Kähönen M, Kööbi T, Mustonen JT, Pörsti IH (2012). Supine and upright haemodynamic effects of sublingual nitroglycerin and inhaled salbutamol: a double-blind, placebo-controlled, randomized study. J Hypertens:30, 297-306.

Takase H, Dohi Y, Toriyama T, Okado T, Tanaka S, Sonoda H, Sato K, Kimura G (2011). Brachial-ankle pulse wave velocity predicts increase in blood pressure and onset of hypertension. Am J Hypertens:24, 667-73.

Tang ZH, Wang L, Zeng F, Zhang K (2014). Association and predictive value analysis for metabolic syndrome on systolic and diastolic heart failure in high-risk patients. BMC Cardiovasc Disord:14, 124.

Targher G, Byrne CD (2013). Clinical Review: Nonalcoholic fatty liver disease: a novel cardiometabolic risk factor for type 2 diabetes and its complications. J Clin Endocrinol Metab:98, 483-95.

Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (1996). Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Eur Heart J: 17, 354-81.

Templin C, Ghadri JR, Diekmann J, Napp LC, Bataiosu DR, Jaguszewski M, Cammann VL, Sarcon A, Geyer V, Neumann CA, Seifert B, Hellermann J, Schwyzer M, Eisenhardt K, Jenewein J, Franke J, Katus HA, Burgdorf C, Schunkert H, Moeller C, Thiele H, Bauersachs J, Tschope C, Schultheiss HP, Laney CA, Rajan L, Michels G, Pfister R,

Ukena C, Bohm M, Erbel R, Cuneo A, Kuck KH, Jacobshagen C, Hasenfuss G, Karakas M, Koenig W, Rottbauer W, Said SM, Braun-Dullaeus RC, Cuculi F, Banning A, Fischer TA, Vasankari T, Airaksinen KE, Fijalkowski M, Rynkiewicz A, Pawlak M, Opolski G, Dworakowski R, MacCarthy P, Kaiser C, Osswald S, Galiuto L, Crea F, Dichtl W, Franz WM, Empen K, Felix SB, Delmas C, Lairez O, Erne P, Bax JJ, Ford I, Ruschitzka F, Prasad A, Luscher TF (2015). Clinical Features and Outcomes of Takotsubo (Stress) Cardiomyopathy. N Engl J Med:373, 929-38.

Teodorovich N, Swissa M (2016). Tilt table test today - state of the art. World J Cardiol:8, 277-82.

Thayer JF, Yamamoto SS, Brosschot JF (2010). The relationship of autonomic imbalance, heart rate variability and cardiovascular disease risk factors. Int J Cardiol:141, 122-31.

Theilade S, Lajer M, Persson F, Joergensen C, Rossing P (2013). Arterial stiffness is associated with cardiovascular, renal, retinal, and autonomic disease in type 1 diabetes. Diabetes Care:36, 715-21.

Tikkakoski AJ, Tahvanainen AM, Leskinen MH, Koskela JK, Haring A, Viitala J, Kähönen MA, Kööbi T, Niemelä O, Mustonen JT, Pörsti IH (2013). Hemodynamic alterations in hypertensive patients at rest and during passive head-up tilt. J Hypertens:31, 906-15.

Towfighi A, Zheng L, Ovbiagele B (2009). Sex-specific trends in midlife coronary heart disease risk and prevalence. Arch Intern Med:169, 1762-6.

Townsend RR, Wilkinson IB, Schiffrin EL, Avolio AP, Chirinos JA, Cockcroft JR, Heffernan KS, Lakatta EG, McEniery CM, Mitchell GF, Najjar SS, Nichols WW, Urbina EM, Weber T (2015). Recommendations for Improving and Standardizing Vascular Research on Arterial Stiffness: A Scientific Statement From the American Heart Association. Hypertension:66, 698-722.

Tsuji H, Larson MG, Venditti FJ, Jr., Manders ES, Evans JC, Feldman CL, Levy D (1996). Impact of reduced heart rate variability on risk for cardiac events. The Framingham Heart Study. Circulation:94, 2850-5.

Turkbey EB, McClelland RL, Kronmal RA, Burke GL, Bild DE, Tracy RP, Arai AE, Lima JA, Bluemke DA (2010). The impact of obesity on the left ventricle: the Multi-Ethnic Study of Atherosclerosis (MESA). JACC Cardiovasc Imaging:3, 266-74.

Tweet MS, Hayes SN, Pitta SR, Simari RD, Lerman A, Lennon RJ, Gersh BJ, Khambatta S, Best PJ, Rihal CS, Gulati R (2012). Clinical features, management, and prognosis of spontaneous coronary artery dissection. Circulation:126, 579-88.

Umetani K, Singer DH, McCraty R, Atkinson M (1998). Twenty-four hour time domain heart rate variability and heart rate: relations to age and gender over nine decades. J Am Coll Cardiol:31, 593-601.

Usselman CW, Stachenfeld NS, Bender JR (2016). The molecular actions of oestrogen in the regulation of vascular health. Exp Physiol:101, 356-61.

Uzu T, Kimura G, Yamauchi A, Kanasaki M, Isshiki K, Araki S, Sugiomoto T, Nishio Y, Maegawa H, Koya D, Haneda M, Kashiwagi A (2006). Enhanced sodium sensitivity and disturbed circadian rhythm of blood pressure in essential hypertension. J Hypertens:24, 1627-32.

Vaile JC, Fletcher J, Al-Ani M, Ross HF, Littler WA, Coote JH, Townend JN (1999). Use of opposing reflex stimuli and heart rate variability to examine the effects of

lipophilic and hydrophilic beta-blockers on human cardiac vagal control. Clin Sci (Lond):97, 585-93; discussion 609-10.
Van Bortel L (2002). Focus on small artery stiffness. J Hypertens:20, 1707-9.
Van Bortel LM, Laurent S, Boutouyrie P, Chowienczyk P, Cruickshank JK, De Backer T, Filipovsky J, Huybrechts S, Mattace-Raso FU, Protogerou AD, Schillaci G, Segers P, Vermeersch S, Weber T (2012). Expert consensus document on the measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity. J Hypertens:30, 445-8.
Van De Water JM, Miller TW, Vogel RL, Mount BE, Dalton ML (2003). Impedance cardiography: the next vital sign technology? Chest:123, 2028-33.
van Eickels M, Grohe C, Cleutjens JP, Janssen BJ, Wellens HJ, Doevendans PA (2001). 17beta-estradiol attenuates the development of pressure-overload hypertrophy. Circulation:104, 1419-23.
van Vliet-Ostaptchouk JV, Nuotio ML, Slagter SN, Doiron D, Fischer K, Foco L, Gaye A, Gogele M, Heier M, Hiekkalinna T, Joensuu A, Newby C, Pang C, Partinen E, Reischl E, Schwienbacher C, Tammesoo ML, Swertz MA, Burton P, Ferretti V, Fortier I, Giepmans L, Harris JR, Hillege HL, Holmen J, Jula A, Kootstra-Ros JE, Kvaloy K, Holmen TL, Mannisto S, Metspalu A, Midthjell K, Murtagh MJ, Peters A, Pramstaller PP, Saaristo T, Salomaa V, Stolk RP, Uusitupa M, van der Harst P, van der Klauw MM, Waldenberger M, Perola M, Wolffenbuttel BH (2014). The prevalence of metabolic syndrome and metabolically healthy obesity in Europe: a collaborative analysis of ten large cohort studies. BMC Endocr Disord:14, 9.
Vanni E, Bugianesi E, Kotronen A, De Minicis S, Yki-Järvinen H, Svegliati-Baroni G (2010). From the metabolic syndrome to NAFLD or vice versa? Dig Liver Dis:42, 320-30.
Vasan RS, Larson MG, Levy D, Evans JC, Benjamin EJ (1997). Distribution and categorization of echocardiographic measurements in relation to reference limits: the Framingham Heart Study: formulation of a height- and sex-specific classification and its prospective validation. Circulation:96, 1863-73.
Veerasamy M, Ford GA, Neely D, Bagnall A, MacGowan G, Das R, Kunadian V (2014). Association of aging, arterial stiffness, and cardiovascular disease: a review. Cardiol Rev:22, 223-32.
Vilmi-Kerälä T, Koivistoinen T, Palomäki O, Uotila J, Palomäki A (2017). Arterial stiffness in fertile women with metabolic syndrome. Ann Med, 1-8.
Vishram JK, Borglykke A, Andreasen AH, Jeppesen J, Ibsen H, Jorgensen T, Palmieri L, Giampaoli S, Donfrancesco C, Kee F, Mancia G, Cesana G, Kuulasmaa K, Salomaa V, Sans S, Ferrieres J, Dallongeville J, Soderberg S, Arveiler D, Wagner A, Tunstall-Pedoe H, Drygas W, Olsen MH (2014). Impact of age and gender on the prevalence and prognostic importance of the metabolic syndrome and its components in Europeans. The MORGAM Prospective Cohort Project. PLoS One:9, e107294.
Vlachopoulos C, Aznaouridis K, Stefanadis C (2010). Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. J Am Coll Cardiol:55, 1318-27.
Vlachopoulos C, Aznaouridis K, Terentes-Printzios D, Ioakeimidis N, Stefanadis C (2012). Prediction of cardiovascular events and all-cause mortality with brachial-ankle elasticity index: a systematic review and meta-analysis. Hypertension:60, 556-62.

von Bibra H, Strohle A, St John Sutton M, Worm N (2017). Dietary therapy in heart failure with preserved ejection fraction and/or left ventricular diastolic dysfunction in patients with metabolic syndrome. Int J Cardiol:234, 7-15.
Voulgari C, Tentolouris N, Dilaveris P, Tousoulis D, Katsilambros N, Stefanadis C (2011). Increased heart failure risk in normal-weight people with metabolic syndrome compared with metabolically healthy obese individuals. J Am Coll Cardiol:58, 1343-50.
Wainwright P, Byrne CD (2016). Bidirectional Relationships and Disconnects between NAFLD and Features of the Metabolic Syndrome. Int J Mol Sci:17, 367.
Wang C, Jackson G, Jones TH, Matsumoto AM, Nehra A, Perelman MA, Swerdloff RS, Traish A, Zitzmann M, Cunningham G (2011a). Low testosterone associated with obesity and the metabolic syndrome contributes to sexual dysfunction and cardiovascular disease risk in men with type 2 diabetes. Diabetes Care:34, 1669-75.
Wang F, Ye P, Luo L, Xiao W, Qi L, Bian S, Wu H, Sheng L, Xiao T, Xu R (2011b). Association of serum lipids with arterial stiffness in a population-based study in Beijing. Eur J Clin Invest:41, 929-36.
Waters WW, Ziegler MG, Meck JV (2002). Postspaceflight orthostatic hypotension occurs mostly in women and is predicted by low vascular resistance. J Appl Physiol:92, 586-94.
Wen W, Peng B, Tang X, Huang HX, Wen X, Hu S, Luo R (2015). Prevalence of High Arterial Stiffness and Gender-specific Differences in the Relationships with Classical Cardiovascular Risk Factors. J Atheroscler Thromb:22, 706-17.
Weng C, Yuan H, Yang K, Tang X, Huang Z, Huang L, Chen W, Chen F, Chen Z, Yang P (2013). Gender-specific association between the metabolic syndrome and arterial stiffness in 8,300 subjects. Am J Med Sci:346, 289-94.
Wenner MM, Stachenfeld NS (2012). Blood pressure and water regulation: understanding sex hormone effects within and between men and women. J Physiol:590, 5949-61.
Westerbacka J, Seppälä-Lindroos A, Yki-Järvinen H (2001). Resistance to acute insulin induced decreases in large artery stiffness accompanies the insulin resistance syndrome. J Clin Endocrinol Metab:86, 5262-8.
Westerbacka J, Vehkavaara S, Bergholm R, Wilkinson I, Cockcroft J, Yki-Järvinen H (1999). Marked resistance of the ability of insulin to decrease arterial stiffness characterizes human obesity. Diabetes:48, 821-7.
White DD, Gotshall RW, Tucker A (1996). Women have lower tolerance to lower body negative pressure than men. J Appl Physiol:80, 1138-43.
WHO (2017). WHO Updates on Cardivascular Diseases. https://www.who.int/en/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds) (12/2018).
Wilenius M, Tikkakoski AJ, Tahvanainen AM, Haring A, Koskela J, Huhtala H, Kähönen M, Kööbi T, Mustonen JT, Pörsti IH (2016). Central wave reflection is associated with peripheral arterial resistance in addition to arterial stiffness in subjects without antihypertensive medication. BMC Cardiovasc Disord:16, 131.
Wilkinson IB, MacCallum H, Flint L, Cockcroft JR, Newby DE, Webb DJ (2000). The influence of heart rate on augmentation index and central arterial pressure in humans. J Physiol:525 Pt 1, 263-70.

Willum Hansen T (2006). Prognostic Value of Aortic Pulse Wave Velocity as Index of Arterial Stiffness in the General Population. Circulation:113, 664-70.
Wilmer NW ORM, Vlachopoulos C. (2011). McDonald´s Blood Flow in Arteries: Theoretical, experimental and clinical principles (6th ed.): Hodder Arnold, London.
Wulsin LR, Horn PS, Perry JL, Massaro JM, D'Agostino RB (2015). Autonomic Imbalance as a Predictor of Metabolic Risks, Cardiovascular Disease, Diabetes, and Mortality. J Clin Endocrinol Metab:100, 2443-8.
Wulsin LR, Horn PS, Perry JL, Massaro JM, D'Agostino RB, Sr. (2016). The Contribution of Autonomic Imbalance to the Development of Metabolic Syndrome. Psychosom Med:78, 474-80.
Xhyheri B, Manfrini O, Mazzolini M, Pizzi C, Bugiardini R (2012). Heart rate variability today. Prog Cardiovasc Dis:55, 321-31.
Xi B, He D, Zhang M, Xue J, Zhou D (2014). Short sleep duration predicts risk of metabolic syndrome: a systematic review and meta-analysis. Sleep Med Rev:18, 293-7.
Xu L, Jiang CQ, Lam TH, Cheng KK, Yue XJ, Lin JM, Zhang WS, Thomas GN (2010). Impact of impaired fasting glucose and impaired glucose tolerance on arterial stiffness in an older Chinese population: the Guangzhou Biobank Cohort Study-CVD. Metabolism:59, 367-72.
Xu S, Wan Y, Xu M, Ming J, Xing Y, An F, Ji Q (2015). The association between obstructive sleep apnea and metabolic syndrome: a systematic review and meta-analysis. BMC Pulm Med:15, 105.
Yahagi K, Davis HR, Arbustini E, Virmani R (2015). Sex differences in coronary artery disease: Pathological observations. Atherosclerosis:239, 260-67.
Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P, Kadowaki T (2001). The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat Med:7, 941-6.
Yeboah J, Blaha MJ, Michos ED, Qureshi W, Miedema M, Flueckiger P, Rodriguez CJ, Szklo M, Bertoni AG (2017). Adult Height, Prevalent Coronary Artery Calcium Score, and Incident Cardiovascular Disease Outcomes in a Multiethnic Cohort. Am J Epidemiol:186, 935-43.
Yki-Järvinen H (2002). Ectopic fat accumulation: an important cause of insulin resistance in humans. J R Soc Med:95 Suppl 42, 39-45.
Yki-Järvinen H (2014). Non-alcoholic fatty liver disease as a cause and a consequence of metabolic syndrome. Lancet Diabetes Endocrinol:2, 901-10.
Yki-Järvinen H, Westerbacka J (2007). Insulin resistance, arterial stiffness and wave reflection. Adv Cardiol:44, 252-60.

10 ORIGINAL PUBLICATIONS

PUBLICATION I

Metabolic syndrome may be associated with increased arterial stiffness even in the absence of hypertension: A study in 84 cases and 82 controls

Kangas P, Tikkakoski AJ, Tahvanainen AM, Leskinen MH, Viitala JM, Kähönen M, Kööbi T, Niemelä OJ, Mustonen JT and Pörsti IH

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Metabolic syndrome may be associated with increased arterial stiffness even in the absence of hypertension: A study in 84 cases and 82 controls

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ABSTRACT

Objective. To evaluate the hemodynamic characteristics of metabolic syndrome (MetS) in the absence and presence of hypertension.

Materials/Methods. Altogether 166 subjects without previously diagnosed cardiovascular disease, diabetes, or antihypertensive medication, were allocated to four groups: control, hypertension only, MetS without hypertension, and MetS with hypertension (mean age 44–46 years). Cut-point for hypertension was blood pressure ≥140/90 mmHg. Other criteria of MetS were as defined by Alberti et al. 2009. Hemodynamic variables were measured using whole-body impedance cardiography and pulse wave analysis.

Results. Pulse wave velocity was higher in hypertensive and normotensive subjects with MetS than controls ($p < 0.05$), and in the hypertensive MetS group than subjects with hypertension only ($p < 0.05$). Aortic pulse pressure was higher in the two hypertensive groups than the two normotensive groups ($p < 0.05$). Systemic vascular resistance index was higher in the hypertensive than normotensive MetS group ($p < 0.05$), and in the group with hypertension alone than in controls ($p < 0.05$). Heart rate was higher in the hypertensive Mets group than in controls and subjects with hypertension only ($p < 0.05$). Cardiac index did not differ, while stroke index was lower in both groups with MetS than groups without MetS. Augmentation pressure was higher in the hypertensive MetS group than in controls and normotensive MetS group ($p < 0.05$).

Conclusions. Pulse wave velocity, an acknowledged marker of arterial stiffness, was associated with MetS even in the absence of hypertension. This emphasizes the importance of the prevention and treatment of MetS.

Abbreviations: AGEs, glycation end-products; AIx, augmentation index; ANOVA, one-way analysis of variance; BMI, body mass index; CI, confidence interval; eGFR, estimated creatinine-based glomerulus filtration rate; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; HT, hypertensive group; HT-MetS, hypertensive metabolic syndrome group; LDL, low-density lipoprotein; MetS, metabolic syndrome; NT-MetS, normotensive metabolic syndrome group; PWA, pulse wave analysis; PWV, pulse wave velocity; SE, standard error of the mean; SVRI, systemic vascular resistance index.

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1. Introduction

Metabolic syndrome (MetS) is a cluster of abnormalities including abdominal obesity, glucose intolerance, dyslipidemias and hypertension. The prevalence of the disorder is expanding worldwide, and for example in North America MetS has been estimated to affect approximately one-quarter of the population [1,2]. MetS confers a 5-fold increase in the risk of type 2 diabetes mellitus [3], and a 2-fold risk of developing cardiovascular disease over the next 5 to 10 years when compared with individuals without the syndrome. The lifetime risk is probably much higher [3].

The mechanisms associating MetS with cardiovascular disease have been examined in many studies, but the underlying pathogenesis is still incompletely understood [2]. Increased arterial stiffness may be a significant link between MetS and excess cardiovascular risk [4,5]. In patients with essential hypertension, higher arterial stiffness is an independent predictor of stroke [6]. Even in the general population increased aortic pulse wave velocity (PWV) independently predicts fatal and non-fatal cardiovascular end-points [7]. Increased aortic pulse pressure [8], increased systemic vascular resistance, and decreased left ventricular stroke index [9] have also been reported to be associated with MetS. Probably due to the unfavorable hemodynamic profile, MetS is associated with impaired left ventricular systolic and diastolic function [10].

Some studies have suggested that like in the case of hypertension, also in MetS elevated blood pressure is the strongest factor determining arterial stiffness [11,12]. A recent report from the Framingham study suggested that although diabetes mellitus was associated with increased risk of cardiovascular events and death, much of the excess risk was attributable to the coexistent hypertension [13]. In the present study we examined how MetS influences hemodynamic variables in the absence and presence of hypertension. The results suggest that MetS may be associated with increased arterial stiffness even in the absence of hypertension.

2. Methods

2.1. Study subjects

Medical doctors and nurses in 4 organizations that provide occupational health-care in the region of Pirkanmaa were contacted and informed about the possibility that subjects examined in routine health inspections could be recruited to participate in the present clinical study on hemodynamics (DYNAMIC-study, clinical trial registration number NCT01742702). An announcement for the recruitment of subjects was also distributed among the personnel of the University of Tampere and Tampere University Hospital, and two announcements were published in a local newspaper. The subjects who responded were recruited in the order that they contacted the research nurse.

The population of the current study was screened from altogether 688 participants, based on criteria mentioned below, and the final study group consisted of 166 Finnish subjects (88 men and 78 women) aged 21–68 years. All participants underwent an interview and physical examination by a medical doctor. The lifestyle habits, family history and medical history were recorded. All subjects were without antihypertensive medication. Subjects with previous myocardial infarction or diagnosed diabetes, coronary heart disease, cardiac insufficiency, atherosclerotic vascular disease, or cerebrovascular disease were excluded. One subject had mildly abnormal electrocardiography suggesting hypertrophic myocardium, but in an ultrasound evaluation by a cardiologist his ejection fraction was normal and the condition was without hemodynamic significance. One subject had previously had mitral insufficiency, but the disorder had been successfully repaired with annuloplasty operation in 2004.

All subjects using medications with potential influences on hemodynamics (like α_1-adrenoceptor blockers for prostate problems, β_2-adrenoceptor agonists, digoxin) were excluded. Five of the included subjects were on statin medication for dyslipidemia (3 subjects in the NT-MetS group and 2 in the HT-MetS group). Altogether 27 of the included female subjects used estrogen, progestin, or their combination, but there were no differences between the four study groups in hormone use ($p = 0.49$). One subject had been diagnosed with anti-phospholipid syndrome and was treated with warfarin, but the patient was physically well and without any symptoms or findings during the recordings. In addition, 5 subjects used intranasal or inhaled corticosteroid for asthma or allergy, and 6 subjects were treated with anti-depressive agents, two subjects used acetylsalicylic acid, and one subject used allopurinol for gout. Also antihistamine products ($n = 3$) and proton pump inhibitors ($n = 8$) were used.

The criteria of Alberti et al. from year 2009 [3] were used for the definition of MetS, and at least three of the following criteria were met: waist circumference ≥94 cm (men) and ≥80 cm (women); triglycerides ≥1.7 mmol/l; HDL <1.0 mmol/l (men) and <1.3 mmol/l (women); systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥85 mmHg; fasting plasma glucose ≥5.6 mmol/l. In the initial analyses the criteria were otherwise applied as above, but since the aim was to examine the effects of hypertension in MetS, the criterion for blood pressure was the accepted cut point of hypertension: systolic blood pressure ≥140 or diastolic blood pressure ≥90 mmHg [14]. Further analyses were performed so that the criterion for hypertension was still ≥140/90 mmHg in subjects without components of MetS, but the cut-off blood pressure in subjects with MetS was ≥130/85 mmHg, i.e. all of the criteria by Alberti et al. were applied for the presence of MetS [3]. In this analysis, blood pressure in all controls and NT-MetS subjects was <130/85 mmHg. Of note, blood pressure level ≥130/85 mmHg has been defined as high normal in the European Society of Hypertension guidelines [14]. Arterial blood pressure was measured continuously during the hemodynamic recordings using a radial tonometric device (see below). The average values of all systolic and diastolic readings during the last 3 min (i.e. recordings of at least 180 cardiac cycles per subject) were used in the analyses, as during this period the signal was most stable. On the basis of the average blood pressures of the tonometric recordings the subjects were classified as being normotensive or hypertensive. Kidney disease was excluded by means of plasma

creatinine determination and urine dipstick and albumin analysis, and primary aldosteronism by determination of plasma renin activity and aldosterone concentration.

The subjects were allocated to 4 groups: 1. healthy controls, 2. hypertension only (normal plasma glucose, triglycerides, HDL, and waist circumference), 3. MetS without hypertension, and 4. MetS with hypertension. The groups were abbreviated as control (n = 58), HT (hypertensive, n = 24), NT-MetS (normotensive MetS, n = 27), and HT-MetS (hypertensive MetS, n = 57). All participants gave written informed consent, and the study was approved by the Ethics Committee of Tampere University Hospital (study code R06086M).

2.2. *Hemodynamic measurements*

Hemodynamic measurements were performed in a quiet, temperature-controlled research laboratory by a trained nurse. The study subjects had refrained from caffeine containing products, smoking and heavy meals for at least 4 h and from alcohol for at least 24 h prior to the investigation. The subjects were resting supine on a table. The electrodes for impedance cardiography were placed on the body surface, a tonometric sensor for pulse wave analysis on the radial pulsation on the left wrist, an oscillometric brachial cuff for blood pressure calibration on the right upper arm. Hemodynamic variables were recorded for 5 min, and the mean values of the last 3 min were used in the analyses. Previously, the good repeatability and reproducibility of these measurements have been demonstrated [15].

2.3. *Pulse wave analysis, PWA*

Blood pressure and pulse wave form were continuously recorded from the radial artery by the use of a tonometric sensor (Colin BP-508T, Colin Medical Instruments, USA), which was fixed on the radial artery pulsation by the use of a wrist band. The blood pressure signal from the radial artery was calibrated on average every 2.5 min by contralateral brachial blood pressure measurements. Aortic blood pressure was derived using the SphygmoCor pulse wave monitoring system (SpygmoCor PWMx, AtCor medical, Australia) by the use of a validated generalized transfer function [16]. Heart rate, aortic pulse pressure, aortic reflection time, augmentation index (AIx, augmented pressure/pulse pressure * 100), and pulse pressure amplification (radial pulse pressure/aortic pulse pressure) were also determined. AIx was adjusted to heart rate 75 beats/min (AIx@75) with the formula of the SphygmoCor software. Due to a technical problem, augmentation pressure and AIx could not be recorded in one subject.

2.4. *Whole-body impedance cardiography*

A whole-body impedance cardiography device (CircMon^R, JR Medical, Tallinn, Estonia), which records the continuous changes in body electrical impedance during a cardiac cycle, was used to determine beat-to-beat heart rate, stroke volume, cardiac output and aortic to popliteal PWV. To calculate the PWV, the software measures the time difference between the onset of the decrease in the whole-body impedance signal and the popliteal artery signal. PWV is then determined from the time difference and the distance between the electrodes. As the present impedance cardiography method has been found to slightly overestimate PWV when compared with Doppler ultrasound measurements, a validated equation was utilized to calculate values that correspond to the ultrasound method (PWV = (PWV impedance * 0.696) + 0.864) [17].

Systemic vascular resistance index (SVRI) was calculated using the blood pressure signal from the radial tonometric sensor and the cardiac index measured by the CircMon^R device. A description of the method and electrode configuration has been previously reported [17–19]. The cardiac output values measured with CircMon^R whole-body impedance cardiography are in good agreement with the values measured by the thermodilution method [18,20].

2.5. *Laboratory tests*

Blood samples were obtained from the antecubital vein after approximately 12 h of fasting. Plasma glucose, triglycerides, total cholesterol, HDL and creatinine were measured using Cobas Integra 800 clinical chemistry analyzer, and plasma insulin using electrochemiluminescence immunoassay on Cobas e 411 analyzer according to the manufacturer's instructions (Roche Diagnostics, Basel, Switzerland). Estimated creatinine-based glomerulus filtration rate (eGFR) was calculated individually using the RULE formula [21], which was chosen because the measured creatinine values were within normal range. LDL was calculated by using Friedewald's formula [22]. In case of three subjects with triglycerides values >4 mmol/l, LDL values were missing. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated according to the formula [plasma fasting insulin (mU/ml) * plasma fasting glucose (mmol/l)/22.5] [23]. A standard 12-lead electrocardiogram was recorded and left ventricular mass evaluated using Cornell voltage QRS duration product with the cut point 2440 mm * ms [14].

2.6. *Statistical analysis*

The one-way analysis of variance (ANOVA) was used to compare the characteristics and hemodynamic variables between the four groups. The univariate statistics were adjusted for possible differences in other variables that could have influenced the outcome by using the variables as covariates in the analyses. The post-hoc test Tukey was used in cases with homogenous variances, and Tamhane's T2 in cases with non-homogenous variances. The skewed distribution of triglycerides and HOMA-IR was corrected using logarithmic transformation before statistical analyses. To compare possible differences in smoking habits, use of estrogen or progestin, and presence of left ventricular hypertrophy between the groups, Pearson Chi-Square or the Fisher's exact test was used. Pearson's or Spearman's correlations were calculated, as appropriate. As both present and previous smoking may influence PWV, all subjects with a smoking history were regarded as smokers and combined to the same group in the analyses. Linear regression

analysis was used to evaluate the associations of different components of MetS with arterial stiffness. Testing was 2-sided and the results in the table were reported as means and standard deviations for normally distributed variables, and medians and lower and upper quartiles for variables with skewed distribution, and numbers of cases and percentages with categorical variables. The results in the figures were depicted as means and standard deviations (SD). P-values < 0.05 were considered statistically significant. All data were analyzed using SPSS 17.0 (SPSS, Chicago, IL, USA).

3. Results

3.1. Study population

The general characteristics of the study subjects are presented in Table 1. Age, plasma creatinine concentration, and eGFR did not differ in the study groups ($p > 0.10$ for all). The prevalence of current or previous smoking ranged from 25% to 52% in the four groups, with numerically, but not quite statistically significantly, highest proportion of smokers in the NT-MetS group ($p = 0.05$). Left ventricular hypertrophy, as evaluated using the Cornell voltage QRS duration product, was detected in 27 subjects (4, 5, 7 and 10 subjects in the control, HT, NT-MetS and HT-MetS groups, respectively), but the differences between the groups were not significant ($p = 0.076$).

As expected, the two MetS groups were characterized by higher body mass index (BMI), waist circumference, plasma total cholesterol, LDL, triglyceride, and fasting glucose concentrations, higher HOMA-IR, and lower plasma HDL concentration, than subjects in the control and HT groups ($p < 0.05$ for all) (Table 1). Mean systolic and diastolic blood pressure during the hemodynamic measurements was higher in the HT and HT-MetS groups than in the two normotensive groups ($p < 0.05$). Mean systolic blood pressure was also slightly higher in the NT-MetS than the control group ($p < 0.05$).

3.2. Hemodynamics of MetS with 140/90 mmHg as the cut-off blood pressure

PWV was higher in both MetS groups (NT-MetS and HT-MetS) than in controls ($p < 0.05$). PWV was also higher in the HT-MetS than the HT group ($p < 0.05$), while the difference between the NT-MetS and HT groups was not significant ($p = 0.073$) (Fig. 1A). The observed differences in PWV remained significant after adjusting for age, sex, height, and smoking habits. There was no significant difference in PWV between the NT-MetS and HT-MetS groups, or between the HT and control groups.

Aortic pulse pressure was higher in both hypertensive groups (HT and HT-MetS) than in the normotensive groups (NT-MetS and control) ($p < 0.05$ for all) (Fig. 1B). No statistically significant differences in AIx@75 were found between the four groups, while augmentation pressure (i.e. amplitude of the reflected pressure wave in mmHg) was higher in the HT-MetS group than in controls and NT-MetS group ($p < 0.05$) (Fig. 1C–D).

SVRI was higher in both hypertensive groups than in controls, and also in the HT-MetS than the NT-MetS group ($p < 0.05$) (Fig. 2A). No significant differences in cardiac index were observed between the four groups ($p > 0.1$ for all) (Fig. 2B), while stroke index was lower in both MetS groups than in the HT and control groups ($p < 0.05$ for all) (Fig. 2C). Heart rate was higher in the HT-MetS group than in controls and the HT group (Fig. 2D). Of note, aortic pulse pressure more strongly correlated with SVRI ($R^2 = 0.221$, $p < 0.05$) than with PWV ($R^2 = 0.072$, $p < 0.05$).

The associations of the different MetS components with PWV were also evaluated using linear regression

Table 1 – Clinical and metabolic characteristics in the study groups with 140/90 mmHg as the cut-off blood pressure.

	Control n = 58	HT n = 24	NT-MetS n = 27	HT-MetS n = 57
Age (years)	44 ± 10	44 ± 14	45 ± 9	46 ± 8
BMI (kg/m^2)	22.8 ± 2.4	22.9 ± 1.7	29.9 ± 3.9*†	30.3 ± 4.6*†
Waist circumference (cm)	79 ± 8	81 ± 8	102 ± 11*†	102 ± 13*†
Systolic blood pressure (mmHg)	119 ± 11	153 ± 13*	126 ± 11*†	151 ± 10*‡
Diastolic blood pressure (mmHg)	69 ± 10	87 ± 7*	75 ± 9†	89 ± 10*‡
Current or previous smoking (n/%)	17/29%	6/25%	14/52%	27/47%
Cornell voltage product in ECG (ms*mm)	1522 ± 584	1803 ± 706	1822 ± 566	1767 ± 547
Total cholesterol (mmol/l)	4.7 ± 0.8	4.9 ± 0.8	5.7 ± 1.3*†	5.7 ± 1.0*†
Triglycerides (mmol/l)	0.8 (0.6-1.1)	0.7 (0.6-0.8)	2.1 (1.7-2.7)*†	1.6 (1.1-2.1)*†‡
HDL (mmol/l)	1.9 ± 0.4	1.9 ± 0.4	1.0 ± 0.2*†	1.4 ± 0.4*†‡
LDL (mmol/l)	2.5 ± 0.8	2.7 ± 0.8	3.7 ± 1.4*†	3.5 ± 0.8*†
Creatinine (μmol/l)	73 ± 14	75 ± 12	72 ± 14	72 ± 14
eGFR (ml/min/1.73 m^2)	111 ± 14	116 ± 17	116 ± 15	112 ± 13
Fasting plasma glucose (mmol/l)	5.0 ± 0.3	5.1 ± 0.3	5.7 ± 0.6*†	5.8 ± 0.4*†
HOMA-IR	1.1 (0.7-1.6)	1.1 (0.8-1.3)	2.8 (2.1-3.5)*†	2.3 (1.6-3.5)*†

Values are means ± SD except the values for smoking, which are the number of cases and percentages, and the values for triglycerides and HOMA-IR, which are shown as medians (lower and upper quartiles) due to skewed distribution. HT, hypertensive subjects without any other components of the metabolic syndrome (MetS); NT-MetS, normotensive subjects with MetS; HT-MetS, hypertensive subjects with MetS; *$p < 0.05$ vs. controls, †$p < 0.05$ vs. HT, ‡$p < 0.05$ vs. NT-MetS; BMI, body mass index; eGFR, estimated glomerulus filtration rate; HOMA-IR, homeostatic model assessment of insulin resistance.

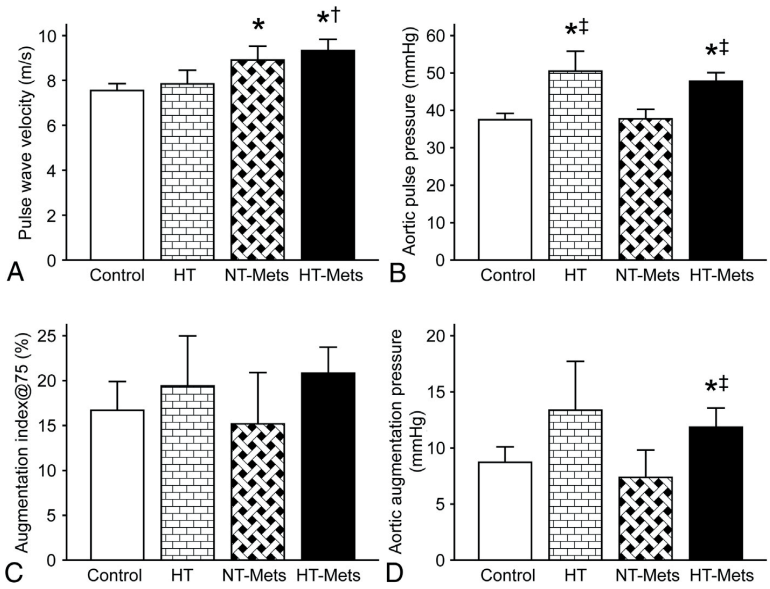

Fig. 1 – Pulse wave velocity (A), aortic pulse pressure (B), augmentation index related to heart rate 75/min (C), and aortic augmentation pressure (D) in the study groups. HT, hypertensive subjects without any other components of the metabolic syndrome (MetS); NT-MetS, normotensive subjects with MetS; HT-MetS, hypertensive subjects with MetS; mean ± CI; *p < 0.05 vs. controls, †p < 0.05 vs. HT, ‡p < 0.05 vs. NT-MetS.

analyses (Table 2), with age, sex, height, waist circumference, smoking status, systolic and diastolic blood pressure, plasma HDL, triglycerides, and glucose as the independent variables (R^2 of the model = 0.461, p < 0.001). The analyses showed that higher age, lower HDL, and higher triglycerides were significantly associated with increased PWV (p < 0.05 for all). The association of systolic blood pressure with PWV was not quite statistically significant (p = 0.057).

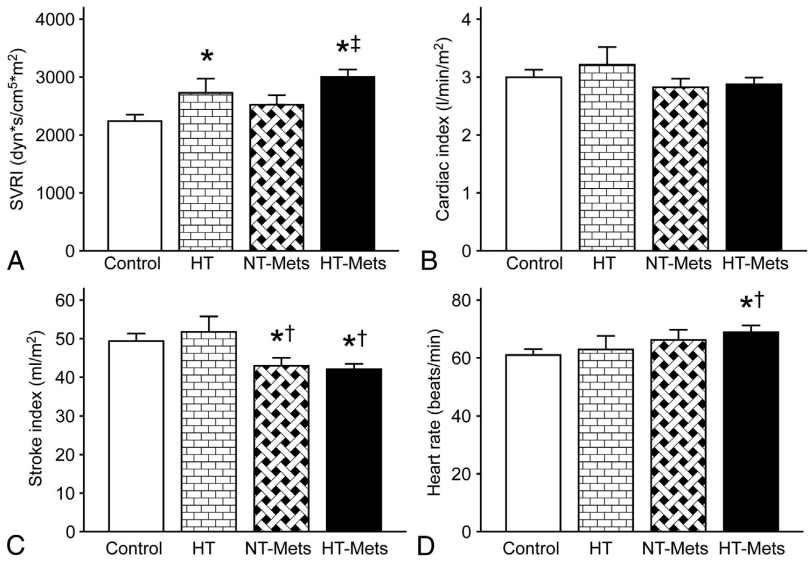

Fig. 2 – Systemic vascular resistance index (SVRI) (A), cardiac index (B), stroke index (C), and heart rate (D) in the study groups. Group abbreviations as in Fig. 1; mean ± CI; *p < 0.05 vs. controls, †p < 0.05 vs. HT, ‡p < 0.05 vs. NT-MetS.

Table 2 – Linear regression analysis of variables associated with pulse wave velocity.

	B	Beta	95% Confidence Interval for B		P - value
			Lower Bound	Upper Bound	
Age	0.075	0.432	0.052	0.098	<0.001
Sex	0.273	0.079	−0.464	1.009	0.466
Height	0.016	0.095	−0.019	0.052	0.366
Waist circumference	−0.001	−0.011	−0.024	0.021	0.913
Current or previous smoking	0.108	0.030	−0.323	0.539	0.622
Systolic blood pressure	0.019	0.205	0.000	0.038	0.057
Diastolic blood pressure	0.004	0.031	−0.024	0.032	0.770
HDL	−0.837	−0.240	−1.470	−0.203	0.010
Triglycerides	0.349	0.172	0.014	0.683	0.041
Fasting plasma glucose	0.324	0.101	−0.164	0.812	0.191

Enter method is used in the linear regression analysis. R square of the model is 0.461. Male = 0, female = 1; non-smoking = 0, current or previous smoking = 1.

3.3. *Arterial stiffness in MetS with 130/85 mmHg as the cut-off blood pressure*

As the blood pressure criterion for the presence of MetS according to Alberti et al. is set to ≥130/85 mmHg [3], additional analyses were performed so that the cut point for normotension in subjects with MetS and controls was <130/85 mmHg. The cut point for hypertension alone remained at ≥140/90 mmHg, according to the European Society of Hypertension guideline [14]. Subsequently, subjects without criteria for MetS and blood pressure values of 131–139 mmHg of systolic, and 85–89 mmHg of diastolic blood pressure, were excluded from the analyses. Thus, the study population consisted of 156 subjects (82 men and 74 women) in these analyses.

The PWV values were (mean ± SD) 7.4 ± 1.0, 7.8 ± 1.4, 8.6 ± 1.6, and 9.3 ± 1.8 m/s in the control (n = 48), HT (n = 24), NT-Mets (n = 14), and HT-Mets (n = 70) groups, respectively. Altogether, the results did not markedly deviate from the original analysis, as PWV was higher in the HT-MetS group than in controls and the HT group ($p < 0.05$). PWV was also still numerically higher in the NT-MetS group (containing 14 subjects) than in controls, but the difference was not quite statistically significant ($p = 0.053$). After adjustment for age, sex, height, and smoking status the statistical significances of the above differences in PWV were not changed. Of note, PWV between the NT-MetS and HT-MetS groups, or between the control and HT groups, did not differ with the lower blood pressure criterion, either.

4. Discussion

In this study we examined the hemodynamic changes associated with MetS in the absence and presence of hypertension. The criterion for hypertension was the accepted cut point of the European Society of Hypertension guideline: systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg [14]. Of note, in the guideline, blood pressure level ≥ 130/85 mmHg (as set in the criteria of MetS) has been defined as high normal [14]. Since the special aim of this study was to examine the impact of hypertension, the higher cut point was primarily chosen. It should be noted that even with this cut-point most of the subjects (68%) with MetS were hypertensive. When using the 140/90 mmHg cut-off pressure, we found that subjects with MetS, regardless of their blood pressure status, had higher PWV when compared with normotensive controls. These results suggest that MetS may result in increased arterial stiffness even in the absence of hypertension. When using the lower 130/85 mmHg cut-off pressure, the difference in PWV between the controls and the NT-Mets groups was not quite significant ($p = 0.053$), but it should be noted that in this additional analysis the size of NT-MetS group was rather small (14 subjects). Importantly, with both of the above cut-off pressures, PWV between the NT-MetS and HT-Mets groups did not differ, which emphasizes the role of the other components of MetS in addition to hypertension in the pathogenesis of arterial stiffening. We also want to stress that arterial stiffness depends critically on the prevailing blood pressure distending the blood vessels [24], and a strength in the present study was that blood pressure and PWV were measured simultaneously during the hemodynamic recordings.

In spite of clear differences in PWV in the current study, we found no differences in AIx (i.e. the proportion of the reflected wave from pulse pressure) between the groups, while aortic pulse pressure was higher in both hypertensive groups when compared with controls and the NT-MetS group. Since SVRI showed a stronger correlation with aortic pulse pressure than PWV, the present results suggest that central pulse pressure is more influenced by SVRI than by arterial stiffness. In addition to peripheral arterial resistance and large artery stiffness, the other known determinants of central pulse pressure are the amplitude and the reflectance point of the pressure wave, and the duration and pattern of left ventricular ejection [25]. It should also be noted that AIx and central pulse pressure are indirect surrogates of arterial stiffness, and that they both are influenced by arterial stiffness, but also by other factors like SVRI. In contrast, aortic PWV, which is measured as the speed of the pressure wave propagation, is a direct and widely acknowledged measure of arterial stiffness [4,25].

The association between MetS and increased arterial stiffness is well known from previous studies [5,12]. Moreover, some reports based on statistical multivariate regression models have suggested that elevated blood pressure would be the strongest factor determining arterial stiffness in MetS

[11,12]. In the present study, we evaluated the role of hypertension by allocating the subjects to different groups on the basis of widely accepted criteria (controls, HT, NT-MetS and HT-Mets) [3,14]. To our knowledge, this is the first report that compared the hemodynamic status between normotensive and hypertensive subjects with MetS.

The present results suggest that MetS may be associated with increased arterial stiffness even in the absence of hypertension. The underlying pathophysiology of increased arterial stiffness in MetS is not completely clear, but several mechanisms have been postulated. An important factor in MetS is abdominal adiposity. Although the pathophysiological processes linking abdominal adiposity to arterial stiffness remain incompletely defined, a widely accepted view is that both the anatomical and metabolic properties of intra-abdominal fat are related to atherosclerosis [11]. Insulin resistance, which was documented in both of the present groups with MetS by means of elevated HOMA-IR, contributes to increased arterial stiffness via several mechanisms [5]. Hyperinsulinemia may increase sympathetic tone, promote sodium reabsorption, activate the renin–angiotensin–aldosterone system, and increase systemic and vascular inflammation [26,27]. The role of elevated blood glucose may also be important, since in diabetic individuals the formation of advanced glycation end-products (AGEs) in the arterial wall is known to cause cross-linking of collagen molecules, which may lead to loss of elasticity and a subsequent increase in arterial stiffness [28]. In addition, low-grade inflammation and endothelial dysfunction, which are closely interrelated, may also explain the increased arterial stiffness related to MetS [5]. There is also evidence that the circulating levels of the carotenoid lycopene are inversely correlated with PWV in male subjects with MetS, suggesting that antioxidant status is associated with arterial stiffness [29]. Also low HDL and high LDL levels have been shown to be associated with aortic stiffness [30]. As MetS is a cluster of abnormalities, it may well be assumed that the above disorders have a synergistic deteriorating effect on arterial stiffness. In the present study, linear regression analysis indicated that lower HDL, higher triglycerides, and higher age were significantly associated with increased arterial stiffness.

We also found that mean stroke index was lower in the groups with MetS, in the absence and presence of hypertension. This may largely be related to the higher BMI and larger body surface area in these subjects. A similar finding has been reported in young adults with MetS [9]. In overweight subjects overactivity of the sympathetic nervous system may also elevate heart rate with a subsequent decrease in stroke volume and left ventricular ejection time [31]. However, impaired left ventricular systolic and diastolic function has previously been found in subjects with MetS [10]. Thus, diminished stroke index in these subjects may also be related to structural and functional alterations in the heart [9].

The hemodynamic changes associated with hypertension in the current study were increased aortic pulse pressure and systemic vascular resistance. Thus, hypertension already in its early stage is associated with elevated central pulse pressure.

In the present study, the influence on blood pressure in linear regression analysis on arterial stiffness was only of borderline significance ($p = 0.057$). Although hypertension has previously been without doubt associated with increased arterial stiffness [4,32], the subjects in the current study with hypertension only, who were also devoid of any other components of MetS, had corresponding PWV values to the normotensive controls. Since all participants were without antihypertensive medication and did not have a history of hypertension, and Cornell voltage product did not differ from normotensive controls, it seems likely that elevated blood pressure in the subjects with hypertension was neither prolonged nor severe. In addition, we want to stress that the subjects in the HT group did not have any other metabolic risk factors in addition to elevated blood pressure. Therefore, they did not represent the typical hypertensive patient characterized by clustering of cardiovascular risk factors [14]. Collectively these findings suggest that increased PWV is not an early feature of hypertension, and that especially the clustering of metabolic risk factors contributes to large arterial stiffening. This corresponds well to the European Society of Hypertension guideline, in which increased arterial stiffness is classified as subclinical target organ damage of hypertension, i.e. consequence of chronically elevated blood pressure [14]. However, recently increased arterial stiffness was suggested to precede the onset of hypertension in 60-year-old subjects [33].

Since impaired renal function is an acknowledged cause of large arterial stiffening [4,31], we estimated renal function by two different approaches (plasma creatinine and eGFR using the RULE formula). As there were no differences in the variables reflecting kidney function between the four groups, we conclude that renal dysfunction was not the explanation for the increased PWV in the groups with MetS.

Our study has several limitations. 1) The observational design does not allow conclusions about causal relationship. 2) The sizes of the groups were relatively small, and the results must be extrapolated with caution. Of note, in the whole population of 688 subjects who participated in the hemodynamic recordings, the prevalence of hypertension in the absence of other components of MetS was low (24 subjects, i.e. 3.5%). 3) The medications used by some of the subjects may have influenced the results. Five subjects were on statin, one subject used warfarin for phospholipid syndrome, and two used acetylsalicylic acid. However, additional analyses were made so that these subjects were excluded, and the results showing an association between NT-MetS and increased arterial stiffness remained the same. There were no significant differences in the use of female hormones between the groups, either. 4) The study groups were not homogenous for smoking habits. Even though smoking status was included in the linear regression analyses, differences in the cumulative dose of smoking between the groups may have influenced the results. 5) The presence of white-coat and masked hypertension may have influenced the results, as the presence of hypertension was determined on the average values of the tonometric recordings. For instance, in 2051 Finnish 45–74 year-old subjects the prevalence of masked hypertension was 14.2% [34], while that of white-coat hypertension was 15.6% [35]. 6) PWV was recorded using the whole-body impedance cardiography method, which has been reported to provide reliable and reproducible

information about PWV when compared with Doppler-ultrasound as the reference method [17]. Normal values for PWV in 799 individuals with ages ranging from 25 to 76 years have also been published, with the conclusion that that whole-body impedance cardiography was a practical method for the evaluation of arterial stiffness [36]. The signal of detected by impedance cardiography depends on the change in electrical resistivity induced by ejection of blood into the ascending aorta with each heartbeat [37], and there may be greater variability in individual PWV values recorded with this technique than with non-invasive devices employing carotid and femoral artery sensors. However, the present PWV values represent average recordings from every cardiac cycle during a 3-min period, in contrast to the ultrasound and tonometric devices, where the recordings are typically derived from 10 consecutive heartbeats. The higher number of PWV recordings can be considered to increase the reliability of the whole-body impedance cardiography data. Nevertheless, hard end-point data concerning the prognostic influence of arterial stiffness measured using whole-body impedance cardiography are still lacking.

In the current study the criteria of Alberti et al. from year 2009 [3] were used for the definition of MetS (except for the criterion of blood pressure). These criteria are relatively tight, and for example in the widely used definition by National Cholesterol Education Program from year 2004, the cut point for waist circumference is ≥102/88 cm (men/women), while in this study the cut point was ≥94/80 cm. When using the criteria of Alberti for defining MetS [3], the prevalence of the disorder is exceedingly high in the Western world. Very often these patients have no medical treatment, especially if they don't have hypertension. MetS is also an expanding disorder worldwide, and a significant risk factor for cardiovascular diseases [2,3]. For better prevention and appropriate treatment, it is important to understand the mechanisms leading to increased risk of cardiovascular disease in MetS.

In conclusion, in the current study we found that central arterial PWV is increased in MetS even in the absence of hypertension. Since increased arterial stiffness is known to be a strong predictor of cardiovascular events [4], the findings of the present study, especially if confirmed in larger prospective studies, emphasize the importance of the prevention and treatment of MetS.

Author contributions

P.K. participated in data collection, reviewed and analyzed the data, and wrote and revised the manuscript. A.J.T participated in data collection and analysis, and contributed to the discussion. A.M.T participated in data collection, contributed to the discussion and reviewed the manuscript. M.H.L participated in data collection and in setting up the database. J.V. constructed the recording system and signal capturing programs and set up the database. M.K. and T.K. contributed to the setting up of recording system and discussion. O.N. participated in the analysis of the laboratory samples and contributed to the discussion. J.T.M contributed to the discussion and reviewed the manuscript. I.H.P contributed to the data collection, setting up of the recording system, signal capturing programs and database, design and management of the research discussion, and reviewed and edited the manuscript. He is also the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Conflict of interest

The authors declare no conflict of interests.

REFERENCES

[1] Ford ES, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among us adults: findings from the Third National Health and Nutrition Examination Survey. JAMA 2002;287:356–9.
[2] Mottillo S, Filion KB, Genest J, et al. The metabolic syndrome and cardiovascular risk a systematic review and meta-analysis. J Am Coll Cardiol 2010;56:1113–32.
[3] Alberti KGMM, Eckel RH, Grundy SM, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation 2009;120:1640–5.
[4] Laurent S, Cockcroft J, Van Bortel L, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur Heart J 2006;27:2588–605.
[5] Stehouwer CDA, Henry RMA, Ferreira I. Arterial stiffness in diabetes and the metabolic syndrome: a pathway to cardiovascular disease. Diabetologia 2008;51:527–39.
[6] Laurent S, Katsahian S, Fassot C, et al. Aortic stiffness is an independent predictor of fatal stroke in essential hypertension. Stroke 2003;34:1203–6.
[7] Willum Hansen T. Prognostic value of aortic pulse wave velocity as index of arterial stiffness in the general population. Circulation 2006;113:664–70.
[8] Emre A, Oz D, Yesilcimen K, et al. Impact of the metabolic syndrome on aortic pulse pressure and ascending aortic pulsatility in patients with angiographically normal coronary arteries. Canadian Journal of Cardiology 2009;25:411–4.

[9] Koivistoinen T, Aatola H, Hutri-Kähönen N, et al. Systemic hemodynamics in young adults with the metabolic syndrome: the Cardiovascular Risk in Young Finns Study. Ann Med 2010;42:612–21.
[10] Gong HP, Tan HW, Fang NN, et al. Impaired left ventricular systolic and diastolic function in patients with metabolic syndrome as assessed by strain and strain rate imaging. Diabetes Res Clin Pract 2009;83:300–7.
[11] Czernichow S, Bertrais S, Blacher J, et al. Metabolic syndrome in relation to structure and function of large arteries: a predominant effect of blood pressurea report from the SU.VI.MAX. vascular study. American Journal of Hypertension 2005;18:1154–60.
[12] Sipilä K, Koivistoinen T, Moilanen L, et al. Metabolic syndrome and arterial stiffness: the Health 2000 Survey. Metabolism 2007;56:320–6.
[13] Chen G, McAlister FA, Walker RL, et al. Cardiovascular outcomes in framingham participants with diabetes: the importance of blood pressure. Hypertension 2011;57:891–7.
[14] Task force members of European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). 2007 Guidelines for the management of arterial hypertension. Journal of Hypertension 2007;25:1105–87.
[15] Tahvanainen A, Koskela J, Tikkakoski A, et al. Analysis of cardiovascular responses to passive head-up tilt using continuous pulse wave analysis and impedance cardiography. Scand J Clin Lab Invest 2009;69:128–37.
[16] Chen CH, Nevo E, Fetics B, et al. Estimation of central aortic pressure waveform by mathematical transformation of radial tonometry pressure. Validation of generalized transfer function. Circulation 1997;95:1827–36.
[17] Kööbi T, Kähönen M, Iivainen T, et al. Simultaneous non-invasive assessment of arterial stiffness and haemodynamics — a validation study. Clin Physiol Funct Imaging 2003;23:31–6.
[18] Kööbi T, Kaukinen S, Ahola T, et al. Non-invasive measurement of cardiac output: whole-body impedance cardiography in simultaneous comparison with thermodilution and direct oxygen fick methods. Intensive Care Med 1997;23:1132–7.
[19] Tahvanainen AM, Tikkakoski AJ, Leskinen MH, et al. Supine and upright haemodynamic effects of sublingual nitroglycerin and inhaled salbutamol: a double-blind, placebo-controlled, randomized study. J Hypertens 2012;30: 297–306.
[20] Kööbi T, Kaukinen S, Turjanmaa VM, et al. Whole-body impedance cardiography in the measurement of cardiac output. Crit Care Med 1997;25:779–85.
[21] Rule AD, Larson TS, Bergstralh EJ, et al. Using serum creatinine to estimate glomerular filtration rate: accuracy in good health and in chronic kidney disease. Ann Intern Med 2004;141:929–37.
[22] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499–502.
[23] Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–9.
[24] Schillaci G, Pucci G, Pirro M, et al. Combined effects of office and 24-h blood pressure on aortic stiffness in human hypertension. J Hypertens 2011;29:869–75.
[25] Laurent S. Surrogate measures of arterial stiffness: do they have additive predictive value or are they only surrogates of a surrogate? Hypertension 2006;47:325–6.
[26] Yki-Järvinen H, Westerbacka J. Insulin resistance, arterial stiffness and wave reflection. Adv Cardiol 2007;44:252–60.
[27] Brillante DG, O'Sullivan AJ, Howes LG. Arterial stiffness in insulin resistance: the role of nitric oxide and angiotensin ii receptors. Vasc Health Risk Manag 2009;5:73–8.
[28] Aronson D. Cross-linking of glycated collagen in the pathogenesis of arterial and myocardial stiffening of aging and diabetes. J Hypertens 2003;21:3–12.
[29] Yeo HY, Kim OY, Lim HH, et al. Association of serum lycopene and brachial-ankle pulse wave velocity with metabolic syndrome. Metabolism 2011;60:537–43.
[30] Wang F, Ye P, Luo L, et al. Association of serum lipids with arterial stiffness in a population-based study in Beijing. Eur J Clin Invest 2011;41:929–36.
[31] Dorresteijn JA, Visseren FL, Spiering W. Mechanisms linking obesity to hypertension. Obes Rev 2012;13:17–26.
[32] Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. J Am Coll Cardiol 2010;55:1318–27.
[33] Kaess BM, Rong J, Larson MG, et al. Aortic stiffness, blood pressure progression, and incident hypertension. JAMA 2012;308:875–81.
[34] Niiranen TJ, Jula AM, Kantola IM, et al. Comparison of agreement between clinic and home-measured blood pressure in the Finnish population: the Finn-HOME Study. J Hypertens 2006;24:1549–55.
[35] Niiranen TJ, Jula AM, Kantola IM, et al. Prevalence and determinants of isolated clinic hypertension in the Finnish population: the Finn-HOME study. J Hypertens 2006;24: 463–70.
[36] Koivistoinen T, Kööbi T, Jula A, et al. Pulse wave velocity reference values in healthy adults aged 26–75 years. Clin Physiol Funct Imaging 2007;27:191–6.
[37] Newman DG, Callister R. The non-invasive assessment of stroke volume and cardiac output by impedance cardiography: a review. Aviat Space Environ Med 1999;70: 780–9.

PUBLICATION II

Increased Cardiac Workload in the Upright Posture in Men: Noninvasive Hemodynamics in Men Versus Women

Kangas P, Tahvanainen A, Tikkakoski A, Koskela J, Uitto M, Viik J, Kähönen M, Kööbi T, Mustonen J and Pörsti I

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ORIGINAL RESEARCH

Increased Cardiac Workload in the Upright Posture in Men: Noninvasive Hemodynamics in Men Versus Women

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Background—Men and women differ in the risk of cardiovascular disease, but the underlying mechanisms are not completely understood. We examined possible sex-related differences in supine and upright cardiovascular regulation.

Methods and Results—Hemodynamics were recorded from 167 men and 167 women of matching age (≈45 years) and body mass index (≈26.5) during passive head-up tilt. None had diabetes mellitus or cardiovascular disease other than hypertension or used antihypertensive medication. Whole-body impedance cardiography, tonometric radial blood pressure, and heart rate variability were analyzed. Results were adjusted for height, smoking, alcohol intake, mean arterial pressure, plasma lipids, and glucose. Supine hemodynamic differences were minor: Men had lower heart rate (−4%) and higher stroke index (+7.5%) than women ($P<0.05$ for both). Upright systemic vascular resistance was lower (−10%), but stroke index (+15%), cardiac index (+16%), and left cardiac work were clearly higher (+20%) in men than in women ($P<0.001$ for all). Corresponding results were observed in a subgroup of men and postmenopausal women (n=76, aged >55 years). Heart rate variability analyses showed higher low:high frequency ratios in supine ($P<0.001$) and upright ($P=0.003$) positions in men.

Conclusions—The foremost difference in cardiovascular regulation between sexes was higher upright hemodynamic workload for the heart in men, a finding not explained by known cardiovascular risk factors or hormonal differences before menopause. Heart rate variability analyses indicated higher sympathovagal balance in men regardless of body position. The deviations in upright hemodynamics could play a role in the differences in cardiovascular risk between men and women.

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Key Words: cardiac output • hemodynamics • sex-specific

The lifetime risk of cardiovascular disease (CVD) is close to 50% for persons aged 30 years without known CVD,[1] and it is the most common cause of death for both men and women.[2] The risk and the outcome of CVD, however, differ between sexes; particularly before middle age, morbidity and mortality caused by CVDs are higher in men.[3] In the Framingham Heart Study, the lifetime risk of coronary heart disease at age 40 years was about 49% for men and 32% for women.[4] These differences in CVD risk between sexes have been attributed to hormonal differences and to the fact that most of the risk factors are more favorable for women.[5]

Distinct differences in cardiovascular regulation have been characterized between sexes. In early adulthood, pulse pressure is higher in men; however, at older ages, pulse pressure becomes higher in women.[6] The myocardial remodeling process in response to aging, pressure and volume overload, and myocardial infarction appears to be more favorable in women than in men.[7] In patients with ischemic heart disease, women may be more prone to myocardial ischemia in response to mental stress, whereas men may show higher increases in blood pressure (BP).[8] Taken together, these findings raise the question of whether further differences in cardiovascular regulation exist between men and women that could influence the hemodynamic load of the heart and subsequently affect the number of cardiovascular end points.

Hypertension is more prevalent in young men than in young women, but after menopause, the risk of hypertension

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is significantly increased in women.[9] The lifetime burden of hypertension on the prevalence of CVDs is substantial.[1] In a survey in Finland performed in 2007, only 37% of the drug-treated hypertensive patients had normal BP (<140/90 mm Hg).[10] The suboptimal control of hypertension raises the question of whether individual characteristics and possible sex-related differences in the regulation of hemodynamics should be more carefully taken into account in the treatment of elevated BP.

Although a major share of the active life is spent in the upright position, most reports on sex-related differences in hemodynamics have focused on resting conditions.[11–14] Only few studies have compared upright hemodynamics between sexes.[15–17] Similar upright increase in peripheral arterial resistance and decrease in cardiac output was reported in 9 men versus 8 women,[15] lower upright splanchnic vasoconstriction but corresponding decrease in cardiac output was reported in 14 women versus 16 men,[17] and corresponding upright increase in forearm vascular resistance was observed in 48 women versus 41 men.[16] In most of the previous studies, the control of cardiac autonomic tone between sexes was evaluated by the use of heart rate variability (HRV). In the majority of these reports, sympathovagal balance was reported to be higher in men, regardless of whether the participants were in supine, sitting, or standing position.[16,18–20]

The previous studies comparing hemodynamics between sexes were performed with small numbers of participants, and the groups have not been uniform in age and body mass index (BMI).[8,15–17] Therapies based on individual sex-related characteristics could improve outcomes in the treatment of CVDs like hypertension,[21] and personalized approaches in the treatment of medical conditions are keenly sought.[22–24] Because only limited information exists about the upright regulation of the cardiovascular system between sexes, we compared the hemodynamic responses to passive head-up tilt in 167 men versus 167 women with corresponding age and BMI. The main findings were verified in a larger group composed of an additional 313 men and 231 women. A total of 480 men and 398 women were examined.

Methods

Study Participants

All participants were in an ongoing study of hemodynamics in volunteers, with the primary aim of examining the hemodynamic changes in primary and secondary hypertension and normotensive control participants in supine and upright positions (DYNAMIC study; ClinicalTrials.gov identifier NCT01742702). Participants were enrolled from the patients treated at Tampere University Hospital. An announcement of recruitment was distributed at Tampere University Hospital, the University of Tampere, offices of local occupational health care providers, and Varala Sports Institute, and 2 announcements were published in a newspaper. Those who agreed to participate were recruited in the order in which their contact information was available to the research nurses.

By the time of the present analysis, 878 participants (480 men and 398 women) had undergone hemodynamic measurements; of those, 615 were without medications with direct influence on cardiovascular function. The remaining participants included medicated patients with hypertension, chronic renal disease, diabetes mellitus, and atherosclerotic vascular disease. All participants were interviewed to record lifestyle habits, family history, and medical history. Clinical examination of cardiovascular status and routine laboratory tests were performed.

For the main analysis of the present study, participants with antihypertensive medication, coronary heart disease, or previous myocardial infarction, diagnosis of diabetes mellitus, cardiac insufficiency, atherosclerotic vascular disease, renal disease, or cerebrovascular disease were excluded. In addition, all participants using medications with potential influence on hemodynamics (eg, α_1-adrenoceptor blockers for prostate problems, β_2-adrenoceptor agonists, digoxin, and topical β-blockers for glaucoma) were excluded.[25] To avoid confounding caused by differences in age and body weight,[26–29] the following inclusion protocol was applied: A female participant was chosen, followed by selection of a male participant with corresponding age (difference in age ≤3 years) and BMI (difference ≤1.5 units). Using this approach, 334 participants aged 21 to 67 years were included (Table 1).

Overall, 114 (34%) of the 334 persons used some medication (Table 2). Forty female participants used systemic estrogen, progestin, or their combination (for contraception or hormone replacement therapy), and 1 participant used tibolone. Eight participants were taking statins, and 11 euthyroid participants were on a stable dose of thyroid hormone. Other medications (acetylsalicylic acid, drugs for mental problems, antihistamines, proton pump inhibitors, intranasal or inhaled corticosteroid for asthma or allergy) were used by the study population. One participant who was physically well and symptomless was treated with warfarin for antiphospholipid syndrome.

Additional analyses were performed in groups in which (1) all participants using systemic female hormones or statins were excluded, (2) all participants were aged ≥55 years, and (3) all 878 participants who participated in the recording of hemodynamics were included. The participants gave written informed consent, and the study was approved by the ethics committee of Tampere University Hospital (study code R06086M).

Table 1. Clinical and Metabolic Characteristics in the Study Groups

	Men (n=167)	Women (n=167)	*P* Value
Age, y	45±12	45±11	0.967
Body mass index, kg/m^2	26.5±3.7	26.6±3.8	0.898
Height, cm	180±6	166±6	<0.001
Weight, kg	88±12	73±11	<0.001
Systolic blood pressure, mm Hg	132±17	127±18	0.006
Diastolic blood pressure, mm Hg	75±12	72±12	0.026
Smoking			
Current	51 (30.5)	46 (27.5)	0.547
Previous	22 (13.2)	17 (10.2)	0.394
Never	94 (56.3)	10 (62.3)	0.265
Alcohol, standard drinks per week	4 (1–10)	2 (0–4)	<0.001
Creatinine, μmol/L	82±12	65±9	<0.001
Cystatin-C, mg/L	0.87±0.14	0.80±0.14	<0.001
eGFR, mL/min per 1.73 m^2	99.2±14.5	99.3±14.0	0.956
Total cholesterol, mmol/L	5.1±1.0	5.1±1.0	0.804
LDL cholesterol, mmol/L	3.1±1.0	2.8±0.9	0.021
HDL cholesterol, mmol/L	1.4±0.3	1.8±0.4	<0.001
Triglycerides, mmol/L	1.3 (0.7–1.5)	1.1 (0.7–1.3)	0.007
Fasting plasma glucose, mmol/L	5.5±0.5	5.3±0.5	<0.001
Cornell voltage product in ECG, ms×mm	1638±615	1621±509	0.779

Values are means±SD except for smoking, which shows the number of participants and percentages, and for alcohol intake and triglycerides, which are shown as medians (lower and upper quartiles) due to skewed distribution. eGFR indicates estimated glomerulus filtration rate[30]; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 2. Medications Used Regularly by the Study Participants (Number of Participants With Each Type of Medication)

Medication	Men (n=167)	Women (n=167)
Acetylsalicylic acid	3	0
Acyclovir	0	1
Allopurinol	0	1
Amitriptyline	0	1
Amoxicillin	0	1
Antidepressant (SSRI or SNRI)	3	12
Antihistamine	2	5
Doxycycline (low dose)	1	0
Ezetimibe	1	0
Female hormones		
Systemic (including tibolone)	0	41
Topical	0	3
Glucosamine	3	3
Intranasal or inhaled corticosteroid	2	6
Isotretinoin	0	1
Letrozole	0	1
Levomepromazine	0	1
Levonorgestrel via intrauterine device	0	16
Liothyronine	0	1
Lymecycline	1	0
Mefloquine	0	1
Melatonin	1	1
Mepacrine	1	0
Nonsteroidal anti-inflammatory drug	1	3
Oxybutynine	0	1
Pramipexole	0	1
Pregabalin	1	1
Proton pump inhibitor	3	4
Quetiapine	0	1
Statin	7	1
Thyroxine	0	10
Valproate	0	1
Vitamin D supplementation	6	11
Warfarin	1	0

SNRI indicates serotonin–norepinephrine reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor.

Hemodynamic Measurements

Hemodynamic measurements were performed in a temperature-controlled laboratory. The participants were advised to refrain from using caffeine-containing products, smoking, and eating heavy meals for at least 4 hours and from drinking alcohol for at least 24 hours prior to the recording. While the participants were resting supine on a tilt table, the electrodes for impedance cardiography were placed on the body surface, a tonometric sensor for radial artery BP on the left wrist (Colin BP-508T; Colin Medical Instruments Corp) and an oscillometric brachial cuff for BP calibration on the right upper arm.[25,26,31] The left arm with the tonometric sensor was abducted to 90° in a support that held the arm at the level of the heart in supine and upright positions. Estimation of central aortic BP was performed by mathematical transformation of radial tonometry pressure with the SphygmoCor pulse wave monitoring system (SpygmoCor PWMx; Atcor Medical) using a validated transfer function.[32]

A whole-body impedance cardiography device (CircMon; JR Medical Ltd) that records changes in body electrical impedance during cardiac cycles was used to determine heart rate, stroke volume, and cardiac output.[25,33–35] The description of the method and electrode configuration was reported previously.[25,33–35] The values were normalized to body surface area and expressed as cardiac index, stroke index, systemic vascular resistance index (SVRI), and left cardiac work index (LCWI). SVRI was calculated from the radial BP signal and cardiac index measured by the CircMon device. LCWI (in kg×m/min per m^2) was calculated using following formula: 0.0143×(mean aortic pressure–pulmonary artery occlusion pressure)×cardiac index. Pulmonary artery occlusion pressure was assumed to be 6 mm Hg (normal), and 0.0143 is the conversion factor of pressure from millimeters of mercury to centimeters of water, volume to density of blood (in kg/L), and centimeters to meters.[36,37] The stroke volume and cardiac output measured using CircMon whole-body impedance cardiography agree with values measured using 3-dimensional ultrasound and the thermodilution method, respectively, and agreement with the latter has been shown in both supine and upright positions.[34,36,38]

An introductory head-up tilt was performed before the recordings. The actual measurement consisted of 3 consecutive 5-minute periods with continuous capture of data: 5 minutes supine, 5 minutes of passive head-up tilt to 60°, and 5 minutes supine. The repeatability and reproducibility of the supine and upright measurements has been shown to be good.[31]

Evaluation of Cardiac Autonomic Tone

HRV analysis from electrocardiograms was used to assess cardiac autonomic tone. The electrocardiograms were recorded by the CircMon device at a 200-Hz sampling rate, and data were analyzed using Matlab software (MathWorks Inc). Normal R-R intervals were recognized, and a beat was considered ectopic if the interval differed >20% from the previous values. The artifacts were processed using the cubic spline interpolation method.[39] Because the data were collected from short-term recordings, the frequency domain method was applied.[40] The following variables were calculated from the recordings in supine (0–5 minutes) and upright (5–10 minutes) positions using the fast Fourier transformation method: (1) total power, (2) power in the low-frequency (LF) range (0.04–0.15 Hz), (3) power in the high-frequency (HF) range (0.15–0.40 Hz), and (4) LF/HF ratio.[40]

Laboratory Tests

Blood samples were obtained after about 12 hours of fasting. Plasma glucose, creatinine, cystatin C, triglyceride, and total and high- and low-density lipoprotein cholesterol concentrations were determined using the Cobas Integra 700/800 or Cobas 6000, module c501 (F. Hoffmann-La Roche Ltd), and blood cell count was performed by an ADVIA 120 or 2120 system (Siemens Healthcare GmbH). In some participants, low-density lipoprotein was calculated using the Friedewald formula.[41] Creatinine- and cystatin C–based estimated glomerular filtration rate was calculated using the Chronic Kidney Disease Epidemiology Collaboration formula.[30] A standard 12-lead electrocardiogram was recorded, and left ventricular mass was evaluated using the Cornell voltage QRS duration product.[21] All laboratory values were missing from 3 participants, and low-density lipoprotein cholesterol values were missing from an additional 2 participants.

Statistical Analyses

The characteristics of men versus women were compared using an independent samples *t* test, and smoking habits (current, previous and never) were compared using the Pearson chi-square test. Hemodynamic differences in supine and upright positions were examined using ANOVA for repeated measures, and the changes in hemodynamic values from supine to upright position (average values of last 3 minutes before and last 3 minutes during the head-up tilt[25]) and HRV differences were analyzed using an independent samples *t* test and ANOVA with possible confounding factors as covariates. Average BP values during the last 3 minutes of the first supine period were applied for the clinical characterization of the BP of participants (Table 1).

The analyses were adjusted for smoking habits, alcohol intake (standard doses per week), fasting plasma glucose, triglycerides, high- and low-density lipoprotein cholesterol, height, and mean arterial pressure, as appropriate (the adjusting variable was not used if it was included in the formula of the variable of interest). HRV analyses were also adjusted for heart rate.[42] In the adjusted analyses, current smoking as cigarettes per day was applied as a continuous variable, whereas in the tables, the smoking habits were described as current, previous, and never smoker. Due to missing data among the main group of interest (334 participants), the number of participants in the adjusted analyses ranged from 311 to 324, with at least 153 men and 158 women in all analyses.

The skewed distributions of triglycerides, total power, LF power, HF power, and LF/HF ratio were logarithmically transformed before statistical analyses. Variables with normal distribution were reported as means and standard deviations (SD) or 95% CIs of the means; skewed distributions were reported as medians, lower and upper quartiles, and range; and categorical variables were reported as numbers of participants and percentages. All testing was 2-sided, and *P*

values <0.05 were considered statistically significant. The data were analyzed using SPSS 17.0 (IBM Corp).

Results

Study Population

The general characteristics of the study participants are presented in Table 1. Because of the inclusion protocol, men and women were well matched for age and BMI. In addition, there were no significant differences in smoking status, estimated glomerular filtration rate,[30] total cholesterol, and Cornell voltage product[21] between the 167 men and 167 women. Men, however, were characterized by somewhat higher systolic and diastolic BP, alcohol intake, and higher fasting plasma creatinine, cystatin C, low-density lipoprotein cholesterol, triglyceride, and glucose concentration and lower high-density lipoprotein cholesterol concentration than women.

Supine and Upright Hemodynamics

Men had higher supine mean arterial pressure, stroke index, and LCWI and lower heart rate than women in unadjusted analyses (Figure 1A–1C and 1E). Supine cardiac index and SVRI did not differ between sexes (Figure 1D and 1F). After adjusting for height, smoking habits, alcohol intake, mean arterial pressure, low- and high-density lipoprotein cholesterol, triglycerides, and glucose, only the differences in supine heart rate and stroke index for men and women remained significant (Figure 1B and 1C).

During passive head-up tilt to 60°, men had higher mean arterial pressure, stroke index, cardiac index, and LCWI (Figure 1A, 1C, 1D, and 1E) and lower SVRI (Figure 1F) than women in unadjusted analyses. Upright heart rate did not differ between men and women (Figure 1B). In adjusted analyses, with the exception of mean arterial pressure, all of the above differences in upright hemodynamics for men and women remained significant (Figure 1A and 1C–1F).

The magnitude of the changes in hemodynamic variables in response to upright posture was also analyzed (see Methods). Unadjusted analyses showed a greater increase in heart rate ($P<0.001$); less decrease in stroke index, cardiac index, and LCWI ($P<0.05$ for all); and less increase in SVRI ($P<0.001$) in response to head-up tilt in men than in women. A similar increase in mean arterial pressure was observed in both sexes ($P=0.812$). In adjusted analyses, the differences in the upright changes in cardiac index, LCWI, and SVRI remained significant ($P<0.01$ for all), whereas the changes in heart rate and stroke index were no longer different between men and women ($P=0.600$ and $P=0.694$, respectively).

HRV in Supine and Upright Positions

In supine and upright positions, there were no significant differences in total power and in power in the LF range in unadjusted analyses (Figure 2A and 2B), whereas men had lower power in the HF range and higher LF/HF ratios than women (Figure 2C and 2D). In adjusted analyses, the upright differences in the HF component were no longer significant (Figure 2C), but supine HF power was lower and both supine and upright LF/HF ratios remained higher in men than in women (Figure 2C and 2D)

Supine and Upright Hemodynamics in Additional Analyses

Three additional analyses of hemodynamic changes in response to head-up tilt were performed: (1) All participants using systemic female hormones and statins were excluded (n=285) (Figure 3); (2) all included participants were aged ≥55 years, and none used systemic female hormones (n=76) (Figure 4); (3) all participants who participated in hemodynamic recordings were included (n=878) (Figure 5). The last group was composed of 615 participants who were without medications with direct influence on cardiovascular function and 263 medicated patients with hypertension, chronic renal disease, diabetes mellitus, or atherosclerotic vascular disease. The outcome of all of these additional analyses was that the sex-related differences in upright hemodynamics were very similar to those observed in the 167 men and 167 women with matching age and BMI. The results clearly showed a higher upright hemodynamic workload for the heart in men (Figures 3 through 5).

Discussion

Several previous studies examined the differences in cardiovascular function between sexes,[6–8,16,18–20,43] but only a few reports compared the upright hemodynamics between sexes.[15–17,44,45] Although head-up tilt for 5 minutes is a short period of observation, we found that upright hemodynamic adaptation differed between men and women. In men, cardiac index and left cardiac work decreased less in the upright position than in women, whereas men also showed lower upright increase in systemic vascular resistance than women. The net outcome was that the upright hemodynamic workload of the heart was higher in men than in women.

To gain functional insight about the cardiovascular system, we applied a head-up tilt protocol to induce changes in autonomic tone, cardiac function, and peripheral arterial resistance in the study participants.[25,26,31,35] Because age and excess body weight have major influences on

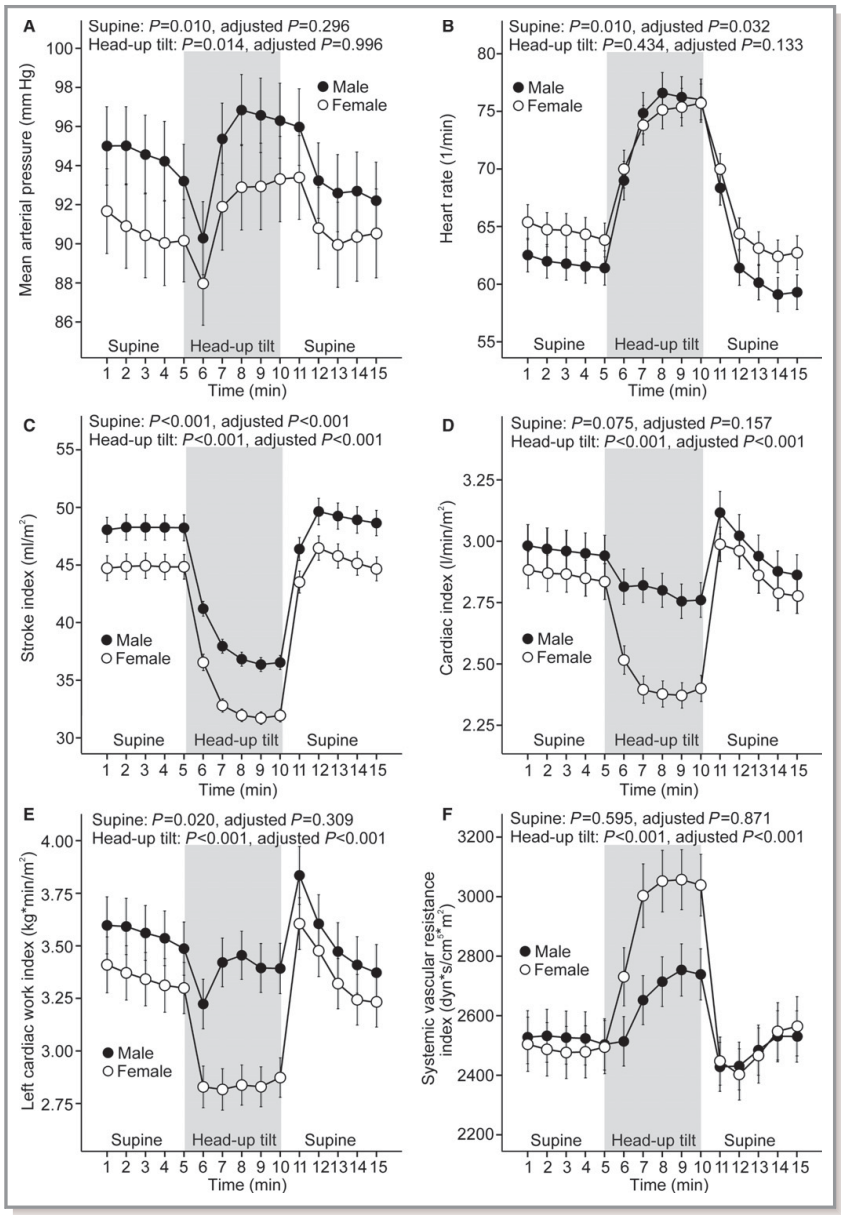

Figure 1. Line graphs show mean arterial pressure (A), heart rate (B), stroke index (C), cardiac index (D), left cardiac work index (E), and systemic vascular resistance index (F) in 167 men and 167 women during supine position and passive head-up tilt (means and 95% CIs of the mean). *P* values denote differences between sexes in unadjusted analysis and in analyses adjusted for low- and high-density lipoprotein cholesterol, triglycerides, glucose, mean arterial pressure, smoking habits, alcohol intake, and height.

hemodynamics,[26–29] we initially performed analyses in 167 men and 167 women with corresponding age and BMI; however, the findings showing higher cardiac workload during head-up tilt in men remained similar in participants not using statins or hormone replacement therapy, in participants aged ≥55 years, and in a large group composed of 878 participants.

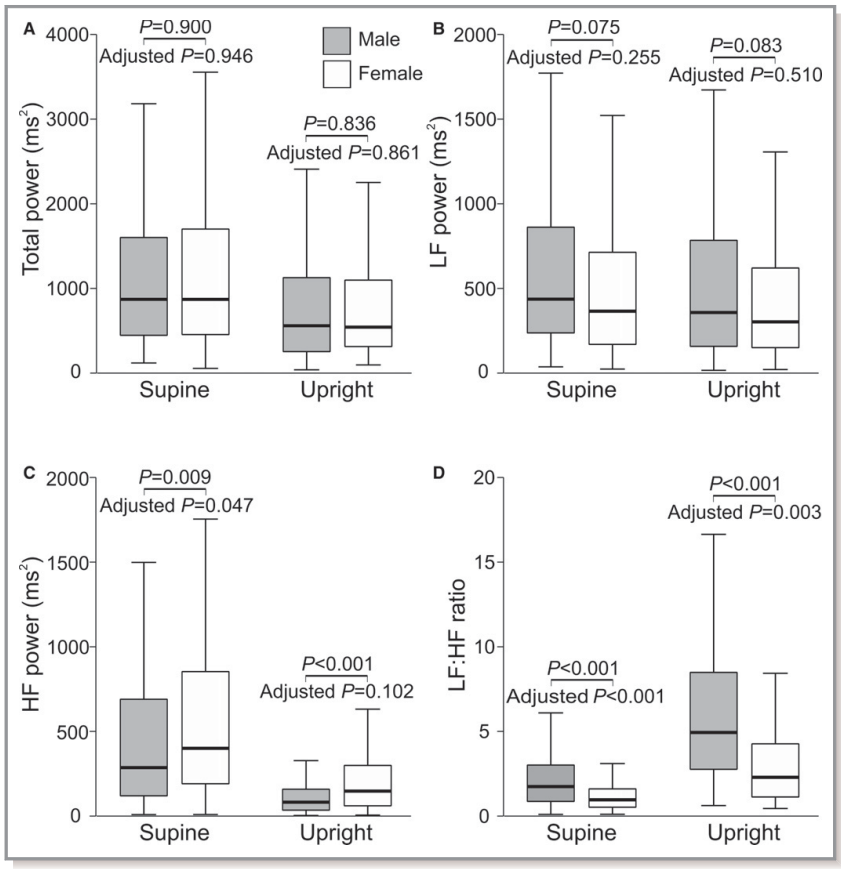

Figure 2. Box plots of heart rate variability in men and women during 5 minutes in supine and upright positions: total power (A), LF power (B), HF power (C), and LF/HF ratio (D) (median [line inside box], 25th to 75th percentile [box], and range [whiskers]; outliers were excluded from the figure but were included in the statistics). *P* values denote differences between sexes in unadjusted analysis and in analyses adjusted for low- and high-density lipoprotein cholesterol, triglycerides, glucose, heart rate,[42] mean arterial pressure, smoking habits, alcohol intake, and height. HR indicates high frequency; LF, low frequency.

We examined cardiac autonomic tone using the frequency domain analyses of HRV.[16,18–20] The HF component reflects cardiac parasympathetic activity,[46–48] whereas the LF component predominantly reflects sympathetic activity, although it contains parasympathetic contributions.[40,46,48] The LF/HF ratio is a marker of sympathovagal balance,[40,46] but this matter remains controversial, and conclusions should be drawn with caution.[47,48] In the present study, the frequency domain analysis of HRV showed higher LF/HF ratios in men both supine and upright, suggesting increased sympathovagal balance. The differences in cardiac autonomic tone largely resulted from decreased HF power, namely, lower cardiac parasympathetic tone in men than women. There has been controversy about whether the indices of HRV are higher in men or in women. In participants aged <30 years, the time domain measures of HRV were higher in men than in women[49]; however, most published reports on frequency domain measures of HRV have found that the values of LF power are higher in men,[18–20,50] whereas the values of HF power are higher in women.[16,19,50] Higher LF/HF ratios during supine and standing positions have been reported in 156 men versus 206 women,[20] and the majority of reports have suggested that sympathovagal balance is higher in men.[15,16,18–20,43,50–52] The present findings stress the role of differences in cardiac parasympathetic regulation between sexes.

Higher sympathovagal balance in men agrees with higher upright cardiac workload but raises questions about lower

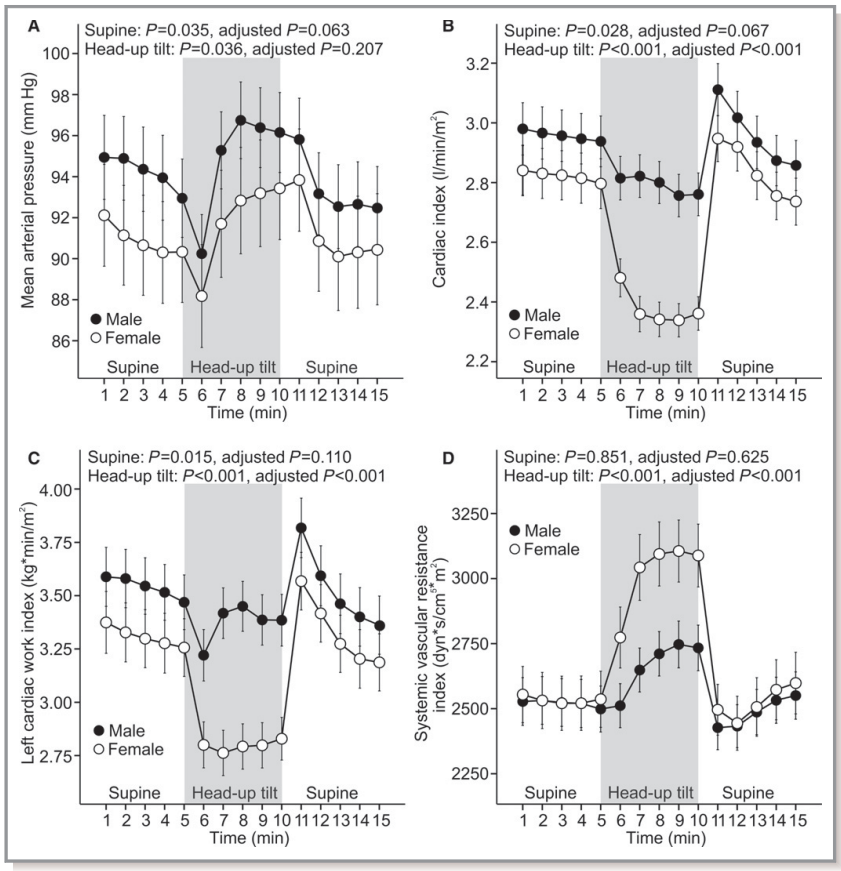

Figure 3. Participants using statins or hormone replacement therapy were excluded. Line graphs show mean arterial pressure (A), cardiac index (B), left cardiac work index (C), and systemic vascular resistance index (D) in 160 men and 125 women during supine position and passive head-up tilt (means and 95% CIs of the mean). *P* values denote differences between sexes in unadjusted analysis and in analyses adjusted for low- and high-density lipoprotein cholesterol, triglycerides, glucose, mean arterial pressure, smoking habits, alcohol intake, age, and body mass index.

upright SVRI in men. Both arteries and veins are innervated by autonomic nerves. Autonomic innervation of the resistance arteries consists of sympathetic and sensory–motor nerves, whereas parasympathetic vascular innervation is nonexistent or very limited.[53] If higher sympathovagal balance would be transmitted to the resistance arteries similarly in men and women, higher cardiac output would result in an excessive upright rise of arterial pressure in men, with subsequent activation of baroreceptors.[54] This would limit the increase in vascular resistance, and the comparison of baroreflexes between men and women is a subject for further studies. Previously, baroreceptor sensitivity during orthostasis has been reported to be higher in men than in women.[15] Although sympathetic nerves are predominantly vasoconstrictor nerves, they can induce vasodilation in skeletal muscles,[53] and the net effect of sympathetic stimulation on peripheral resistance depends on the circulatory balance between skeletal muscles and other organs. Studies of muscle sympathetic nerve activity have shown that the association of sympathetic tone with hemodynamics is not straightforward.[14,15] In younger men and women (aged <40 years), no relationship was found between muscle sympathetic nerve activity and BP, although a clear relationship was observed in older participants (aged ≥40 years).[14] In younger men, muscle sympathetic nerve activity was positively related to peripheral vascular resistance and negatively related to cardiac output, whereas in younger women, no relationship was found between muscle sympathetic nerve activity and peripheral vascular resistance

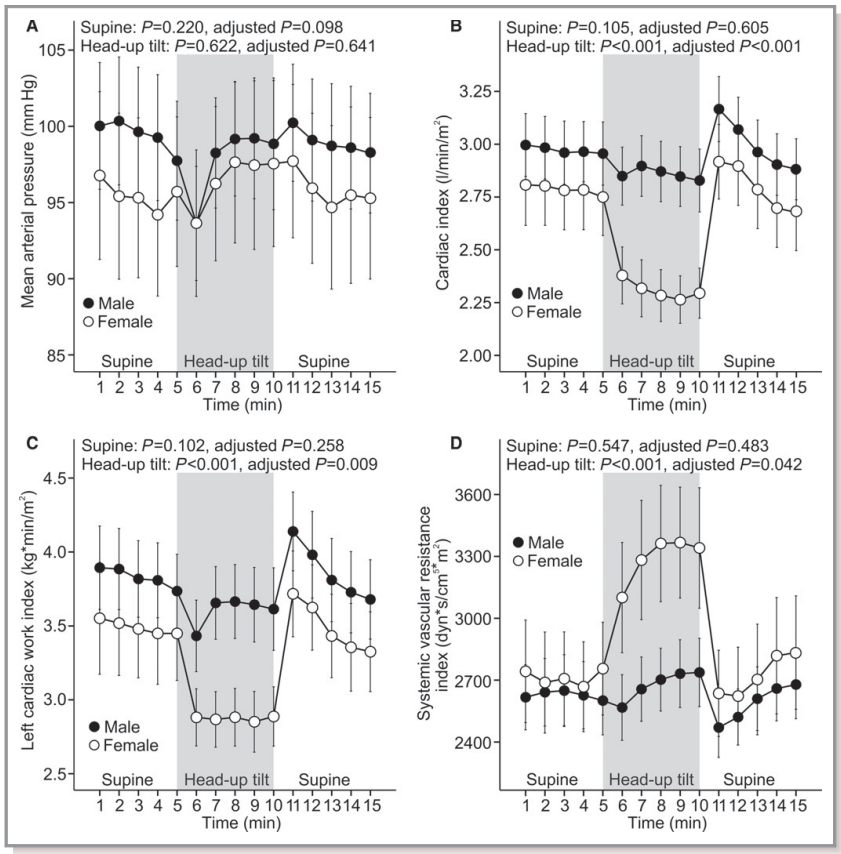

Figure 4. All included participants aged ≥55 years. Line graphs show mean arterial pressure (A), cardiac index (B), left cardiac work index (C), and systemic vascular resistance index (D) in 43 men and 33 women (no hormone replacement therapy in use) during supine position and passive head-up tilt (means and 95% CIs of the mean). *P* values denote differences between sexes in unadjusted analysis and in analyses adjusted for low- and high-density lipoprotein cholesterol, triglycerides, glucose, mean arterial pressure, smoking habits, alcohol intake, age, and body mass index.

or cardiac output.[14] Finally, autonomic innervation is not the sole regulator of resistance arterial tone; endothelium-dependent mechanisms, myogenic regulation, and humoral factors also contribute to vascular resistance.[53,55]

The present findings provide another putative explanation for the higher risk of cardiac events in men. Coronary heart disease and myocardial infarction account for a third to half of the initial presentations of CVD, and male sex particularly predisposes to myocardial infarction in those aged <60 years.[56] The more unfavorable upright hemodynamic load of the heart could lead to earlier clinical manifestation of coronary heart disease in men than in women. Of note, the coronary findings in those who experienced sudden death also differ between sexes. In 442 cases of sudden coronary death, the prevalence of acute thrombosis and plaque rupture was higher in men than in women (53% versus 46% and 71% versus 33%, respectively), whereas plaque erosion as a cause of thrombosis was less frequent in men than in women (24% versus 58%).[57] Finally, even the prognosis of heart failure has been reported to be worse in men than in women.[58,59] This is not explained by the etiology of the heart failure or by differences in left ventricular ejection fraction.[60] Differences in the upright hemodynamic load of the heart could partially explain some of the aforementioned differences in cardiovascular risk between men and women.

An obvious difference between men and women is hormonal function, and both endogenous and exogenous sex hormones have effects on the vasculature.[9,61]

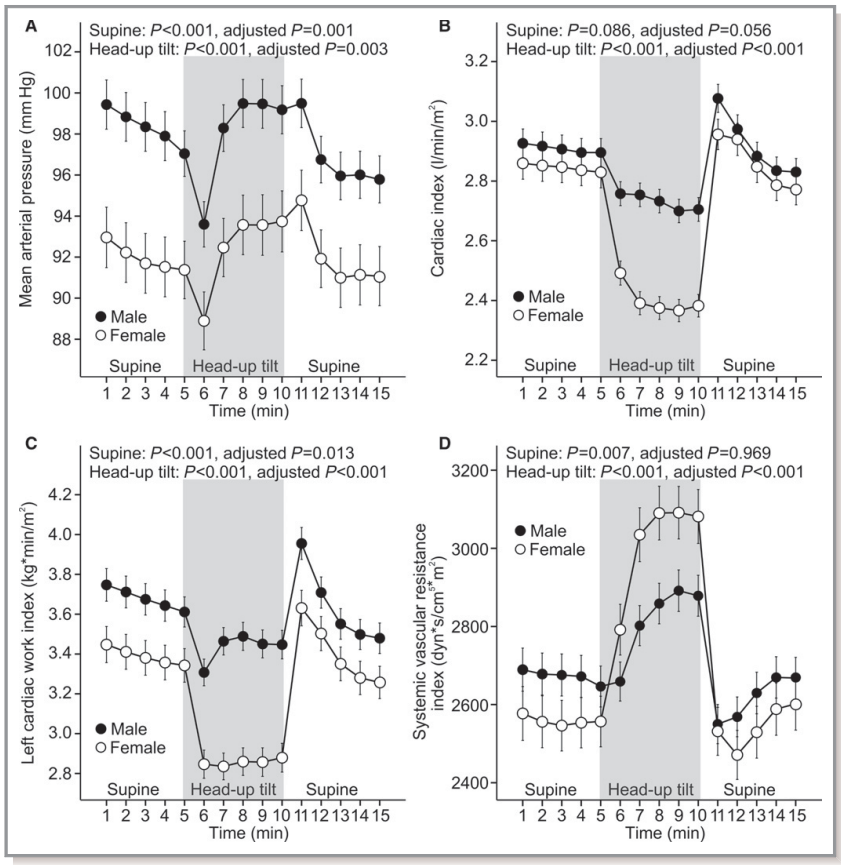

Figure 5. Overall, 878 participants were examined. Line graphs show mean arterial pressure (A), cardiac index (B), left cardiac work index (C), and systemic vascular resistance index (D) in 480 men and 398 women during supine position and passive head-up tilt (312 men and 303 women were without medications with direct cardiovascular actions; means and 95% CIs of the mean). *P* values denote differences between sexes in unadjusted analysis and in analyses adjusted for low- and high-density lipoprotein cholesterol, triglycerides, glucose, mean arterial pressure, smoking habits, alcohol intake, age, and body mass index. In adjusted analyses, the numbers of participants ranged from 402 to 411 for men and from 332 to 342 for women.

Progesterone, for example, has vasodilatory or vasoconstrictive effects depending on the location of the vessel and the level of exposure.[9] Exogenous progestins have androgenic properties compared with endogenous progesterone, and baroreflex sensitivity during oral contraceptive pill use may differ from that during the normal menstrual cycle.[9] Estradiol may inhibit renin release, whereas testosterone may activate the renin–angiotensin system.[61] Estrogens may even acutely enhance endothelium-dependent vasodilation in postmenopausal women.[62] In the present study, the upright differences in hemodynamics between men and women were not confined to premenopausal women but were consistent in participants of postmenopausal age. Consequently, higher left cardiac work in the upright position cannot be explained solely by differences in the sex hormones of men and women.

The sex differences in CVD risk factors and end points are known to diminish with increasing age.[5] Although the incidence of myocardial infarction increases in postmenopausal women, after age 70 years, heart failure and stroke are the most common initial presentations of CVD in both sexes.[56] A major predisposing factor to heart failure and stroke is the increased prevalence of hypertension.[1,9] The withdrawal of estrogen increases the age-related cardiovascular risk in postmenopausal women, but chronological aging

processes acting on top of biological differences between men and women likely play a more important role in the increase of cardiovascular risk.[63]

Another difference between men and women is body height. An inverse association between height and the risk of CVD has been found.[64,65] Hemodynamically, height is correlated with higher systolic pressure amplification from central to peripheral arteries and prolonged return of the reflected pressure wave form the peripheral circulation to the aorta.[64,66] Subsequently, shorter body height in women results in less peripheral systolic pressure amplification than in men, with lower peripheral but not central systolic pressure. In premenopausal women, greater arterial distensibility partially offsets the effects of shorter body height, but after menopause, arterial stiffness increases and does not compensate for the smaller stature of women, resulting in higher pressure wave reflections in central arteries.[64,66] In the present study, the estimation of aortic BP was performed using a pulse wave monitoring system that takes into account the reflected pressure waves, and the influences of the reflected waves were included in the estimation of aortic mean pressure that was used for the calculation of left cardiac work.

Our study has several limitations. The noninvasive recordings of cardiac output require mathematical equations and simplification of physiology,[38] but invasive measurements are not justified without a clinical reason. The present methods have been validated against invasive methods,[32,34,67] and we have no reason to suspect that the recordings would be less reliable in the upright position between sexes. Although participants taking medications with direct influence on hemodynamics were excluded from the main group composed of 334 participants, the medications used by the participants may have influenced the results. Selection bias also may have influenced the results in the group composed of 334 participants; however, it is unlikely that the results were observed merely by chance because the outcome in 878 participants (of whom 263 had CVD and were on various medications) correspondingly showed higher upright cardiac load in men than in women. The average BMI (in kg/m^2) in the study population was ≈26.5, which corresponds well to the average BMI in Finnish men (27.4) and women (26.9) in a recent survey.[68] Finally, the present analyses were adjusted for smoking habits, BP, lipid profile and glucose, and the groups of men and women had similar BMIs and ages. Consequently, the sex-related differences in upright hemodynamics were not explained by the generally known cardiovascular risk factors.

In conclusion, we found clear differences in upright hemodynamics between men and women. In men, the upright position was associated with higher workload of the heart, whereas in women, the most marked hemodynamic change was a significant rise in peripheral arterial resistance. These hemodynamic differences were not explained by the generally known cardiovascular risk factors like smoking, alcohol use, lipid or glucose disorders, or level of BP. The findings of the current study emphasize that upright hemodynamics should receive special attention when examining cardiovascular differences between men and women. The observed differences in upright hemodynamics could play a role in the higher risk of cardiac events in men than in women.

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Disclosures

None.

References

1. Rapsomaniki E, Timmis A, George J, Pujades-Rodriguez M, Shah AD, Denaxas S, White IR, Caulfield MJ, Deanfield JE, Smeeth L, Williams B, Hingorani A, Hemingway H. Blood pressure and incidence of twelve cardiovascular diseases: lifetime risks, healthy life-years lost, and age-specific associations in 1.25 million people. *Lancet*. 2014;383:1899–1911.
2. Laslett LJ, Alagona P Jr, Clark BA III, Drozda JP Jr, Saldivar F, Wilson SR, Poe C, Hart M. The worldwide environment of cardiovascular disease: prevalence, diagnosis, therapy, and policy issues: a report from the American College of Cardiology. *J Am Coll Cardiol*. 2012;60:S1–S49.
3. Kannel WB, Wilson PW. Risk factors that attenuate the female coronary disease advantage. *Arch Intern Med*. 1995;155:57–61.
4. Lloyd-Jones DM, Larson MG, Beiser A, Levy D. Lifetime risk of developing coronary heart disease. *Lancet*. 1999;353:89–92.
5. Jousilahti P, Vartiainen E, Tuomilehto J, Puska P. Sex, age, cardiovascular risk factors, and coronary heart disease: a prospective follow-up study of 14 786 middle-aged men and women in Finland. *Circulation*. 1999;99:1165–1172.
6. Skurnick JH, Aladjem M, Aviv A. Sex differences in pulse pressure trends with age are cross-cultural. *Hypertension*. 2010;55:40–47.
7. Piro M, Della Bona R, Abbate A, Biasucci LM, Crea F. Sex-related differences in myocardial remodeling. *J Am Coll Cardiol*. 2010;55:1057–1065.
8. Samad Z, Boyle S, Ersboll M, Vora AN, Zhang Y, Becker RC, Williams R, Kuhn C, Ortel TL, Rogers JG, O'Connor CM, Velazquez EJ, Jiang W. Sex differences in platelet reactivity and cardiovascular and psychological response to mental stress in patients with stable ischemic heart disease: insights from the REMIT study. *J Am Coll Cardiol*. 2014;64:1669–1678.
9. Wenner MM, Stachenfeld NS. Blood pressure and water regulation: understanding sex hormone effects within and between men and women. *J Physiol*. 2012;590:5949–5961.
10. Kastarinen M, Antikainen R, Peltonen M, Laatikainen T, Barengo NC, Jula A, Salomaa V, Jousilahti P, Nissinen A, Vartiainen E, Tuomilehto J. Prevalence,

awareness and treatment of hypertension in Finland during 1982–2007. *J Hypertens*. 2009;27:1552–1559.

11. Denton KM, Hilliard LM, Tare M. Sex-related differences in hypertension: seek and ye shall find. *Hypertension*. 2013;62:674–677.
12. Gatzka CD, Kingwell BA, Cameron JD, Berry KL, Liang YL, Dewar EM, Reid CM, Jennings GL, Dart AM. Gender differences in the timing of arterial wave reflection beyond differences in body height. *J Hypertens*. 2001;19:2197–2203.
13. Hart EC, Charkoudian N, Wallin BG, Curry TB, Eisenach JH, Joyner MJ. Sex differences in sympathetic neural-hemodynamic balance: implications for human blood pressure regulation. *Hypertension*. 2009;53:571–576.
14. Hart EC, Joyner MJ, Wallin BG, Charkoudian N. Sex, ageing and resting blood pressure: gaining insights from the integrated balance of neural and haemodynamic factors. *J Physiol*. 2012;590:2069–2079.
15. Shoemaker JK, Hogeman CS, Khan M, Kimmerly DS, Sinoway LI. Gender affects sympathetic and hemodynamic response to postural stress. *Am J Physiol Heart Circ Physiol*. 2001;281:H2028–H2035.
16. Barnett SR, Morin RJ, Kiely DK, Gagnon M, Azhar G, Knight EL, Nelson JC, Lipsitz LA. Effects of age and gender on autonomic control of blood pressure dynamics. *Hypertension*. 1999;33:1195–1200.
17. Jarvis SS, Florian JP, Curren MJ, Pawelczyk JA. Sex differences in vasoconstrictor reserve during 70 deg head-up tilt. *Exp Physiol*. 2010;95:184–193.
18. Huikuri HV, Pikkujämsa SM, Airaksinen KE, Ikäheimo MJ, Rantala AO, Kauma H, Lilja M, Kesäniemi YA. Sex-related differences in autonomic modulation of heart rate in middle-aged subjects. *Circulation*. 1996;94:122–125.
19. Stolarz K, Staessen JA, Kuznetsova T, Tikhonoff V, State D, Babeanu S, Casiglia E, Fagard RH, Kawecka-Jaszcz K, Nikitin Y. Host and environmental determinants of heart rate and heart rate variability in four European populations. *J Hypertens*. 2003;21:525–535.
20. Barantke M, Krauss T, Ortak J, Lieb W, Reppel M, Burgdorf C, Pramstaller PP, Schunkert H, Bonnemeier H. Effects of gender and aging on differential autonomic responses to orthostatic maneuvers. *J Cardiovasc Electrophysiol*. 2008;19:1296–1303.
21. Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Bohm M, Christiaens T, Cifkova R, De Backer G, Dominiczak A, Galderisi M, Grobbee DE, Jaarsma T, Kirchhof P, Kjeldsen SE, Laurent S, Manolis AJ, Nilsson PM, Ruilope LM, Schmieder RE, Sirnes PA, Sleight P, Viigimaa M, Waeber B, Zannad F. 2013 ESH/ESC Guidelines for the management of arterial hypertension: the Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *J Hypertens*. 2013;31:1281–1357.
22. Cooper-DeHoff RM, Johnson JA. Hypertension pharmacogenomics: in search of personalized treatment approaches. *Nat Rev Nephrol*. 2016;12:110–122.
23. Mann SJ, Ernst ME. Personalizing the diuretic treatment of hypertension: the need for more clinical and research attention. *Curr Hypertens Rep*. 2015;17:542.
24. Te Riet L, van Esch JH, Roks AJ, van den Meiracker AH, Danser AH. Hypertension: renin-angiotensin-aldosterone system alterations. *Circ Res*. 2015;116:960–975.
25. Tikkakoski AJ, Tahvanainen AM, Leskinen MH, Koskela JK, Haring A, Viitala J, Kähönen MA, Koobi T, Niemelä O, Mustonen JT, Pörsti IH. Hemodynamic alterations in hypertensive patients at rest and during passive head-up tilt. *J Hypertens*. 2013;31:906–915.
26. Tahvanainen A, Leskinen M, Koskela J, Ilveskoski E, Nordhausen K, Oja H, Kähönen M, Kööbi T, Mustonen J, Pörsti I. Ageing and cardiovascular responses to head-up tilt in healthy subjects. *Atherosclerosis*. 2009;207:445–451.
27. Recio-Rodriguez JI, Gomez-Marcos MA, Patino-Alonso MC, Agudo-Conde C, Rodriguez-Sanchez E, Garcia-Ortiz L, Vasorisk-group. Abdominal obesity vs general obesity for identifying arterial stiffness, subclinical atherosclerosis and wave reflection in healthy, diabetics and hypertensive. *BMC Cardiovasc Disord*. 2012;12:3.
28. Russo C, Jin Z, Homma S, Rundek T, Elkind MS, Sacco RL, Di Tullio MR. Effect of obesity and overweight on left ventricular diastolic function: a community-based study in an elderly cohort. *J Am Coll Cardiol*. 2011;57:1368–1374.
29. Safar ME, Czernichow S, Blacher J. Obesity, arterial stiffness, and cardiovascular risk. *J Am Soc Nephrol*. 2006;17:S109–S111.
30. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, Kusek JW, Manzi J, Van Lente F, Zhang YL, Coresh J, Levey AS. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med*. 2012;367:20–29.
31. Tahvanainen A, Koskela J, Tikkakoski A, Lahtela J, Leskinen M, Kähönen M, Nieminen T, Kööbi T, Mustonen J, Pörsti I. Analysis of cardiovascular responses to passive head-up tilt using continuous pulse wave analysis and impedance cardiography. *Scand J Clin Lab Invest*. 2009;69:128–137.
32. Chen CH, Nevo E, Fetics B, Pak PH, Yin FC, Maughan WL, Kass DA. Estimation of central aortic pressure waveform by mathematical transformation of radial tonometry pressure, Validation of generalized transfer function. *Circulation*. 1997;95:1827–1836.
33. Kööbi T, Kähönen M, Iivainen T, Turjanmaa V. Simultaneous non-invasive assessment of arterial stiffness and haemodynamics - a validation study. *Clin Physiol Funct Imaging*. 2003;23:31–36.
34. Kööbi T, Kaukinen S, Ahola T, Turjanmaa VM. Non-invasive measurement of cardiac output: whole-body impedance cardiography in simultaneous comparison with thermodilution and direct oxygen Fick methods. *Intensive Care Med*. 1997;23:1132–1137.
35. Tahvanainen AM, Tikkakoski AJ, Leskinen MH, Nordhausen K, Kähönen M, Kööbi T, Mustonen JT, Pörsti IH. Supine and upright haemodynamic effects of sublingual nitroglycerin and inhaled salbutamol: a double-blind, placebo-controlled, randomized study. *J Hypertens*. 2012;30:297–306.
36. Koskela JK, Tahvanainen A, Haring A, Tikkakoski AJ, Ilveskoski E, Viitala J, Leskinen MH, Lehtimaki T, Kahonen MA, Koobi T, Niemela O, Mustonen JT, Porsti IH. Association of resting heart rate with cardiovascular function: a cross-sectional study in 522 Finnish subjects. *BMC Cardiovasc Disord*. 2013;13:102.
37. Gorlin R, Mc MI, Medd WE, Matthews MB, Daley R. Dynamics of the circulation in aortic valvular disease. *Am J Med*. 1955;18:855–870.
38. Kööbi T, Kaukinen S, Turjanmaa VM, Uusitalo AJ. Whole-body impedance cardiography in the measurement of cardiac output. *Crit Care Med*. 1997;25:779–785.
39. Peltola MA. Role of editing of R-R intervals in the analysis of heart rate variability. *Front Physiol*. 2012;3:148.
40. Task Force of the European Society of Cardiology, the North American Society of Pacing and Electrophysiology. Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Eur Heart J*. 1996;17:354–381.
41. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. 1972;18:499–502.
42. Monfredi O, Lyashkov AE, Johnsen AB, Inada S, Schneider H, Wang R, Nirmalan M, Wisloff U, Maltsev VA, Lakatta EG, Zhang H, Boyett MR. Biophysical characterization of the underappreciated and important relationship between heart rate variability and heart rate. *Hypertension*. 2014;64:1334–1343.
43. Smetana P, Malik M. Sex differences in cardiac autonomic regulation and in repolarisation electrocardiography. *Pflugers Arch*. 2013;465:699–717.
44. White DD, Gotshall RW, Tucker A. Women have lower tolerance to lower body negative pressure than men. *J Appl Physiol*. 1996;80:1138–1143.
45. Convertino VA. Gender differences in autonomic functions associated with blood pressure regulation. *Am J Physiol*. 1998;275:R1909–R1920.
46. Xhyheri B, Manfrini O, Mazzolini M, Pizzi C, Bugiardini R. Heart rate variability today. *Prog Cardiovasc Dis*. 2012;55:321–331.
47. Cooke WH, Hoag JB, Crossman AA, Kuusela TA, Tahvanainen KU, Eckberg DL. Human responses to upright tilt: a window on central autonomic integration. *J Physiol*. 1999;517(Pt 2):617–628.
48. Eckberg DL. Sympathovagal balance: a critical appraisal. *Circulation*. 1997;96:3224–3232.
49. Umetani K, Singer DH, McCraty R, Atkinson M. Twenty-four hour time domain heart rate variability and heart rate: relations to age and gender over nine decades. *J Am Coll Cardiol*. 1998;31:593–601.
50. Dutra SG, Pereira AP, Tezini GC, Mazon JH, Martins-Pinge MC, Souza HC. Cardiac autonomic modulation is determined by gender and is independent of aerobic physical capacity in healthy subjects. *PLoS ONE*. 2013;8:e77092.
51. Evans JM, Ziegler MG, Patwardhan AR, Ott JB, Kim CS, Leonelli FM, Knapp CF. Gender differences in autonomic cardiovascular regulation: spectral, hormonal, and hemodynamic indexes. *J Appl Physiol*. 2001;91:2611–2618.
52. Cheng YC, Vyas A, Hymen E, Perlmuter LC. Gender differences in orthostatic hypotension. *Am J Med Sci*. 2011;342:221–225.
53. Storkebaum E, Carmeliet P. Paracrine control of vascular innervation in health and disease. *Acta Physiol (Oxf)*. 2011;203:61–86.
54. Bernardi L, Spallone V, Stevens M, Hilsted J, Frontoni S, Pop-Busui R, Ziegler D, Kempler P, Freeman R, Low P, Tesfaye S, Valensi P. Methods of investigation for cardiac autonomic dysfunction in human research studies. *Diabetes Metab Res Rev*. 2011;27:654–664.

55. Luksha L, Agewall S, Kublickiene K. Endothelium-derived hyperpolarizing factor in vascular physiology and cardiovascular disease. *Atherosclerosis*. 2009;202:330–344.
56. George J, Rapsomaniki E, Pujades-Rodriguez M, Shah AD, Denaxas S, Herrett E, Smeeth L, Timmis A, Hemingway H. How does cardiovascular disease first present in women and men? Incidence of 12 cardiovascular diseases in a contemporary cohort of 1,937,360 people. *Circulation*. 2015;132:1320–1328.
57. Yahagi K, Davis HR, Arbustini E, Virmani R. Sex differences in coronary artery disease: pathological observations. *Atherosclerosis*. 2015;239:260–267.
58. Martinez-Selles M, Doughty RN, Poppe K, Whalley GA, Earle N, Tribouilloy C, McMurray JJ, Swedberg K, Kober L, Berry C, Squire I. Gender and survival in patients with heart failure: interactions with diabetes and aetiology. Results from the MAGGIC individual patient meta-analysis. *Eur J Heart Fail*. 2012;14:473–479.
59. Parashar S, Katz R, Smith NL, Arnold AM, Vaccarino V, Wenger NK, Gottdiener JS. Race, gender, and mortality in adults > or =65 years of age with incident heart failure (from the Cardiovascular Health Study). *Am J Cardiol*. 2009;103:1120–1127.
60. O'Meara E, Clayton T, McEntegart MB, McMurray JJ, Pina IL, Granger CB, Ostergren J, Michelson EL, Solomon SD, Pocock S, Yusuf S, Swedberg K, Pfeffer MA. Sex differences in clinical characteristics and prognosis in a broad spectrum of patients with heart failure: results of the Candesartan in Heart failure: Assessment of Reduction in Mortality and morbidity (CHARM) program. *Circulation*. 2007;115:3111–3120.
61. Orshal JM, Khalil RA. Gender, sex hormones, and vascular tone. *Am J Physiol Regul Integr Comp Physiol*. 2004;286:R233–R249.
62. Gilligan DM, Badar DM, Panza JA, Quyyumi AA, Cannon RO III. Acute vascular effects of estrogen in postmenopausal women. *Circulation*. 1994;90:786–791.
63. Merz AA, Cheng S. Sex differences in cardiovascular ageing. *Heart*. 2016;102:825–831. doi: 10.1136/heartjnl-2015-308769.
64. Smulyan H, Marchais SJ, Pannier B, Guerin AP, Safar ME, London GM. Influence of body height on pulsatile arterial hemodynamic data. *J Am Coll Cardiol*. 1998;31:1103–1109.
65. Batty GD, Shipley MJ, Gunnell D, Huxley R, Kivimaki M, Woodward M, Lee CM, Smith GD. Height, wealth, and health: an overview with new data from three longitudinal studies. *Econ Hum Biol*. 2009;7:137–152.
66. London GM, Guerin AP, Pannier B, Marchais SJ, Stimpel M. Influence of sex on arterial hemodynamics and blood pressure. Role of body height. *Hypertension*. 1995;26:514–519.
67. Boer P, Roos JC, Geyskes GG, Mees EJ. Measurement of cardiac output by impedance cardiography under various conditions. *Am J Physiol*. 1979;237: H491–H496.
68. Peltonen M, Harald K, Männistö S, Saarikoski L, Peltomäki P, Lund L, Sundvall J, Juolevi A, Laatikainen T, Aldén-Nieminen H, Luoto R, Jousilahti P, Salomaa V, Taimi M, Vartiainen E. The National FINRISK 2007 Study. *Publications of the National Public Health Institute, B34/2008* (*with English summary*). 2008. https://www.julkari.fi/bitstream/handle/10024/78146/2008b34.pdf.

Increased Cardiac Workload in the Upright Posture in Men: Noninvasive Hemodynamics in Men Versus Women

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PUBLICATION III

Changes in hemodynamics associated with metabolic syndrome may be more pronounced in women than in men

Kangas P, Tikkakoski A, Kettunen J, Eräranta A, Huhtala H, Kähönen M, Sipilä K, Mustonen J and Pörsti I

Submitted

PUBLICATION IV

Metabolic syndrome is associated with decreased heart rate variability in a sex-dependent manner: a comparison between 252 men and 249 women

Kangas P, Tikkakoski A, Uitto M, Viik J, Bouquin H, Niemelä O, Mustonen J, Pörsti I

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Metabolic syndrome is associated with decreased heart rate variability in a sex-dependent manner: a comparison between 252 men and 249 women

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Summary

Impaired heart rate variability (HRV) is associated with increased risk of cardiovascular disease, but evidence regarding alterations of HRV in metabolic syndrome (MetS) remains elusive. In order to examine HRV in MetS, we subjected 501 volunteers without atherosclerosis, diabetes or antihypertensive medication, mean age 48 years, to passive head-up tilt. The subjects were divided to control men (n = 131), men with MetS (n = 121), control women (n = 191) and women with MetS (n = 58) according to the criteria by Alberti *et al.* (Circulation, 2009, 120, 1640). In unadjusted analyses (i) men and women with MetS had lower total power and high-frequency (HF) power of HRV than controls whether supine or upright ($P<0{\cdot}05$ for all). (ii) Supine low-frequency (LF) power of HRV was lower in men ($P = 0{\cdot}012$) but not in women ($P = 0{\cdot}064$) with MetS than in controls, while men and women with MetS had lower upright LF power of HRV than controls ($P <0{\cdot}01$ for both). (iii) The LF:HF ratio did not differ between subjects with and without MetS. After adjustment for age, smoking habits, alcohol intake, height, heart rate and breathing frequency, only the differences in upright total power and HF power of HRV between women with MetS and control women remained significant ($P<0{\cdot}05$). In conclusion, reduced total and HF power of HRV in the upright position may partially explain why the relative increase in cardiovascular risk associated with MetS is greater in women than in men. Additionally, the present results emphasize that the confounding factors must be carefully taken into consideration when evaluating HRV.

Introduction

Metabolic syndrome (MetS), a disorder characterized by a cluster of unfavourable changes in lipid profile, blood pressure, glucose metabolism and waist circumference is very common, especially in the Western countries. MetS is related to an increased risk of diabetes and cardiovascular disease (CVD) (Alberti *et al.*, 2009), while CVDs are the most important factor causing mortality worldwide (Mendis & Norrving, 2011). Consequently, MetS has been under active investigation during the last decades. However, the mechanisms linking MetS with increased risk of CVD are still incompletely understood (Mottillo *et al.*, 2010).

Imbalance in the autonomic nervous system with sympathetic overdrive has been linked with MetS (Grassi, 2006). An applicable, non-invasive method for the evaluation of cardiac autonomic tone is the measurement of heart rate variability (HRV). Decreased vagal activity, as indicated by HRV, has been shown to predict mortality in both high-risk and low-risk populations (Thayer *et al.*, 2010; Wulsin *et al.*, 2015). According to a recent systematic review (Stuckey *et al.*, 2014), numerous cross-sectional studies have revealed impaired cardiac autonomic function associated with MetS. Furthermore, a longitudinal study showed that low HRV increased the odds of developing MetS during 12 years of follow-up (Wulsin *et al.*, 2016).

In previous studies, MetS was associated with lower indices of HRV, but the results have not been consistent (Stuckey *et al.*, 2014). In many reports, the changes of cardiac autonomic tone in MetS seemed to be more pronounced in women than in men (Koskinen *et al.*, 2009; Stuckey *et al.*, 2014, 2015). Several studies suggested that the association of MetS with cardiovascular end points is also stronger in women than in men (Hunt *et al.*, 2004; Schillaci *et al.*, 2006). This raises the question whether the sex-related differences in autonomic tone in MetS could play a role to explain these differences.

In addition to various medical conditions, also age (Bonnemeier *et al.*, 2003), sex (Koenig & Thayer, 2016), heart rate (HR) (Billman, 2013a; Sacha, 2014), circadian rhythm (Bonnemeier *et al.*, 2003) and breathing frequency (Stolarz *et al.*, 2003; Billman, 2011) can influence HRV. In many studies, evaluating HRV in MetS these factors were somewhat neglected, and this may explain some of the discrepancies in the published results (Stuckey *et al.*, 2014). According to a review published in 2014 (Stuckey *et al.*, 2014), only three reports had separated the analyses by sex, while only one study in young adults had adjusted the HRV results for HR (Koskinen *et al.*, 2009), although increased HR is characteristic of subjects with MetS (Mancia *et al.*, 2007). To our knowledge, no HR-adjusted results of HRV in MetS have been published thereafter.

Importantly, many antihypertensive medications like beta-blockers (Vaile *et al.*, 1999), calcium channel blockers (Karas *et al.*, 2005), angiotensin receptor blockers (Karas *et al.*, 2005; Okano *et al.*, 2009) and angiotensin-converting enzyme inhibitors (Karas *et al.*, 2005) can influence HRV. Hypertension is very common in MetS, and in many studies evaluating HRV in MetS, subjects with antihypertensive medication were included (Stuckey *et al.*, 2014), and this has probably also contributed the discrepancy in the published results.

In the current study, we examined changes in HRV associated with MetS separately in men and women. None of the subjects used antihypertensive agents or other medications with direct cardiovascular influences, and the differences between HR and breathing frequency were also taken into account. As upright position is characterized by differences in the haemodynamic responses between men and women (Kangas *et al.*, 2016), we examined HRV during supine and upright positions.

Methods

Study subjects

This study is part of an ongoing investigation of haemodynamics in the University of Tampere (DYNAMIC-study, ClinicalTrials.gov identifier NCT01742702). The procedures for recruiting of participants and data collection have been previously described (Kangas *et al.*, 2016). The population of the current study was screened from 1091 subjects. The exclusion criteria were diagnosed atherosclerosis, cardiac insufficiency or cerebrovascular disease, diabetes, and use of antihypertensive drugs or other medications having influences on haemodynamics (like β-blocker eye drops for glaucoma, β_2-adrenoceptor agonists, α_1-adrenoceptor blockers for prostate problems and digoxin). After the exclusion process, 501 subjects aged 19–72 years were eligible for the study population. Clinical cardiovascular status was examined, lifestyle habits, medical history, family history and use of medicines were recorded, and laboratory tests were taken before the haemodynamic recordings.

For the definition of MetS, the criteria of Alberti *et al.* from 2009 (Alberti *et al.*, 2009) were used, so that three or more of the following criteria were met: waist circumference ≥ 94 cm (men) and ≥ 80 cm (women); high-density lipoprotein cholesterol (HDL-C) <1·0 mmol l^{-1} (men) and <1·3 mmol l^{-1} (women); triglycerides ≥ 1·7 mmol l^{-1}; fasting plasma glucose ≥ 5·6 mmol l^{-1}; systolic BP ≥ 130 mmHg and/or diastolic BP ≥ 85 mmHg. The study subjects were allocated to four groups: men without MetS (M-control, n = 131), men with MetS (M-MetS, n = 121), women without MetS (W-control, n = 191) and women with MetS (W-MetS, n = 58).

Although none of the subjects used antihypertensive agents, medications not affecting haemodynamics were allowed. Altogether, 186 subjects (37%) used some medication. Thirteen had statins for dyslipidemia, 77 female participants (31%) used systemic female hormones (hormone replacement therapy or contraception). In the use of female hormones, no difference was found between W-control and W-MetS groups (P = 0·761). Additionally, some other drugs (e.g. antihistamines, thyroid hormones, proton pump inhibitors, acetylsalicylic acid, selective serotonin re-uptake inhibitors and intranasal or inhaled corticosteroids) were used by the participants. One subject had anti-phospholipid syndrome without any symptoms or findings, and he was receiving warfarin.

Measuring heart rate variability during passive head-up tilt test

The participants were instructed to refrain from smoking, caffeine-containing products and heavy meals for at least 4 h, and from alcohol for at least 24 h prior to the recordings. The recordings were performed in a temperature-controlled laboratory by a trained research nurse during two consecutive 5-min periods with continuous capture of data: 5 min of supine recordings followed by 5 min of passive head-up tilt to >60°. The actual recordings were preceded by an introductory head-up tilt. The HRV values of the last 3 min of the supine and the upright periods were used in the analyses, since the signal of these periods was most stable (Tikkakoski *et al.*, 2013). Continuous radial tonometric blood pressure values were recorded during the measurements, and the average systolic and diastolic values of the last supine 3 min were used for the definition of MetS. The detailed description of

the measurement protocol has been previously published (Kangas *et al.*, 2013; Koskela *et al.*, 2013).

For assessing cardiac autonomic tone, HRV analysis from a single channel electrocardiogram (ECG) was used. The ECG was recorded by the CircMon^{R} device (CircMon; JR Medical Ltd) with 200 Hz sampling rate, and Matlab software (MathWorks Inc., Natick, Massachusetts, USA) was used for data analyses. Normal R-R intervals were recognized, and if the interval differed more than 20% from the previous values, the beat was considered ectopic. The processing of artefacts was performed using the cubic spline interpolation method (Peltola, 2012). Since the data was collected from short-term recordings, the frequency domain method was applied (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). The following HRV variables were calculated using the fast Fourier transformation: (i) total power, (ii) power in the low-frequency (LF) range (0·04–0·15 Hz), (iii) power in the high-frequency (HF) range (0·15–0·40 Hz) and (iv) LF:HF ratio. The recordings were performed in both supine and upright positions. Breathing frequency was captured from the CircMon^{R} data, the focus being on the last 3 min of the supine and upright phases of recording.

Laboratory tests

Blood samples were obtained after ~12 h of fasting, and plasma total cholesterol, HDL-C and low-density lipoprotein cholesterol (LDL-C), triglycerides, glucose, creatinine and cystatin C were determined using the Cobas Integra 700/800 or Cobas 6000 (Roche Diagnostics, Basel, Switzerland), and insulin using electrochemiluminescence immunoassay (Cobas e 422, Roche Diagnostics). A standard 12-lead electrocardiogram was recorded and Cornell voltage QRS duration product was calculated for evaluating left ventricular mass (ESC, 2007). Insulin sensitivity was estimated using the Quantitative insulin-sensitivity check index (QUICKI) (Katz *et al.*, 2000). Estimated glomerulus filtration rate (eGFR) was calculated using the CKD-EPI formula (Inker *et al.*, 2012).

Statistical analyses

The skewed distributions of total power, LF power, HF power, LF:HF ratio and triglycerides were logarithmically transformed before the statistical analyses. The original values of the HRV variables are represented in the Figures. The statistical analyses were performed separately in men and women. In the comparisons of HRV variables and the characteristics between control and MetS groups, the independent samples of t-test were used. Pearson chi-square test was applied to compare smoking habits (current, previous and never) and the use of female hormones between the study groups, while Mann–Whitney U-test was used for the comparisons of alcohol intake due to its skewed distribution.

To assess the influence of different confounding factors on the HRV results, three separate models of adjustment were crafted. The results adjusted for (i) age, smoking (current smoking amount), alcohol intake and height; (ii) model 1 plus HR (Billman, 2013a; Sacha, 2013; Monfredi *et al.*, 2014); (iii) model 2 plus breathing frequency (Brown *et al.*, 1993; Billman, 2013b). In the adjusted analyses, analysis of covariance (ANCOVA) was used with the above variables as covariates.

HRV data were captured from all 501 participants. However, some of the additional data were missing: Information about alcohol intake was missing from 14 and about smoking from five subjects. LDL-C value was missing from four, and ECG from one subject. QUICKI could not be calculated from 47 participants. Due to technical problems, breathing frequency was not obtained from 77 subjects in the supine position and from 76 subjects in the upright position. Altogether, 482 (96%) subjects were included in the adjusted models 1 and 2, while 407–408 (81%) subjects were included in the adjusted model 3.

All testing was 2-sided, and the results in the table were reported as means and standard deviations for normally distributed variables, and medians and lower and upper quartiles for variables with skewed distribution. For categorical variables, numbers of cases and percentages are shown. P-values <0·05 were considered significant. The statistical analyses were performed using IBM SPSS Statistics (software version 24, Armonk, New York, USA).

Results

Study population

The characteristics of the study participants are presented in Table 1. The MetS and the control groups did not differ in age or in alcohol use ($P > 0·1$ for all). In women, the proportion of previous smokers was higher in the MetS group than in the control group ($P = 0·047$), but the proportions of current smokers were similar in the MetS and control groups, in both women and men ($P>0·1$). As expected, female and male subjects with MetS had higher systolic and diastolic blood pressure, BMI and waist circumference; and higher fasting plasma glucose, total cholesterol, triglycerides, LDL-C ($P<0·001$ for all), and lower HDL-C and QUICKI ($P<0·001$ for all) than the control subjects. Creatinine was higher in the W-control group than in the W-MetS group ($P = 0·004$), but eGFR did not differ between the W-MetS and the W-control groups, or between the M-MetS and the M-control groups ($P>0·1$ for both). Both women and men with MetS had higher Cornell voltage product in ECG than controls ($P<0·05$).

HRV in supine and upright positions

Unadjusted supine analyses: Supine total power (Fig. 1a) and HF power (Fig. 1b) of HRV were lower in the MetS groups than in the control groups ($P<0·05$ for analyses in men and in women). The M-MetS group had lower supine LF power (Fig. 2a) than the M-control group ($P = 0·012$), while in

Table 1 Clinical and metabolic characteristics in the study groups.

Variable	M-control	M-MetS	*P*-value	W-control	W-MetS	*P*-value
Number of subjects	131	121		191	58	
Age (years)	48 ± 10	49 ± 9	0·457	47 ± 9	49 ± 10	0·113
BMI (kg m^{-2})	26 ± 3	30 ± 4	<0·001	25 ± 4	30 ± 5	<0·001
Waist circumference (cm)	95 ± 10	106 ± 8	<0·001	85 ± 12	98 ± 14	<0·001
Systolic blood pressure (mmHg)	130 ± 16	144 ± 15	<0·001	125 ± 18	142 ± 19	<0·001
Diastolic blood pressure (mmHg)	75 ± 11	84 ± 10	<0·001	72 ± 12	81 ± 13	<0·001
Smoking status						
Never smoked (n/%)	70/53	57/47	0·316	118/62	28/48	0·067
Current smoker (n/%)	21/16	18/15	0·800	26/14	8/14	0·972
Previous smoker (n/%)	40/31	46/38	0·211	47/25	22/38	0·047
Alcohol intake (standard doses per week)	4 (1–9)	4 (2–11)	0·410	2 (0–3)	2 (0–4)	0·697
Creatinine (μmol l^{-1})	82 ± 12	82 ± 11	0·551	66 ± 9	62 ± 9	0·004
eGFR (ml min^{-1} 1·73 m^{-2})	95 ± 13	96 ± 13	0·842	95 ± 13	98 ± 13	0·122
Fasting plasma glucose (mmol l^{-1})	5·4 ± 0·4	5·9 ± 0·5	<0·001	5,2 ± 0·4	5·8 ± 0·5	<0·001
Total cholesterol (mmol l^{-1})	5·2 ± 0·9	5·6 ± 1·1	<0·001	5,1 ± 1·0	5·6 ± 0·9	<0·001
Triglycerides (mmol l^{-1})	1·0 (0·7 - 1·4)	1·7 (1·1 - 2·3)	<0·001	0·9 (0·6 - 1·2)	1·5 (1·0 - 2·0)	<0·001
High-density lipoprotein cholesterol (mmol l^{-1})	1·5 ± 0·3	1·2 ± 0·3	<0·001	1·9 ± 0·4	1·5 ± 0·4	<0·001
Low-density lipoprotein cholesterol (mmol l^{-1})	3·2 ± 0·9	3·7 ± 1·0	<0·001	2·8 ± 0·9	3·4 ± 0·8	<0·001
Quantitative insulin sensitivity check index	0·365 ± 0·046	0·343 ± 0·042	<0·001	0·372 ± 0·042	0·339 ± 0·032	<0·001
Cornell voltage product in ECG (ms × mm)	1624 ± 823	1807 ± 584	0·044	1543 ± 523	1739 ± 506	0·012

M-control, men without MetS; M-MetS, men with MetS; W-control, women without MetS; W-MetS, women with MetS; BMI, body mass index; eGFR, estimated glomerulus filtration rate (Inker *et al.*, 2012).
Values are means ± SD except the values for smoking, which are the number of cases and percentages, and the values for triglycerides and alcohol intake, which are shown as medians (lower and upper quartiles) due to skewed distribution.

women supine LF power did not show a significant difference between the two groups (P = 0·064). The supine LF:HF ratio (Fig. 2b) did not differ between the MetS and the control groups (P>0·1).

Unadjusted upright analyses: Upright total power (Fig. 1a), HF power (Fig. 1b) and LF power (Fig. 2a) were all different in the comparisons between the M-MetS and the M-control groups and between the W-MetS and the W-control groups (P<0·05 for all). The upright LF:HF ratio (Fig. 2b) did not differ between the MetS and the control groups (P>0·1).

When the results were adjusted for the confounding factors, the differences between the MetS and the control groups were not as clear as in the unadjusted analyses. The detailed comparisons with the three models of adjustment with the exact P-values are presented in the Figs. 1 and 2. The results of model 1, with adjustments for age, smoking habits, alcohol intake and height, paralleled well the unadjusted results. In model 2 with additional adjustments for HR, the differences between the MetS and the control groups in supine total power and HF power in women and supine LF power in men were no longer significant.

In model 3, where the HRV results were also adjusted for breathing frequency, only the differences in upright total power (Fig. 1a), and upright HF power (Fig. 1b), between the MetS and the control groups remained statistically significant, and these differences were only found in women (P<0·05 for both). In men, all of the adjusted P-values in model 3 were not significant (P≥0·105).

Discussion

The present study demonstrates sex-dependent characteristics of cardiac autonomic tone in MetS using the frequency domain analyses of HRV. In HRV indices, the HF component represents cardiac parasympathetic activity (Eckberg, 1997; Cooke *et al.*, 1999; Xhyheri *et al.*, 2012), while the LF component predominantly reflects sympathetic activity, although it contains parasympathetic contributions (Eckberg, 1997; Xhyheri *et al.*, 2012). The interpretation of LF:HF ratio remains somewhat controversial. It has been suggested to represent sympathovagal balance (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Xhyheri *et al.*, 2012), but this interpretation has been criticized, and conclusions should be drawn with caution (Eckberg, 1997; Cooke *et al.*, 1999; Billman, 2013b).

Impaired cardiac autonomic tone is associated with cardiovascular risk (Tsuji *et al.*, 1996; Schuster *et al.*, 2016; Patel *et al.*, 2017) and mortality (Thayer *et al.*, 2010; Wulsin *et al.*, 2015). Several studies have reported changes in the HRV indices in MetS (Stuckey *et al.*, 2014), and disturbances in autonomic nervous tone have been suggested as an important link between MetS and cardiovascular diseases (Grassi, 2006). However, although the HRV indices are known to be affected by multiple factors, aspects like HR, sex and breathing frequency have been somewhat neglected in many reports evaluating the association between MetS and HRV (Stuckey *et al.*,

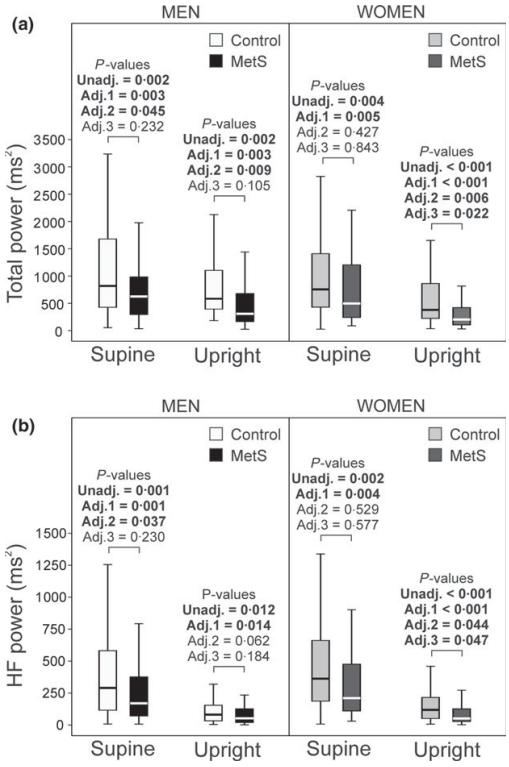

Figure 1 Box plots of total power (a) and high-frequency (HF) power (b) of heart rate variability in the study groups (median [line inside box], 25th to 75th percentile [box], and range [whiskers]; outliers were excluded from the Figure, but were included in the statistics). Supine and upright P values in unadjusted analyses and in analyses adjusted for (i) age, smoking (current smoking amount), alcohol intake and height; (ii) model 1 plus heart rate; (iii) model 2 plus breathing frequency. Numbers of subjects in the groups of men without metabolic syndrome (MetS), men with MetS, women without MetS and women with MetS, respectively: unadjusted n = 131, n = 121, n = 191 and n = 58; adjusted models 1 and 2 n = 125, n = 120, n = 180 and n = 57; adjusted model 3 n = 110, n = 90; n = 160 and n = 47–48.

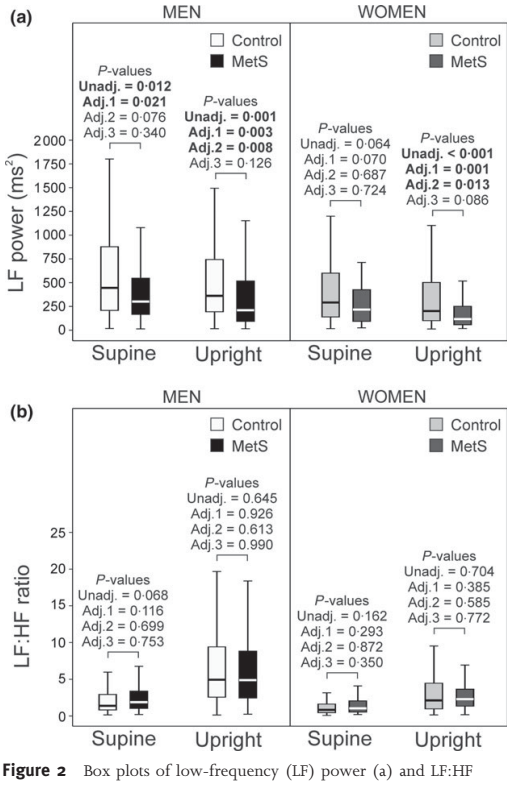

Figure 2 Box plots of low-frequency (LF) power (a) and LF:HF ratio (b) in the study groups (median [line inside box], 25th–75th percentile [box], and range [whiskers]; outliers were excluded from the Figure, but were included in the statistics). Supine and upright P values in unadjusted analyses and in analyses adjusted for (i) age, smoking (current smoking amount), alcohol intake and height; (ii) model 1 plus heart rate; (iii) model 2 plus breathing frequency. Number of subjects in the unadjusted and adjusted models as in Figure 1.

2014). In the current study, we found that MetS was associated with lower total power, HF power and LF power of HRV in both women and men. These findings are in accordance with previous studies. When the results were adjusted for age, smoking habits, alcohol intake, height, HR and breathing frequency, the differences between the MetS and the control groups diminished. Even after all adjustments, the total power and HF power, measured in the upright position, were still lower in women with MetS than in control women. Similar results were not found in men.

HR has a significant influence on HRV. Higher HRV represents higher parasympathetic nervous system activity, which leads to slower HR (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Thus, there is a physiological association between HR and HRV indices. In addition, a non-linear relationship exists between the heart period (R-R intervals) and HR, causing a mathematical dependence between HRV and HR (Sacha & Pluta, 2008; Sacha, 2014). Furthermore, the impact of HR on the prognostic value of HRV has been found to differ between sexes: as the HRV indices became more dependent on HR, the predictive power of spectral indices increased for cardiac death in men, whereas the prognostic power of the indices decreased in women (Sacha *et al.*, 2014). Monfredi *et al.* have concluded that HRV is primarily dependent on HR and it cannot be used in any simple way to assess autonomic nerve activity to the heart (Monfredi *et al.*, 2014). Therefore, all studies concerning HRV should carefully correct for differences in HR before drawing conclusions (Monfredi *et al.*, 2014). In comparisons between sexes, women have presented with greater vagal activity as

indexed by the HF power, but higher HR is also characteristic for women (Koenig & Thayer, 2016). Therefore, the relationship between the HRV indices and HR is not straightforward. In the present study, HR was taken into account in the adjusting process, and this clearly influenced the results.

Breathing frequency is another important factor that influences the HRV indices (Stolarz *et al.*, 2003; Billman, 2011). HRV increases when respiratory frequency decreases, while HRV decreases when the tidal volume of ventilation decreases (Brown *et al.*, 1993). Furthermore, mechanical factors that occur during respiration (stretch of the atria that results from changes in thoracic pressure and cardiac filling) influence HRV independent of changes in cardiac autonomic nerve activity (Billman, 2013b). Several studies have shown that slow, paced breathing strengthens vagal activity and modifies HRV (Howorka *et al.*, 2013; Prinsloo *et al.*, 2013; Kromenacker *et al.*, 2018; Li *et al.*, 2018). In the current study, the influence of breathing on the HRV indices was taken into account by adjusting the results for breathing frequency.

The underlying pathophysiology for the association of MetS with impaired HRV is not clear, but some factors have been suggested. Insulin resistance is commonly found in MetS, and several studies have revealed an association of insulin resistance with impaired HRV (Rodriguez-Colon *et al.*, 2010; Hillebrand *et al.*, 2015; Saito *et al.*, 2015). However, a study that examined HRV in subjects with and without MetS and comprised 220 subjects, found that insulin resistance was associated with HR but not with HRV (Stuckey *et al.*, 2015). Inflammatory markers, like interleukin-6, have been shown to inversely associate with HRV (Brunner *et al.*, 2002; Haensel *et al.*, 2008). Also increased plasma leptin level seems to associate with a shift of the sympathovagal balance towards sympathetic predominance (Paolisso *et al.*, 2000), and this relationship was found to be stronger in women than in men (Flanagan *et al.*, 2007). Both interleukin-6 and leptin are associated with obesity, and influence in the pathogenesis of MetS (Kaur, 2014). When the influence of the different MetS components (waist circumference, HDL-C, triglycerides, fasting glucose and blood pressure) on HRV were evaluated in a large cohort of men, all components had a strong, linear association (Hemingway *et al.*, 2005), but the strongest association was found between HRV and waist circumference (Hemingway *et al.*, 2005; Stuckey *et al.*, 2015). However, a study with 2441 participants emphasized the role of glycemic status above all other components of MetS as a cause for the impaired HRV (Jarczok *et al.*, 2013).

In the current study, the association of MetS with reduced HRV indices was found to be stronger in women than in men. This is in good concordance with previous studies (Stuckey *et al.*, 2014, 2015). The tilt test challenges the autonomic nervous system (Avolio & Parati, 2011; Teodorovich & Swissa, 2016), thus it is logical that the differences between MetS and control groups found in women were accentuated in the upright position. The reason why women with MetS seem to have relatively more disturbances in HRV than men with MetS remains unknown. However, hormonal factors may play an important role in this process.

Our study has some limitations. (i) The observational design does not allow conclusions about causal relationship. (ii) The collection of the breathing frequency data was not complete from all participants, and the depth of respiration was not measured. (iii) The information about the hormonal status like menstrual cycle and testosterone level could have given additional information, as hormonal factors potentially influence HRV. However, the use of exogenous female hormones was reported, and no difference was found between the W-MetS and W-control groups. (iv) Subjects using medications that are known to influence HRV were excluded, but it remains uncertain whether the medications that were used by some of the participants had an effect on the results. (v) The criteria by Alberti *et al.*(Alberti *et al.*, 2009) were used for the definition of MetS, instead of the definition by National Cholesterol Education Program (NCEP, 2001). Using the criteria of Alberti *et al.*, healthier subjects are defined as MetS patients. Of note, despite this MetS definition used in the current study, the results showed impaired HRV in women with MetS.

In conclusion, when the confounding factors were taken into account, the MetS-related changes in HRV seemed to be more pronounced in women than in men, especially in the upright position. It is possible that changes in cardiac autonomic tone, presented as lower total power and lower HF power of HRV, may contribute to the previously reported greater relative increase in cardiovascular risk in women than in men with MetS (Hunt *et al.*, 2004; Iglseder *et al.*, 2005; Schillaci *et al.*, 2006).

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Conflict of interest

The authors declare that the research was performed in the absence of any commercial or financial relationship that could be considered a potential conflict of interest.

References

Alberti KGMM, Eckel RH, Grundy SM, *et al.* Harmonizing the Metabolic Syndrome: a Joint Interim Statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation* (2009); **120**: 1640–1645.

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

Billman GE. Heart rate variability - a historical perspective. *Front Physiol* (2011); **2**: 86.

Billman GE. The effect of heart rate on the heart rate variability response to autonomic interventions. *Front Physiol* (2013a); **4**: 222.

Billman GE. The LF/HF ratio does not accurately measure cardiac sympatho-vagal balance. *Front Physiol* (2013b); **4**: 26.

Bonnemeier H, Richardt G, Potratz J, *et al.* Circadian profile of cardiac autonomic nervous modulation in healthy subjects: differing effects of aging and gender on heart rate variability. *J Cardiovasc Electrophysiol* (2003); **14**: 791–799.

Brown TE, Beightol LA, Koh J, *et al.* Important influence of respiration on human R-R interval power spectra is largely ignored. *J Appl Physiol (1985)* (1993); **75**: 2310–2317.

Brunner EJ, Hemingway H, Walker BR, *et al.* Adrenocortical, autonomic, and inflammatory causes of the metabolic syndrome: nested case-control study. *Circulation* (2002); **106**: 2659–2665.

Cooke WH, Hoag JB, Crossman AA, *et al.* Human responses to upright tilt: a window on central autonomic integration. *J Physiol* (1999); **517**(Pt 2): 617–628.

Eckberg DL. Sympathovagal balance: a critical appraisal. *Circulation* (1997); **96**: 3224–3232.

Flanagan DE, Vaile JC, Petley GW, *et al.* Gender differences in the relationship between leptin, insulin resistance and the autonomic nervous system. *Regul Pept* (2007); **140**: 37–42.

Grassi G. Sympathetic overdrive and cardiovascular risk in the metabolic syndrome. *Hypertens Res* (2006); **29**: 839–847.

Haensel A, Mills PJ, Nelesen RA, *et al.* The relationship between heart rate variability and inflammatory markers in cardiovascular diseases. *Psychoneuroendocrinology* (2008); **33**: 1305–1312.

Hemingway H, Shipley M, Brunner E, *et al.* Does autonomic function link social position to coronary risk? The Whitehall II study *Circulation* (2005); **111**: 3071–3077.

Hillebrand S, Swenne CA, Gast KB, *et al.* The role of insulin resistance in the association between body fat and autonomic function. *Nutr Metab Cardiovasc Dis* (2015); **25**: 93–99.

Howorka K, Pumprla J, Tamm J, *et al.* Effects of guided breathing on blood pressure and heart rate variability in hypertensive diabetic patients. *Auton Neurosci* (2013); **179**: 131–137.

Hunt KJ, Resendez RG, Williams K, *et al.* National Cholesterol Education Program versus World Health Organization metabolic syndrome in relation to all-cause and cardiovascular mortality in the San Antonio Heart Study. *Circulation* (2004); **110**: 1251–1257.

Iglseder BMD, Cip PMD, Malaimare LMD, *et al.* The metabolic syndrome is a stronger risk factor for early carotid atherosclerosis in women than in men. *Stroke* (2005); **36**: 1212–1217.

Inker LA, Schmid CH, Tighiouart H, *et al.* Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med* (2012); **367**: 20–29.

Jarczok MN, Li J, Mauss D, *et al.* Heart rate variability is associated with glycemic status after controlling for components of the metabolic syndrome. *Int J Cardiol* (2013); **167**: 855–861.

Kangas P, Tikkakoski AJ, Tahvanainen AM, *et al.* Metabolic syndrome may be associated with increased arterial stiffness even in the absence of hypertension: a study in 84 cases and 82 controls. *Metabolism* (2013); **62**: 1114–1122.

Kangas P, Tahvanainen A, Tikkakoski A, *et al.* Increased cardiac workload in the upright posture in men: noninvasive hemodynamics in men versus women. *J Am Heart Assoc* (2016); **5**: e002883.

Karas M, Lacourciere Y, LeBlanc AR, *et al.* Effect of the renin-angiotensin system or calcium channel blockade on the circadian variation of heart rate variability, blood pressure and circulating catecholamines in hypertensive patients. *J Hypertens* (2005); **23**: 1251–1260.

Katz A, Nambi SS, Mather K, *et al.* Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab* (2000); **85**: 2402–2410.

Kaur J. A comprehensive review on metabolic syndrome. *Cardiol Res Pract* (2014); **2014**: 943162.

Koenig J, Thayer JF. Sex differences in healthy human heart rate variability: a meta-analysis. *Neurosci Biobehav Rev* (2016); **64**: 288–310.

Koskela JK, Tahvanainen A, Haring A, *et al.* Association of resting heart rate with cardiovascular function: a cross-sectional study in 522 Finnish subjects. *BMC Cardiovasc Disord* (2013); **13**: 102.

Koskinen T, Kähönen M, Jula A, *et al.* Metabolic syndrome and short-term heart rate variability in young adults. The cardiovascular risk in young Finns study. *Diabet Med* (2009); **26**: 354–361.

Kromenacker BW, Sanova AA, Marcus FI, *et al.* Vagal mediation of low frequency heart rate variability during slow yogic breathing. *Psychosom Med* (2018); **80**: 581–587.

Li C, Chang Q, Zhang J, *et al.* Effects of slow breathing rate on heart rate variability and arterial baroreflex sensitivity in essential hypertension. *Medicine (Baltimore)* (2018); **97**: e0639.

Mancia G, Bombelli M, Corrao G, *et al.* Metabolic syndrome in the Pressioni Arteriose Monitorate E Loro Associazioni (PAMELA) study: daily life blood pressure, cardiac damage, and prognosis. *Hypertension* (2007); **49**: 40–47.

Mendis SPP, Norrving B (eds.). *Global atlas on cardiovascular disease prevention and control.* (2011). World Health Organization, Geneva, Switzerland.

Monfredi O, Lyashkov AE, Johnsen AB, *et al.* Biophysical Characterization of the Underappreciated and Important Relationship Between Heart Rate Variability and Heart Rate. *Hypertension* (2014); **64**: 1334–1343.

Mottillo S, Filion KB, Genest J, *et al.* The metabolic syndrome and cardiovascular risk a systematic review and meta-analysis. *J Am Coll Cardiol* (2010); **56**: 1113–1132.

NCEP. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III). *JAMA* (2001); **285**: 2486–2497.

Okano Y, Tamura K, Masuda S, *et al.* Effects of angiotensin II receptor blockers on the relationships between ambulatory blood pressure and anti-hypertensive effects, autonomic function, and health-related quality of life. *Clin Exp Hypertens* (2009); **31**: 680–689.

Paolisso G, Manzella D, Montano N, *et al.* Plasma leptin concentrations and cardiac autonomic nervous system in healthy

subjects with different body weights. *J Clin Endocrinol Metab* (2000); **85**: 1810–1814.

Patel VN, Pierce BR, Bodapati RK, *et al.* Association of holter-derived heart rate variability parameters with the development of congestive heart failure in the cardiovascular health study. *JACC Heart Fail* (2017); **5**: 423–431.

Peltola MA. Role of editing of R-R intervals in the analysis of heart rate variability. *Front Physiol* (2012); **3**: 148.

Prinsloo GE, Derman WE, Lambert MI, *et al.* The effect of a single session of short duration biofeedback-induced deep breathing on measures of heart rate variability during laboratory-induced cognitive stress: a pilot study. *Appl Psychophysiol Biofeedback* (2013); **38**: 81–90.

Rodriguez-Colon SM, Li X, Shaffer ML, *et al.* Insulin resistance and circadian rhythm of cardiac autonomic modulation. *Cardiovasc Diabetol* (2010); **9**: 85.

Sacha J. Why should one normalize heart rate variability with respect to average heart rate. *Front Physiol* (2013); **4**: 306.

Sacha J. Interaction between heart rate and heart rate variability. *Ann Noninvasive Electrocardiol* (2014); **19**: 207–216.

Sacha J, Pluta W. Alterations of an average heart rate change heart rate variability due to mathematical reasons. *Int J Cardiol* (2008); **128**: 444–447.

Sacha J, Barabach S, Statkiewicz-Barabach G, *et al.* Gender differences in the interaction between heart rate and its variability - how to use it to improve the prognostic power of heart rate variability. *Int J Cardiol* (2014); **171**: e42–e45.

Saito I, Hitsumoto S, Maruyama K, *et al.* Heart rate variability, insulin resistance, and insulin sensitivity in Japanese adults: the toon health study. *J Epidemiol* (2015); **25**: 583–591.

Schillaci G, Pirro M, Pucci G, *et al.* Different impact of the metabolic syndrome on left ventricular structure and function in hypertensive men and women. *Hypertension* (2006); **47**: 881–886.

Schuster AK, Fischer JE, Thayer JF, *et al.* Decreased heart rate variability correlates to increased cardiovascular risk. *Int J Cardiol* (2016); **203**: 728–730.

Stolarz K, Staessen JA, Kuznetsova T, *et al.* Host and environmental determinants of heart rate and heart rate variability in four European populations. *J Hypertens* (2003); **21**: 525–535.

Stuckey MI, Tulppo MP, Kiviniemi AM, *et al.* Heart rate variability and the metabolic syndrome: a systematic review of the literature. *Diabetes Metab Res Rev* (2014); **30**: 784–793.

Stuckey MI, Kiviniemi A, Gill DP, *et al.* Associations between heart rate variability, metabolic syndrome risk factors, and insulin resistance. *Appl Physiol Nutr Metab* (2015); **40**: 734–740.

Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *J Hypertens* (2007); **25**: 1105–1187.

Task Force of the European Society of Cardiology, and the North American Society of Pacing and Electrophysiology. Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. *Eur Heart J* (1996); **17**: 354–381.

Teodorovich N, Swissa M. Tilt table test today - state of the art. *World J Cardiol* (2016); **8**: 277–282.

Thayer JF, Yamamoto SS, Brosschot JF. The relationship of autonomic imbalance, heart rate variability and cardiovascular disease risk factors. *Int J Cardiol* (2010); **141**: 122–131.

Tikkakoski AJ, Tahvanainen AM, Leskinen MH, *et al.* Hemodynamic alterations in hypertensive patients at rest and during passive head-up tilt. *J Hypertens* (2013); **31**: 906–915.

Tsuji H, Larson MG, Venditti Jr FJ, *et al.* Impact of reduced heart rate variability on risk for cardiac events. The Framingham heart study. *Circulation* (1996); **94**: 2850–2855.

Vaile JC, Fletcher J, Al-Ani M, *et al.* Use of opposing reflex stimuli and heart rate variability to examine the effects of lipophilic and hydrophilic beta-blockers on human cardiac vagal control. *Clin Sci (Lond)* (1999); **97**: 585–593.; discussion 609-10.

Wulsin LR, Horn PS, Perry JL, *et al.* Autonomic imbalance as a predictor of metabolic risks, cardiovascular disease, diabetes, and mortality. *J Clin Endocrinol Metab* (2015); **100**: 2443–2448.

Wulsin LR, Horn PS, Perry JL, *et al.* The contribution of autonomic imbalance to the development of metabolic syndrome. *Psychosom Med* (2016); **78**: 474–480.

Xhyheri B, Manfrini O, Mazzolini M, *et al.* Heart rate variability today. *Prog Cardiovasc Dis* (2012); **55**: 321–331.