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Whole Body Impedance Cardiography and Continuous Pulse Wave Analysis in the Measurement of Human Haemodynamics during Passive Head-up Tilt

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LIST OF ORIGINAL COMMUNICATIONS

This dissertation is based on following original communications, which are referred to in the text by roman numerals I-V:

I   Anna Tahvanainen, Jenni Koskela, Antti Tikkakoski, Jorma Lahtela, Miia Leskinen, Mika Kähönen, Tuomo Nieminen, Tiit Kööbi, Jukka Mustonen and Ilkka Pörsti: Analysis of cardiovascular responses to passive head-up tilt using continuous pulse wave analysis and impedance cardiography. Scandinavian Journal of Clinical and Laboratory Investigation 2009; 69: 128-137.

II   Anna Tahvanainen, Antti Tikkakoski, Miia Leskinen, Klaus Nordhausen, Mika Kähönen, Tiit Kööbi, Jukka Mustonen and Ilkka Pörsti: Supine and upright haemodynamic effects of sublingual nitroglycerin and inhaled salbutamol – a double-blind, placebo-controlled, randomized study. Journal of Hypertension, in press.

III   Anna Tahvanainen, Miia Leskinen, Jenni Koskela, Erkki Ilveskoski, Juha Alanko, Mika Kähönen, Tiit Kööbi, Lauri Lehtimäki, Eeva Moilanen, Jukka Mustonen and Ilkka Pörsti: Non-invasive measurement of the haemodynamic effects of inhaled salbutamol, intravenous L-arginine and sublingual nitroglycerin. British Journal of Clinical Pharmacology 2009; 68: 23-33.

IV   Anna Tahvanainen, Jenni Koskela, Miia Leskinen, Erkki Ilveskoski, Klaus Nordhausen, Mika Kähönen, Tiit Kööbi, Jukka Mustonen and Ilkka Pörsti: Reduced systemic vascular resistance in healthy volunteers with presyncopal symptoms during a nitrate-stimulated tilt-table test. British Journal of Clinical Pharmacology 2011; 71: 41-51.

V   Anna Tahvanainen, Miia Leskinen, Jenni Koskela, Erkki Ilveskoski, Klaus Nordhausen, Hannu Oja, Mika Kähönen, Tiit Kööbi, Jukka Mustonen and Ilkka Pörsti: Ageing and cardiovascular responses to head-up tilt in healthy subjects. Atherosclerosis 2009; 207: 445-451.

ABBREVIATIONS

AASI	Ambulatory arterial stiffness index
ACh	Acethylcholine
AIx	Augmentation index
ANOVA	One-way analysis of variance
BMI	Body mass index
BP	Blood pressure
C1	Capacitative compliance
C2	Reflective compliance
Ca^{2+}	Calcium
CI	Cardiac index
CO	Cardiac output
eNOS	Endothelial nitric oxide synthase
FMD	Flow mediated dilatation
GFR	Glomerular filtration rate
HR	Heart rate
HRV	Heart rate variability
ICG	Impedance cardiography
ICG_{WB}	Whole-body impedance cardiography
ICG_{TH}	Thoracic impedance cardiography
K^{+}	Potassium
MetS	Metabolic syndrome
MSNA	Muscle sympathetic nerve activity
Na^{+}	Sodium
NO	Nitric oxide
NTG	Nitroglycerin
PP	Pulse pressure
PWV	Pulse wave velocity
SEM	Standard error of the mean
SI	Stroke index
SV	Stroke volume
SVR	Systemic vascular resistance
SVRI	Systemic vascular resistance index

ABSTRACT

Regulation of blood pressure is a complex interplay between several haemodynamic mechanisms, but only resting blood pressure and heart rate are commonly measured in clinical practice to determine a patient's haemodynamic status. However, the value of single blood pressure measurements as diagnostic tests has recently been questioned. In addition, changes in for example arterial compliance, endothelial function, or the regulation of vascular resistance can already be measured before clinical manifestations of cardiovascular disease.

The aim of the present study was to test the repeatability and reproducibility of a comprehensive haemodynamic measurement protocol in both supine position and during head-up tilt. In addition, the effects on two largely endothelium-dependent agents, inhaled salbutamol and intravenous L-arginine, and one endothelium-independent agent, sublingual nitroglycerin, were investigated, and the haemodynamic mechanism of nitrate-induced presyncope were examined. Moreover, the age-related haemodynamic changes, supine and upright, in normotensive subjects were clarified. Finally, the application was tested in patients with either essential hypertension or metabolic syndrome.

Non-invasive haemodynamic measurements were performed by applying whole body impedance cardiography, continuous pulse wave analysis and plethysmographic finger blood pressure measurements in supine position and during head-up tilt. Thus, peripheral and central blood pressure, indices of pulse wave reflection, pulse wave velocity, vascular resistance, and cardiac function could be simultaneously assessed. Repeated measurements were performed to test the repeatability and reproducibility of the method, and to study the drug effects in a placebo-controlled manner. All study subjects, except for the subjects included in the testing of the method's clinical application, were normotensive and had no medication affecting cardiovascular status.

The measurement protocol was repeatable and reproducible in both supine position and during head-up tilt. Inhaled salbutamol decreased blood pressure, vascular resistance and augmentation index, while heart rate and cardiac output were increased. Sublingual nitroglycerin clearly decreased blood pressure, vascular

resistance, augmentation index and pulse wave velocity, and increased heart rate and cardiac output. Importantly, the haemodynamic effects of nitroglycerin were enhanced during the head-up tilt, while the salbutamol effects were more evident in the supine position. Intravenous L-arginine resulted in decreased blood pressure and increased aortic reflection time only during the tilt. During nitrate-induced presyncope, reduced vascular resistance was observed. Increasing age from 20 to 60 years was associated with increased arterial stiffness and wave reflection, while no changes were observed in cardiac function or vascular resistance. A more pronounced decrease in central systolic blood pressure in response to tilt was associated with increased age and arterial stiffness. Subjects with metabolic syndrome had higher heart rate and arterial stiffness than hypertensive subjects, despite similar elevation of blood pressure and vascular resistance. These results suggest more widespread changes in cardiovascular status than just elevated blood pressure in metabolic syndrome.

In conclusion, the present measurement protocol enables a detailed characterization of patients' haemodynamic profile, including the assessment of the role of the endothelium in vascular responsiveness. The divergent effects of research drugs in different postures, and the age-related changes in haemodynamic responsiveness to head-up tilt underlie the importance to study upright haemodynamics.

TIIVISTELMÄ

Hyvin moni rakenteellinen ja toiminnallinen tekijä osallistuu ihmisen verenpainetason säätelyyn. Kliinisessä työssä tyydytään kuitenkin usein verenpaineen ja syketason määrittämiseen levossa, vaikka kertamittausten arvo kohonneen verenpaineen diagnostiikassa onkin viime aikoina kyseenalaistettu. Myös muutoksia mm. endoteelin toiminnassa, valtimokomplianssissa ja ääreisverenkierron vastuksen säätelyssä on mahdollista todeta jo ennen tautitapahtumien ilmaantumista. Tutkimuksen tarkoituksena oli kehittää toistettava ja monipuolinen sydän- ja verenkiertoelimistön mittausmenetelmä, jossa hemodynaamisia muuttujia mitataan sekä makuulla että kallistuskokeen aikana. Lisäksi tutkittiin kahden pääasiassa endoteelivälitteisen lääkeaineen, inhaloidun salbutamolin ja suonensisäisen L-arginiinin, ja yhden endoteelista riippumattoman, sublinguaalisen nitroglyseriinin, hemodynaamisia vaikutuksia, sekä nitroglyseriinin aiheuttaman presynkopeen hemodynaamisia mekanismeja. Tutkimuksessa selvitettiin myös ikääntymiseen liittyviä sydän- ja verenkiertoelimistön muutoksia. Menetelmää testattiin metabolista oireyhtymää ja essentielliä verenpainetautia saitastavilla potilailla.

Kajoamattomat hemodynamiikan mittaukset suoritettiin sekä levossa että passiivisen kallistuskokeen aikana koko kehon impedanssikardiografiaa, jatkuvaa pulssiaaltoanalyysiä ja pletysmografista sormiverenpaineen mittausta käyttäen. Samanaikaisesti mitattavia muuttujia olivat perifeerinen ja aorttatason verenpaine, pulssiaallon heijastumisesta kertovat suureet, pulssiaallon etenemisnopeus, ääreisverenkierron vastus sekä sydämen isku- ja minuuttitilavuus. Toistettuja mittauksia suoritettiin menetelmän toistettavuuden ja lumelääkekontrolloitujen lääkevasteiden määrittämiseksi. Kaikilla tutkimushenkilöillä, lukuunottomatta metabolista oireyhtymää tai verenpainetautia sairastavia, oli normaali verenpaine, eikä heillä ollut käytössä hemodynamiikkaan vaikuttavia lääkeaineita.

Mittaukset olivat toistettavia sekä makuulla että kallistuskokeen aikana. Salbutamoli-inhalaatio laski verenpainetta, ääreisvastusta ja augmentaatioindeksiä, sekä nosti sykettä ja sydämen minuuttitilavuutta. Sublinguaalinen nitroglyseriini laski huomattavasti verenpainetta, ääreisvastusta, augmentaatioindeksiä ja pulssiaallon etenemisnopeutta, ja syke sekä sydämen minuuttitilavuus kasvoivat. Salbutamolin

aiheuttamat muutokset korostuivat makuuasennossa, kun taas vaste nitroglyseriinille on selvempi kallistuskokeen aikana. Suonensisäinen L-arginiini aiheutti verenpaineen laskun ja pulssiaallon takaisinheijastuma-ajan kasvun kallistuskokeen aikana.

Nitroglyseriinin aiheuttaman presynkopeen aikana havaittiin selvästi vähentynyt ääreisverenkierron vastus. Valtimojäykkyys ja pulssiaallon takaisinheijastuminen lisääntyivät iän myötä, mutta ääreisvastus ja sydämen pumppaustoiminta eivät muuttuneet. Suurempi aorttatason systolisen verenpaineen lasku kallistuskokeessa liittyi korkeampaan ikään ja suurempaan pulssiaallon etenemisnopeuteen. Metabolista oireyhtymää sairastavilla potilailla oli korkeampi syketaso ja pulssiaallon etenemisnopeus kuin verenpainetautia sairastavilla, vaikka verenpainetaso ja ääreisvastus eivät eronneet merkittäväst. Tulos osoittaa, että tutkituilla potilasryhmillä on verenpainetasosta riippumattomia eroja verenkiertoelimistön rakenteessa ja toiminnassa.

Mittausmenetelmä on kajoamaton ja toistettava, ja mahdollistaa entistä tarkemman sydän- ja verenkiertoelimistön toiminnan määrittämisen. Metelmän avulla voidaan arvioida myös endoteelin osuutta hemodynamiikan säätelyssä. Tulokset korostavat pystyasennossa suoritettavien mittausten tärkeyttä, koska lääkevasteet ja ikääntymiseen liittyvät muutokset olivat erilaisia makuulla ja kallistuskokeessa.

INTRODUCTION

Elevated blood pressure (BP) and related cardiovascular complications are the leading causes of morbidity and mortality in the modern world (Ezzati et al. 2002). Thus, effective antihypertensive medication is widely available, and the target level of BP is set lower than ever. However, a significant part of hypertensive patients fail to reach the intended BP level despite active treatment. For example, only 50% of the subjects with elevated BP in single measurements were aware of the disorder, and only 25-30% of hypertensive patients receiving antihypertensive medication reached the BP goal in a Finnish population study (Antikainen et al. 2006). Besides hypertension, also other measurable haemodynamic factors, like increased arterial stiffness or impaired endothelial function, have been associated with poor cardiovascular outcome (Laurent et al. 2001, Fichtlscherer et al. 2004).

Currently, the diagnosis of hypertension is based on the average values of the repeated clinic or home BP measurements, and ambulatory 24-hour BP recording including several measurements is applied to selected patients (Mancia et al. 2007). However, according to a recent meta-analysis, single BP measurements have insufficient sensitivity and specificity as a diagnostic test of hypertension when compared with ambulatory recordings (Hodgkinson et al. 2011). Importantly, the knowledge of patients' BP and heart rate (HR) level only, either from single resting measurements or from ambulatory recordings, provides limited information about the functional haemodynamic status.

The haemodynamic changes resulting in elevated BP may differ substantially between patients and disorders. Systemic vascular resistance (SVR) is considered to be elevated in essential hypertension (Omvik et al. 2000), while changes in fluid- and electrolyte balance are common in chronic kidney disease (Luke 1998). In addition, decreased large arterial compliance is associated with various cardiovascular disorders, as well as to normal vascular ageing (McEniery et al. 2005, Greenwald 2007). Antihypertensive medication aims to reduce vascular resistance, left ventricular preload, and to improve arterial compliance, but the treatment decisions and the assessment of treatment effects are solely based on the BP level. The assessment of individual haemodynamic profile beyond elevated BP could aid the

physicians to design and follow-up the antihypertensive treatment. For example, measurement of cardiac function and vascular resistance by non-invasive impedance cardiography (ICG) before antihypertensive treatment has been shown to improve the treatment results when compared with conventionally selected antihypertensive medication (Smith et al. 2006).

Traditionally, patients are instructed to rest about 15 minutes before BP measurement (Mancia et al. 2007). However, humans spend a great proportion of the waking hours in the upright position or in locomotion, which questions the value of measurements performed only at rest. Previously, the haemodynamic response to physical challenge has been shown to predict cardiovascular outcome. For example, the BP response to two-step exercise test strongly predicted the future incidence of hypertension in Japanese men (Tsumura et al. 2002). Since the change in body position from supine to upright induce significant changes in for example blood volume distribution, autonomic nervous tone and cardiac function, it can be considered as a simple exercise test (Avolio and Parati 2011).

The aim of the present study was to develop and test a comprehensive haemodynamic measurement protocol. The method includes the determination of peripheral and central BP, cardiac function, vascular resistance, arterial compliance, indices of pulse wave reflection and global endothelial function. Besides the measurements performed in the supine position, passive orthostatic challenge is included to the protocol to assess functional haemodynamic status.

REVIEW OF THE LITERATURE

1. Haemodynamic determinants of blood pressure

    1.1. Cardiac function and vascular resistance

As defined in basic physiology, arterial pressure is a balance between blood flow into and out of the arteries. Blood flow into the aorta is equal to cardiac output (CO) from the left ventricle, while blood flow out of the arteries is mainly influenced by resistance to blood flow in arterioles, SVR (Guyton and Hall 2006). CO is defined as a product of HR and stroke volume (SV). Stimulation of adrenergic and cholinergic receptors of autonomic nervous system influences the HR, while SV is defined by the force of ventricular contraction and intravascular fluid volume. Multiple mechanisms including structural, neural, humoral and renal factors control the level of SVR locally, regionally and systematically (Guyton and Hall 2006). Several haemodynamic factors that have an influence on either of the basic determinants of BP, CO or SVR, are discussed below.

    1.2. Arterial pulse wave form and reflection

        1.2.1. Pulse wave form and augmentation

Pulse wave recorded from an artery is a sum of forward pressure wave originating from the left ventricle and backward pressure wave reflecting from various reflection sites in the circulation. Main sites for wave reflection are branching points in the arterial tree, sites where arterial distensibility or lumen cross-sectional area changes, and peripheral arterioles with high resistance (Pedley 1980, O'Rourke 1982, Latham et al. 1987, Nichols and O'Rourke 1998). Various landmarks are recognizable from the pressure waveform, as depicted in Figure 1. Start of the reflected wave can be identified in the pressure waveform as an inflection point. In young, compliant arteries the velocity of the pulse wave is low and the reflected wave tends to return during diastole. In stiffer arteries, the wave reflection occurs already during systole while aortic valve is still open. The contribution of the reflected wave to systolic pressure is called augmentation pressure (pressure difference of the first and second

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systolic peaks in the pulse waveform). Augmentation index (AIx) is defined as a ratio between augmentation pressure and pulse pressure (PP) (Nichols and O'Rourke 1998).

Figure 1. Pulse wave form
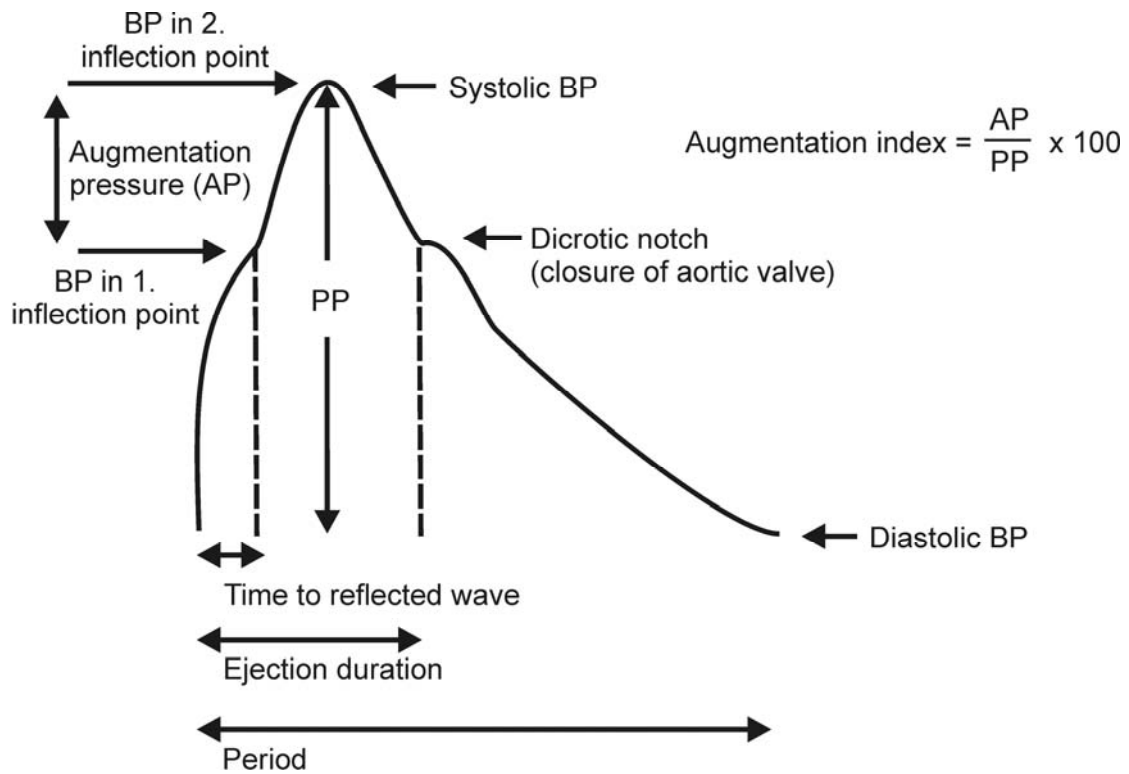
AP=augmentation pressure, BP= blood pressure, PP=pulse pressure

1.2.2. Amplification

The shape of the pressure pulse originating from the left ventricle is modified as it travels through the arterial tree. The pulse waveform features alter, and the amplitude increases toward periphery. In young, compliant arteries the systolic peak of the peripheral waveform is generally narrower and higher than that of the central arteries, while mean BP and diastolic BP remain almost steady (Latham et al. 1985, Pauca et al. 1992). This difference in systolic BP and in PP between central and peripheral sites is known as amplification (Figure 2). In peripheral arteries, the pulse wave reflection sites are closer than in the larger arteries which amplify the pressure wave (Latham et al. 1985). Additionally, pulse wave travels faster in stiffer peripheral arteries further increasing the amplification. As the large arteries stiffen, for example with ageing,

the amplification phenomenon decreases, thus central and peripheral systolic BPs converges.

Figure 2: Variation of pressure wave form along arterial tree; amplification. Modified from Protogerou et al. 2007.
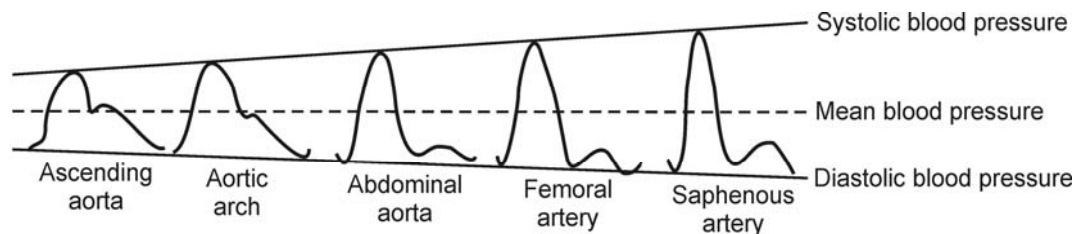
1.3. Arterial stiffness

1.3.1. Overview

Arterial distensibility stands for the change in volume or cross-sectional area of a vessel for a given change in pressure during cardiac cycle, and arterial stiffness is defined by a reduction in arterial distensibility. Young, compliant arteries cushion the arterial pulsations so that the flow in the capillary level remains continuous (O'Rourke and Hashimoto 2007). Reduced arterial distensibility transfers pulsatile flow further to the periphery, which has harmful influences in the microcirculation (O'Rourke and Safar 2005). Stiffening of the arteries leads to increased pulse wave velocity (PWV) and earlier wave reflection, thus increasing systolic BP and PP and decreasing perfusion of the myocardium during the diastole, and increasing the load to left ventricle (Nichols and O'Rourke 1998, Mitchell et al. 2004, O'Rourke and Hashimoto 2007). Additionally, arterial stiffness increases the risk of atherosclerotic plaque rupture (Van Bortel 2002). Due to stiffening, arteries undergo a process of remodelling to reduce wall stress, leading to intima–media thickening (Dao et al. 2005). Moreover, endothelial nitric oxide (NO) production is reduced as the shear stress rate falls with arterial stiffening, which increases the risk for atheroma formation (Soucy et al. 2006). Based on these facts, increased arterial stiffness has been recognized as an independent risk factor for cardiovascular mortality and

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morbidity both in healthy subjects and in subjects with various medical disorders (Laurent et al. 2001, Blacher et al. 2002, Safar et al. 2002a, Sutton-Tyrrell et al. 2005). Harmful effects of arterial stiffening have been presented in Figure 3.

Figure 3. Typical arterial pulse wave forms in compliant (A) and stiff (B) arteries.
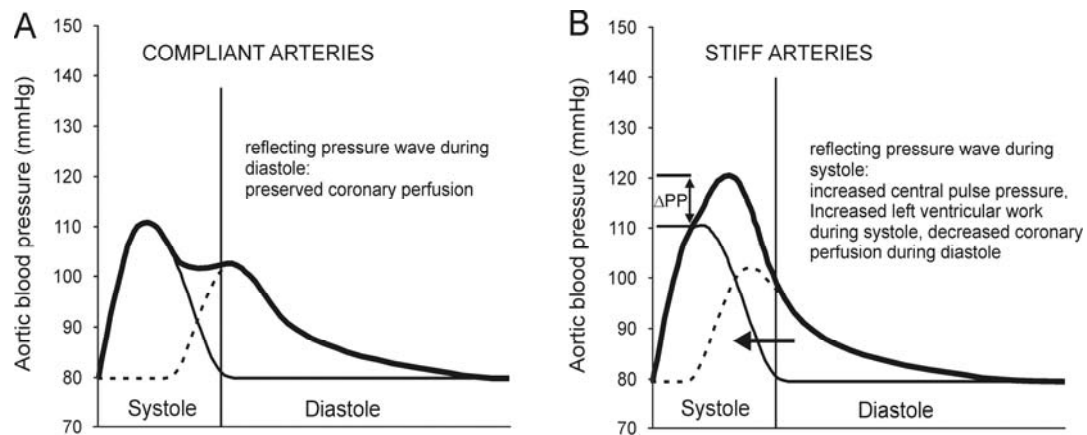
Thin black line=forward pressure wave; dashed line=reflecting pressure wave; bold line=combined pressure wave

1.3.2. Determinants of arterial stiffness

Structure of the arterial wall
The molecular, cellular and histological structure of the arterial wall is an important determinant of arterial stiffness and it greatly varies along arterial tree. The predominant elastic materials of the arterial wall are elastin and collagen, while third important component of the arterial wall, smooth muscle, cannot be regarded as true elastic material (Nichols and O'Rourke 1998). In the proximal, large arteries elastin is the dominant component, while in the distal arteries collagen dominates. The elastic modulus of collagen is significantly higher than that of elastin, which results to increased arterial stiffness in distal arteries (Armentano et al. 1991). As a result, PWV increases from 4-5 m/s in the aorta to 8-9 m/s in the peripheral arteries (Latham et al. 1985, Nichols and O'Rourke 1998).

Age
Ageing is associated with progressive structural and functional changes in the cardiovascular system. With increasing age large arteries dilate and stiffen as a result

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of thickening of the intima, reduced elastin content and vascular smooth muscle cell number, and increased collagen composition in the vessel wall (Greenwald 2007, O'Rourke and Hashimoto 2007). The elastin lamellae undergo structural changes with age, showing signs of fragmentation, disorganisation and calcification (O'Rourke 1976). These changes are apparent especially in large arteries, while aging does not seem to affect the mechanical properties of smaller, muscular arteries to the same extent probably due to different morphology (Smulyan et al. 1983, Kawasaki et al. 1987, Bortolotto et al. 1999). The relationship between large arterial stiffening and age has documented to be non-linear with an acceleration of age-related aortic stiffening after the 5th decade of life (McEniery et al. 2005).

Cardiovascular risk factors and disorders
Several cardiovascular risk factors and diseases are known to cause similar morphological changes in arterial wall as ageing, further accelerating arterial stiffening. Hypertension has been shown to be associated with increased large artery stiffness when compared with age-matched normotensive subjects (Bouthier et al. 1985, Liu et al. 1989, Benetos et al. 2002, Greenwald 2007). On the other hand, the incidence of hypertension is increased in normotensive subjects with greater large arterial stiffness (Liao et al. 1999, Dernellis and Panaretou 2005). Different stages of chronic kidney disease has been linked to large and peripheral muscular arterial stiffening (Wang et al. 2005, Briet et al. 2006, Lacy et al. 2006), and an inverse relationship between aortic and radial arterial stiffness and glomerular filtration rate (GFR) has been documented in hypertensive subjects with normal renal function (Schillaci et al. 2006). Furthermore, level of fasting plasma glucose has been shown to correlate with indices of arterial stiffness in population studies (Salomaa et al. 1995, Sipila et al. 2007), and the association between arterial stiffness and Type 1 and 2 diabetes has been repeatedly demonstrated (Oxlund et al. 1989, McVeigh et al. 1993, Taniwaki et al. 1999, Devereux et al. 2000). An association between lipid profile abnormalities and arterial stiffening has been demonstrated in some studies (Riggio et al. , Juonala et al. 2005), while others have shown no correlation (Saba et al. 1999). In addition, other classic risk factors of cardiovascular disease, like increased waist circumference, body mass index (BMI) and smoking have been linked to large arterial stiffening (Liang et al. 2001, Schillaci et al. 2005).

1.4. Autonomic nervous system

It is widely acknowledged that the autonomic nervous system and its sympathetic arm have an important role in the short-term regulation of BP via baroreflex mechanisms responding to rapid changes in arterial pressure (Cowley et al. 1973). However, the role of sympathetic activation in the long-term regulation of BP is a more controversial issue. Sympathetic outflow, estimated by the measurement of muscle sympathetic nerve activity (MSNA), is not clearly associated with baseline BP levels since high MSNA levels have also been demonstrated in normotensive subjects (Charkoudian et al. 2005), and the rise in MSNA associated to elevated BP is only modest (Floras and Hara 1993). In younger men, an inverse relationship between MSNA and both CO and adrenergic sensitivity has been suggested as an explanation for the overlap in MSNA distribution between normotensive and hypertensive subjects (Charkoudian et al. 2005, Charkoudian et al. 2006). In addition, high MSNA is associated with increased endothelial NO release in young (21-33 years) men suggesting that endothelium-mediated vasodilatation might compensate for the sympathetically driven vasoconstriction (Charkoudian et al. 2006). Moreover, sympathetic activity increases with ageing and the relationship between BP and MNSA becomes more evident in individuals aged over 40 years (Narkiewicz et al. 2005). However, the above mentioned mechanisms explaining interindividual variability in MNSA could not be demonstrated in older subjects (Hart et al. 2009).

The balance between sympathetic and parasympathetic tone can also be evaluated by measuring HR variability (HRV) at rest or in response to physical or pharmacological stimuli (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996). Low HRV representing decreased parasympathetic activity is associated with hypertension and with the risk of developing elevated BP (Schroeder et al. 2003). In addition, decreased HRV predicts mortality in both high risk and low risk populations (Tsuji et al. 1994, Gerritsen et al. 2001).

1.5. Vascular endothelium

1.5.1. Role of the endothelium in vascular regulation

Endothelium responds to various physical and chemical signals in the circulation by releasing factors that regulate vascular permeability, platelet and leucocyte adhesion and aggregation, thrombosis, smooth muscle cell proliferation and vascular tone (Landmesser et al. 2004, Rumbaut et al. 2005). An important pathway for endothelium-mediated vascular smooth muscle cell relaxation is the activation of the enzyme endothelial NO synthase (eNOS) and subsequent release of NO, which in turn stimulates gyanylate cyclase in vascular smooth muscle inducing vasorelaxation. Essential for this reaction is the increase in calcium (Ca^{2+}) concentration within the endothelial cells. Moreover, release of prostacyclin from the endothelial cells stimulates adenylate cyclase in the vascular smooth muscle leading to vasorelaxation via hyperpolarization (Feletou and Vanhoutte 2006). In addition, several NO and prostacyclin-independent pathways of endothelium-derived vascular relaxation via hyperpolarization of smooth muscle have been recognized (Feletou and Vanhoutte 2006, Deanfield et al. 2007). Briefly, these pathways include an increase in intracellurar Ca^{2+} concentration, activation of calcium-activated potassium (K^+) channels, and subsequent hyperpolarization of endothelial cells. Hyperpolarization is spread to the underlying smooth muscle via myo-endothelial gap junctions or via increased K^+ concentration in the intercellural space (Feletou and Vanhoutte 2006). Such mechanisms can compensate for the loss of NO-mediated vasodilatory capacity during various pathophysiological conditions (Halcox et al. 2001). Besides vasodilatory effects, endothelium produces vasoconstrictors like endothelin, prostaglandin $F_{2\alpha}$ and thromboxane, and converts angiotensin I to vasoconstrictive angiotensin II (Saye et al. 1984, Kinlay et al. 2001) thus having a diverse role in vascular tone regulation. The most important pathways for endothelium-deriver relaxation and contraction are presented in Figure 4, and a schematic view of endothelium-derived hyperpolarization of smooth muscle is depicted in Figure 5.

Figure 4. Most important pathways for endothelium-derived vascular smooth muscle cell relaxation and contraction.
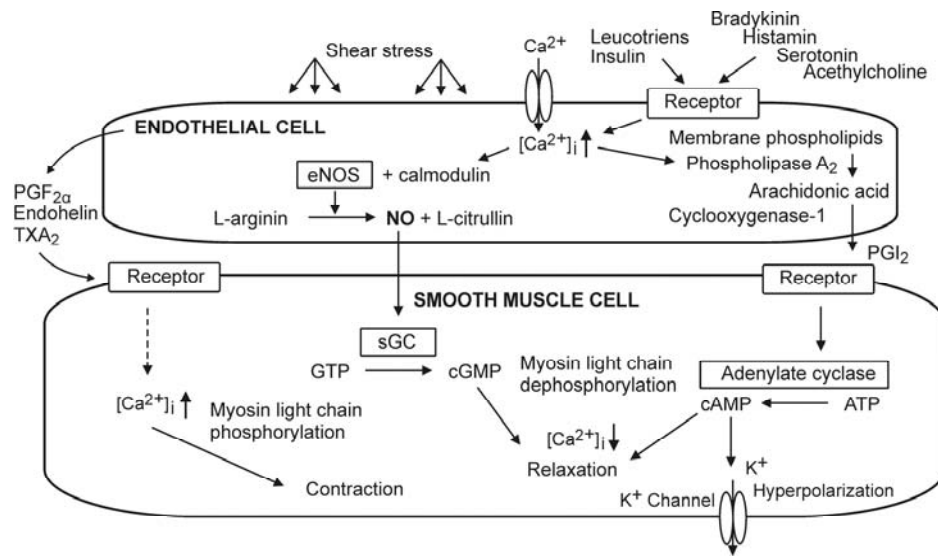
PG $F_{2\alpha}$=prostaglandin $F_{2\alpha}$, TXA_2=thromboxane, eNOS=endothelial nitric oxide synthase, PGI_2= prostacyclin, sGC=soluble guanylate cyclase, GTP=guanosine triphosphate, cGMP=cyclic guanosine monophosphate, ATP=adenosine triphosphate, cAMP=cyclic adenosine monophosphate

Figure 5. Hyperpolarization of endothelial and vascular smooth muscle cells.
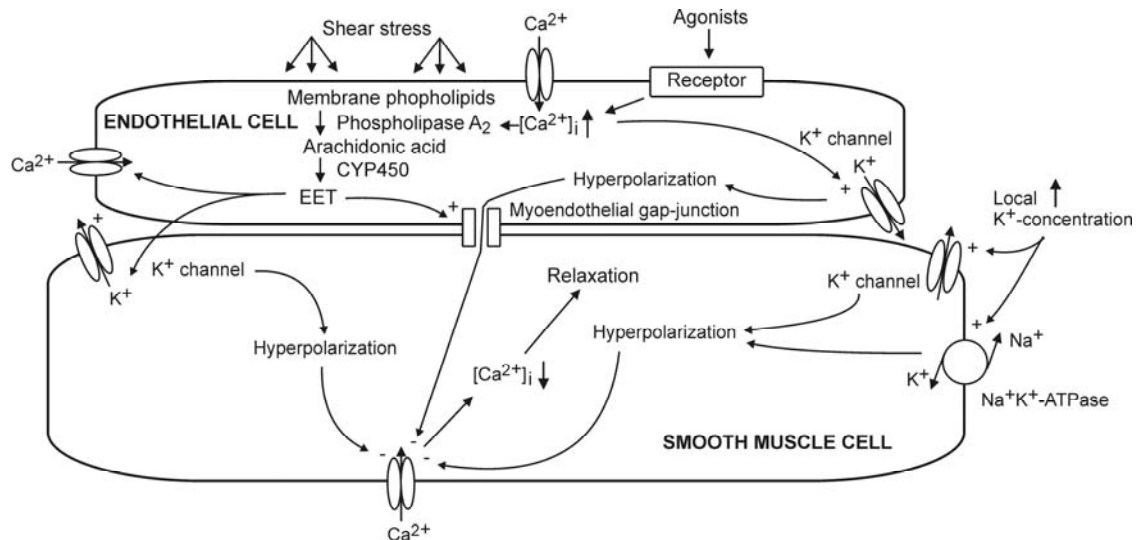
EET=epoxyeicosatrienoic acid

1.5.2. Endothelial dysfunction

The present view is that endothelial dysfunction is characterized by excessive production of reactive oxygen species, which reduce the bioavailability of endothelium-derived NO and other vasodilatory autacoids (Rhee 2006). As a result, expression and synthesis of proinflammatory and prothrombotic factors and adhesion molecules are increased, and the healthy regulation of vascular tone is disturbed (Drexler 1997). Impaired endothelial function has been repeatedly demonstrated in clinically healthy subjects with cardiovascular risk factors, which indicates that the changes in endothelial function already appear in the early stages of atherosclerosis (Celermajer et al. 1994, Ross 1999, Hashimoto et al. 2000). Furthermore, endothelial dysfunction has been linked to various cardiovascular diseases including hypertension, dyslipidaemia, coronary artery disease, peripheral arterial disease and diabetes (Creager et al. 1990, Neunteufl et al. 1997, Perticone et al. 1998, Yu et al. 2001), as well as to future cardiovascular events in subjects with atherosclerotic risk factors, stable ischaemic heart disease and acute coronary syndromes (Perticone et al. 2001, Fichtlscherer et al. 2004). In hypertensive subjects, improved endothelial function with antihypertensive medication has been correlated with a more favourable prognosis (Modena et al. 2002).

1.6. Fluid and electrolyte balance – role of the kidneys

Sodium (Na^+) and other anions serve as principal effective osmoles holding water in the extracellular space, while K^+ acts to hold water within the cells. Thus, the control of Na^+ balance is essential for the maintenance of the extracellular fluid volume and BP, and it is known that excessive dietary Na^+ intake is associated with increased BP (He and MacGregor 2004). The mechanism pressure natriuresis - pressure diuresis can explain how the kidney can alter the level of Na^+ and water excretion in response to changes in renal arterial pressure (Guyton 1989a). Increased free radical generation and oxidative stress in the renal medulla may lead to dysfunction in the pressure natriuresis system, thus resulting in Na^+ and volume retention, which subsequently predisposes to the development of hypertension (Cowley 2008). In addition, a decrease in renal BP stimulates the synthesis and release of renin from the juxtaglomerular cells thus activating the renin-angiotensin-aldosterone system, which

finally leads to increased Na$^+$ reabsorbtion in the renal tubule. Although multiple physiological mechanisms act in response to altered body fluid balance, normally only a change in Na$^+$ and water excretion by the kidneys can bring fluid balance and arterial pressure back to normal levels, thereby emphasizing the role of kidneys in long-term control of BP (Guyton 1989b).

2. Measurement of haemodynamics and haemodynamic reactivity in humans

2.1. Overview

Cardiovascular complications related to elevated BP and other disorders are the leading causes of mortality and morbidity in the modern world (Ezzati et al. 2002). As discussed previously, various changes in the cardiovascular system can result in elevated BP. However, in clinical practice only BP and HR are traditionally determined when hypertensive subjects are examined. In addition, the traditional measurements are only performed in resting conditions with the subject supine or seated, even though humans spend a great proportion of the day in the upright position or in locomotion. Ideally, the personal characteristics of the cardiovascular system should be determined, so that possible treatment decisions of elevated BP could be based on physiological facts, not merely on assumptions. Indeed, it has been demonstrated in hypertensive subjects that when the cardiovascular status of a patient group was examined in more detail by the use of non-invasive CO and SVR determinations, target BP levels could be reached more often than in a conventionally treated control study group (Smith et al. 2006). Below, the main methods available for the measurement of human haemodynamics are discussed.

2.2. Measurement of cardiac output

2.2.1. Fick Principle and indicator dilution techniques

In 1870, Adolph Fick demonstrated the first technique for CO measuring based on the assumption that oxygen consumption of the body is equal to the product of blood flow (CO) and arterio-venous oxygen content difference (Cournand et al. 1945). However, the direct Fick technique is laborious and prone to errors, requiring pulmonary arterial

catheterization and determination of several variables under stable conditions. The Fick principle can also be applied to other gasses that diffuse through the lungs, especially carbon dioxide (Gueret et al. 2006).

Several indicator dilution techniques have been introduced in the measurement of CO so that an indicator is injected and detected downstream of the injection site. Dilution signal is processed and CO is computed as a ratio of the indicator dose and area under dilution curve. Intermittent pulmonary thermodilution is based on the conservation of thermal energy with a bolus of cold saline as an indicator, and the technique has been accepted as a clinical standard for CO measurement (Jansen et al. 2001). Continuous measurement of CO following the principles of thermodilution can be performed using a pulmonary arterial cathether with an embedded heating filament (Yelderman 1990). The filament releases thermal pulses every 30-60 seconds and the change in blood temperature is detected downstream. However, these methods are a source of potential serious complications due to pulmonary arterial catheterization. In the transpulmonary thermodilution method cold saline is injected to central vein and the dilution curve is detected from femoral, axillary or brachial artery (Pauli et al. 2002). As a less invasive technique, transpulmonary thermodilution is more widely applicable. Finally, injection of small dose of lithium chloride into peripheral vein and the detection of dilution curve from the peripheral artery relies on the same principles mentioned above (Costa et al. 2008).

2.2.2. Invasive and non-invasive pulse contour methods

There are several commercially available invasive techniques that indirectly estimate the CO from arterial pressure pulsation (Table 1). In general, arterial pressure waveform is obtained using an intra-arterial pressure sensor in a peripheral artery and conducted to a CO-device. Using specific algorithms, individual demographic data or calibration measurements with indicator dilution technique, SV and subsequently CO is calculated from the pressure wave curve. Pulse contour analysis systems have been validated against indicator dilution techniques in several patient groups, and naturally the methods utilizing calibration with indicator dilution method have documented the best agreement with reference techniques. In general, pulse contour derived measurements track the changes in CO relatively well despite the potential error in

baseline values (de Wilde et al. 2009). In a recent meta-analysis by Peyton and Chong (2010) 59 studies with non-calibrated and calibrated pulse contour CO measurements in haemodynamically stable patients were evaluated. Altogether 24 studies were included in the meta-analysis, and the percentage error for mean bias was 41.3% against thermodilution, while the commonly accepted limit for percentage error should be less than 30 %.

Arterial pressure wave input for pulse contour analysis of CO can also be captured non-invasively for example using plethysmographic finger BP measurements, which track the changes in intra-arterial pressure well (Wesseling et al. 1985). However, the systematic difference between intra-arterial and finger BP must be taken into consideration in CO determination (Guelen et al. 2008). Recently, two non-invasive pulse contour methods with different equations used in the CO determination were evaluated against invasive pulse contour methods and thermodilution (Bogert et al. 2010). One method calculated SV by dividing the area under the systolic portion of the arterial pressure curve by the aortic input impedance, while the other generated aortic flow curves constructed from pressure waveforms. The results demonstrated that the first method but not the second, had a good agreement with thermodilution derived CO-values. Thus, comparison of different methods and software version is not without concerns. Major limitations of pulse contour CO are listed in Table 1.

2.2.3. Ultrasound methods

CO can be estimated using Doppler ultrasound derived aortic blood flow velocity and cross-sectional area of the aorta, obtained either transthoracically or transoesophagially. However, ultrasound methods are highly operator-dependent and time-consuming, and major potential sources of errors include position and angle of the transducer and movements of the subject. Older studies with ultrasound techniques have documented poor agreement for baseline values in comparison with references methods, but the changes in CO have been tracked well (Lavandier et al. 1985, Schmid et al. 1993). Studies performed with newer equipment have shown better agreement, as stated in a recent meta-analysis (Dark and Singer 2004).

Table 1. Arterial pulse contour analysis methods for cardiac output (CO) determination

Technique	Trademark	Invasiveness	Calibration	References	Limitations*
Calibrated continuous arterial pulse contour	PiCCO LiDCO	++ (PiCCO) + (LiDCO)	Transpulmonary thermodilution Lithium dilution	Halvorsen et al. 2007 Mora et al. 2011	• Based on mathematical models • Variation in arterial signal quality • Rapid changes in vascular tone • Variety of techniques and software versions • Peripheral arterial pressure instead of central pressure
Pressure recording analytical method	PRAM	++	Independent CO measurement eg. by intermittent thermodilution	Scolletta et al. 2005	
Noncalibrated continuous arterial pulse contour	Vigileo	+	No external calibration Demographic data used in equation	Scheeren et al. 2008	
Invasive modelflow	Modelflow	+ (noncalibrated) ++ (calibrated)	Demographic data used in equation / independent CO measurement	Wesseling et al. 1993	
Non-invasive modelflow	Modelflow Nexfin CO	-(finger BP as an input signal)	Demographic data used in equation	Bogert et al. 2010	

+peripheral arterial catheterization, ++ peripheral and central arterial catheterization. *The limitations column concerns all methods.

2.2.4. Bioimpedance

Impedance cardiography (ICG) technique to determine CO has been available since 1960's (Kubicek et al. 1966). A constant, high frequency and low alternating electrical current is applied to the body through superficial current electrodes and the generated voltage is measured with other electrodes (Kööbi et al. 1997b). The impedance is then derived according to Ohm's law where resistance equals voltage over current. Changes in bioimpedance signal in response to current are related to cardiac events and blood flow, since electrical current passes through the tissues with the lowest impedance, i.e. blood and plasma (Baker 1989). Already during its early days, the technique has been validated against invasive CO measurements under various conditions causing changes in CO (Balasubramanian et al. 1978, Boer et al. 1979). The assessment of SV from the changes in impedance requires mathematical algorithms and the base equation has been modified for different electrode configurations and current magnitudes. In addition, some equations include correction factors for example for sex, weight and haematocrit (Lababidi et al. 1971, Kööbi et al. 1997b, Cotter et al. 2004). ICG is a completely non-invasive and thus widely applicable method, which enables the determination of CO also in outpatients, to whom potential risks of arterial catheterization are not justified.

In thoracic impedance cardiography (ICG$_{TH}$) paired electrodes are placed on the neck and chest, mainly detecting the change in impedance in the aorta and pulmonary artery. In another approach, whole body impedance cardiography (ICG$_{WB}$), electrodes are placed on the extremities proximally to wrists and ankles, and the changes in the impedance along the whole arterial tree are detected (Kööbi et al. 1997b, Cotter et al. 2004). The electrode configuration in both of the ICG techniques is presented in Figure 6. Both techniques have been repeatedly validated against reference methods with varying results, newer software versions and developed equations showing better agreement when compared with the earlier versions. For example, Van De Water et al. (2003) compared three equations for ICG$_{TH}$ derived CO with thermodilution CO and documented marked improvement in agreement when using the latest version instead of the older ones. In addition, several reports have documented good agreement of ICH$_{WB}$ derived CO with thermodilution (Kööbi et al. 1997b, Cotter et al. 2004, Paredes et al. 2006). However, criticism against ICG derived CO

determination has also been presented due to over-simplification of human physiology by mathematical equations (Geerts et al. 2011). The major advances and limitations of ICG technique are presented in Table 2.

Figure 6. Electrode configuration in whole body impedance cardiography (A) and thoracic impedance cardiography (B).
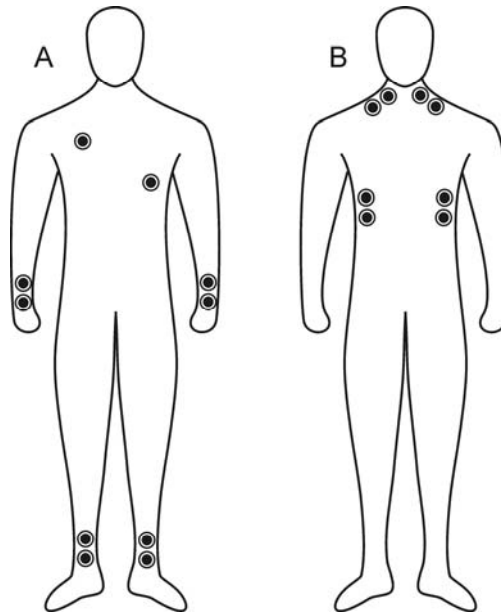
Table 2. Major advantages and limitations of impedance cardiography technique for cardiac output monitoring.

Advances	Limitations
Completely non-invasive	Based on a mathematical model
Safe	Heterogeneity of equations
Inexpensive	Ignores individual anatomy
Easy and fast to perform	Variation in validation results
Repeatable	Motion artefacts
Operator-independent	Unreliable during irregular cardiac rhythm
Widely applicable	and in patients with valvular diseases

2.3. Non-invasive assessment of central blood pressure

BP and PP in central arteries are better predictors of cardiovascular outcome in several populations when compared with peripheral BP, and despite similar effects on peripheral BP, different treatments may have divergent influence on central pressure. For example, in the CAFE (Conduit Artery Function Evaluation) study amlodipine/perindopril based treatment was compared with atenolol/thiazide based treatment (Williams et al. 2006). Despite the similar reduction of peripheral BP, subjects in the amlodipine/perindopril treatment arm had lower central BP and less cardiovascular events during the 4-year follow-up. Higher central BP and PP have been shown to be associated with the risk of restenosis after percutaneous transluminal coronary angioplasty (Nakayama et al. 2000) and with the severity of coronary artery disease (Jankowski et al. 2004). In addition, central BP is better associated with the reduction in left ventricular mass after pharmacological treatment than peripheral BP (Hashimoto et al. 2007).

Central BP can be estimated non-invasively by arterial applanation tonometry, which is performed by placing a tonometric pressure sensor on an artery. Commonly, a pen like sensor is used to record 10-20 consecutive cardiac cycles, but also continuous recording is possible with a sensor fixed on the artery with e.g. wrist band. Central pressure wave can also be obtained directly from the carotid artery, and besides applanation tonometry, echotracking device can be used to record arterial distension waves produced by variation in BP (Van Bortel et al. 2001). Applanation tonometry can be also performed on a peripheral artery, typically radial or digital, and a mathematical transfer function is applied to estimate central pressure wave from the peripheral one. A generalized transfer function which is validated against invasive central BP measurements is commercially available in the Sphygmocor system (Karamanoglu et al. 1993), while other functions have also been developed and validated for pulse waves obtained from radial or digital artery (Chen et al. 1997, Hope et al. 2003). Sharman et al. (2006) measured central pressure with radial applanation tonometry and invasive arterial catheterization and found good agreement and high correlation for the measurements both at rest and during supine cycling. The recorded arterial pressure wave is usually calibrated by brachial BP measurements assuming equal pressure at the two measurement sites, which is the major limitation

of the transfer functions. However, the method is widely applicable, easy and fast to perform and operator-independent. Figure 7 depicts typical radial and derived aortic waveforms from a young study subject obtained with radial applanation tonometry.

Figure 7. A typical measured radial waveform and derived aortic waveform of a young, normotensive subject.
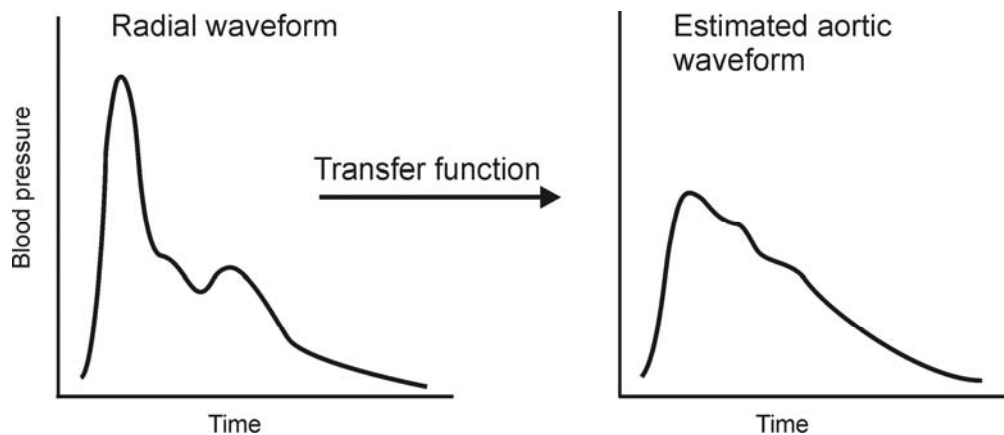
2.4. Non-invasive assessment of arterial stiffness

2.4.1. Pulse pressure

Pulse pressure (PP) is defined as a difference between systolic and diastolic BP and is influenced by arterial compliance (Dart and Kingwell 2001). Even though PP is considered as a simple measure of arterial stiffness, also many other factors like wave reflection, contribute to the level of PP. In addition, the rise in PP can be secondary to the rise in SV without a change in arterial distensibility (Alfie et al. 1999). Moreover, PP is directly related to mean arterial pressure and thus lowering BP decreases PP without a parallel decrease in arterial stiffness. An increase in PP has also been documented during β-adrenergic stimulation without a change in PWV, a measure of arterial stiffness (Lemogoum et al. 2004). Importantly, PP measured at peripheral sites is not equal to central PP due to the amplification phenomenon (see above), and pathophysiological conditions and drugs may change central PP without changing peripheral PP. The discrepancy is emphasized in young subjects with compliant arteries and diminished with ageing and arterial stiffening (Nichols and O'Rourke 1998).

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Both peripheral and central PP predicts cardiovascular morbidity and mortality in various populations. The predictive value of PP has been demonstrated in general population (Panagiotakos et al. 2005), healthy individuals (Franklin et al. 1999, Lee et al. 1999), treated and untreated hypertensive subjects (Fang et al. 1995, Millar et al. 1999), patients with left ventricular hypertrophy (Domanski et al. 1999) and subjects with type I or II diabetes (Cockcroft et al. 2005).

2.4.2. Pulse wave velocity

Velocity of the pulse wave traveling along the arterial tree has been described as a simple, non-invasive and reproducible method, and carotid-femoral PWV is considered as the golden standard in the assessment of large arterial stiffness (Laurent et al. 2006). Theoretical basis of PWV measurement takes into account the density of blood, thickness and diameter of the artery, and Young's elastic modulus, which is a physical parameter representing the stiffness of elastic materials (Franck 1920, Bramwell and Hill 1922).

In practice, PWV is calculated as a distance divided by the traveling time of the pulse wave between two measurement sites. PWV can be measured from various locations, but the measurement of aortic PWV is the most clinically relevant, since the large arteries are responsible for most of the pathophysiological events of arterial stiffening (Boutouyrie et al. 2002). However, recently it has been demonstrated that also brachial-ankle PWV reflects similar characteristics than aortic PWV, and is associated with cardiovascular risk (Tsuchikura et al. 2010).

The distance between the two pulse assessment points is usually measured by tape over the body surface. To determine the pulse transit time, pulse waveforms are obtained from two locations, usually from carotid and femoral arteries to define aortic PWV. The foot of the pulse waveform, i.e. end of the diastole, is commonly identified when determining the pulse transit time since that part of the waveform is least affected by the wave reflection. The waveforms can be obtained using several methods. Mechanotransducers placed on the skin simultaneously assess the arterial pressure pulse at different sites, and a correlation algorithm is utilized to determine

the transit time (Asmar et al. 1995). Pressure waveforms can be determined subsequently from different sites using applanation tonometry, and electrocardiogram is recorded parallel to define the timing between the waveform foots. The distension waveforms can be determined using echotracking devices or continuous Doppler probes (Millasseau et al. 2000, Cruickshank et al. 2002). PWV can also be determined with an ICG_{WB} device (CircMon^R). Briefly, the distal impedance is recorded from a popliteal artery at knee joint level, and the active electrode is placed on the lateral side of the knee and the reference electrode on the calf below knee. When the PP wave enters the aortic arch and the diameter of the aorta changes, the whole-body impedance decreases, and this can be measured by the voltage electrodes. To calculate the PWV value, the CircMon^R software measures the time difference between onset of the decrease in impedance in the whole-body impedance signal and the popliteal artery signal (Kööbi et al. 1997a, Kööbi et al. 2003).

PWV estimation includes potential errors in both distance and waveform determinations. The measurement of the distance over the body surface is overestimated in obese subjects, and includes an assumption that the aorta is straight. As determining the timing, the identification of pulse waveform foot may fail, and several different points can be chosen to indicate the start of the pulse (Chiu et al. 1991). In addition, the femoral pressure waveform may be difficult to determine in subjects with metabolic syndrome, obesity, diabetes or peripheral arterial disease (Van Bortel et al. 2002).

Increased PWV has been associated with cardiovascular mortality and morbidity in several studies. The prognostic value has been demonstrated both in general populations (Mitchell et al. 2010) and in hypertension, diabetes and in end stage renal disease (Laurent et al. 2001, Cruickshank et al. 2002, Pannier et al. 2005). However, it is not completely clear whether high PWV truly precedes disease or is it solely associated with a potentially coexisting cardiovascular disorder.

2.4.3. Augmentation index

As discussed in section 1.3, AIx can be determined from the arterial pressure wave as a ratio of augmentation pressure and PP (Figure 1). Central AIx is determined non-

invasively from the estimated central pressure wave obtained e.g. by radial applanation tonometry using pulse wave analysis softwares. The principle of central pulse wave estimation using transfer functions is described in section 2.3.

AIx is influenced by the shape and amplitude of volume wave from the left ventricle, magnitude and timing of the reflecting wave and arterial stiffness as well as by gender, age and height (Kingwell and Gatzka 2002). Thus, AIx cannot be regarded as a true indicator of arterial stiffness, but an index of wave reflection including arterial compliance. Importantly, a significant linear relationship between AIx and HR has been demonstrated so that AIx falls approximately 4 % for every 10 beats/min increase in HR (Wilkinson et al. 2000a), since decreased duration of systole shifts the reflected wave towards diastole. Therefore, AIx corrected to HR 75 has been recently more widely used (Vyssoulis et al. 2010).

As expected, a good correlation between AIx and aortic PWV has been demonstrated since the pulse wave travels faster in stiff arteries thus increasing the augmentation pressure (Yasmin and Brown 1999, Lemogoum et al. 2004, Filipovsky et al. 2005). Increased AIx has been demonstrated in patients with type I diabetes (Wilkinson et al. 2000b), hypercholesterolemia (Wilkinson et al. 2002b), hypertension (Scuteri et al. 2001) and end stage renal disease (Safar et al. 2002b). Furthermore, London et al. (2001) have shown that increased AIx in patients with end stage renal disease is associated with cardiovascular and total mortality, and the same has been observed in patients with coronary artery disease (Weber et al. 2005). However, in a cohort of the Framingham Heart Study, PWV, but not AIx was associated with cardiovascular morbidity (Mitchell et al. 2010)

2.4.4. Ambulatory arterial stiffness index

A novel measure of arterial stiffness, ambulatory arterial stiffness index (AASI), is defined as a relationship between diastolic and systolic BP during 24-hour BP monitoring, which usually contains approximately 70 brachial cuff BP recordings (Li et al. 2006). The technique is based on a hypothesis that increased arterial stiffness influences the height of diastolic BP and its relationship to systolic BP, and it is thought that AASI mainly reflects the mechanical properties of small arteries

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(Benetos and Lacolley 2006). In practice, the regression slope of diastolic BP on systolic BP is calculated by plotting the individual values of systolic and diastolic BP measurements obtained during the 24-hour BP monitoring. The numeric value of AASI is then computed using the formula AASI = 1 – gradient of the regression slope, and arterial stiffness increases as the gradient approaches 0 and AASI approaches 1(Li et al. 2006). This principle is based on an assumption that in a compliant artery both systolic and diastolic BP tend to increase in a parallel fashion, while in a stiff artery, the increase in systolic BP is accompanied by a lesser increase, or even by a decrease, in diastolic BP.

In healthy subjects, a significant correlation between AASI and aortic PWV has been demonstrated (Li et al. 2006). Leoncini et al. (2006) demonstrated that AASI is associated with target organ damage in patients with primary hypertension. In another study, AASI predicted cardiovascular mortality after adjustment for traditional risk factors, but lost its predictive value after adjustment for PP (Dolan et al. 2006). This technique is relatively novel and requires further validation.

2.4.5. Diastolic pulse contour method

It is considered that stiffening of the small arteries alters the amplitude and timing of reflected waves obtained in the larger vessels, which can be interpreted from computer analysis of the diastolic pressure decay of the pressure wave (Cohn et al. 1995). Applying a modified Windkessel model, assessment of capacitative compliance (C1, large artery compliance) and reflective compliance (C2, small artery compliance) is enabled (Cohn et al. 1995). Pressure wave can be measured e.g. by applanation tonometry or finger plethysmography. Different algorithms exists, but in general C1 is calculated as a ratio between a change in volume and change in pressure during diastolic decay, and C2 as a same ratio during oscillations around diastolic decay (Cohn et al. 1995, Woodman et al. 2005). Romney and Lewanczuk (2001) have documented that both C1 and C2 were reduced in type 1 diabetics before diagnosed microvascular complications. In a population sample of 870 subjects, decreased C2 was an independent predictor of cardiovascular events and death (Grey et al. 2003). However, the validity of C1 and C2 as an indexes of systemic arterial stiffness have been questioned, since poor correlations between aortic PWV and C1 or C2, and

between C1 or C2 measured from radial and tibial arteries, have been documented (Manning et al. 2002, Woodman et al. 2005)

2.4.6.   Assessment of local arterial stiffness

Local compliance in an artery can be determined by obtaining the changes in vessel diameter and BP during cardiac cycle, and all superficial arteries, especially common carotid, femoral, brachial and radial arteries are suitable sites for such measurements. The measurements are commonly performed using ultrasound systems, and simultaneous BP measurement e.g. with tonometry is preferably also performed (Perret et al. 1991, Giannattasio et al. 2008). In addition, local stiffness of deeper arteries, like aorta, can be determined using magnetic resonance imaging (Mohiaddin et al. 1989). As an advantage, these methods provide a direct measure of local stiffness without using any models of circulation. However, no assumptions concerning systemic arterial stiffness can be made based on these measurements, since arterial compliance may vary even within the same vessel (Reneman et al. 1992). Furthermore, ultrasound methods demand technical expertise and take time, and are highly operator-dependent.

2.5.   Assessment of endothelial function

2.5.1.   Invasive methods

2.5.1.1. Coronary angiography

Angiographic measurement of coronary vessel diameter after intracoronary infusion of endothelium-dependent and -independent agents in increasing concentrations is used to assess coronary endothelial function. Acetylcholine (ACh) is most widely used as an endothelium-dependent vasodilator substance, while administration of nitroglycerin (NTG) is performed to assess endothelium-independent vasorelaxation (Table 3). In healthy vessels, both substances induce vasodilatation, while paradoxical vasoconstriction in response to ACh has been documented in atherosclerotic coronary vessels (Ludmer et al. 1986).

Infusion of adenosine or papaverine induces endothelium-independent vasorelaxation especially in the coronary microvasculature and increases blood flow (Table 3). Adenosine also serves as a partially endothelium-dependent vasodilator via the release of NO and endothelium-hyperpolarizing factors (Hein and Kuo 1999). Increased blood flow and shear stress result in endothelial release of NO and vasodilatation in the proximal coronary tree, which can be measured to assess endothelial function (Cox et al. 1989). The measurement of the changes in vessel diameter mainly reflects vasomotion in large coronary vessels, while endothelial function of the coronary microvasculature can be assessed by measuring changes in coronary blood flow in response to stimuli using intracoronary Doppler probe (Treasure et al. 1993). As an invasive technique, angiography is only available for selected patient groups and is not applicable for repeated measurements. Furthermore, it only reflects the possible pathophysiological changes in one vascular bed.

Table 3. Most common pharmacological substances used in studies assessing endothelial and vascular smooth muscle function in the control of vascular tone in humans.

Stimuli	Administration	Action
Endothelium-dependent		
Acetylcholine	Infusion	Release of NO, PGI2, EDHF(s)
Metacholine	Infusion	Release of NO, PGI2, EDHF(s)
Bradykinin	Infusion	Release of NO, PGI2, EDHF(s)
Substance P	Infusion	Release of NO, PGI2, EDHF(s)
Adenosine	Infusion	Release of NO, PGI2, EDHF(s)
Salbutamol	Inhalation/infusion	Release of NO, PGI2, EDHF(s)
L-arginine	Infusion	Substrate for endothelial NO synthesis
L-NMMA	Infusion	Inhibition of endothelial NO synthesis
Endothelium-independent		
Nitroglycerin	Infusion/sublingual	Smooth muscle cell relaxation via cGMP
Papaverine	Infusion	Smooth muscle cell relaxation via PDE inhibition
Sodium nitroprusside	Infusion	Smooth muscle cell relaxation via cGMP
Adenosine	Infusion	Smooth muscle cell relaxation via cAMP

NO=nitric oxide, PGI2=prostacyclin, EFHF(s)=endothelium derived hyperpolarizing factor(s), L-NMMA= L^G-monomethyl L-arginine. PDE=phosphodiesterase

2.5.1.2. Venous occlusion plethysmography

Venous occlusion plethysmography is based on the measurement of the tissue volume change after occlusion of venous return induced by cuff inflation proximal to the site of evaluation, usually in the forearm (Hokanson et al. 1975, Fichtlscherer et al. 2004). The occlusion pressure only prevents venous outflow without modifying the arterial inflow. Measurement of volume change is usually performed with strain gauge placed around the examination area (Hokanson et al. 1975). Response in blood flow (volume change) to intra-arterial infusion of endothelium-dependent and independent agents is used to assess local vasodilatation, and ideally the measurement of blood flow also in the contralateral arm should be performed to verify potential systemic effects of drug infusion. Impaired increase in ACh-induced forearm blood flow has been demonstrated in subjects with cardiovascular risk factors (Panza et al. 1990), and it is an independent predictor of cardiovascular events in subjects with hypertension and coronary artery disease (Heitzer et al. 2001, Perticone et al. 2001). However, the invasive nature of the method limits its use in clinical settings.

2.5.2. Non-invasive methods

2.5.2.1. Flow mediated dilatation

The endothelium responds to increased blood flow and shear stress by releasing NO and other vasodilator substances, which leads to flow mediated vasodilatation (FMD). FMD is usually measured from brachial, and sometimes from femoral or radial arteries, and the increase in blood flow is usually induced by short period (e.g. 2-5 min) of ischemia with BP cuff inflation above systolic BP. Cuff release leads to reactive hyperaemia, and subsequent vasodilatation (change in arterial diameter proximally to the ischaemic segment) is documented with ultrasound from straight, non-branching segment of the artery (Celermajer et al. 1992). Commonly, also a single dose of sublingual NTG or other systemic vasodilator is administrated to assess endothelium-independent vasodilation (Celermajer et al. 1994).

The FMD technique is non-invasive and relatively simple. However, obtaining the change in arterial diameter with ultrasound requires good technical skills and training,

although available computerized edge-detection and wall-tracking software systems have improved the reproducibility (Woodman et al. 2001). In addition, variations in study protocols (e.g. time of cuff inflation, site for ultrasound measurement, dose of NTG) limit the direct comparison of the results of published studies.

A significant association between impaired FMD and cardiovascular risk factors has been documented already in the preclinical phase of cardiovascular disease (Celermajer et al. 1994). In patients with peripheral arterial disease, impaired FMD was an independent predictor of cardiovascular events (Gokce et al. 2003). In addition, in a large population of young adults (The Cardiovascular Risk in Young Finns Study), a strong inverse relationship has been documented between FMD and carotid intima media thickness (Juonala et al. 2004).

2.5.2.2. Peripheral arterial tonometry and pulse contour analysis

AIx, an index of wave reflection, is derived from arterial waveform obtained with applanation tonometry, as described previously. Vasodilatation in small arteries decreases the proportion of the reflected wave influencing systolic BP and thus leads to the reduction of AIx. Accordingly, changes in AIx in response to β_2-adrenoceptor agonist administration, most commonly inhaled salbutamol, have been used to assess the role of endothelium in vascular responsiveness (Hayward et al. 2002). Importantly, changes AIx after salbutamol but not after NTG, have been blunted by L^G-monomethyl L-arginine (eNOS inhibitor) infusion, suggesting that the effect of salbutamol is significantly mediated through the endothelial NO release (Hayward et al. 2002, Wilkinson et al. 2002a).

Pulse wave form can also be obtained from digital pulse volume using a finger photopletysmography (pulse contour analysis, see section 2.2.2.). Changes in reflection index, a measure of vascular tone, are automatically calculated as the relative amplitude of the forward wave and reflected wave component from the pulse contour analysis. Recently, changes in fingertip peripheral arterial tonometry signal recorded before and after proximally placed BP cuff inflation (reactive hyperemia) have been introduced as a method to evaluate endothelial vasodilator function. This method is patented as EndoPAT, and the outcome variable called PAT-ratio is

calculated as the ratio of signal amplitude before and after cuff occlusion. Acceptable correlation between PAT-ratio and FMD has been documented in some studies (Kuvin et al. 2003), while others have reported poor correlation and relation to different risk factor profiles between PAT-ratio and FMD (Hamburg et al. 2011, Schnabel et al. 2011). Thus, it has been discussed that the methods might reflect different cardiovascular pathologies.

Blunted response to endothelial stimuli in above mentioned measures have been documented in several clinical conditions, and some studies have shown a relationship between decreased vasodilator response and increased mortality or morbidity (Table 4). However, due to the novelty of these techniques, further data is needed to study the association between impaired functional responses and cardiovascular disease and effects of various treatments.

The major advantage of tonometry and pulse contour analysis in assessing endothelial function is their non-invasive nature. Moreover, when compared with the non-invasive FMD, these measurements are easier and faster to perform, and do not rely on the skills of the examiner as much as FMD. However, it is not completely clear whether the changes in the observed responses to various stimuli are uniformly endothelium-dependent, since arterial wave reflection is a complex interplay between several factors. In addition, mathematical transfer functions and equations are not without concerns, as discussed previously.

Table 4. Studies of the relationship between endothelial function assessed with pulse wave analysis, pulse contour analysis or fingertip peripheral arterial tonometry, and disease or outcome.

Study	Method	Stimuli	Subjects	Finding
Chowienczyk et al. 1999	PCA	Salbutamol	Type II diabetes (n=20)	Blunted decrease in wave reflection
Hayward et al. 2002	PWA	Salbutamol	Coronary artery disease (n=12)	Blunted decrease in AIx
Wilkinson et al. 2002a	PWA	Salbutamol	Hypercholesterolemia (n=27)	Blunted decrease in AIx
Covic et al. 2003	PWA	Salbutamol	Renal transplant recipient (n=20)	Blunted decrease in AIx
Bonetti et al. 2004	PAT-ratio	Reactive hyperemia	Abnormal response to intracoronary Ach (n=55)	Decreased PAT-ratio
Lind et al. 2005	PWA	Terbutaline	Elderly (n=1016)	Association between response and risk score*
Kals et al. 2006	PWA	Salbutamol	Peripheral arterial disease (n=24)	Blunted decrease in AIx and PWV
Waring et al. 2006	PWA	Salbutamol	Uncomplicated hypertension (n=20)	Preserved decrease in AIx
Wallberg-Jonsson et al. 2008	PWA	Salbutamol	Rheumatoid arthritis (n=30)	Blunted decrease in AIx and RI
Kohler et al. 2008	PWA	Salbutamol	Obstructive sleep apnoea (n=64)	Blunted decrease in AIx
Peled et al. 2009	PAT-ratio	Reactive hyperemia	Pulmonary arterial hypertension (n=38)	Decreased PAT-ratio
Gordon et al. 2009	PCA	Salbutamol	Coronary artery disease (n=9)	Blunted decrease in wave reflection
Rubinshtein et al. 2010	PAT-ratio	Reactive hyperemia	Outpatients with chest pain (n=329)	Decreased PAT-ratio as a predictor of CV events
Duffy et al. 2011	PWA	Salbutamol	Critically ill patients (n=94)	Blunted decrease in AIx as a predictor of mortality

PCA=pulse contour analysis, PWA= pulse wave analysis, PAT-ratio=ratio of fingertip peripheral arterial tonometry signal before and during reactive hyperemia, AIx=augmentation index, RI=reflection index, computed as the relative amplitude of the forward wave and reflected wave components. *Framingham risk score.

2.5.2.3. Laser Doppler flowmetry

Laser Doppler flowmetry is based on the reflection of a laser beam: light undergoes changes in wavelength when it hits moving blood cells. The magnitude and frequency distribution of the changes in wavelength are related to the number and velocity of blood cells. The technique enables the monitoring of changes in skin microvascular blood flow during post-occlusive hyperaemia, local heating of the skin or ACh iontophoresis (Gush and King 1991). However, none of these stimuli probably induce solely endothelial NO mediated response, but might represent a summation of complex responses involving neural, metabolic and NO-independent endothelial vasodilators. For example, it has been documented that ACh mediated dilatation of human skin vasculature is unchanged or only modestly attenuated following eNOS inhibition (Holowatz et al. 2005). Since skin serves as a crucial thermoregulatory organ, there is a great interindividual variation in local basal blood. In addition, lack in protocol standardization limits the comparison between the published studies.

2.5.2.4. Other methods

Recently, coronary endothelial function (change in arterial diameter or blood flow in response to stimuli) has been non-invasively measured applying magnetic resonance imaging, computed tomography and positron emission tomography (Prior et al. 2007, Terashima et al. 2008). Endothelial function can be assessed also by measuring the levels of molecules of endothelial origin in circulating blood. These include direct products of endothelial cells that change with endothelial activation, such as measures of NO biology, inflammatory cytokines, adhesion molecules, regulators of thrombosis, as well as markers of endothelial damage and repair.

2.6. Haemodynamic response to upright posture

2.6.1. Head-up tilt table test

Change in posture from supine to upright leads to significant haemodynamic alterations, and can thus be regarded as a challenge to cardiovascular system. Most important changes are possibly the effects of gravity on fluid compartments resulting

in increased venous pooling of blood and decreased CO, and the subsequent activation of autonomic control mechanisms (baroreflex) to maintain BP. Additionally, peripheral vascular resistance increases, and the compliance of large conduit arteries changes in a pressure-dependent manner. As a result, arterial wave form morphology is different in supine and upright positions. Recently, a decrease in AIx from supine to upright posture has been documented with a parallel increase in vascular resistance, even though the increase in resistance is generally considered to enhance wave reflection (Davis et al. 2011). This possibly implies that the main sites for wave reflection are located in larger conduit arteries instead of microvasculature with high resistance, but the issue requires further studies. Characteristic changes in haemodynamic variables in response to tilting are presented in Table 5.

Table 5. Typical changes in haemodynamic variables in response to head-up tilt

Variable	Change in response to tilt	Explanation
Systolic BP	↔ / ↓	Maintained by compensatory mechanisms
Diastolic BP	↔ / ↑	Maintained by compensatory mechanisms
Stroke volume	↓	Increased venous pooling
Heart rate	↑	Increased sympathetic activity
Cardiac output	↓	Increased venous pooling
Vascular resistance	↑	Increased sympathetic activity
Augmentation index	↓	Decreased wave reflection

In addition to research settings, head-up tilt-table testing is used as a diagnostic tool in the examination of syncope and orthostatic hypotension. However, no generally accepted standard protocol exits, and for example different tilt-angles from 60 to 90 degrees have been used, and the duration of the tilt-phase has varied. HR, BP and electrocardiogram are generally recorded, preferably continuously, while also additional measurements performed for example with ICG, echo-Doppler devices, or applanation tonometry have been utilized (Stewart 2000, Cybulski et al. 2004). Neurogenic orthostatic hypotension from autonomic failure is generally evident within the first 5 to 15 minutes of tilting, while more prolonged protocols are

sometimes needed to demonstrate reflex or vasovagal syncope, which are at times further delayed or require a pharmacological challenge to appear.

2.6.2. Nitroglycerin-stimulated tilt table test

Tilt-table test without pharmacological stimuli in the diagnostics of vasovagal syncope is relatively time-consuming, which has led to the introduction of nitrate-stimulated tilt table test (Raviele et al. 1994). The precise mechanism of nitrate-stimulated vasovagal syncope is still under debate. Traditionally, venodilatation and venous pooling of blood into the lower extremities and splanchnic vasculature, and subsequently reduced left ventricular preload, have been regarded as major haemodynamic effects of small-dose NTG in humans, while arterial dilatation is thought to occur to a lesser extend (Mason and Braunwald 1965, Raviele et al. 1994). However, a reduction in wave reflection and central rather than peripheral BP has been documented after sublingual NTG in patients undergoing cardiac catheterization (Kelly et al. 1990).This implies that small doses of NTG dilate arteries resulting in reduced left ventricular afterload.

Conflicting results concerning nitrate-induced changes in haemodynamics during tilt-table testing have also been reported. In some studies, nitrate induced presyncope has been considered to be CO-mediated without a decrease in SVR (Gisolf et al. 2004). However, another report demonstrated a decrease in SVR without a marked change in HR or cardiac filling during NTG-induced presyncope (Mitro and Hijova 2006). Besides direct haemodynamic alterations, changes in autonomic tone, neurohormonal substances and central nervous system function may be involved in nitrate-induced syncope (Aerts 2003).

Several protocols for NTG stimulated tilt table test exit. Commonly, a non-stimulated phase of head-up tilting for 5-60 minutes precedes the NTG administration and the head-up tilt is continued for 10-50 minutes or until presyncopal symptoms appear (Aerts 2003). Recently, a protocol without a preceding passive tilt phase was also introduced with good sensitivity and specifity (Aerts and Dendale 2005). NTG is commonly administrated in the upright posture (Aerts et al. 1999), but some protocols have included the lowering of the tilt table before drug administration (Raviele et al.

1994). Both intravenous and sublingual NTG and isosorbidedinitrate have been used at clinically relevant doses (e.g. sublingual NTG 0.25-0.8 mg) (Aerts et al. 1999, Oraii et al. 1999). However, it has not been investigated, how the differences in the protocols affect the sensitivity or specificity of the test.

AIMS OF THE STUDY

The aim was to establish a comprehensive measurement protocol, during which both supine and upright haemodynamics are measured, and which could be utilized in future investigations comparing cardiovascular function between healthy subjects and patients.

Specific aims of the study were:

1. To test repeatability and reproducibility of the haemodynamic measurement protocol utilizing ICG, plethysmographic finger BP measurements, and continuous pulse wave analysis in supine position and during passive head-up tilt.
2. To examine the effects of the largely endothelium-dependent vasodilators, salbutamol and L-arginine, and the endothelium-independent vasodilator NTG, on supine and upright haemodynamics in healthy volunteers.
3. To compare the underlying haemodynamic mechanisms in subjects without and with presyncopal symptoms during NTG-stimulated tilt table test.
4. To compare haemodynamic responsiveness to upright posture in different age groups with normal BP.
5. To test the application in patients with either essential hypertension or metabolic syndrome in comparison with control subjects.

SUBJECTS AND METHODS

1. Study subjects

Number of subjects and basic characteristics in all studies are shown in Table 6. The population of studies I and II consisted of 35 (19 female and 16 male) healthy normotensive volunteers. Two of the study subjects dropped out because of individual reasons after 2/4 haemodynamic measurements, and in the Study I the data from 33 (17 female and 16 male) subjects could be analysed. In the Study I, the repeatability and reproducibility of the protocol, and haemodynamic response to tilt table test was examined. In the Study II, haemodynamic effects of sublingual NTG and inhaled salbutamol were studied in a double-blind, randomized manner in supine position and during tilt.

The population of Study III consisted of 9 (4 female and 5 male) healthy, normotensive subjects, and the haemodynamic effects of sublingual NTG, inhaled salbutamol and intravenous L-arginine was analysed. Six of the study subjects underwent alveolar NO concentration measurement to verify salbutamol-induced release of NO.

In the Study IV, haemodynamics of 21 (16 female, 5 male) normotensive subjects with presyncopal symptoms during nitrate stimulated tilt table test was analysed in comparison with 21(16 female, 5 male) age-, gender- and BMI-matched normotensive control subjects without presyncopal symptoms. Three subjects experienced a brief total loss of consciousness during tilt in the presence of NTG, and their data was analysed separately. Additional measurements were performed to 19 (11 female, 8 male) of the above mentioned subjects using alternative measurement protocol during which NTG was administrated in the upright position and the head-up tilt phase was longer than in the initial protocol. Of these subjects, ten experienced presyncopal symptoms while 9 did not. Two subjects classified to the presyncope group in the alternative protocol were originally in the non-syncope group (when NTG was administered in supine position), and *vice versa*. Thus, 79% of the subjects retained their classification whether NTG was given supine or during the head-up tilt.

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In the Study V, 179 (109 female, 70 male) normotensive subjects were examined. Subjects were divided to four age groups: 20-29 years (n=34, 21 female, 13 male), 30-39 years (n=40, 24 female, 16 male), 40-49 years (n=53, 30 female, 23 male) and 50-59 years (n=52, 34 female, 18 male). Haemodynamics in supine position and during head-up tilt was compared between the age groups.

In Studies I-V, only normotensive (BP<140/85 mmHg) subjects over 18 years of age were examined. None of the subjects had medication for cardiovascular disorders. Exclusion criterion for head-up tilt table test was standing systolic $BP \leq 90$ mmHg, and for research drug administration allergy to the compounds.

To test the application in patients, 19 subjects with metabolic syndrome (MetS), 19 age-matched ($\pm$ 4 years) subjects with essential hypertension, and 19 age-matched control subjects were examined (mean ages 48$\pm$2, 47$\pm$2 and 48$\pm$2, respectively). All subjects were males, and none had medication for cardiovascular disease. Subjects with MetS met the following inclusion criteria: office $BP \geq 135/85$ mmHg, waist circumference ≥ 102 cm, triglycerides ≥ 1.7 mmol/l, fasting plasma glucose ≥ 5.6 mmol/l or 2 hour value in oral glucose tolerance test ≥ 7.8 mmol/l. Subjects with essential hypertension had office $BP \geq 135/85$ mmHg and control subjects < 135/85 mmHg, but other criteria mentioned above were not fulfilled.

2. Medical examination and laboratory analyses

All subjects underwent physical examination, and lifestyle habits, family history for cardiovascular disease, and medical history were documented. Present and previous smoking history was reported, including the duration of smoking in years and the amount of cicarettes in a day. Alcohol consumption was reported as restaurant doses per week. Blood and urine samples were obtained after a minimum of 12-h fast, and a standard 12-lead electrocardiogram was recorded. The following laboratory tests were included: basic blood count, plasma C-reactive protein, K^+, Na^+, calcium, creatinine, uric acid, fasting glucose, alanine aminotransferase, alkaline phosphatase, fasting lipid profile and cystatin-C. Urine cell count, over-night urine albumin excretion (microalbuminuria) and 24-hour urine K^+ and Na^+ excretion were also studied.

Table 6. Study population in Studies I-V.

Variable	Study I	Study II	Study III	Study IV			Study V
				Presyncope	Control	Additional	
Number of subjects	33	35	9	21	21	19	179
Age	34.5 ± 1.5	34.0 ± 1.4	32.0 ± 2.5	43.2 ± 2.6	43.3 ± 2.5	41.5 ± 2.6	42.1 ± 0.8
Female/male	17 / 16	19 / 16	4 / 5	16 / 5	16 / 5	11 / 8	109 / 70
Body mass index (kg/m^2)	23.2 ± 0.5	23.1 ± 0.4	24.5 ± 1.0	24.1 ± 0.7	23.5 ± 0.5	24.8 ± 0.7	25.6 ± 0.3
Smoking (present/previous/never)	4 / 5 / 24	4 / 5 / 26	0 / 2 / 7	1 / 6 / 14	2 / 6 / 13	0 / 7 / 12	15 / 30 / 134
Office systolic blood pressure mmHg	125 ± 2	125 ± 2	124 ± 2	126 ± 3	127 ± 3	127 ± 4	132 ± 2
Office diastolic blood pressure mmHg	82 ± 2	81 ± 2	80 ± 4	79 ± 2	83 ± 3	83 ± 2	85 ± 1
Office heart rate	66 ± 2	66 ± 2	60 ± 5	62 ± 3	61 ± 3	62 ± 4	66 ± 1

3. Haemodynamic measurements

3.1. Measurement protocol

Haemodynamic measurements were performed by trained nurses in a standard research laboratory. The subjects were instructed to avoid caffeine containing products, smoking and heavy meal for at least 4 h and alcohol for at least 24 h prior to the investigation. Subjects were lying on a tilt-table, and the electrodes for ICG were placed on the body surface, a tonometric sensor for pulse wave analysis on the radial pulsation to the left wrist with a wrist band, a brachial cuff for BP calibration to the right upper arm, and a plethysmographic cuff for finger BP measurement to the right middle finger. An introductory head-up tilt was performed before actual measurements to familiarize the subjects with tilting. Measurement protocols in each study are presented in Figures 8, 9 and 10. In general, 5-minute phases in supine or upright (60 degrees head-up tilt) positions followed each other and the haemodynamic data was captured continuously. Studies I and II consisted of four measurements performed on separate days, while in the Study III, five measurements on different days were performed. In the studies IV and V, and when testing the application in patients, single measurements were performed to all subjects. In Study IV, 19/42 subjects underwent an additional measurement with a different protocol (Figure 10). The research drugs (studies II, III and IV) were administrated in supine position, except for the additional measurements in the Study IV, in which sublingual NTG was administrated during tilt. In addition, in this alternative protocol the head-up tilt phases lasted longer, for 15-20 minutes. If the subject reported presyncopal symptoms during the head-up tilt (dizziness, light-headedness, sweating, nausea), and the research nurse observed progressively falling BP, the tilt table was returned to the horizontal position before the intended length of head-up tilt was fulfilled.

Figure 8. Measurement protocol in Studies I and V and in the clinical application with patients.
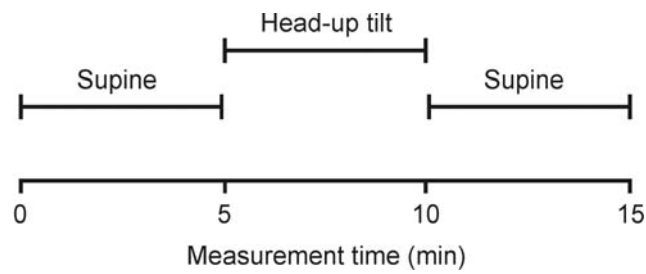
Figure 9. Measurement protocol in Studies II, III and IV
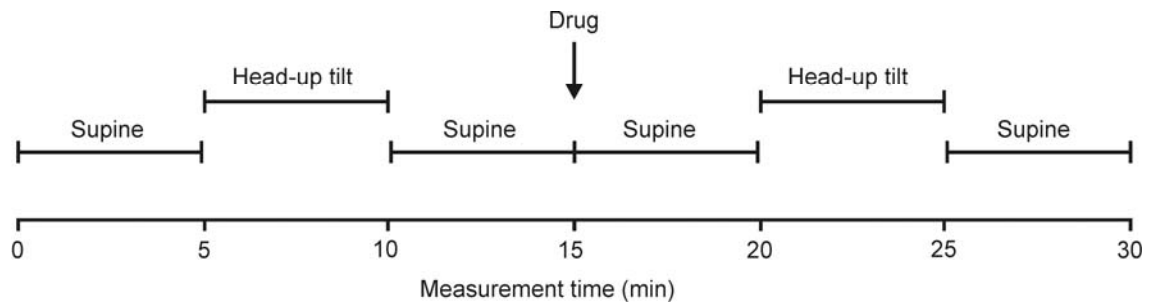
Figure 10. Measurement protocol in Study IV (additional measurements)
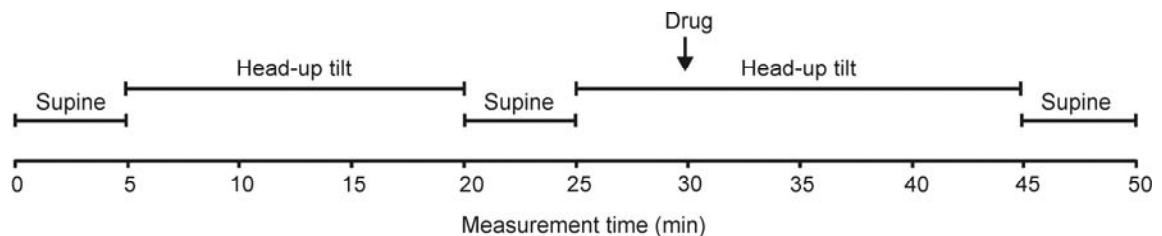
3.2. Research drugs

Research drugs were used in the Studies II, III and IV. In the studies II and III, inhaled salbutamol, sublingual NTG and corresponding placebo compounds were administrated after a 15-minute measurement in the absence of drugs (Figure 9). Placebo inhalation and 400 mg salbutamol inhalation (placebo for Ventolin® and Ventolin®, respectively; GlaxoSmithKline, Uxbridge, UK) were given with a spacer device (Volumatic; Allen & Hanbury's, Uxbridge, UK) in a blinded fashion. The 400-mg salbutamol dose was chosen on the basis of test experiments with the present

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study protocol and previously published work on the effects of inhaled salbutamol on haemodynamics (Hayward et al. 2002, McEniery et al. 2006).

Sublingual placebo resoriblet and 0.25 mg NTG (Nitro resoriblet; Orion Pharma, Espoo, Finland) were also administrated in a blinded fashion. The placebo resoriblets were professionally manufactured by the University Pharmacy, Helsinki, Finland. The NTG dose was chosen on the basis of test experiments with the present study protocol, and on previous reports using the same dose (Hayward et al. 2002).

During the 5th measurement in the Study III, an intravenous 20-G cannula was placed in a brachial vein and a slow saline infusion was started. L-arginine hydrochloride 20 mg ml^{-1} (B. Braun Melsungen Ag, Melsungen, Germany) was diluted in 100 ml of saline, and infusion at the dose 10 mg kg^{-1} min^{-1} was started at 15 min and continued for 10 min. Previously, intravenous L-arginine infusion at the rate of 3.33 mg kg^{-1} min^{-1} has induced vasodilation in the renal vasculature in humans (Delles et al. 2003), and at rate of 0.3 g min^{-1} it has enhanced the acute haemodynamic effects of 50 mg of losartan (Koifman et al. 2006). Thus, the present dosage is considered sufficient to induce cardiovascular changes. In the Study IV, sublingual 0.25 mg NTG was administrated in supine and upright positions, as described above.

3.3. Exhaled alveolar nitric oxide measurement (Study III)

To verify the salbutamol-induced release of NO, NO concentration from the alveolar air was measured in 6/9 of the subjects in Study III using a Sievers NOA 280 analyser (Sievers Instruments, Boulder, CO, USA) at three exhalation flow rates (100, 200 and 300 ml s^{-1}). The measurements were performed three times before (0, 10 and 20 min) and twice after (10 and 20 min) the placebo and salbutamol 400-mg inhalations. Exhaled NO output (concentration x flow rate) was plotted against exhalation flow rate and a linear regression was set. The slope and intercept of the regression line are approximates of alveolar NO concentration and bronchial NO flux, respectively (Lehtimaki et al. 2001). The exhalation flow rates were computer-controlled using an adjustable flow restrictor, and the subjects maintained the exhalation pressure between 5 and 20 cmH$_2$O.

3.4. Pulse wave analysis

Radial BP and pulse wave form were continuously determined from the radial pulsation by a tonometric sensor (Colin BP-508T[R], Colin Medical Instruments Corp., USA), which was fixed on the radial pulse with a wrist band. The radial BP signal was calibrated approximately every 2.5 minutes by a brachial cuff BP measurement. Continuous aortic BP was derived with the SphygmoCor pulse wave monitoring system (SpygmoCor PWMx, Atcor medical, Australia) using the previously validated generalized transfer function (Chen et al. 1997)(Figure 11A). In addition to aortic and radial BP and HR, PP, aortic reflection time, AIx and ejection duration were determined.

3.5. Whole-body impedance cardiography

A ICG_{WB} device (CircMon[R], JR Medical Ltd., Tallinn, Estonia) and plethysmographic BP recordings from fingers (Finapress[R], Ohmeda, Englewood, Colorado, USA) were used to determine beat-to-beat HR, SV, CO and PWV. CO and SV were related to the estimated body surface area to derive cardiac index (CI, $l/min/m^2$) and stroke index (SI, ml/m^2). A pair of current electrodes was placed on the distal parts of the extremities, just proximal of the wrists and ankles. Voltage electrodes were placed proximal of the current electrodes so that the distance between the centres of the electrodes was 5 cm (see Figure 6). The distal impedance was recorded from a popliteal artery at knee joint level, and the active electrode was placed on the lateral side of the knee and the reference electrode on the calf 20 cm below knee (Figure 11C).

When the pressure wave enters the aortic arch and the diameter of the aorta changes, the whole-body impedance decreases, and this can be measured by the voltage electrodes on the distal parts of the extremities. To calculate the PWV value, the CircMon[R] software measures the time difference between onset of the decrease in impedance in the whole-body impedance signal and the popliteal artery signal. The PWV can be determined from the time difference and the distance between the electrodes (Kööbi et al. 1997a, Kööbi et al. 2003).

Systemic vascular resistance index (SVRI) was calculated using the BP signal from the radial tonometric sensor and the CI measured by the CircMon[R] device. The CO values measured with CircMon[R] ICG$_{WB}$ are in good agreement with the values measured by the thermodilution method, in both supine position and during head-up tilt (Kööbi et al. 1997a, Kööbi et al. 2003). PWV was not assessed during the head-up tilt due to less accurate timing of left ventricular ejection during reduced SV (Kööbi et al. 2003).

Figure 11. An example of the original signals exported from the Sphygmocor[R] and Circmon[R] systems. 10 consecutive tonometric radial and derived aortic pulse waves and average BP values from the Sphygmocor[R] system (A); ECG, impedance 1 and 2 (ICG1 and 2) and plethysmographig finger blood pressure (BP) signals and the values during the first cardiac cycle from the Circmon[R] system (B).
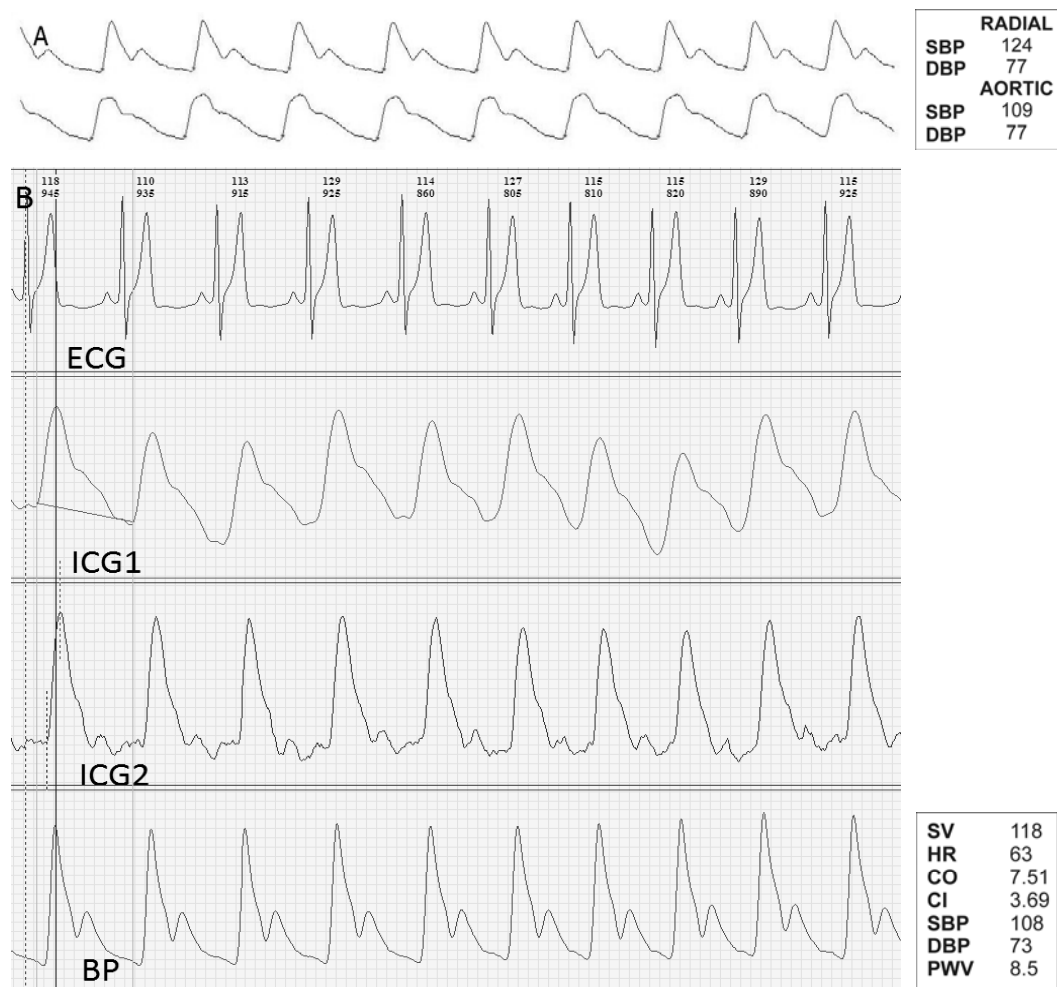
4. Statistical methods

Studies I and III

Statistical analyses were carried out by SPSS software version 11.5 (SPSS Inc., Chicago, Ill., USA) and p-values <0.05 were considered statistically significant. One-way analysis of variance (ANOVA), ANOVA for repeated measurements and paired samples T-test were applied to compare the measurements on different visits and different body positions. Values were expressed as mean ± standard error of the mean (SEM). In the statistical analysis, two averages of each 5-minute study phase were used (averages of the 2nd and 5th minute of the phase).

In the Study I, the associations between AIx and other variables were investigated using Pearson's coefficient of correlation test. The repeatability (two consecutive measurements) and reproducibility (four measurements on different days) were analysed using two statistical methods: 1) Repeatability and reproducibility indexes (R) were calculated from the formula $R = 1 - MS_W / MS_T$, in which MS_W and MS_T are square sums indicating variation within a subject and in the total study population, respectively. R ranges from 0 to 1, with 0 indicating no consistency and 1 complete consistency. 2) Repeatability and reproducibility values (limits of agreement) were also calculated as recommended by Bland and Altman (1986), showing the range as ± 2 standard deviations, which covered 95 % of the observed differences between repetitive measurements.

Due to the small number of subjects in the Study III, the study power was analysed using the PS 3.0.2 power and sample size calculations program (Vanderbilt Biostatistics, Nashville, TN, USA). A change in SVRI > 400 $dyn*s/cm^5*m^2$ in the supine position was chosen as the main outcome variable (such a change was observed after both salbutamol and NTG). The number of subjects (n = 9) was found to have a power of 92% to detect a significant difference (400 $dyn*s/cm^5*m^2$) between the salbutamol and the placebo effect (α-level 0.05).

Study II

The software package R 2.10.1 was used in the statistical analyses and p-values <0.05 were considered statistically significant (Nordhausen et al. 2011). All one-minute

averages of the study protocol were used in the statistical analysis. Robust nonparametrical methods were applied since the sample size was relatively small and the tonometric measurements were prone to temporary deviations. To evaluate the repeatability of the four different measurements, a multivariate Friedman test based on spatial signs was applied on the values of the first 15 minutes (study phase in the absence of research drugs). To study drug and placebo effects, a spatial sign test was applied to the differences of the values during the first 15 minutes (0-15 min), and the values during the last 15 minutes (16-30 min) of each subject. To compare if the drug and corresponding placebo effects differed, a spatial sign test was in turn computed for the difference of the aforementioned differences. To test if the drug effect depends on body position, differences of the estimated drug effects between the different postures were compared. For the spatial sign tests the p-values were based on the permutation test version based on 1000 replications. Corresponding point estimates for the effects spatial medians were computed.

Study IV

The software package R 2.10.1 was used (Nordhausen et al. 2011). The general difference between controls and cases was evaluated using a multivariate Wilcoxon signed-rank test based on marginal signed-ranks. Due to the small sample size a permutation version of the test was applied using 1000 replications. For further interpretation the univariate sample quartiles (quartile 1 – median – quartile 3) were reported and exact marginal Wilcoxon signed rank tests results were given.

Study V

The software package R 2.8.1 was used (2008), and robust and nonparametric methods were applied. Univariate one-way ANOVA was performed using the Kruskal–Wallis test and independence in two-way contingency tables was evaluated using Fisher's exact test. To evaluate haemodynamic differences between the four age groups, stratified multivariate c-sample tests were performed. The test applied here was the affine equivariant version of the spatial sign test and as corresponding point estimate the affine equivariant version of the spatial median was reported (Oja and Randles 2004). Stratification was done separately for sex, smoking, alcohol consumption level and for a binary indicator based on a canonical analysis between other confounding variables (baseline BP, AIx, PWV, aortic reflection time, waist and

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hip circumference, cholesterol status, hematocrit, C-reactive protein, estimated GFR, Cornell voltage product) and the response. A robust linear MM-regression was used to model the reaction of a subject to the tilt-table test. The model selection was performed using the smallest complete subset and successively removing the explanatory variable with the highest p-value (>0.05), while always keeping age as the variable of interest in the model. All explanatory variables entered the model linearly and no interactions were considered. The final model was refitted using the largest possible subset of the data and the model assumptions graphically evaluated. A difficulty of the model fitting was the correlation between several explanatory variables (multicollinearity problem, i.e. an explanatory variable in the final model might rather stand for a group of variables).

Clinical application with patients
SPSS software version 17.0 was used, and p-values <0.05 were considered statistically significant. ANOVA, ANOVA for repeated measurements, and paired samples T-test were applied to compare the groups. Values were expressed as mean ± standard error of the mean (SEM). In the statistical analyses, one-minute averages of each 5-minute study phase were used.

5. Ethical aspects

All subjects gave a written informed consent. The study complies with the declaration of Helsinki, and was approved by the ethical committee of the Pirkanmaa Hospital District.

RESULTS

1. Repeatability and reproducibility of the measurement protocol

In the Study I, repeatability (2 consecutive measurements) and reproducibility (4 measurements on separate days) were analysed using the statistical methods mentioned above both in supine position and during head-up tilt, and the values are presented in Table 7. The repeatability and reproducibility index varies from 0 to 1, values approaching 1 indicating high consistency. Thus, values presented in Table 7 demonstrate good repeatability and reproducibility except for the repeatability of HR measurements. In addition, limits of agreement according to Bland and Altman (1986) also showed good repeatability and reproducibility, comparable with previous reports on repeatability of haemodynamic measurements (Kööbi et al. 1997a, Kööbi et al. 1997b, Filipovsky et al. 2000). Figure 12 represents the repeatability (Bland and Altman limit of agreement) of AIx, CO and SVRI measurements in supine position and during head-up tilt.

In the Study II, all one-minute averages of the study protocol were used in the statistical analyses, and applied statistical methods were more sophisticated than in Study I. Thus, the analysis of reproducibility (measurements on 4 separate days, first 15 minutes of each measurement) was repeated. Consistent with the Study I, all measured variables showed good reproducibility during the 15-minute measurement (supine and during head-up tilt) in the absence of research drugs (p<0.05).

Table 7. Repeatability and reproducibility of haemodynamic measurements in supine position and during head-up tilt (Study I).

Variable	Repeatability (supine/tilt)		Reproducibility (supine/tilt)	
	Bland & Altman	Index R	Bland & Altman	Index R
Tonometry				
Radial systolic BP (mmHg)	10/12	0.91/0.85	15/15	0.77/0.78
Aortic systolic BP (mmHg)	10/14	0.90/0.74	14/16	0.74/0.74
Radial diastolic BP (mmHg)	10/12	0.87/0.79	12/13	0.78/0.78
Aortic diastolic BP (mmHg)	10/12	0.87/0.79	12/13	0.79/0.78
Aortic pulse pressure (mmHg)	5/8	0.88/0.66	9/11	0.61/0.46
Aortic augmentation index (%)	5/6	0.95/0.94	12/13	0.78/0.69
Aortic reflection time (ms)	20/11	0.85/0.87	25/27	0.79/0.47
Ejection Duration (ms)	12/15	0.96/0.95	28/41	0.74/0.57
Impedance cardiography				
Stroke volume (ml)	4/3	0.99/0.99	15/8	0.88/0.92
Heart rate (beats/min)	23/29	0.22/0.22	15/18	0.67/0.66
Cardiac output (l/min)	0.62/0.45	0.92/0.97	1.48/1.22	0.70/0.81
SVRI (dyn*s/cm^5*m^2)	349/390	0.93/0.91	937/993	0.58/0.54
Pulse wave velocity (m/s)	0.40/-	0.99/-	1.45/-	0.87/-

SVRI= systemic vascular resistance index, R= repeatability and reproducibility index

Figure 12. Bland-Altman plots for the comparison of two consecutive measurements (repeatability) in supine position and during head-up tilt. Augmentation index (A and B), cardiac output (C and D) and systemic vascular resistance index (SVRI, E and F).
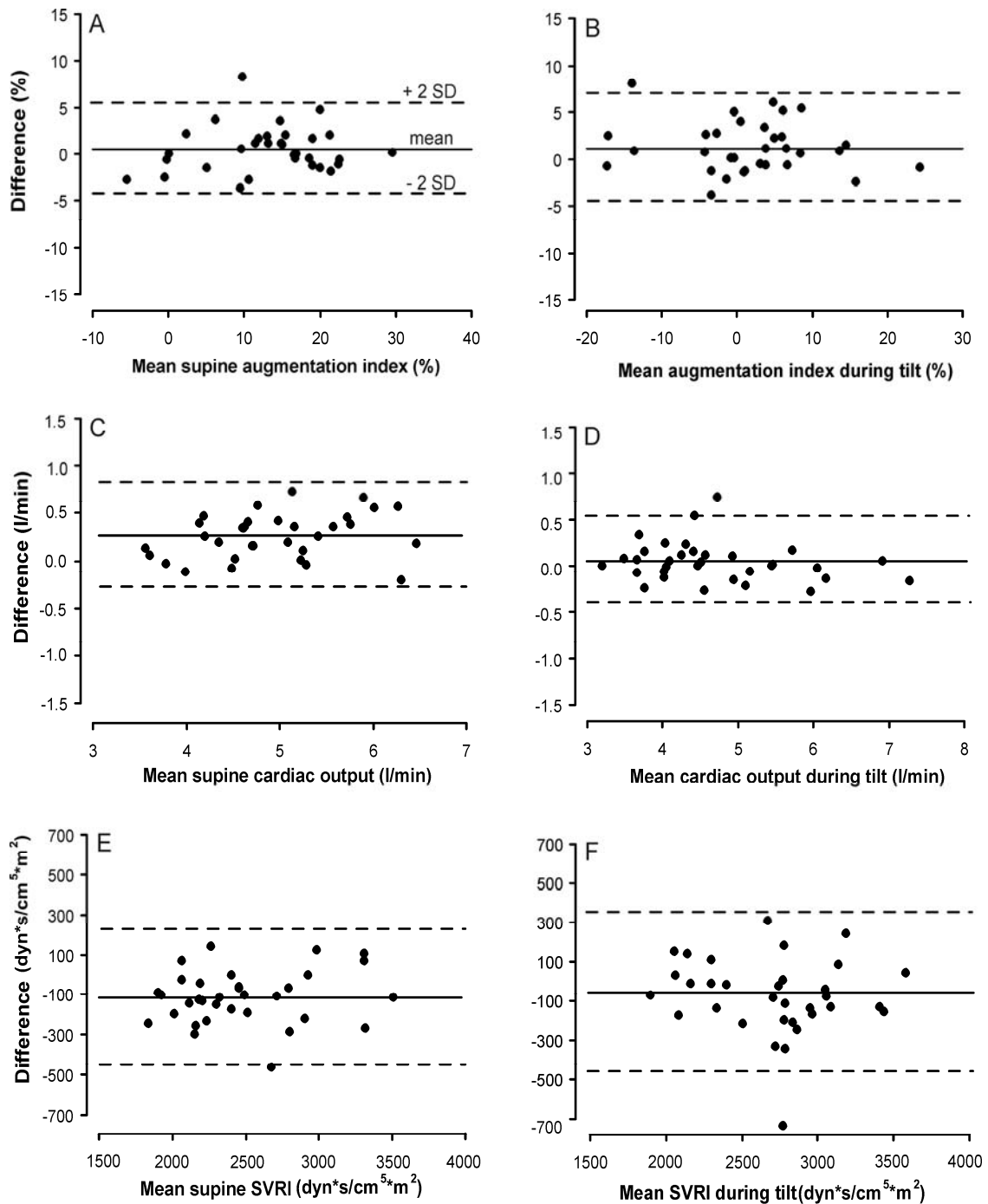
2. Heamodynamic response to passive head-up tilt

Studies I-V included a head-up tilt without pharmacological stimuli and the average changes in haemodynamic variables in response to tilting were similar. In general, all of the variables changed significantly during the head-up tilt. Table 8 presents the average direction of the change in the haemodynamic variables, and as an example the percentage change in the Study I.

Table 8. Average direction of the change in haemodynamic variables in response to head-up tilt in the absence of research drugs (all studies), and mean change in the Study I.

| Variable	Average change	Change in Study I (%)
Tonometry		
Radial systolic BP	↓	-2.1 ± 0.5
Aortic systolic BP	↓	-2.9 ± 0.4
Radial diastolic BP	↑	5.2 ± 0.7
Aortic diastolic BP	↑	5.6 ± 0.7
Aortic pulse pressure	↓	-23.7 ± 1.0
Aortic augmentation index	↓	-11.8 ± 0.5
Aortic reflection time	↓	-5.9 ± 0.6
Ejection duration	↓	-20.7 ± 0.5
Impedance cardiography		
Stroke volume	↓	-24.6 ± 0.8
Heart rate	↑	24.4 ± 1.2
Cardiac output	↓	-3.4 ± 0.6
SVRI	↑	14.3 ± 1.3

BP=blood pressure, SVRI=systemic vascular resistance index. Values are mean ± SEM

3. Association between augmentation index and other haemodynamic variables

Association between AIx and other haemodynamic variables was examined in the Study I. Supine AIx correlated with aortic systolic BP (r=0.423), aortic reflection time

(r=-0.647), PWV (r=0.287), and age (r=0.480). During the head-up tilt, AIx correlated significantly with ejection duration (r=0.504), HR (r=-0.339) and age (r=0.408). Both AIx and SV decreased in response to head-up tilt (Figures 13A-13B), but the correlation between the magnitude of the change between these two variables was not significant (p=0.131). The change in AIx during head-up tilt correlated with the change in aortic systolic BP (r=0.469), aortic pulse pressure (r=0.606), ejection duration (r=0.374) and HR (r=-0.445) (Figures 13C-13F).

Figure 13. Individual changes from supine to head-up tilt in stroke volume (A) and augmentation index (B) and the correlation between the change in augmentation index and in aortic systolic BP (C), aortic pulse pressure (D), heart rate (E) and ejection duration (D) in response to head-up tilt.
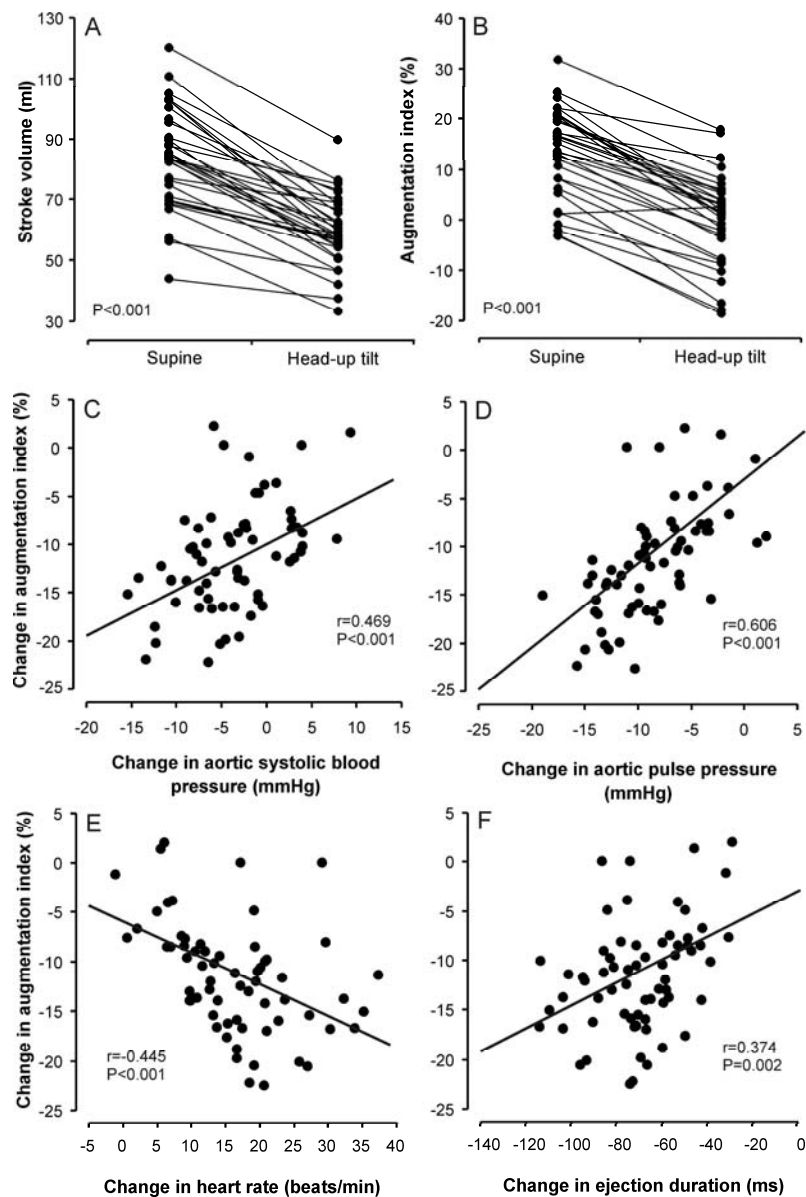
4. Haemodynamic response to placebo and pharmacological stimuli

4.1. Placebo

In the Study II, sublingual NTG and placebo resoriblet, and salbutamol and placebo inhalations were administrated in a blinded, randomized fashion. Administration of placebo simultaneously tests the habituation of a subject to the measurement protocol during the same session. The effect of placebo drugs on haemodynamics was analysed by calculating the difference between the measurements before (0-15 min) and after (16-30 min) the placebo drug administration (study protocol in Figure 9). Small but statistically significant changes in some of the haemodynamic variables were observed. After placebo resoriblet, radial systolic BP (-1.9 mmHg, p=0.010), aortic and radial diastolic BP (-2.0 mmHg and -2.1 mmHg, p=0.005 and 0.040, respectively) and CI (-0.08 l/min/m^2, p=0.010) were lower when compared with the preceding phase. After placebo inhalation, radial and aortic systolic BP (-2.0 mmHg and -2.2 mmHg, p<0.001 for both) and HR (-1.0 /min, p<0.001) were lower when compared with the measurements before placebo inhalation. Other variables did not change significantly. Figure 14 represents the median of the change in aortic systolic and diastolic BP, HR and CI in response to placebo drug administration.

Figure 14. Change in aortic systolic BP (A, B), aortic diastolic BP (C, D), heart rate (E, F) and cardiac index (G, H) in response to placebo resoriblet and placebo inhalation administration. Median (bold line) and individual curves (gray lines).
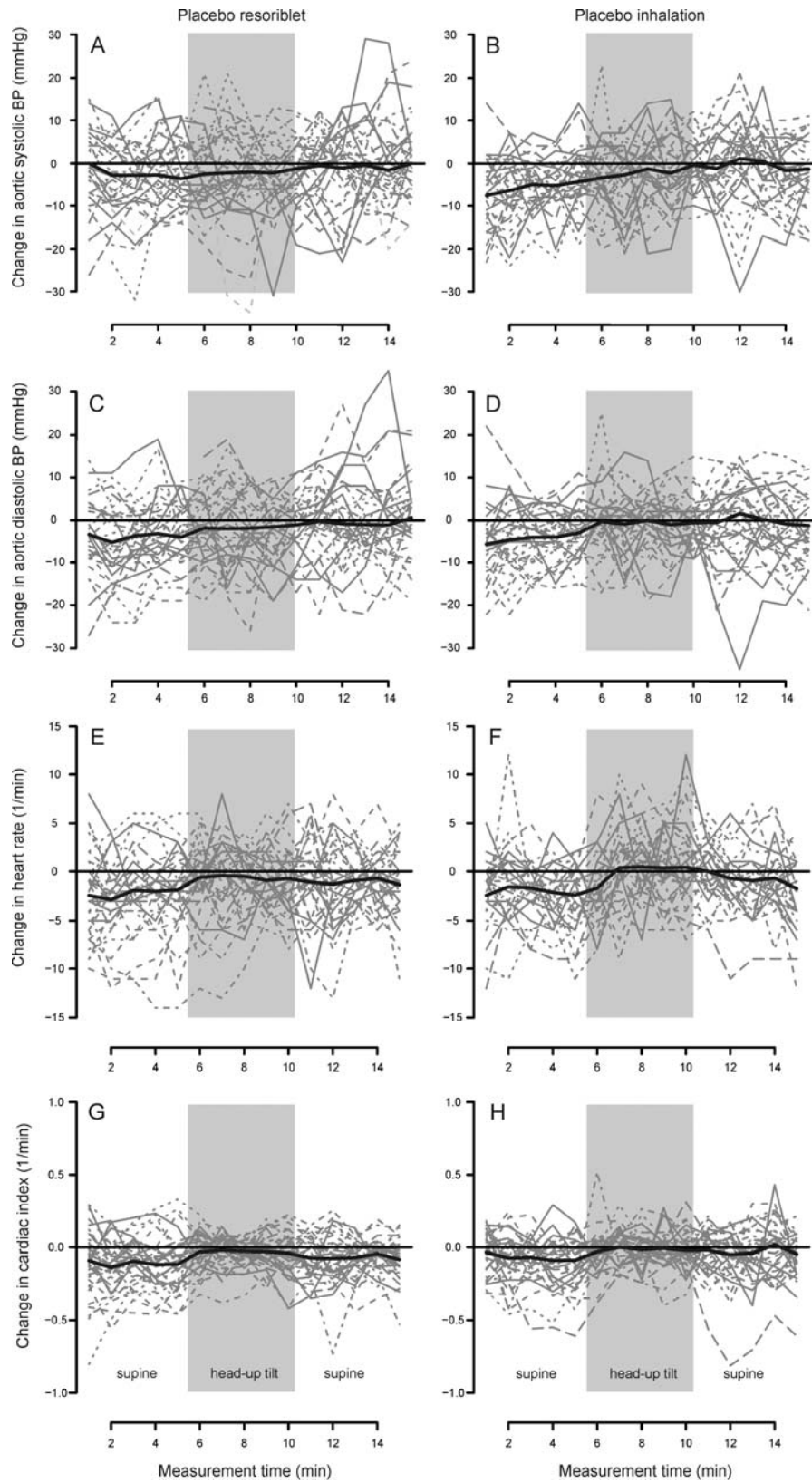
4.2. Sublingual nitroglycerin

In Study II sublingual 0.25 mg NTG was administrated in a placebo controlled fashion. The possible effect of placebo resoriblet was taken into account in the statistical analysis: if significant changes were observed both after placebo and NTG resoriblet administration, it was calculated whether the NTG effect differed from the placebo effect. In response to NTG, aortic BP and PP, finger BP and radial diastolic BP and PP decreased. HR, CI, SI and aortic reflection time markedly increased, while SVRI, AIX and PWV clearly decreased (<0.05).

The effect of NTG on most variables was different in supine and upright positions. The effect on radial and aortic diastolic BP, finger BP, aortic and radial PP, HR, SVRI, CI and AIx was greater during tilt when compared with supine values ($p<0.040$ for all). During the last supine phase the effect of NTG on aortic and radial BP and PP, HR, CI, SVRI, AIx and aortic reflection time was less marked when compared with the first supine phase after drug administration ($p<0.040$ for all). Mean values of radial mean BP, AIx, aortic reflection time, HR, CO and SVRI during NTG, salbutamol and corresponding placebo drug administration are presented in Figure 15. The statistical analysis is based on the magnitude of the difference observed in variables before and after drug administration.

In the Study III, the effect of sublingual 0.25 mg NTG was compared with placebo resoriblet effect. NTG decreased supine and upright SVRI ($-6.7\pm1.7\%$ and $-7.8\pm5.8\%$) and increased supine and upright CO ($+10.6\pm5.6\%$ and $+20.5\pm6.2\%$). Supine AIx was decreased ($-18.7\pm3.2\%$), while HR ($+40.4\pm7.5\%$) and aortic reflection time ($+1.2\pm3.4\%$) were increased during the tilt when compared with the placebo effects ($p<0.05$ for all). NTG induced a small but significant reduction in PWV ($-5.7\pm2.4\%$) when compared with placebo resoriblet ($0.9\pm1.7\%$) ($p<0.05$).

Figure 15. Mean values of radial mean BP (A), augmentation index (B), aortic reflection time (C), heart rate (D), cardiac output (E) and systemic vascular resistance index (F) before and after research drug or placebo administration (Study II). Mean±SEM.
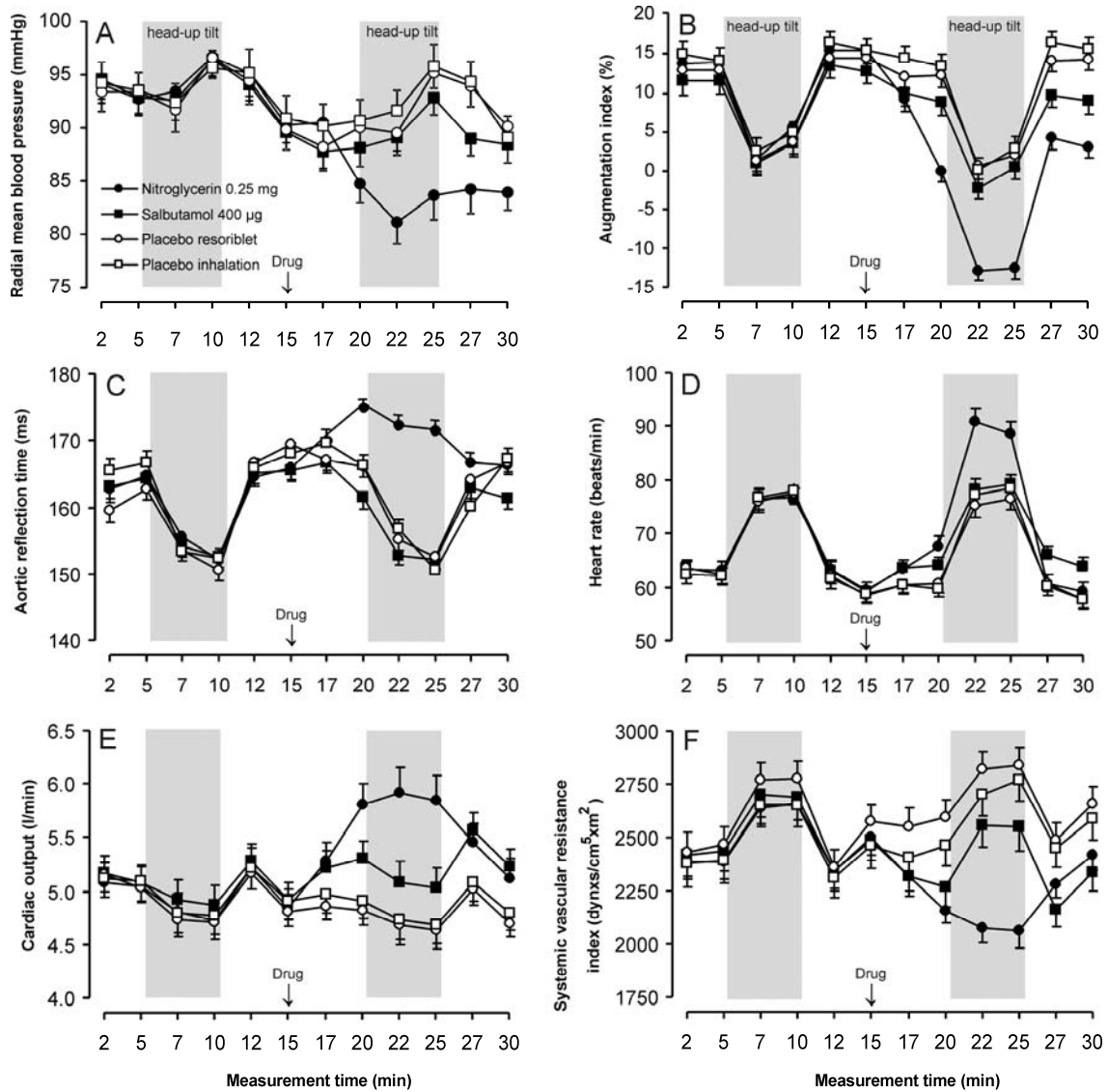
4.3. Inhaled salbutamol

The effect of 400 µg salbutamol inhalation was also analysed in a placebo controlled fashion. Salbutamol administration induced significant changes in many variables, but in general the changes were less marked than those observed after NTG administration (Figure 15). Radial and aortic BP, aortic PP, finger diastolic BP, SVRI and AIx decreased, while HR and CI increased in response to salbutamol ($p<0.05$ for

all). The placebo-controlled effect of salbutamol on HR and CI was greater during the last supine phase when compared with the tilt-phase and the first supine phase after drug administration ($p<0.040$ for all). The effect on radial systolic BP and finger BP was greater during the last supine phase when compared with the first supine phase ($p<0.040$). Mean values of radial mean BP, AIx, aortic reflection time, HR, CO and SVRI during NTG, salbutamol and corresponding placebo drugs administration are presented in Figure 15.

In the Study III, the effect of inhaled salbutamol was compared with placebo inhalation effect. In response to salbutamol, supine SVRI decreased by $-9.2\pm2.6\%$ ($p<0.05$ when compared with placebo), while SVRI during head up tilt was numerically, but not statistically significantly lower with salbutamol than with placebo. Supine HR increased by $+8.6\pm2.5\%$ ($p<0.05$ compared with placebo inhalation). In the supine position after the head-up tilt AIx was significantly lower after salbutamol when compared with placebo (numerical values $5.5\pm2.4\%$ vs. $14.1\pm3.2\%$, respectively). During the head-up tilt aortic systolic BP after salbutamol inhalation was lower than after placebo inhalation (106 ± 2 mmHg vs. 113 ± 2 mmHg).

To document the salbutamol-induced synthesis and release of NO, exhaled alveolar NO concentration was measured in six study subjects both after placebo and salbutamol inhalations. Alveolar NO concentration in these subjects was 1.55 ± 0.17 parts per billion before salbutamol inhalation, while the concentration was 1.81 ± 0.19 parts per billion before placebo inhalation. After salbutamol inhalation alveolar NO concentration increased by 19%, whereas after placebo inhalation the concentration decreased by 10% ($p=0.01$). Figure 16 depicts the change in alveolar NO concentration after salbutamol and placebo inhalations.

Figure 16. Change (%) in alveolar nitric oxide concentration after salbutamol and placebo inhalations.
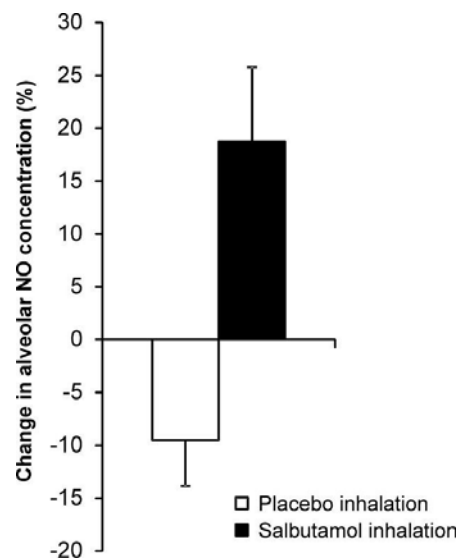
4.4. Intravenous L-arginine

The haemodynamic effects of L-arginine infusion were compared with the preceding phase during the same measurement session, during which only saline was infused. The modest haemodynamic effects of L-arginine infusion were only observed during the head-up tilt (Table 9). Differences in comparison with saline infusion were detected in aortic and radial BP and aortic reflection time: BP was decreased ($p<0.05$) while time to reflection was numerically increased ($p=0.162$). However, aortic reflection time during head-up tilt with L-arginine infusion was significantly longer than with placebo resoriblet, placebo inhalation and salbutamol inhalation ($p<0.05$ for all). L-arginine had no significant effects in supine position on any of the studied variables.

Table 9. Mean values in the upright position during saline and L-arginine infusions.

Variable	Saline infusion head-up tilt	L-Arginine infusion head-up tilt
Tonometry		
Radial systolic BP (mmHg)	127 ± 2	121 ± 2*
Aortic systolic BP (mmHg)	111 ± 2	105 ± 2*
Radial diastolic BP (mmHg)	79 ± 1	74 ± 2*
Aortic diastolic BP (mmHg)	80 ± 2	74 ± 2*
Augmentation index (%)	4.2 ± 2.8	3.2 ± 2.6
Aortic reflection time (ms)	162 ± 4	170 ± 4†
Ejection duration (ms)	277 ± 8	290 ± 9
Impedance cardiography		
Stroke volume (ml)	68 ± 5	71 ± 5
Heart rate (beats/min)	68 ± 3	65 ± 2
Cardiac output (l/min)	4.58 ± 0.27	4.55 ± 0.24
SVRI (dyn*s/cm^5*m^2)	3052 ± 174†	2920 ± 166

BP=blood pressure, SVRI= systemic vascular resistance index

* $p<0.05$ when compared with saline infusion. Mean ± SEM; †$p<0.05$ when compared with salbutamol inhalation, placebo inhalation and placebo resoriblet

5. Haemodynamics during NTG induced presyncope

The haemodynamics of 21 subjects with presyncopal symptoms during nitrate stimulated tilt table test were compared with 21 age-, gender- and BMI-matched control subjects. Control subjects were selected from normotensive study subjects measured in the haemodynamic recording study project of our research group. The only haemodynamic information used in the selection was the lack of presyncopal symptoms after NTG administration. Three subjects encountered a brief total loss of consciousness during the head-up tilt in the presence of NTG, and their data was also analysed separately.

The haemodynamics of the study and control groups did not differ before NTG administration (in supine position or during head-up tilt) or during the first 5 minutes in supine position after NTG. The study protocol (Figure 9) was carried out completely in the control group, while the head-up tilt in the presence of NTG was aborted after 3.4 ± 0.2 minutes in the presyncope group. During the last minute prior tilt-back, subjects in the presyncope group had lower aortic and radial BP and SVRI, and longer aortic reflection time and ejection duration when compared with the control group. The decrease in HR during tilt was significantly greater in the presyncope group when compared with controls, but the absolute values during the last minute in the upright position did not differ. Other variables did not significantly differ between the groups. The values during the last minute prior tilt-back in the presence of NTG are presented in Table 10.

Table 10. Mean values during the last minute before tilt-back during the nitroglycerin stimulated tilt table test.

Variable	Control n=21	Presyncope n=21
Tonometry		
Radial systolic BP (mmHg)	121 ± 3	$98 \pm 4*$
Aortic systolic BP (mmHg)	104 ± 3	$86 \pm 3*$
Radial diastolic BP (mmHg)	76 ± 2	$62 \pm 3*$
Aortic diastolic BP (mmHg)	77 ± 2	$63 \pm 3*$
Aortic pulse pressure (mmHg)	27 ± 1	24 ± 2
Aortic augmentation index (%)	-4 ± 2	-9 ± 3
Aortic reflection time (ms)	161 ± 4	$199 \pm 8*$
Ejection duration (ms)	251 ± 5	$277 \pm 10*$
Impedance cardiography		
Stroke index (ml/m^2)	39 ± 1	39 ± 2
Heart rate (beats/min)	84 ± 3	81 ± 3
Cardiac index (l/min/m^2)	3.3 ± 0.1	3.1 ± 0.1
SVRI (dyn*s/cm^5*m^2)	1944 ± 99	$1607 \pm 77*$

BP=blood pressure, SVRI=systemic vascular resistance index

* $p<0.05$ when compared with control. Mean±SEM

Subjects with a brief loss of consciousness

Three subjects underwent a total loss of consciousness after reporting presyncopal symptoms, but the consciousness was rapidly restored after tilt-back. Due to the small number of subjects, their data was analysed separately, and was not compared with the other groups. During the tilt with NTG in these subjects, a decrease in SVRI and BP was documented first, with CI remaining constant (180-60 seconds before the tilt-back). Just before the loss of consciousness a further, but a moderate decrease in SVRI, and a clear decrease in CO, HR and BP was documented (30-0 seconds before the tilt back).

Additional measurements

Measurements with an alternative study protocol (Figure 10) were performed to 19 study subjects to investigate whether the administration of NTG in different body position would change the haemodynamic pattern observed during presyncope. In addition, the head-up tilt phases were longer (15-20 min) when compared with the initial protocol (5 min). It was documented that SVRI and BP (radial and aortic mean BP, aortic PP) decreased and aortic reflection time increased in the presyncope group, while CI was preserved at a higher level than it was before NTG administration. During the last minute in the upright position, SVRI was significantly lower (P = 0.001) and aortic reflection time longer (P = 0.049) in the presyncope group when compared with the non-syncope group, while there were no statistically significant differences in other variables. These results support the initial finding of decreased vascular resistance as the principal haemodynamic alteration during NTG-induced presyncope.

6. Impact of age on supine and upright haemodynamics

The four age groups had some differences in the physical characteristics and laboratory values. BMI and waist circumference of subjects aged 20-29 years were lower than in 40-49 year-old and 50-59 year-old subjects, and those of 30-39 year-old subjects were lower than in 50-59 year-old subjects (p<0.05). Estimated GFR was higher in 20-29 year-old subjects when compared with 40-49 and 50-59 year-old subjects, and in 30-39 year-old subjects when compared with 50-59 year-old subjects (p<0.05). Plasma LDL-cholesterol increased with age (p<0.05). The differences were

taken into account in the statistical analysis, since BMI, cholesterol status and estimated GFR were considered as confounding factors in multivariate analysis.

The haemodynamic measurements of four different age groups in supine position and during head-up tilt are presented in Figure 17 (aortic BP and PP, AIx, aortic reflection time, HR, CI and SVRI). All of the comparisons were adjusted for several confounding factors in multivariate analysis (see statistical methods). Aortic and radial systolic BP and PP increased significantly with age, even after full adjustment for the confounders (Figure 17A and 17C, $p<0.05$). Aortic and radial diastolic BP increased with age after the adjustment for sex, but not after the adjustment for other confounders (Figure 17B). A decrease in aortic reflection time and an increase in AIx were documented with age, and the difference was significant after the adjustment for confounding variables (Figure 17D and 17E, $p<0.05$ for both). A general difference between the groups in the fully adjusted statistical model was observed in HR, but the mean or median values did not show a systematic direction of the difference with age (Figure 17F). In addition, there was a general age-related difference in SVRI, but after adjustment for sex or other confounding variables the difference was no more statistically significant (Figure 17G). PWV increased significantly with age before and after adjustment for confounders, and the correlation between PWV and age is shown in Figure 18 ($r=0.623$, $p<0.001$). CI or SI did not differ by age (Figure 17H).

All study subjects were further divided to subgroups according to their smoking status and alcohol consumption level. Smoking status or alcohol consumption did not have an influence on BP. HR differed by the smoking status and the level of alcohol consumption ($p<0.05$), so that the smokers and subjects consuming more alcohol had a higher HR during the head-up tilt but not in the supine position. Smoking status did not have an influence on the level of augmentation or the time of pulse wave reflection, but aortic reflection time differed by the level of alcohol consumption ($p<0.05$) The difference was only observed during the tilt, so that lower alcohol consumption corresponded to a lesser decrease in aortic reflection time. CI, SI, SVRI or PWV did not differ by smoking status or the level of alcohol consumption.

Figure 17. Aortic systolic and diastolic BP (A, B), aortic pulse pressure (C), augmentation index (D), aortic reflection time (E), heart rate (F), systemic vascular resistance index (G) and cardiac index (H) in different age groups.
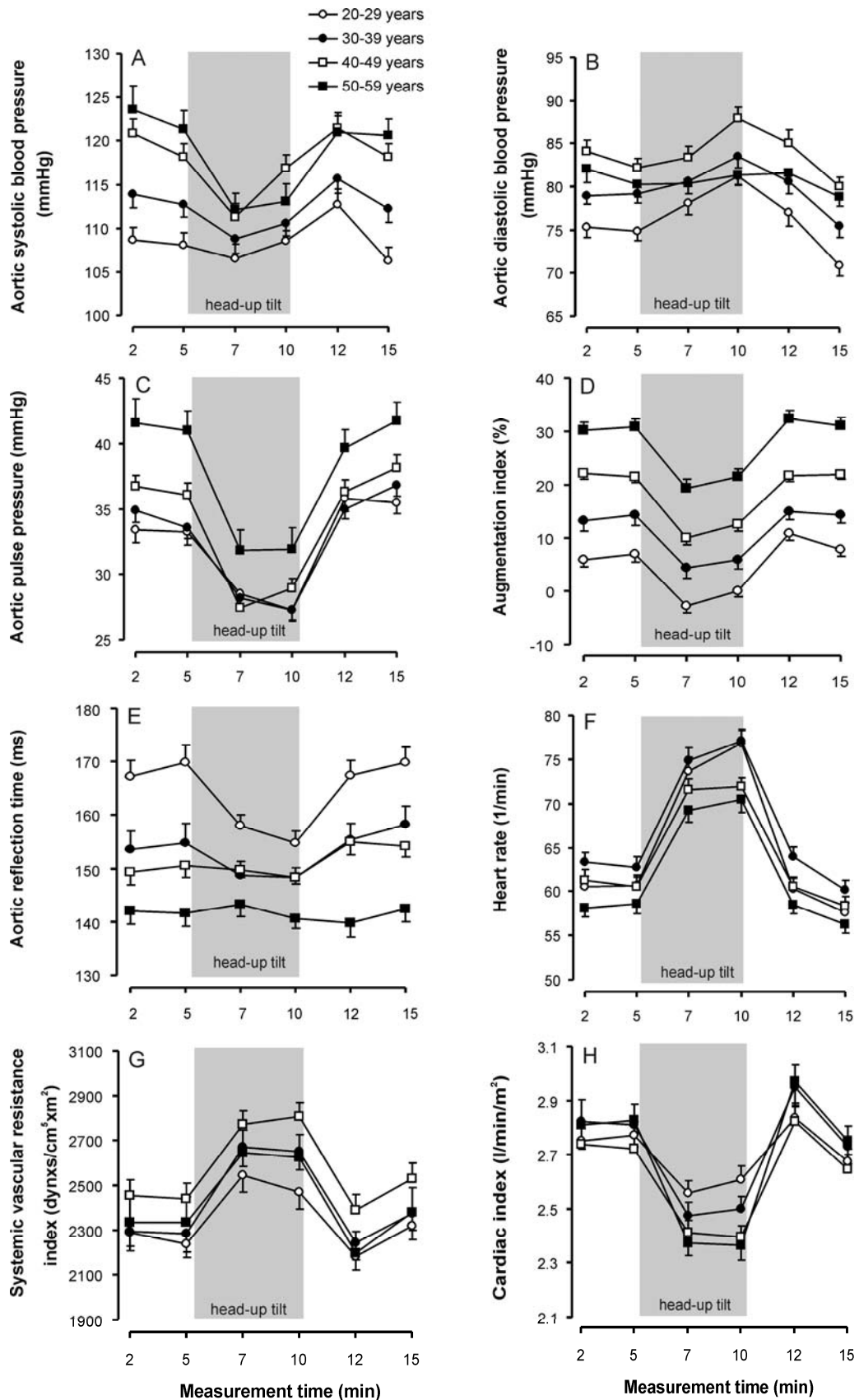
Figure 18. Correlation between age and pulse wave velocity.
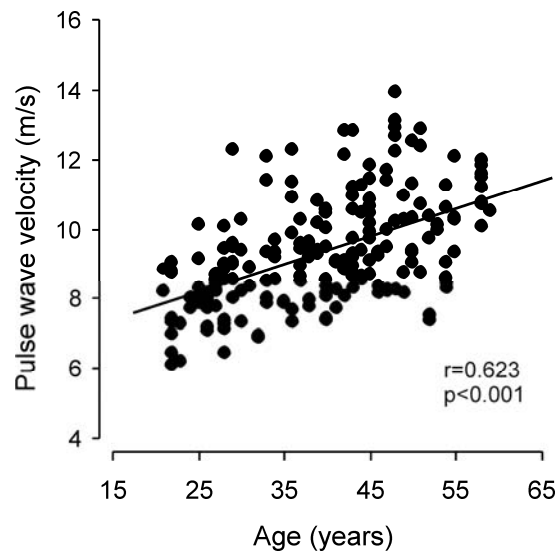
Linear regression analysis was applied to study whether the changes in haemodynamic variables in response to head-up tilt differed by age. The age of 50-59 years, PWV and baseline of aortic systolic BP were significant explanatory factors for the change in aortic systolic BP during the head-up tilt ($p<0.007$), with older subjects showing a more pronounced decrease when compared with younger ones. Figure 19 presents the median change in aortic systolic BP in response to head-up tilt in the different age groups. However, age was not an explanatory factor for the observed changes in the other variables in the regression analysis, but the influence of the baseline level of each variable (i.e. the value in the first measurement point) remained significant in all final models. For example, the change in aortic reflection time (Figure 17E) clearly differed between the different age groups. However, in the regression analysis age was not, but the baseline value of the reflection time and PWV were significant explanatory factors for the change ($p<0.05$).

Figure 19. Median change (bold line) in aortic systolic blood pressure during head-up tilt in different age groups (interquartile range ± SD; outliers defined by circles).
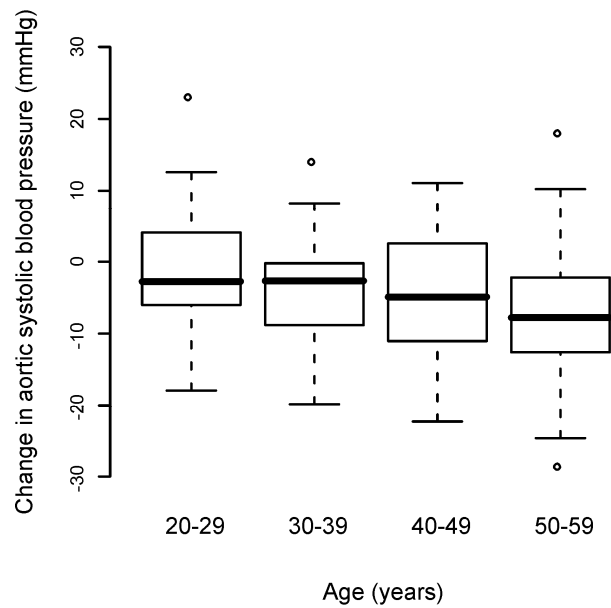
7. Subjects with essential hypertension or metabolic syndrome

The characteristics of the subjects are presented in Table 11. In the haemodynamic measurements, subjects with MetS and hypertension had higher aortic and radial BP and SVRI when compared with controls (Figure 21). Subjects with MetS had higher HR and lower SI than the other groups, but CI did not differ between the groups (Figure 21). AIx or AIx adjusted to HR 75 beats/min did not differ between the groups. Control subjects had lower aortic PP than subjects with MetS, and longer aortic reflection time than hypertensive subjects in the supine position (p<0.05 for both). PWV of the subjects with MetS was significantly higher when compared with the other groups (Figure 20).

Table 11. Study population

Variable	Control	Hypertensive	MetS
Number of subjects	19	19	19
Age (years)	48±2	47±2	48±2
Body mass index (kg/m^2)	24.4±0.5	25.7±0.6	32.3±1.0*
Waist circumference (cm)	91±2	93±2	112±2*
Office systolic BP (mmHg)	126±1	159±5†	165±4†
Office diastolic BP (mmHg)	81±1	99±2†	103±2†
Office heart rate (1/min)	61±1	67±2	75±3*
Fasting plasma glucose (mmol/l)	5.3±0.1	5.3±0.1	6.2±0.1*
Uric acid (µmol/l)	268±30	315±21	417±14*
Alanine aminotransferase (U/l)	23±2	30±3	53±5*
Cholesterol (mmol/l)	5.2±0.2	5.0±0.2	5.8±0.2‡
LDL cholesterol (mmol/l)	3.2±0.2	3.0±0.2	3.5±0.2
HDL cholesterol (mmol/l)	1.47±0.09	1.50±0.09	1.09±0.06*
Triglycerides (mmol/l)	1.02±0.06	1.03±0.09	2.95±0.30*

*p<0.05 when compared with hypertensive and controls. †p<0.05 when compared with controls, ‡p<0.05 when compared with hypertensive

Figure 20. Supine pulse wave velocity in in controls and in subjects with either essential hypertension or metabolic syndrome.
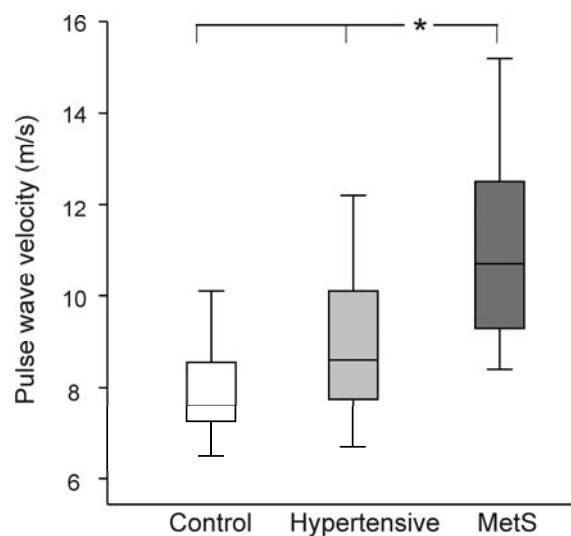
MetS=Metabolic syndrome. *p<0.05.

Figure 21. Aortic systolic and diastolic BP (A, B), heart rate (C), systemic vascular resistance index (D), cardiac index (E) and stroke index (F) in controls and in subjects with either essential hypertension or metabolic syndrome. *p<0.05.
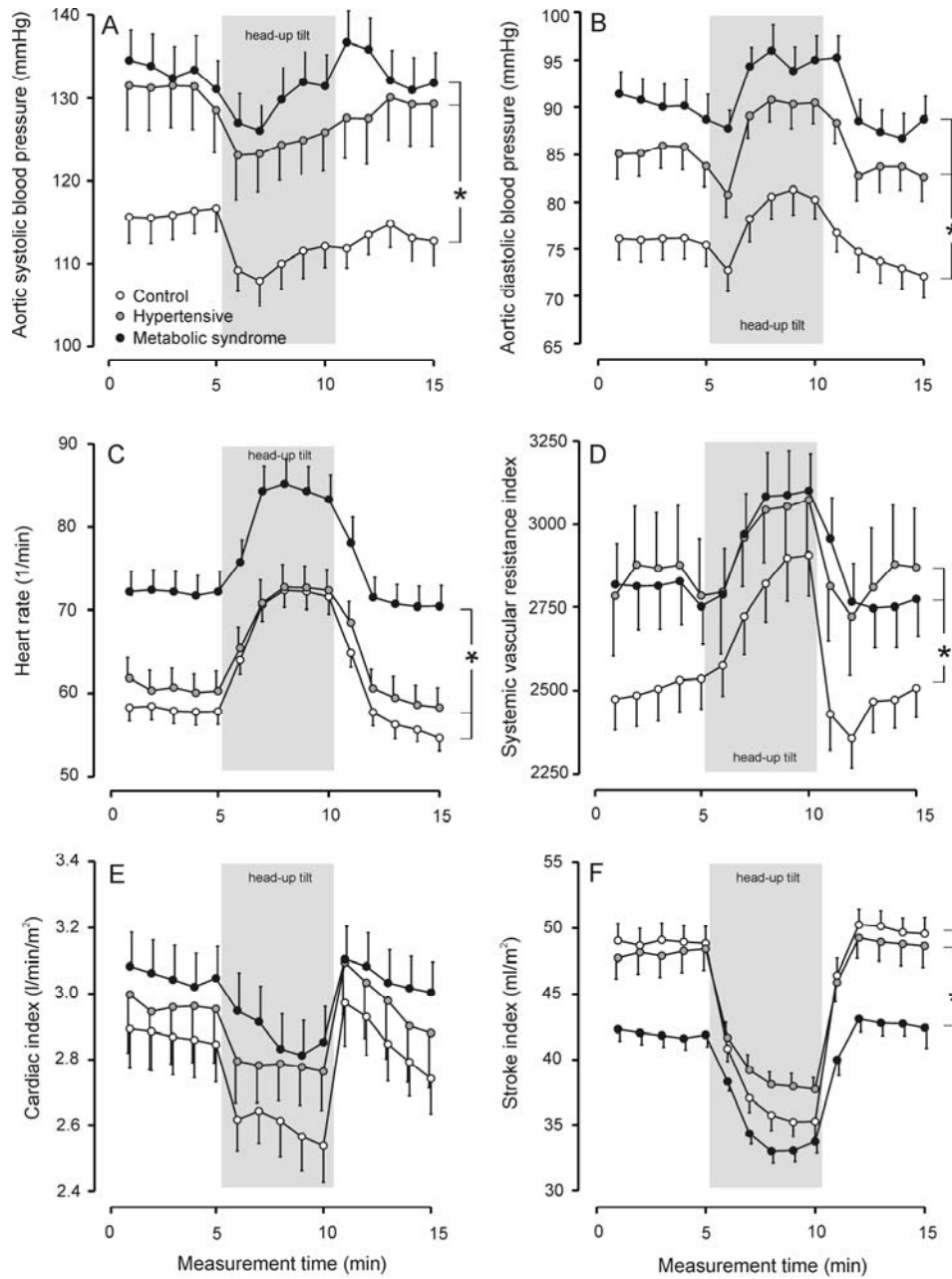
DISCUSSION

1. Subjects

The present investigations were designed to set up a novel measurement protocol for the study of cardiovascular function, and this was achieved by combining methods that were commercially available and had been previously used in many published reports. To avoid possible confounding factors induced by i.e. cardiovascular pathology, only normotensive subjects were included in the studies I-V. Finally, additional measurements were performed in subjects with essential hypertension or MetS to test the application in patients. None of the study subjects were taking medication for cardiovascular diseases.

The subjects included in the present studies were all under the age of 60 years, and thus the results must be applied to elderly subjects with caution. More advanced age is known to be associated with enhanced large arterial stiffening thus increasing central and peripheral BP, PWV and arterial wave reflection (McEniery et al. 2005). Haemodynamic profile of aged individuals during tilt table test may be clearly different from that of the younger ones, as already suggested by the results in the Study V examining subjects less than 60 years of age.

Recent reports have shown several haemodynamic differences between sexes (Gatzka et al. 2001, Shim et al. 2011). For example, higher AIx, higher central PP and lower PP amplification have been documented in women when compared with men (Shim et al. 2011). Only in the Study V the results were adjusted for sex, while in the Study IV gender-matched control-case pairs were examined. In the Studies I-III the data of male and female subjects was analysed together due to relatively small number of subjects. This can be considered as a limitation, and in future studies it is of importance to analyse the results of male and female subjects separately.

2. Methods and measurement protocol

Impedance cardiography

ICG_{WB} is an easy, completely non-invasive and continuous method to measure several haemodynamic variables, and the possibility of inter-user related errors is minimal. As a criticism, ICG technology utilizes mathematical equations to derive the haemodynamic variables, and different softwares may use different algorithms and yield different results. However, good agreement of ICG-derived CO with thermodilution derived values has been demonstrated (Kööbi et al. 1997b, Cotter et al. 2004, Paredes et al. 2006). It is also of note that the validation measurements of the CircMon[R] software used in the present study have also been performed during head-up tilt with good results (Kööbi et al. 1997b).

Pulse wave analysis

Peripheral and estimated central BP and indices of pulse wave reflection were non-invasively measured with pulse wave analysis system. In the present study a tonometric sensor for continuous radial pulse wave detection was used, and the probe was fixed on the radial artery with a wrist band. In previous reports, radial waveform has usually been detected with a pen-like tonometric sensor, and the operator holding the probe has selected only 10-20 successful cardiac cycles for the analyses (Hayward et al. 2002). Importantly, the sensor used in the present study enables continuous measurement, and the fixation of the probe significantly reduces measurement errors related to sensor movements. To ensure the reliability of the central BP results, the system was also calibrated approximately every 2.5 minutes.

The SphygmoCor pulse wave analysis system utilizes a generalized transfer function to derive central BP from the radial BP signal. The use of transfer functions on measured peripheral signal has raised critical conversation about their validity. SphygmoCor system utilizes the only commercially available generalized aorto-radial transfer function, while several similar transfer functions have been developed and published by research groups (Fetics et al. 1999, Hope et al. 2003). Even though the functions involve mathematical equations with slight differences, it is unlikely that they produce significantly different results due to the relatively wide confidence intervals associated with these methods, as stated also by O'Rourke (2007), an

innovator of the pulse wave analysis technique. The estimation of the aortic waveform using generalized transfer function has been validated against invasive aortic BP measurements with good results in several studies (Karamanoglu et al. 1993, Chen et al. 1997, Fetics et al. 1999). However, also poor association between estimated and measured aortic pressures has been reported (Cloud et al. 2003). Hope et al. (2002) documented a relatively poor agreement when using the same generalized transfer function for the total study population, but the agreement was improved when using a gender-specific transfer function separately in male and female subjects. The results above suggest that the development of an accurate transfer function in an on-going process, and it must be taken into account when interpreting the results.

Taken together, the knowledge of aortic BP greatly improves the characterization of a haemodynamic profile. However, invasive measurement of central BP is not justified in subjects without a clinical reason, and despite its limitations, the use of a transfer function on radial BP signal provides a non-invasive, easy and widely applicable method for central BP estimation.

Measurement protocol

As a distinction from previous studies on human haemodynamics, the measurements in the present study were performed in both supine position and during head-up tilt. The change in body position, a relatively simple physiological challenge, produced marked haemodynamic changes in all measured variables, and the response to sublingual NTG and inhaled salbutamol was also different in different postures. The repeatability and reproducibility of the measurement protocol, both supine and during tilt was comprehensively examined with good results.

The change in body posture from supine to upright induces significant changes in blood distribution, arterial resistance and autonomic nervous system, and orthostatic challenge can thus be regarded as an exercise test addressing cardiovascular reactivity (Avolio and Parati 2011). Importantly, it can be argued that measurements performed only at rest provide very limited haemodynamic information. Previous reports have shown that the changes in haemodynamic variables in response to physical challenge have also prognostic value. Hypertension was more likely to develop to Japanese men with enhanced BP response to a simple 4-minute 2-step exercise test during a 16-year

follow-up (Tsumura et al. 2002). In addition, patients with an abnormal BP response during a treadmill exercise test have increased risk of cardiovascular complications at elective vascular surgery (de et al. 2011). With the present measurement protocol it is possible to examine how the functional change in response to head-up tilt differs in subjects with various cardiovascular pathologies, and this could also provide prognostic information. Since the change in body posture leads to clear changes in autonomic nervous tone, the lack of assessment of sympathetic activity in the present study must be considered as a limitation.

3. Determinants of augmentation index

As the AIx was introduced, it was generally considered as an easily available marker of arterial stiffness. However, recently it has been described as an indicator of central wave reflection, arterial stiffness being one of its determinants. In addition to age, arterial compliance and BP, AIx is influenced by cardiac function and sites of wave reflection (Wilkinson et al. 2002a). In the present study (Study I), AIx at rest correlated with aortic systolic BP, age and aortic reflection time, consistent with previous studies (Wilkinson et al. 2002b, Sakurai et al. 2007). AIx also correlated with PWV at rest, but the relatively weak correlation stresses the view that other determinants in addition to arterial stiffness markedly influence AIx.

Both AIx and SV decreased in response to head-up tilt, thus showing that the changes in body position and cardiac function have a major influence on the level of augmentation. The magnitude of the decrease in AIx during head-up tilt correlated significantly with the changes in aortic systolic BP and PP, ejection duration and HR. An increase in HR is known to decrease the duration of ventricular ejection and systole, shifting the reflecting wave towards diastole, which subsequently causes the reduction of systolic BP and AIx. Previously, AIx has been reduced by 4–5 % for each 10 beats/min increment in HR during pacing (Wilkinson et al. 2000a). Thus, the increase in HR during tilt in the present study (~15beats/min) is not sufficient to explain the observed decrease in AIx (~11%), and also other mechanisms, like changes in stroke index, must be involved.

4. Effect of pharmacological stimuli on supine and upright haemodynamics

The haemodynamic effects of sublingual NTG and inhaled salbutamol were assessed placebo-controlled (Studies II and III), so that the small but significant placebo-induced changes in some variables were taken into consideration in the analysis of drug effects (chapter 5.3.1.) This approach further strengthened the findings.

Sublingual nitroglycerin
Small doses of sublingual NTG have been traditionally considered as venodilators, leading to increased venous pooling and subsequently reduced left ventricular preload. The effect on arterial tone has been considered to be lesser, and evident only at higher doses of NTG (Mason and Braunwald 1965). However, the results of the present study strongly imply that a small 0.25 mg dose of sublingual NTG induces arterial dilatation, since a marked decrease in SVRI and an increase in aortic reflection time were observed. In addition, the study provided no evidence of increased venous pooling after NTG. On the contrary, CO was clearly increased. NTG also decreased AIx, probably via the decrease in SVRI and subsequent reduction in PWV, and delayed the return of the reflected pressure wave. Autonomic nervous tone was not assessed in the present study, but in addition to its direct vasodilatory effect NTG can also sensitize the baroreflex arc, thus leading to reduced sympathetic tone and decreased SVRI (Zanzinger 1999). Results of a thorough statistical analysis showed, that the effect of NTG on many variables was enhanced during the head-up tilt when compared to the effect in the supine position. These changes may be explained by the alterations in baroreflex sensitivity, neurohormonal changes, and the effect of gravity during active vascular smooth muscle relaxation during the tilt.

The mechanisms of nitrate-induced presyncope have remained controversial in the literature. Again, increased venous pooling has been suggested in several reports (Gisolf et al. 2004, Verheyden et al. 2007), while only few studies have documented decreased vascular resistance during presyncope (Mitro and Hijova 2006). In the Study IV, a clear decrease in SVRI and increase in aortic reflection time during presyncope was observed, while CO was maintained at a higher level than before NTG administration. Only in three subjects with a brief total loss of consciousness, the final measurements preceding the collapse were characterized by decreased CO

and HR, which may imply a central sympatho-inhibitory effect of NTG during the final phase of the tilt test.

The present study protocol differed from the majority of the previous reports, since NTG was administrated supine instead of upright position. However, a decrease in SVRI, but not in CO, was also documented in the additional measurements in which NTG was administrated during the tilt. Since indices of arterial stiffness or wave reflection (AIx, PWV, aortic reflection time) did not differ between the presyncope and control groups, it can be argued that the mechanical properties on large arteries did not explain the increased sensitivity to NTG. However, as a difference of the previous reports on haemodynamics during presyncope (Gisolf et al. 2004, Verheyden et al. 2007), the subjects of the present study had not reported a history of unexplained syncope. In the future, the protocol should also be evaluated in syncopal subjects.

Inhaled salbutamol

The important role of vascular endothelium in haemodynamic regulation has also led to the development of methods assessing endothelial function *in vivo*. An ideal method would be easy, non-invasive and repeatable, and applicable to the whole arterial tree. In 2002, Wilkinson et al. (2002) introduced a method, in which changes in the tonometric measures of arterial wave, especially in AIx, after salbutamol inhalation were used as a measure of global endothelial function. A decrease of ~9 % in AIx was documented after salbutamol inhalation, while no significant change in AIx was observed when salbutamol was administrated after eNOS inhibitor infusion. Based mostly on these results, it has been considered that the salbutamol-induced changes in AIx are largely endothelium-mediated. Since its introduction, the method has been widely applied with similar results (Hayward et al. 2002). However, salbutamol-induced β_2-adrenoceptor stimulation may also result in changes in cardiac function, which has not been commonly measured.

An increase in alveolar NO concentration after salbutamol inhalation was documented when compared with placebo, verifying the salbutamol increased release of NO. The measurements were only performed in a small number of subjects (n=6), but as the result was clear, no further measurement were performed. It should be noted that in the haemodynamic measurements inhaled salbutamol not only decreased AIx, but also

reduced BP and SVR. In addition, HR and CO were increased, and the effect was greater in supine position when compared to tilt. This emphasizes the importance of the analyses of endothelial function to be performed also in the upright position. As PWV was not changed, this implies that the observed decrease in AIx indeed resulted from decreased and delayed wave reflection in response to decreased SVR, and not from changed large arterial compliance.

Intravenous L-arginine

L-arginine, a semi-essential amino acid, acts as a precursor for eNOS, thus inducing a largely endothelium-dependent vasodilatation. Measurable vasodilatation in response to L-arginine administration has been reported in both healthy subjects and in subjects with various cardiovascular diseases, but it should be noted that the results have remained rather controversial. For example, Drexler et al. (1994) demonstrated that improvement of coronary endothelial function with L-arginine was more likely in vessels with normal wall morphology(Drexler et al. 1994), while another report documented a more pronounced vasodilator effect of L-arginine in stenosed coronary arteries when compared with healthy vessel segments (Tousoulis et al. 1998). It has been stated that eNOS should be saturated with physiological levels of L-arginine, and not to be dependent on extracellular substrate supply. However, excess availability of L-arginine may overcome the effects of asymmetric dimethylaginine (ADMA), an endogenous molecule with eNOS inhibitory effects (Leiper and Vallance 1999). Elevated concentrations of ADMA are present in patients with vascular diseases, which explain the effects of L-arginine in these patients (Perticone et al. 2005). In healthy subjects, the vasodilatory effects of L-arginine may also be mediated via endocrine mechanisms, i.e. stimulation of growth hormone and insulin secretion (Merimee et al. 1967).

In the present study, the relatively large dose of L-arginine should have been sufficient to induce cardiovascular changes, when compared with previous reports examining L-arginine effects (Delles et al. 2003, Koifman et al. 2006). However, the haemodynamic effects of L-arginine in healthy subjects were modest and documented only in the upright position, during which BP vas decreased and aortic reflection time increased. The time to reflected wave increases with the decrease in arterial compliance, and the reflected wave is shifted towards diastole. However, L-arginine

did not lower AIx, probably due similar effects on augmentation pressure and PP, the determinants of AIx. Since SVRI did not change after L-arginine, documented effects can be considered to have resulted from increased large arterial compliance.

5. Ageing and haemodynamics

Ageing is associated with structural and functional changes in the vasculature, including large arterial dilatation and stiffening (Greenwald 2007, O'Rourke and Hashimoto 2007). However, age-related changes in smaller arteries are considered not to be as profound as in the larger ones. In concert with the view above, results of the present study (Study V) showed a clear age-related increase in radial and aortic systolic BP and PP, PWV, AIx and a decrease in aortic reflection time, while no systematic age-related difference was observed in HR, SVRI or CO. Diastolic BP was lower in 50-59 year-old subjects when compared with the 40-49 year-old subjects, which may be explained by the increased stiffness in the older subjects, and shifting of the reflected pressure wave towards systole. AIx and PWV differed significantly already in younger age groups, while aortic PP was increased only after the age of 50 years (Figure 17C). This suggests that PP is a relatively inaccurate marker of arterial stiffness in younger subjects. Indeed, the relationship between large arterial stiffening and age has been documented to be non-linear, with an acceleration of aortic stiffening after the 5th decade of life (McEniery et al. 2005). In the present study, the correlation between age and AIx or PWV seemed to be linear, probably due to the lack of older subjects (>60 years).

The incidence of orthostatic hypotension increases with age (Rutan et al. 1992), but the underlying haemodynamic mechanisms are not completely clear. For example, increased arterial stiffness, increased venous pooling of blood and attenuated HR response due to decreased baroreflex sensitivity have been suggested as mechanisms of impaired haemodynamic regulation (Monahan et al. 2001, Mattace-Raso et al. 2006, Verheyden et al. 2007). Increased arterial stiffness at sites rich in baroreceptors may further decrease baroreflex sensitivity (Monahan et al. 2001).

In the Study V, adjusted regression models revealed that the age of 50-59 years was a significant explanatory factor for enhanced decrease in aortic systolic BP during tilt.

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In addition, baseline PWV, the golden standard of arterial stiffness, explained the more pronounced change. Moreover, aortic reflection time decreased numerically more during the head-up tilt in younger than older subjects, and baseline PWV was a significant explanatory factor for the change. These results imply that increased arterial stiffness with ageing can explain the age-related haemodynamic differences in the responses to head-up tilt. In the present study, no systematic differences in HR or SVRI responses to tilt were observed, suggesting preserved baroreflex sensitivity. Futhermore, contrary to the view of increased venous pooling of blood in the older subjects (Verheyden et al. 2007), the decrease in CO during tilt did not differ with ageing in the present study. It is of note that the subjects of the present study were relatively young, although comparable with some of the previous studies with different findings (Monahan et al. 2001, Verheyden et al. 2007). In the future the measurements should be repeated in older subjects. In addition, a longitudinal study examining individual age-related changes would provide more detailed information about the age-associated changes than the present, cross-sectional view.

6. Clinical implications

Several haemodynamic factors that have an influence on BP or on individual cardiovascular risk profile, are not routinely assessed in clinical practice. For example, increased arterial stiffness and impaired endothelial function have been associated with increased cardiovascular mortality and morbidity, and BP measured from central arteries serves as a better predictor of cardiovascular outcome than peripheral BP. In addition, the determination of functional haemodynamic status utilizing a physiological challenge to the cardiovascular system could provide more crucial information than the measurements performed only at rest.

To test the present application in patients, subjects with essential hypertension or MetS were examined in comparison with normal subjects. The BP level or SVRI of the two patient groups did not significantly differ from each other. However, subjects with MetS had higher HR and lower SI than the other groups, demonstrating differences in the regulation of cardiac function. In addition, despite very similar elevation of BP, subject with Mets had significantly higher PWV when compared with hypertensive subjects. These results demonstrate that the other components of

MetS in addition to hypertension clearly contribute to the increased arterial stiffness in these subjects. These preliminary results also strengthen the view that distinct haemodynamic alterations beyond mere elevation of BP can be found in different cardiovascular disorders.

The present measurement protocol provides a repeatable and an easy method for the detailed characterization of patients' haemodynamic profile. The method is applicable to almost all patients, since it is completely non-invasive and safe. In the future, the haemodynamic profile of patients with various cardiovascular disorders, both supine and upright, is going to be further evaluated. In addition, the prognostic value of the measured variables and the responses to head-up tilt can be analysed after follow-up measurements. Furthermore, the effects of interventions, both pharmacological and non-pharmacological, can individually be determined.

SUMMARY AND CONCLUSIONS

The present non-invasive haemodynamic measurement protocol is a novel combination of methods, utilizing both physiological and pharmacological challenges to cardiovascular system. In the future, the method can be applied to patients in clinical practice to gather detailed functional haemodynamic information.

The results of the present study can be summarized as follows:

1. Haemodynamic measurements with non-invasive ICG, continuous pulse wave analysis and plethysmographic finger BP measurements were highly repeatable and reproducible both in supine position and during passive orthostatic challenge. Head-up tilt as a physiological challenge induced significant changes in almost all measured variables.

2. A small dose of sublingual NTG resulted in marked haemodynamic responses especially during the head-up tilt. Inhaled salbutamol induced several haemodynamic changes, and some of the effects were enhanced in the supine position, while the modest effects of intravenous L-arginine could only be observed during the tilt. The present results show that the administration of inhaled salbutamol combined to the measurement protocol can be applied to evaluate the role of the endothelium in cardiovascular responsiveness. Divergent drug effects in different body positions underline the importance of upright measurements.

3. A significant decrease in SVRI was observed during NTG-induced presyncope when compared with control subjects without presyncopal symptoms. Contrary to the previously accepted view, no evidence of increased venous pooling and a subsequent decrease in cardiac output could be observed.

4. Ageing was associated with increased arterial stiffening and wave reflection, while no age-related changes were observed in vascular resistance or cardiac function. In addition, the observed age-related changes in the responses to head-up tilt could be largely explained by the increased large arterial stiffness.

5. Haemodynamic profile of subjects with essential hypertension or MetS clearly differed from that of the control subjects. In addition, subjects with MetS had higher HR and PWV than hypertensive subjects, demonstrating differences in the regulation of cardiac function, and increased arterial stiffness in MetS. These results support the view of the clinical applicability of the present non-invasive functional examination of the cardiovascular system.

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ORIGINAL ARTICLE

Analysis of cardiovascular responses to passive head-up tilt using continuous pulse wave analysis and impedance cardiography

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Objective. To non-invasively measure central haemodynamics, arterial stiffness, cardiac function and vascular resistance, with the subject in the supine position and during head-up tilt, in order to examine the haemodynamic changes associated with alterations in the augmentation index, and to investigate repeatability and reproducibility of the measurement protocol. ***Material and methods.*** Thirty-three healthy volunteers (21–51 years) were investigated using continuous pulse wave analysis from the radial artery with a tonometric sensor, whole-body impedance cardiography and plethysmographic blood pressure (BP) recordings from the fingers. The measurements were performed with the subject supine and during passive head-up tilt, and repeated during the same session and on four separate days. ***Results.*** During the head-up tilt, diastolic BP (5.2 ± 0.6 %), heart rate (27.6 ± 1.9 %) and vascular resistance (12.5 ± 1.7 %) increased (all $p < 0.05$), while systolic BP (-3.2 ± 0.6 %), aortic pulse pressure (-23.3 ± 1.4 %), augmentation index (-11.6 ± 0.7 %), aortic reflection time (-7.0 ± 1.0 %), ejection duration (-21.4 ± 0.7 %), stroke volume (-26.1 ± 1.2 %) and cardiac output (-5.0 ± 1.5 %) decreased (all $p < 0.05$). Augmentation index at rest correlated with aortic systolic BP ($r = 0.423$), aortic reflection time ($r = -0.647$), pulse wave velocity ($r = 0.287$) and age ($r = 0.480$). The change in augmentation index during head-up tilt correlated with the change in aortic systolic BP ($r = 0.469$), aortic pulse pressure ($r = 0.606$), ejection duration ($r = 0.374$) and heart rate ($r = -0.445$). According to Bland-Altman and repeatability index analyses, repeatability and reproducibility of the measurements were good during the same session and on separate days. ***Conclusions.*** Combined pulse wave analysis and impedance cardiography with the subject in the supine position and during head-up tilt is a repeatable and reproducible method for comprehensive investigation of the cardiovascular function.

Keywords: Arterial stiffness; cardiac function; haemodynamics; systemic vascular resistance

Introduction

Hypertension and decreased arterial compliance are major risk factors for target organ damage in various cardiovascular disorders, and early detection of a haemodynamic high-risk profile is an important measure by which to decrease the incidence of complications [1,2]. In addition to indirect determination of brachial blood pressure (BP), non-invasive pulse wave analysis, which provides information about central pressures in the circulation, has improved possibilities to identify subjects with increased cardiovascular risk [1,3,4]. At present, the augmentation index (AIx), i.e. the amplitude of the reflected pressure wave divided by pulse pressure, is an acknowledged measure of central wave reflection [2,5]. However, parallel recordings of peripheral

arterial resistance and cardiac output have seldom been combined with pulse wave analysis, although these variables are important determinants of AIx and BP [6].

Pulse wave analysis is usually performed with a pen-like sensor from 10 consecutive heart beats, with the study subject resting quietly in the supine position [7–9]. However, early alterations in arterial compliance, wave reflection and cardiac function may become more apparent during physical challenge, since some reports have found that enhanced BP response to exercise is a predictor of the development of hypertension [10,11]. There have been very few studies on pulse wave analysis during physical or orthostatic challenge, but a recent report found excellent concordance between invasively and

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non-invasively measured central pressures during a cycling test [12].

The treatment of hypertension has become increasingly active and the recommended BP targets lower, which underscores the need for BP and haemodynamics to be measured with subjects in the upright position [13,14]. The purpose of this study was to examine haemodynamics using continuous recordings of central wave reflections, cardiac function and systemic vascular resistance in healthy volunteers both at rest and during passive orthostatic challenge, and to test the reproducibility and repeatability of the measurement protocol.

Methods

Study subjects

The study population comprised 33 (17 F and 16 M) healthy, normotensive individuals aged between 21 and 51 years. All of the subjects underwent physical examination by a medical doctor. During the interview, lifestyle habits, family history of cardiovascular disease and medical history were recorded. All participants gave written informed consent, and the study was approved by the Ethics Committee of Tampere University Hospital (approval no. R06086M).

Experimental protocol

Haemodymamic measurements were carried out by a trained nurse in a quiet, temperature-controlled, research laboratory. The study subjects had refrained from taking caffeine-containing products and from smoking or eating a heavy meal in at least the 4 h prior to the study, and from alcohol in at least the 24 h prior to the investigation, but a light breakfast or meal was allowed 1–3 h before the measurements. The subjects rested supine on a tilt-table, with the standard electrodes for whole-body impedance cardiography placed on the body surface; the tonometric sensor for pulse wave analysis on the radial pulsation to the left wrist and oscillometric brachial cuff for BP calibration to the right upper arm. Before the actual measurement, an introductory head-up tilt was done to familiarize the study subject with the tilt. The actual measurement was haemodynamic data captured continuously during three consecutive 5-min intervals. For the first 5 min, the subjects rested supine on the tilt-table, followed by 5 min of head-up tilt to 60 degrees. After 5 min standing, the tilt-table was returned to the horizontal position for another 5 min. To test the repeatability of the method, the same protocol was repeated immediately (5 min supine–5 min standing–5 min supine). To elucidate

the reproducibility of the method, the study subjects underwent the same haemodynamic measurements on four separate days during a maximum period of 4 weeks (visits 1–4).

Pulse wave analysis

Radial BP and pulse wave form were determined from the radial pulsation using a tonometric sensor (Colin BP-508T, Colin Medical Instruments Corp., USA) fixed on the radial pulse with a wrist band. A continuous radial BP signal was calibrated every 2.5 min by a brachial BP measurement. Continuous aortic BP was estimated with the SphygmoCor pulse wave monitoring system (SpygmoCor PWMx; AtCor Medical, Australia) using the previously validated generalized transfer function [15]. Ejection duration, aortic reflection time and AIx (augmented pressure/ pulse pressure*100) were determined.

Whole-body impedance cardiography

A whole-body impedance cardiography device (CircMon®; JR Medical Ltd., Tallinn, Estonia), which records the continuous changes in body electrical impedance during a cardiac cycle, and plethysmographic BP recordings from fingers (Finapres, Ohmeda, Englewood, Colorado, USA) were used to determine beat-to-beat heart rate, stroke volume, cardiac output, systemic vascular resistance index (SVRI) and pulse wave velocity (PWV) [16–19]. A pair of current electrodes was placed on the distal parts of the extremities, just proximal of the wrists and ankles. Voltage electrodes were placed proximal of the current electrodes, so that the distance between the centres of the electrodes was 5 cm. The distal impedance was recorded from a popliteal artery at knee joint level, and the active electrode was placed on the lateral side of the knee and the reference electrode on the calf – the distance between the electrodes being 20 cm. When the pulse pressure wave enters the aortic arch and the diameter of the aorta changes, the whole-body impedance decreases, and this can be measured by the voltage electrodes on the distal parts of the extremities. To calculate the PWV value, the CircMon software measures the time difference between onset of the decrease ('foot') in impedance in the whole-body impedance signal and the popliteal artery signal. The PWV can be determined from the time difference and the distance between the electrodes.

The cardiac output values measured with CircMon® whole-body impedance cardiography are in good agreement with those measured by the thermodilution method, with the subject in both in the supine position and during head-up tilt [18]. A

detailed description of the method and electrode configuration has been reported previously [17–19]. PWV was not assessed during head-up tilt because of less accurate timing of left ventricular ejection during reduced stroke volume.

Laboratory analyses

Blood samples were obtained after a minimum of 12-h fast in the morning for laboratory analyses, and a standard 12-lead electrocardiogram was recorded. Plasma sodium, potassium, calcium, glucose, creatinine, triglycerides and total, high-density and low-density lipoprotein cholesterols were determined by Cobas Integra 700/800 (F. Hoffmann-LaRoche Ltd, Basle, Switzerland) and blood cell count by ADVIA 120 or 2120 (Bayer Health Care, Tarrytown, NY, USA). Creatinine clearance was estimated using the Cockcroft–Gault formula [20].

Statistical analysis

To compare the measurements performed on the same day and on four different days (visits 1–4), one-way analysis of variance (ANOVA) and ANOVA for repeated measurements were applied. Repeatability (two consecutive measurements) and reproducibility (four measurements on different days) were analysed using two statistical methods: 1) Repeatability and reproducibility indexes (R) were calculated utilizing one-way ANOVA: the outcome ranges from 0 to 1, with 0 indicating no consistency and 1 complete consistency (expressed as %). R was calculated from the formula $R = 1 - MS_w/MS_T$, in which MS_w and MS_T are square sums indicating variation within a subject and in the total study population, respectively. 2) Repeatability and reproducibility values were also calculated as recommended by Bland & Altman [21], showing the range as ± 2 standard deviations (SD), which covered 95 % of the observed differences between repetitive measurements. To compare the haemodynamic values with the subject in the supine position versus the standing position, the paired samples t-test was utilized. The associations between the AIx and other variables were investigated using Pearson's coefficient of correlation test. P-values < 0.05 were considered statistically significant. Values were expressed as mean $\pm$ standard error of the mean (SEM) and the data were analysed using SPSS 11.5 for Windows (SPSS Inc., Chicago, Ill., USA).

Results

Study population

Demographic data and basic laboratory values of the study population are given in Table I. None of the

Table I. Study population ($n = 33$).

Variable	Mean $\pm$ SEM
Age	33.9 ± 1.4
Female/male	17/16
Body mass index (kg/m^2)	23.2 ± 0.5
Waist circumference (cm)	
Male	87.4 ± 1.4
Female	79.1 ± 2.7
Smoking status (present/previous/never)	4/5/24
Plasma	
Fasting glucose (mmol/L)	5.1 ± 0.1
Total cholesterol (mmol/L)	4.4 ± 0.1
HDL cholesterol (mmol/L)	1.7 ± 0.1
LDL cholesterol (mmol/L)	2.2 ± 0.1
Triglycerides (mmol/L)	1.0 ± 0.1
Creatinine (μmol/L)	77.5 ± 1.9
Potassium (mmol/L)	3.90 ± 0.01
Sodium (mmol/L)	139.9 ± 0.3
Calcium (mmol/L)	2.26 ± 0.01
Hematocrit (%)	41.9 ± 0.8
Estimated creatinine clearance (mL/min/1.73 m^2)	111.3 ± 5.7

SEM = standard error of the mean.

study subjects had a medical history of cardiovascular disease or elevated BP. The use of medication was minimal: 6 female subjects were taking an oestrogen/progesterone combination for contraception. Four of the subjects were present smokers, while five had a previous smoking history. Lipid profile, fasting glucose, electrolytes and kidney function were all within the normal range.

Haemodynamics of patients – supine position and head-up tilt

The haemodynamic changes in response to head-up tilt were reproducible on four separate days (Figure 1) and during two consecutive measurements (not shown). All of the variables changed significantly with the subject in the upright position ($p < 0.05$ versus supine values; Table II): diastolic BP ($+5.2 \pm 0.6$ %), heart rate ($+27.6 \pm 1.9$ %) and vascular resistance increased ($+12.5 \pm 1.7$ %), while systolic BP (-3.2 ± 0.6 %), aortic pulse pressure (-23.3 ± 1.4 %), augmentation index (-11.6 ± 0.7 %), aortic reflection time (-7.0 ± 1.0 %), ejection duration (-21.4 ± 0.7 %), stroke volume (-26.1 ± 1.2 %) and cardiac output (-5.0 ± 1.5 %) decreased. Radial systolic BP was significantly higher than aortic systolic BP ($p < 0.05$), while there was no difference between radial and aortic diastolic BP (Table II).

Association between augmentation index and other haemodynamic variables

Supine AIx correlated with aortic systolic BP ($r = 0.423$, $p = 0.001$), aortic reflection time
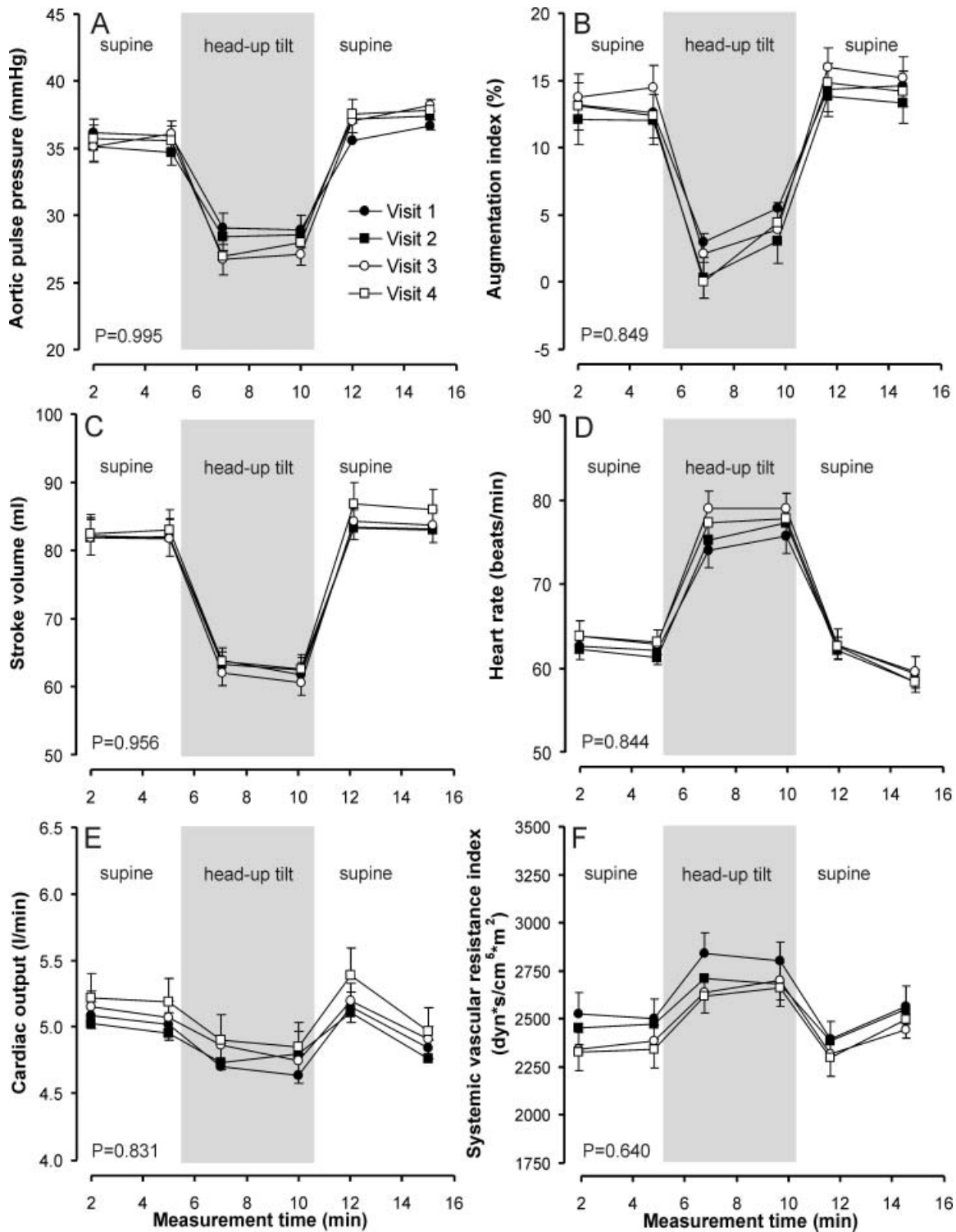
Figure 1. Mean values of aortic pulse pressure (A), augmentation index (B), stroke volume (C), heart rate (D), cardiac output (E) and systemic vascular resistance index (F) of hemodymamic measurements performed on four separate days (visits 1–4). Each subject ($n=33$) underwent all four 15-min measurements and a 5-min head-up tilt was performed between 5 and 10 min. *P*-values derived from ANOVA for repeated measurements.

($r=-0.647$, $p=0.000$), PWV ($r=0.287$, $p=0.024$) and age ($r=0.480$, $p=0.005$). During the head-up tilt, AIx correlated significantly with ejection duration ($r=0.504$, $p=0.000$), heart rate ($r=-0.339$, $p=0.007$), SVRI ($r=0.335$, $p=0.007$) and age ($r=0.408$, $p=0.005$).

Table II. Haemodynamic variables, repeatability and reproducibility values supine and during head-up tilt.

| | | | Repeatability: two consecutive measurements | | Reproducibility: four separate days | |
| | | | Bland-Altman | R (%) | Bland-Altman | R (%) |
	Supine	Tilt	Supine/Tilt	Supine/Tilt	Supine/Tilt	Supine/Tilt
Tonometry						
Radial systolic BP (mmHg)	127 ± 2	$125 \pm 3*$	10/12	90/85	15/15	77/78
Aortic systolic BP (mmHg)	113 ± 2	$109 \pm 1*$	10/14	90/74	14/16	74/74
Radial diastolic BP (mmHg)	76 ± 2	$80 \pm 1*$	10/12	87/79	12/13	79/78
Aortic diastolic BP (mmHg)	77 ± 2	$80 \pm 1*$	10/12	87/79	12/13	79/79
Aortic pulse pressure (mmHg)	36 ± 1	$28 \pm 1*$	5/8	88/67	9/11	62/46
Aortic augmentation index (%)	13.7 ± 1.4	$1.5 \pm 1.4*$	5/6	95/95	12/13	78/70
Aortic reflection time (ms)	166 ± 3	$153 \pm 2*$	20/11	86/87	25/27	79/48
Ejection duration (ms)	334 ± 3	$263 \pm 3*$	12/15	96/96	28/41	74/57
Impedance cardiography						
Stroke volume (mL)	84 ± 3	$62 \pm 2*$	4/3	99/99	15/8	89/93
Heart rate (beats/min)	61 ± 1	$77 \pm 2*$	23/29	22/23	15/18	67/66
Cardiac output (L/min)	5.00 ± 0.14	$4.75 \pm 0.16*$	0.62/0.45	92/97	1.48/1.22	70/82
Vascular resistance index (dyn*s/cm^5*m^2)	2478 ± 82	$2741 \pm 78*$	349/390	93/91	937/993	59/54
Pulse wave velocity (m/s)	8.9 ± 0.2	–	0.40/–	99/–	87/–	87/–

Mean $\pm$ standard error of the mean. $*p < 0.05$ compared to supine values. Repeatability and reproducibility values by Bland and Altman, and index R.

Both AIx and stroke volume decreased in response to head-up tilt, but correlation between the magnitude of the change between these two variables was not significant ($p = 0.131$). The change in AIx during head-up tilt correlated with the change in aortic systolic BP ($r = 0.469$, $p = 0.000$), aortic pulse pressure ($r = 0.606$, $p = 0.000$), ejection duration ($r = 0.374$, $p = 0.002$) and heart rate ($r = -0.445$, $p = 0.000$).

Two consecutive measurements: repeatability

The repeatability values for the haemodynamic variables are given in Table II. The repeatability index, reflecting the variation between two consecutive measurements, was good in both the supine position and during head-up tilt for all other variables with the exception of heart rate, for which it was only 22–23 % (Table II). Repeatability of the measurements according to Bland & Altman is given in Table II and Figures 2 and 3, and the limits of agreement are good compared with the previous studies on repeatability of haemodynamic measurements [9,17,18].

Measurements on four separate days: reproducibility

The reproducibility values are given in Table II. The reproducibility index, which describes the variation in the measurements performed on four separate days, was lower than the repeatability index. Still, reproducibility was good, with values varying from 59 % to 89 % with the subject in the supine position and from 46 % to 93 % during head-up tilt (Table II).

Reproducibility according to Bland & Altman was also good (Table II).

Discussion

The purpose of the present study was to examine haemodynamics both at rest and during orthostatic challenge, and to test the repeatability and reproducibility of the measurement protocol. The present non-invasive approach provides information about arterial stiffness, central haemodynamics, cardiac function (heart rate, stroke volume, cardiac output, ejection duration) and systemic vascular resistance, all of which are important regulators of BP. All of the measured variables changed significantly in response to head-up tilt, and the repeatability and reproducibility of the method was good [9,17,18].

The present treatment strategies of hypertension with lower treatment goals than ever, especially in high-risk patients with complicated diabetes or kidney disease, emphasize the importance of the measurement of BP and haemodynamics with the subject in the upright position [13,14]. Here we used passive head-up tilt to induce the challenge to the cardiovascular system, during which diastolic BP increased slightly (5 %), while systolic BP decreased slightly (−2 %). However, major changes were observed in heart rate, stroke volume, systemic vascular resistance and AIx. It is notable that BP response to a simple 4-min two-step exercise test has been found to be a strong predictor of hypertension in a Japanese population-based study, suggesting that

Figure 2. Repeatability at rest: Bland-Altman plots for the comparison of two consecutive measurements showing the differences against the mean value with the subject in the supine position. Augmentation index (A), aortic pulse pressure (B), cardiac output (C), systemic vascular resistance index (D), heart rate (E) and stroke volume (F).

prognostic changes in BP may be evident even during a relatively minor physical challenge [10]. In more rigorous treadmill or ergometer tests, enhanced

exercise-induced reactivity of BP has been shown to predict new-onset hypertension [11]. It remains to be determined whether the changes in haemodynamic
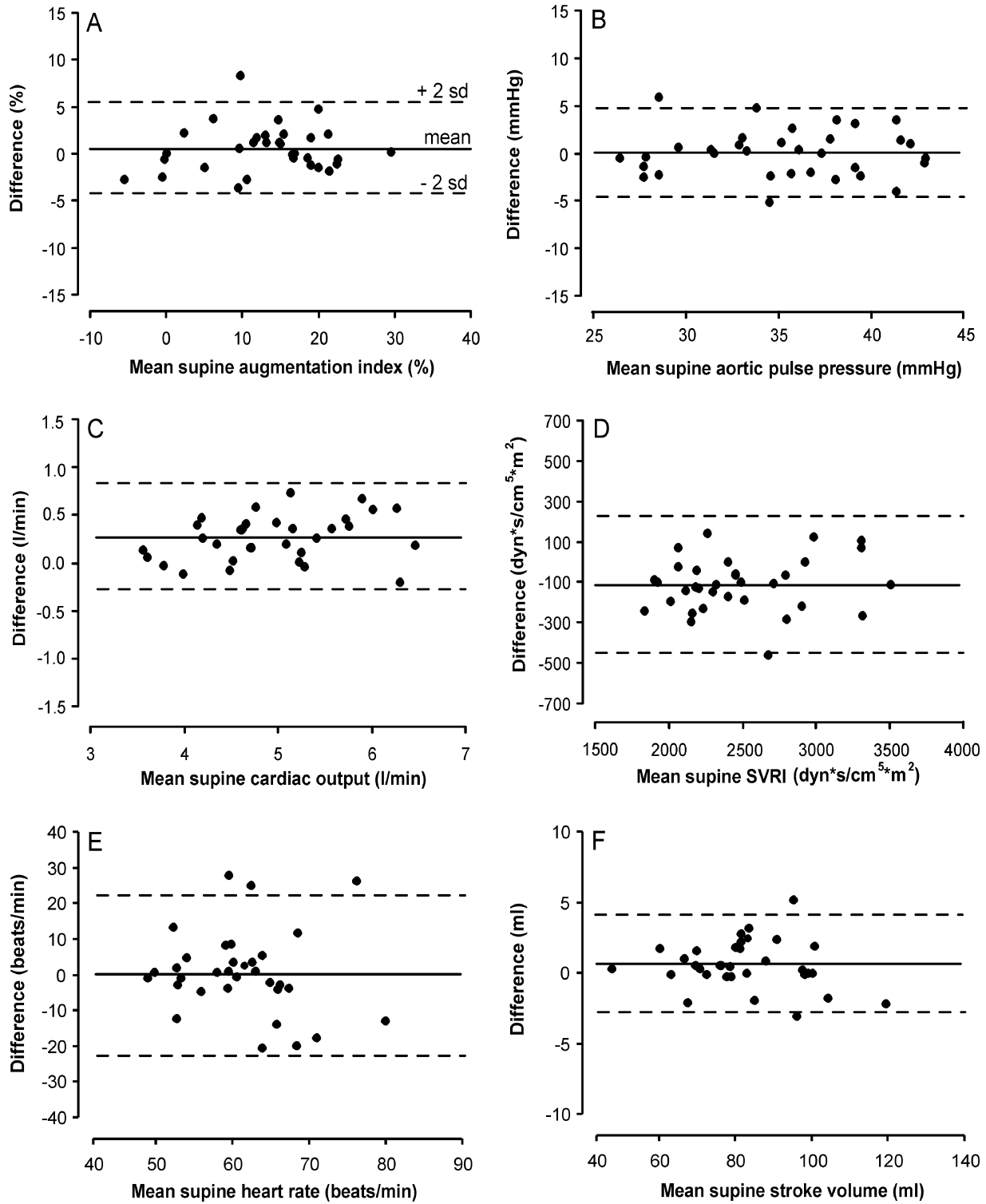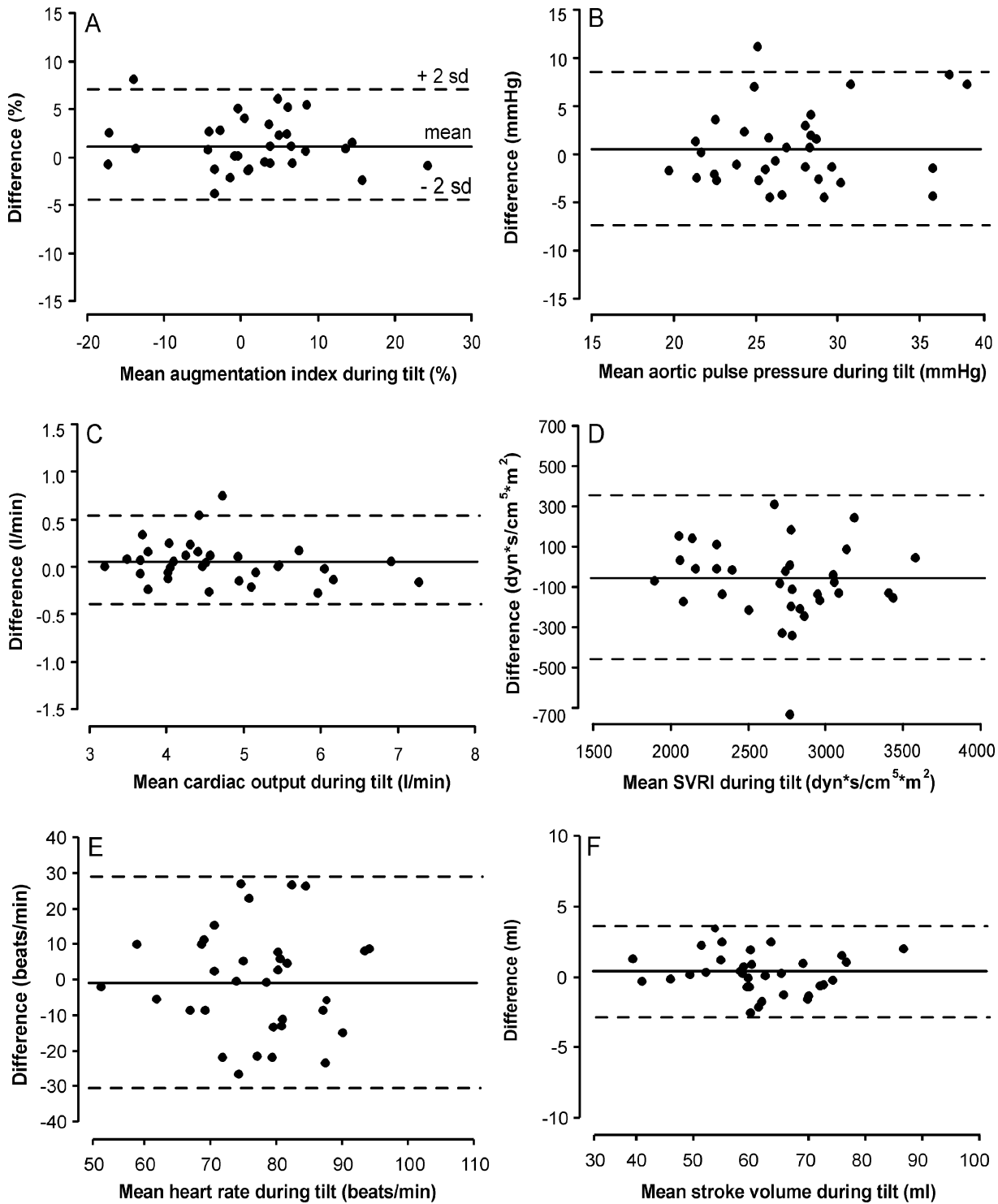
Figure 3. Repeatability during orthostatic challenge: Bland-Altman plots for the comparison of two consecutive measurements showing the differences against the mean value during the head-up tilt. Augmentation index (A), aortic pulse pressure (B), cardiac output (C), systemic vascular resistance index (D), heart rate (E) and stroke volume (F).

variables induced by an orthostatic challenge provide significant prognostic information, and whether these changes can be used in the guidance of the treatment of cardiovascular disease.

Decreased arterial compliance is an acknowledged risk factor for cardiovascular morbidity and mortality [22]. Local arterial stiffness and vessel wall properties, for example from carotid or brachial

sites, can be defined by the use of ultrasound methods [23]. Regional arterial stiffness, like carotid-femoral and aortic PWV, can be analysed by means of arterial tonometry, mechanotransducer methods and echo-tracking devices [24–26]. At present, the AIx, derived from arterial waveform consisting of incident and reflecting wave, is one of the most commonly used indicators of central wave reflection and arterial compliance. The major determinants of AIx are BP, PWV and cardiac function. In addition, age has a major influence on all of the aforementioned variables, while AIx is also affected by the site of wave reflection [27,29]. However, the role of AIx as marker of arterial stiffness has recently been questioned due to a lack of correlation between invasive PWV and AIx [29–31]. In the present study, we utilized continuous pulse wave analysis and determination of the AIx, combined with the analyses of cardiac function and vascular resistance both at rest and during orthostatic challenge. AIx at rest correlated with aortic systolic BP, age and aortic reflection time, consistent with previous studies [29,32]. AIx also correlated with PWV at rest, but the relatively weak correlation underlies the importance of the other determinants affecting AIx.

In response to head-up tilt, both AIx and stroke volume decreased, showing that the changes in body position and cardiac function, especially heart rate, have a major influence on the level of central pressure augmentation. The magnitude of the decrease in AIx during head-up tilt correlated significantly with the changes in aortic systolic BP and pulse pressure, ejection duration and heart rate. An increase in heart rate is known to decrease the duration of ventricular ejection and systole, and shift the reflecting wave towards diastole, which subsequently causes the reduction of systolic BP and AIx. Previously, AIx has been reduced by 4–5 % for each 10 beats/min increment in heart rate during pacing [32,33]. In the present study, the heart rate increased by approximately 14–17 beats/min, which alone could explain a 6–8 % decrease in AIx. However, we observed a 12 % decrease in AIx during the head-up tilt, and in addition to the change in heart rate also the reduction in stroke volume may have influenced the level of augmentation.

Analysis of repeatability and reproducibility using the Bland-Altman and repeatability index methods included the variation of the measured variable in the entire study population and within a single study subject [34]. Good repeatability and reproducibility of radial pulse wave analysis [9,35] and impedance cardiography [17,36] at rest have been described previously. In contrast to the previous reports, we utilized a combination of

techniques and the measurements were continuous with the subject in both the supine position and during head-up tilt. The recordings carried out on four separate days were in correspondence (Table II); and successive recordings on the same day were also highly repeatable (two consecutive measurements). Heart rate was the only variable showing relatively poor repeatability (Table II) and a wide scale of variation (Figures 2E and 3E). This probably contributed to the observed variation in cardiac output (Figures 2C and 3C), and it could be argued that the changes resulted from the rapid responsiveness of heart rate to various external and internal stimuli.

In conclusion, combined pulse wave analysis and impedance cardiography enabled the measurement of several haemodynamic variables during a simple orthostatic challenge. We found that the observed changes in haemodynamics induced by passive head-up tilt were highly repeatable and reproducible.

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Non-invasive measurement of the haemodynamic effects of inhaled salbutamol, intravenous L-arginine and sublingual nitroglycerin

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Haemodynamic effects of endothelial stimuli induced by salbutamol and L-arginine in humans have been previously studied predominantly at rest in selected vascular beds.
- We studied the effects of salbutamol, L-arginine and nitroglycerin on cardiac and vascular function by continuous recording of pulse wave analysis and impedance cardiography both in the supine position and during passive head-up tilt.

WHAT THIS STUDY ADDS

- The divergent effects of the research drugs in supine position and during head-up tilt indicate that human haemodynamics should also be studied in the upright position.
- Since inhaled salbutamol induced more pronounced changes in haemodynamics, it provides a clinically more applicable tool than infused L-arginine for the assessment of endothelial function in humans.

AIMS

To examine the effects of salbutamol and L-arginine, two compounds acting largely on the endothelium, and the endothelium-independent agent nitroglycerin on blood pressure, arterial compliance, cardiac function and vascular resistance.

METHODS

Continuous radial pulse wave analysis, whole-body impedance cardiography, and plethysmographic blood pressure from fingers in the supine position and during head-up tilt were recorded in nine healthy subjects. Data were captured before and after L-arginine (10 mg mg^{-1} min^{-1}) or saline infusion, salbutamol (400 µg) or placebo inhalation, and sublingual nitroglycerin (0.25 mg) or placebo resoriblet.

RESULTS

The results of all measurements were comparable before drug administration. The effects of inhaled salbutamol were apparent in the supine position: systemic vascular resistance ($-9.2 \pm 2.6\%$) and augmentation index ($-4.0 \pm 1.5\%$) decreased, and heart rate ($8.6 \pm 2.5\%$) and cardiac output ($8.8 \pm 3.1\%$) increased. L-arginine had no clear effects on supine haemodynamics, but during head-up tilt blood pressure was moderately decreased and reduction in aortic reflection time prevented, indicating improved large arterial compliance. Nitroglycerin reduced supine vascular resistance ($-6.7 \pm 1.8\%$) and augmentation index ($-7.4 \pm 1.6\%$), and increased cardiac output ($+9.2 \pm 2.7\%$). During head-up tilt, nitroglycerin increased cardiac output ($+10.6 \pm 5.6\%$) and heart rate ($+40 \pm 7.5\%$), decreased vascular resistance ($-7.8 \pm 5.8\%$) and augmentation index ($-18.7 \pm 3.2\%$), and prevented the decrease in aortic reflection time.

CONCLUSIONS

Inhaled salbutamol predominantly changed supine haemodynamics, whereas the moderate effects of L-arginine were observed during the head-up tilt. In contrast, small doses of nitroglycerin induced major changes in haemodynamics both supine and during the head-up tilt. Altogether, these results emphasize the importance of haemodynamic measurements in both the supine and upright positions.

Introduction

The endothelium controls vascular tone via various mechanisms including the release of nitric oxide (NO), prostacyclin, and several pathways causing vascular smooth muscle cell hyperpolarization [1]. In addition, the endothelium actively regulates vascular permeability, platelet and leucocyte adhesion and aggregation, and thrombosis [2, 3]. Impairments in these mechanisms maintaining vascular homeostasis lead to functional manifestations, which has emphasized the importance of studying endothelial function *in vivo*. The present treatment strategies of hypertension with lower treatment goals than ever, especially in high-risk patients with complicated diabetes or kidney disease, stress the importance of the measurement of blood pressure (BP), haemodynamics and endothelial function also in the upright position [4, 5]

Peripheral endothelial function can be measured invasively by venous occlusion plethysmography, and noninvasively by measuring arterial diameter by ultrasound systems after ischaemia or warming-induced increase in blood flow [flow-mediated dilatation (FMD)] [6, 7]. Repeatability of the plethysmographic measurements is good, but as an invasive technique it is not convenient for multiple measurements. FMD requires good technical skills, and the lack of universal standards in the measurement protocol makes comparison between different laboratories difficult [8]. The responsiveness of coronary arteries to different endothelium-dependent stimuli has been evaluated by observing changes in artery diameter [9] or coronary blood flow [10, 11]. However, these methods are invasive and laborious, and demand high technical expertise.

Pulse wave analysis (PWA) is a non-invasive, repeatable technique that analyses the arterial pulse wave form providing information about arterial compliance [12]. Recently, the changes in PWA-derived measure of wave reflection and arterial stiffness, the augmentation index (AIx), after β_2-adrenoceptor agonist-induced endothelial stimulation have been used to assess the role of endothelium in vascular responsiveness [13, 14]. Another possibility to stimulate the endothelium is the administration of the semi-essential amino acid L-arginine, which serves as a precursor for endogenous NO synthesis. L-arginine administration has been shown to improve endothelium-dependent vasodilation in animals [15, 16] and humans [17, 18]. However, the effect of endothelial stimulation on systemic vascular resistance or cardiac function has seldom been studied [19, 20].

The purpose of this study was to evaluate the haemodynamic effects of inhaled salbutamol and infused L-arginine in comparison with the endothelium-independent vasodilator nitroglycerin. For a comprehensive analysis we applied continuous PWA combined with the measurement of cardiac output and systemic vascular resistance using whole-body impedance cardiography both in the supine position and during passive orthostatic challenge. The results suggest that inhaled salbutamol induces changes in supine haemodynamics, the moderate effects of L-arginine are observed during head-up tilt, whereas already a small dose nitroglycerin influences haemodynamics in both the supine and upright positions.

Methods

Study subjects

The study population consisted of nine healthy (five male, four female), normotensive individuals aged 25–44 years. All participants gave written informed consent and thereafter underwent a physical examination performed by a physician. During the interview, lifestyle habits, family history of cardiovascular disease, and medical history were documented. The study was approved by the Ethics Committee of Tampere University Hospital and the National Agency of Medicines, Finland, and it complies with the Declaration of Helsinki.

Laboratory analyses

Blood and urine samples were obtained after a minimum of 12 h fast in the morning for laboratory analyses, and a standard 12-lead electrocardiogram was recorded. Plasma sodium, potassium, calcium, glucose, creatinine, triglyceride, and total, high-density and low-density lipoprotein cholesterol concentrations were determined by Cobas Integra 700/800 (F. Hoffmann-LaRoche Ltd, Basel, Switzerland), and blood cell count by ADVIA 120 or 2120 (Bayer Health Care, Tarrytown, NY, USA). Creatinine clearance was estimated using the Cockroft–Gault formula [21].

Haemodynamic measurement protocol

Haemodynamic measurements were performed in a quiet, temperature-controlled research laboratory by a trained nurse on five separate days. 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 tilt-table, and the electrodes for impedance cardiography were placed on the body surface, the tonometric sensor for PWA on the radial pulsation to the left wrist, and oscillometric brachial cuff for BP calibration to the right upper arm. Before the actual measurement, an introductory head-up tilt was performed to familiarize the study subject with the method.

The actual measurement consisted of six consecutive 5-min intervals, during which haemodynamic data were captured continuously. For the first 5 min, subjects were resting supine on the tilt table, followed by 5 min of head-up tilt to 60°, then the tilt table was returned to the horizontal position for another 5 min. During this period no research drugs were given, but saline was infused before the head-up tilt with L-arginine. After that the

research drug (L-arginine, salbutamol, placebo inhalation, nitroglycerin or placebo resoriblet) was administered, the same protocol was repeated (5 min supine–5 min head-up tilt–5 min supine). The results of the haemodynamic measurements during the first 15 min on the five separate recording days did not differ either in the supine position or during the orthostatic challenge before the test drug administration (data not shown).

Research drugs

Administration of L-arginine, salbutamol, placebo inhalation, nitroglycerin and placebo resoriblet was performed on five separate measurements on different days. Placebo inhalation and 400 µg salbutamol inhalation (placebo for Ventolin® and Ventolin®, respectively; GlaxoSmithKline, Uxbridge, UK) were given with a spacer device (Volumatic; Allen & Hanbury's, Uxbridge, UK) in a blinded fashion. The 400-µg salbutamol dose was chosen on the basis of test experiments with the present study protocol and previously published work on the effects of inhaled salbutamol on haemodynamics [13, 14, 22].

Sublingual placebo resoriblet and 0.25 mg nitroglycerin (Nitro resoriblet; Orion Pharma, Espoo, Finland) were also administrated in a blinded fashion. The placebo resoriblets were professionally manufactured by the University Pharmacy, Helsinki, Finland, to resemble very closely the commercial nitroglycerin resoriblets in both appearance and taste. The nitroglycerin dose was chosen on the basis of test experiments with the present study protocol, and a previous report using the same dose [13].

In the fifth measurement, an intravenous (i.v.) 20-G cannula was placed in a brachial vein and a slow saline infusion was started. L-arginine hydrochloride 20 mg ml^{-1} (B. Braun Melsungen Ag, Melsungen, Germany) was diluted in 100 ml of saline, and infusion at the dose 10 mg kg^{-1} min^{-1} was started at 15 min and continued for 10 min. This administration protocol resulted in a relatively high dose of infused L-arginine in 10 min. This dose can be considered sufficient to induce cardiovascular changes, as i.v. L-arginine infusion at the rate of (i) 3.33 mg kg^{-1} min^{-1} has induced vasodilation in the renal vasculature in humans [23], (ii) 0.3 g min^{-1} has enhanced the acute haemodynamic effects of 50 mg of losartan [24], and (iii) 0.5 g min^{-1} has increased skeletal muscle glucose clearance in humans, probably via increased NO production [25]. On the basis of previous work, L-arginine has rarely caused side-effects at infusion rates that do not exceed 1 g min^{-1} [26], and the dose of 1 g min^{-1} of L-arginine has been used in many studies [27–29].

Pulse wave analysis

Radial BP and pulse wave form were continuously determined from the radial pulsation by a tonometric sensor (Colin BP-508T; Colin Medical Instruments Corp., San Antonio, TX, USA), which was fixed on the radial pulse with a wrist band. The radial BP signal was calibrated every 2.5 min by a brachial BP measurement. Continuous aortic BP was derived with the SphygmoCor pulse wave monitoring system (SpygmoCor PWMx; AtCor Medical, West Ryde, Australia) using the previously validated generalized transfer function [12]. Ejection duration, aortic reflection time and AIx (augmented pressure/pulse pressure × 100) were determined.

Whole-body impedance cardiography

A whole-body impedance cardiography device (CircMon^R; JR Medical Ltd., Tallinn, Estonia), which records the continuous changes in body electrical impedance during a cardiac cycle, and plethysmographic BP recordings from fingers (Finapres; Ohmeda, Englewood, CO, USA) were used to determine beat-to-beat heart rate, stroke volume (ml), cardiac output (l min^{-1}), systemic vascular resistance index (SVRI, systemic vascular resistance/body surface area, dyn × s cm^{-5} × m^2) and pulse wave velocity (PWV) [30–32]. The cardiac output values measured with CircMon^R whole-body impedance cardiography are in good agreement with the values measured by the thermodilution method, both in the supine position and during head-up tilt [32]. A detailed description of the method and electrode configuration has been previously reported [30–32]. PWV was not assessed during the head-up tilt due to less accurate timing of left ventricular ejection during reduced stroke volume.

Exhaled alveolar nitric oxide measurement after salbutamol inhalation

To verify the salbutamol-induced production of NO, we measured NO concentration from the alveolar air in six of the study subjects using a Sievers NOA 280 analyser (Sievers Instruments, Boulder, CO, USA) at three exhalation flow rates (100, 200 and 300 ml s^{-1}) [33, 34]. The measurements were performed three times before (0, 10 and 20 min) and twice after (10 and 20 min) the placebo and salbutamol 400-µg inhalations, and alveolar NO concentration was calculated as previously described [33, 34]. Briefly, exhaled NO output (concentration × flow rate) was plotted against exhalation flow rate and a linear regression was set. The slope and intercept of the regression line are approximates of alveolar NO concentration and bronchial NO flux, respectively. The exhalation flow rates were computer-controlled using an adjustable flow restrictor, and the subjects maintained the exhalation pressure between 5 and 20 cmH$_2$O, as described previously [34, 35].

Statistical analysis

Values are expressed as mean ± standard error of the mean (SEM) and the data were analysed using SPSS 11.5 for Windows (SPSS Inc., Chicago, IL, USA). To compare haemodynamic measurements, one-way analysis of variance (ANOVA) and ANOVA for repeated measurements were applied (RANOVA). P-values < 0.05 were considered statistically significant. The study power was analysed using the

PS 3.0.2 power and sample size calculations program (Vanderbilt Biostatistics, Nashville, TN, USA). A change in SVRI > 400 dyn × s cm^{-5} × m^2 in the supine position was chosen as the main outcome variable (such a change was observed after both salbutamol and nitroglycerin). When using the variables of the salbutamol measurements in the power calculations, the present number of subjects ($n = 9$) was found to have a power of 92% to detect a significant difference ($\delta = 400$ dyn × s cm^{-5} × m^2) between the salbutamol and the placebo effect (α-level 0.05).

Results

Study population
The basic characteristics and laboratory values of the study population were all within the normal range. The mean age was 32 ± 2.5 years, body mass index 24.5 ± 1.0 kg m^{-2}, and waist circumference 80 ± 5 cm in women and 91 ± 3 cm in men. None of the study subjects had a medical history of cardiovascular disease or elevated BP, and none of the subjects was a present smoker, while two subjects had a previous smoking history. Plasma lipid profile, fasting glucose, electrolytes, and kidney function were all within the normal range. None of the study subjects reported any adverse effects related to the research drug administration.

Haemodynamic effects of L-arginine, salbutamol and nitroglycerin in supine position
The average values of the first 5 min of the haemodynamic recordings following drug administration in the supine position are shown in Tables 1–3 for L-arginine, salbutamol and nitroglycerin, respectively. The average results of the second and fifth minute during each recording phase (5 min supine–5 min tilt–5 min supine) are depicted in Figures 1 and 2.

L-arginine infusion did not have any significant haemo-dynamic effects in the supine position before the head-up tilt (Table 1, Figures 1 and 2). In contrast, before the head-up tilt salbutamol induced a 9.2 ± 2.6% decrease in SVRI (Table 2) and during the fifth minute an 8.6 ± 2.5% increase in heart rate (Figure 2) ($P < 0.05$ for both compared with placebo inhalation). During the fifth minute following nitroglycerin administration, supine cardiac output was increased (+9.2 ± 2.7%) and SVRI was reduced (−6.7 ± 1.8%) ($P < 0.05$ for both compared with placebo resoriblet) (Figure 1).

During the second minute in the supine position after the head-up tilt aortic mean BP was lower during L-arginine infusion than during saline infusion, but L-arginine infusion did not have any other effects on BP values or supine haemodynamics (Table 1, Figure 1). In the supine position after the head-up tilt AIx was significantly lower after salbutamol (AIx value 5.5 ± 2.5%) and nitroglycerin (AIx −1.4 ± 2.5%) when compared with placebo inhalation (AIx 14.1 ± 3.2%), placebo resoriblet (AIx 12.1 ± 3.7%) and with L-arginine infusion (AIx 11.4 ± 2.6%) ($P < 0.05$ for all comparisons) (Figure 2). SVRI in the supine position after the second head-up tilt was reduced after salbutamol when compared with placebo inhalation (5-min averages 2105 ± 89 and 2546 ± 73 dyn × s cm^{-5} × m^2, respectively, $P = 0.026$) (Figure 1).

Supine PWV values were not significantly different after L-arginine infusion, salbutamol inhalation or nitroglycerin administration when compared with the respective controls (Tables 1–3). However, nitroglycerin resoriblet induced a small but significant reduction in PWV (−5.7 ± 2.4%) when compared with placebo resoriblet (0.9 ± 1.7%) ($P < 0.05$).

Table 1

Average haemodynamic effects of L-arginine (10 mg kg^{-1} min^{-1}) and saline infusion during the first 5 min in the supine position and 5 min of head-up tilt (mean ± SEM)

	Saline infusion		L-arginine infusion	
Tonometry	Supine	Head-up tilt	Supine	Head-up tilt
Radial SBP (mmHg)	131 ± 3	127 ± 2	127 ± 3	121 ± 2*
Aortic SBP (mmHg)	116 ± 3	111 ± 2†	110 ± 3	105 ± 2*
Radial DBP (mmHg)	76 ± 2	79 ± 1	73 ± 3	74 ± 2*
Aortic DBP (mmHg)	77 ± 2	80 ± 2†	74 ± 3	74 ± 2*
Augmentation index (%)	11.8 ± 3.3	4.2 ± 2.8†	9.5 ± 3.2	3.2 ± 2.6†
Aortic reflection time (ms)	171 ± 6	162 ± 4†	169 ± 8	170 ± 4*
Ejection duration (ms)	342 ± 4	277 ± 8†	347 ± 5	290 ± 9†
Impedance cardiography				
Stroke volume (ml)	100 ± 9	68 ± 5†	98 ± 8	71 ± 5†
Heart rate (beats min^{-1})	58 ± 3	68 ± 3†	55 ± 2	65 ± 2†
Cardiac output (l min^{-1})	5.71 ± 0.44	4.58 ± 0.27†	5.30 ± 0.39	4.55 ± 0.24†
Systemic vascular resistance index (dyn × s cm^{-5} × m^2)	2385 ± 191	3052 ± 174†	2521 ± 162	2920 ± 166†
Pulse wave velocity (m s^{-1})	8.66 ± 0.17	–	8.63 ± 0.16	–

*$P < 0.05$ compared with corresponding value during saline infusion. †$P < 0.05$ compared with corresponding supine values. SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 2

Average haemodynamic effects of 400 µg inhaled salbutamol during the first 5 min in the supine position and 5 min of head-up tilt (mean ± SEM)

	Placebo inhalation		Salbutamol inhalation	
Tonometry	Supine	Head-up tilt	Supine	Head-up tilt
Radial SBP (mmHg)	126 ± 3	129 ± 2	123 ± 2	124 ± 2
Aortic SBP (mmHg)	111 ± 3	112 ± 2	105 ± 2	106 ± 2*
Radial DBP (mmHg)	73 ± 2	80 ± 2†	70±2	75 ± 2†
Aortic DBP (mmHg)	74 ± 2	82 ± 2†	70 ± 2	75 ± 2†
Augmentation index (%)	12.7 ± 3.7	−0.4 ± 4.4†	6.0 ± 2.7	−1.9 ± 2.5†
Aortic reflection time (ms)	172 ± 10	158 ± 5	168 ± 8	158 ± 4†
Ejection duration (ms)	342 ± 7	270 ± 7†	343 ± 5	272 ± 9†
Impedance cardiography				
Stroke volume (ml)	91 ± 6	66 ± 5†	90 ± 5	71 ± 4†
Heart rate (beats min^{-1})	54 ± 2	71 ± 3†	61 ± 2*	71 ± 2†
Cardiac output (l min^{-1})	4.78 ± 0.18	4.65 ± 0.38	5.44 ± 0.26	5.08 ± 0.34
Systemic vascular resistance index (dyn × s cm^{-5} × m^2)	2449 ± 61	2769 ± 176	2148 ± 96*	2448 ± 139†
Pulse wave velocity (m s^{-1})	8.40 ± 0.29	–	8.54 ± 0.18	–

*$P < 0.05$ compared with corresponding value during placebo inhalation. †$P < 0.05$ compared with corresponding supine values. SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 3

Average haemodynamic effects of 0.25 mg nitroglycerin resoriblet during the first 5 min in the supine position and 5 min of head-up tilt (mean ± SEM)

	Placebo resoriblet		Nitroglycerin resoriblet	
Tonometry	Supine	Head-up tilt	Supine	Head-up tilt
Radial SBP (mmHg)	122 ± 3	121 ± 2	124 ± 1	116 ± 3†
Aortic SBP (mmHg)	106 ± 3	105 ± 2	107 ± 2	99 ± 2†
Radial DBP (mmHg)	68 ± 3	75 ± 2†	70 ± 2	69 ± 3
Aortic DBP (mmHg)	69 ± 3	76 ± 2†	71 ± 2	71 ± 2
Augmentation index (%)	8.1 ± 3.9	−0.9 ± 5.3†	3.0 ± 3.5	−15.8 ± 1.6*†
Aortic reflection time (ms)	178 ± 10	156 ± 6	173 ± 7	175 ± 7*
Ejection duration (ms)	344 ± 5	269 ± 9†	330 ± 6*	247 ± 10†
Impedance cardiography				
Stroke volume (ml)	90 ± 6	66 ± 5†	94 ± 7	76 ± 6†
Heart rate (beats min^{-1})	55 ± 2	70 ± 3†	62 ± 3*	85 ± 3*†
Cardiac output (l min^{-1})	4.89 ± 0.26	4.64 ± 0.47	5.74±0.36*	6.34 ± 0.48*
Systemic vascular resistance index (dyn × s cm^{-5} × m^2)	2438 ± 148	2663 ± 155	2089 ± 119	1955 ± 189*
Pulse wave velocity (m s^{-1})	8.47 ± 0.12	–	7.89 ± 0.28	–

*$P < 0.05$ compared with corresponding value during placebo resoriblet. †$P < 0.05$ compared with corresponding supine values. SBP, systolic blood pressure; DBP, diastolic blood pressure.

Haemodynamic effects of L-arginine, salbutamol and nitroglycerin during the head-up tilt

The average values of the haemodynamic recordings during the 5-min head-up are shown in Tables 1–3, and the average results of the second and fifth minute in Figures 1 and 2.

During the head-up tilt aortic and radial BP were reduced with L-arginine when compared with saline infusion (Table 1). Aortic mean BP was also lower during L-arginine infusion (85 ± 2 mmHg) than during saline infusion (90 ± 1 mmHg, $P = 0.02$). During the head-up, aortic reflection time was numerically but not statistically significantly longer during L-arginine than saline infusion ($P = 0.162$, Table 1, Figure 2), and the respective changes in

aortic reflection time were not significantly different either (8.0 ± 8.8% *vs.* −6.8 ± 2.4%, respectively, $P = 0.147$). However, in additional comparisons aortic reflection time was longer during the head-up tilt with L-arginine infusion (170 ± 4 ms) than during placebo inhalation (153 ± 4 ms, $P = 0.02$), placebo resoriblet (155 ± 6 ms, $P = 0.045$) and salbutamol inhalation (158 ± 4 ms, $P = 0.047$, Table 3). These results suggest that the reduction in aortic reflection time during head-up tilt was prevented during L-arginine infusion.

During the head-up tilt aortic systolic BP after salbutamol inhalation was lower than after placebo inhalation (Table 2, $P = 0.024$), while there was no significant difference in radial systolic, aortic and radial diastolic, or mean BP (Table 2). Although SVRI was numerically lower during
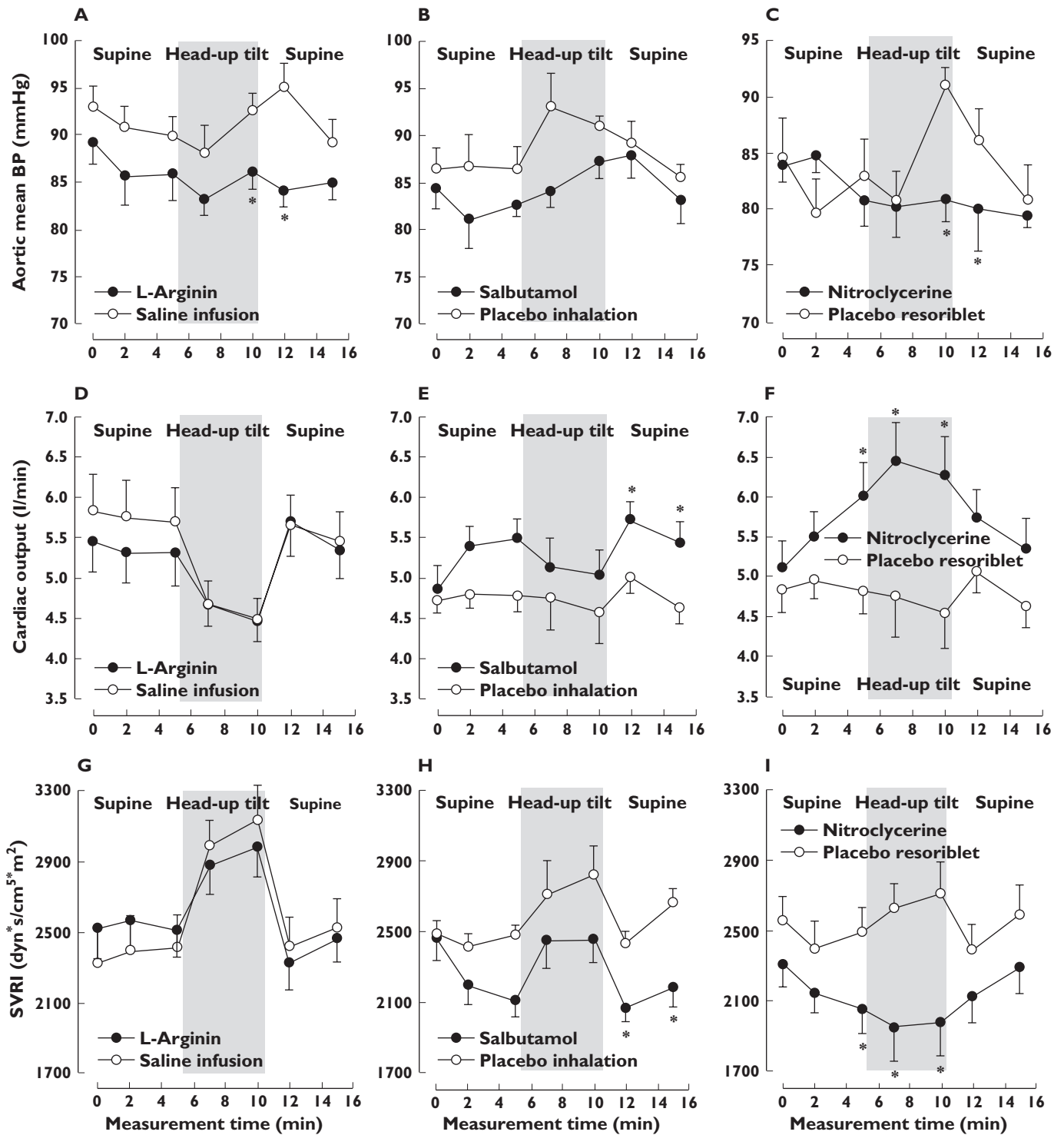
Figure 1

Mean values for aortic mean arterial pressure (a–c), cardiac output (d–f) and systemic vascular resistance index (SVRI) (g–i) after L-arginine infusion (10 mg kg^{-1} min^{-1}), saline infusion, salbutamol inhalation (400 µg), placebo inhalation, nitroglycerin resoriblet (0.25 mg) and placebo resoriblet. Research drug was administered at measurement time 0 and head-up tilt was performed from 5 to 10 min. *$P < 0.05$ vs. saline/placebo, ANOVA

Figure 2

Mean values for heart rate (a–c), augmentation index (d–f) and aortic reflection time (g–i) after L-arginine infusion (10 mg kg^{-1} min^{-1}), saline infusion, salbutamol inhalation (400 µg), placebo inhalation, nitroglycerin resoriblet (0.25 mg) and placebo resoriblet. Research drug was administrated at measurement time 0 and head-up tilt was performed from 5 to 10 min. *$P < 0.05$ vs. saline/placebo, ANOVA
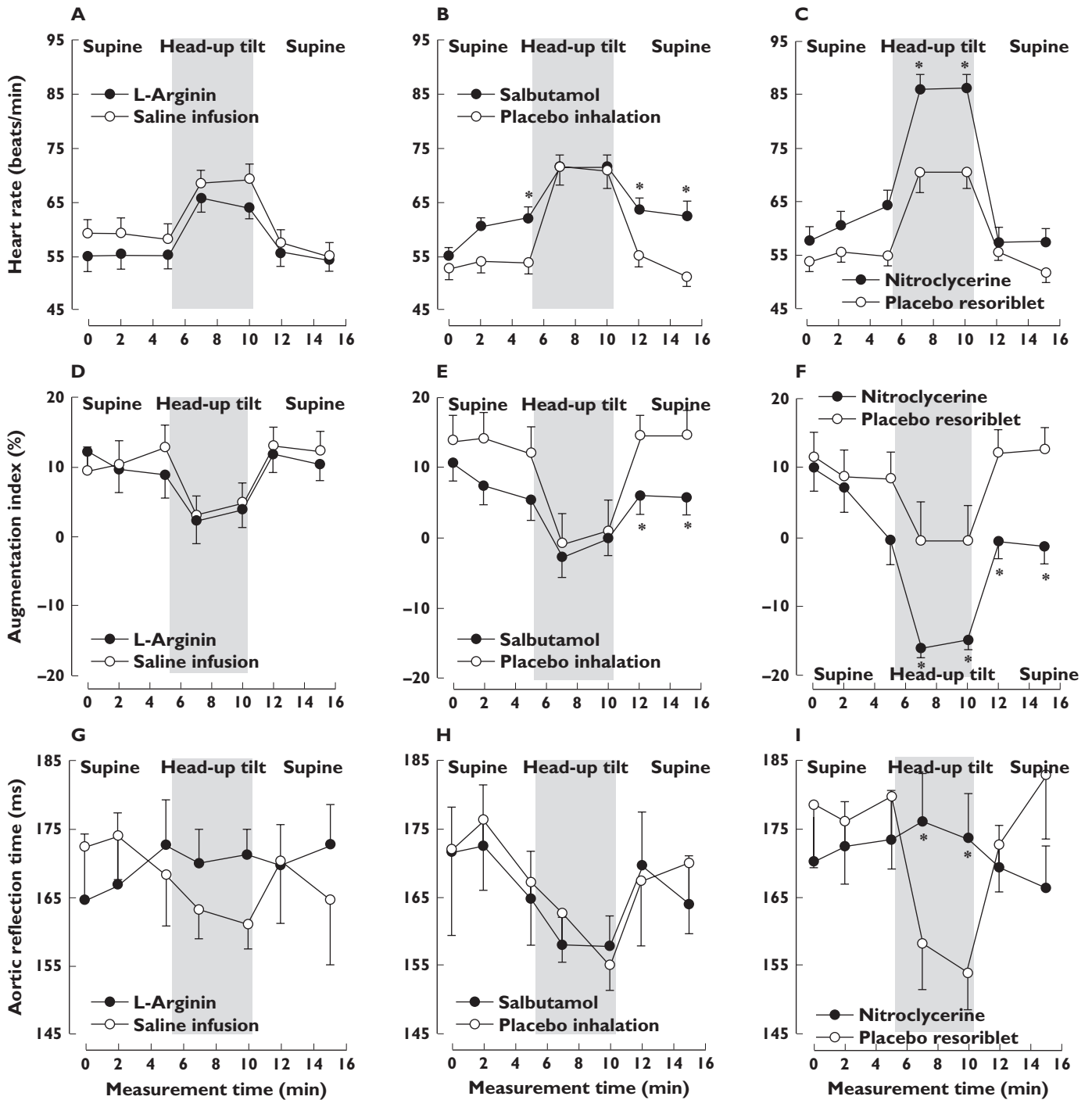
the head-up tilt with salbutamol than with placebo inhalation, the difference was not significant ($P = 0.196$) (Table 2, Figure 1).

During the head-up tilt, aortic and radial diastolic and systolic BP were not reduced with nitroglycerin when com-

pared with placebo resoriblet (Table 3), but during the fifth minute of the head-up tilt aortic mean BP was lower with nitroglycerin than with placebo resoriblet (80 ± 2 vs. 91 ± 2 mmHg, $P = 0.002$, Figure 1). However, during the head-up tilt the other effects of 0.25 mg sublingual nitroglycerin on

haemodynamics were very clear (Table 3, Figures 1 and 2): cardiac output was higher in comparison with placebo resoriblet, and also higher than during L-arginine infusion and placebo inhalation ($P < 0.05$ for all), SVRI was lower when compared with placebo resoriblet, placebo inhalation and L-arginine infusion ($P < 0.05$), heart rate was higher and AIx was lower in comparison with all other measurements ($P < 0.05$), and aortic reflection time was longer than with placebo resoriblet ($P = 0.023$).

Exhaled nitric oxide concentrations after salbutamol

Alveolar NO concentration in six of the study subjects was 1.55 ± 0.17 parts per billion before salbutamol inhalation, while the concentration was 1.81 ± 0.19 parts per billion before placebo inhalation. After salbutamol inhalation alveolar NO concentration increased by 19%, whereas after placebo inhalation the concentration decreased by 10% ($P = 0.01$).

Discussion

Here we evaluated the use of two pharmacological compounds acting on the endothelium and the endothelium-independent agent nitroglycerin on non-invasive haemodynamics in healthy volunteers. The study has shown that inhaled salbutamol decreased systemic vascular resistance and AIx and increased heart rate and cardiac output, whereas L-arginine only resulted in a decrease of BP during the head-up tilt. In contrast, nitroglycerin markedly decreased systemic vascular resistance, AIx and BP, and increased heart rate, cardiac output and aortic reflection time.

Although the observed haemodynamic changes with L-arginine and salbutamol were moderate, the combination of PWA and impedance cardiography provided data that would have remained uncovered when using only one of these methods. The present non-invasive measurement protocol provides continuous haemodynamic information about central wave reflection and BP, arterial compliance, systemic vascular resistance and cardiac function in both the supine and upright positions. Thus, beat-to-beat changes in haemodynamics can be revealed, which provides benefits when compared with single tonometric measurements using a pen-like sensor, the approach of which has been applied in the majority of recent PWA studies [14, 36]. The present approach also enables more thorough assessment of vascular responsiveness than methods like FMD in the upper arm arteries, which examine only a section of the cardiovascular system. It is important to notice that the effects of endothelial stimulation on arterial tone may depend on the vascular bed studied [37, 38].

The semi-essential amino acid L-arginine serves as a precursor for endothelial nitric oxide synthase (NOS).

L-arginine administration has been reported to induce vasodilation in healthy subjects and patients with cardiovascular disease [17–19]. L-arginine has rarely caused side-effects at doses <30 g during a 30-min infusion (i.e. at a maximum rate of $1 \, g \, min^{-1}$) [26], which favours its use in clinical research. However, the beneficial effects of L-arginine do not seem to be due to extracellular substrate supply for endothelial NOS, as the enzyme should be saturated with physiological L-arginine levels [39]. In contrast, excess substrate availability can overcome the inhibition of endothelial NOS activity due to accumulation of false L-arginine derivatives during cardiovascular disease states, the mechanism of which can explain the vasodilatory effects of L-arginine. Furthermore, L-arginine has been reported to improve vasodilation in healthy humans with low concentrations of endothelial NOS-inhibitory substances [40, 41], and in this case other endocrine mechanisms, i.e. stimulation of growth hormone and insulin secretion, may play a role [42, 43].

In the present study the haemodynamic effects of L-arginine infusion were modest. The BP-lowering influence was seen only during the head-up tilt, during which the normal decrease in aortic reflection time was abolished by L-arginine. As the compliance of the arteries increases, the aortic reflection time lengthens and the reflected wave shifts towards diastole, resulting in decreased systolic BP. Since L-arginine did not decrease systemic vascular resistance, the BP effect probably resulted from increased compliance in large arteries. In spite of the reduced BP and prolonged aortic reflection time induced by L-arginine, AIx decreased correspondingly during the head-up tilt in response to L-arginine, saline infusion, salbutamol and placebo. The reduction in AIx in the upright position cannot be attributed to changes in large arterial compliance, but may result from a more pronounced decrease in the augmentation pressure than pulse pressure. Thus, AIx is not always a reliable indicator of arterial compliance but merely an indicator of central wave reflection.

Although β_2-adrenoceptors mediate vasorelaxation at the level of vascular smooth muscle, the stimulation of β_2-adrenoceptors is known to increase endothelial release of NO and cause largely endothelium-mediated vascular relaxation [44, 45]. Therefore, the effect of the β_2-adrenoceptor agonist salbutamol on the PWA-derived measure of wave reflection and arterial stiffness, the AIx, has been applied as a method to evaluate the influence of endothelial stimulation in the whole arterial tree [13, 46]. Inhaled salbutamol has induced an 8–12% decrease in the AIx in healthy subjects, but in many studies the effects on other haemodynamic variables have not been determined [13, 14, 46]. As a standard methodological approach, the AIx has been derived from 10 consecutive heart beats every 5 min for up to 20 min, which provides a relatively narrow window of observation when compared with continuous PWA recording. In the present study, the inhalation

of 400 µg salbutamol reduced systemic vascular resistance and BP and increased heart rate already before the decrease in AIx was observed. These results favour the use of continuous recording of haemodynamics in the assessment of vascular responsiveness, and indicate that additional methods besides PWA increase the reliability of the analysis.

Administration of sublingual nitroglycerin was included in the study as an endothelium-independent vasodilator, and even a small 0.25-mg dose of nitroglycerin resulted in major haemodynamic changes in comparison with salbutamol and L-arginine. Traditionally, venodilation and venous pooling of blood into the lower extremities and splanchnic vasculature, and subsequently reduced left ventricular preload, have been regarded as major haemodynamic effects of nitroglycerin in humans, whereas arterial dilation is thought to occur to a lesser extent [47, 48]. However, in the present study nitroglycerin induced a clear decrease in systemic vascular resistance and an increase in cardiac output and aortic reflection time, strongly suggesting reduced arterial resistance.

In the present study, alveolar NO concentration was increased by 19% after salbutamol inhalation. Alveolar NO concentration could be increased via three mechanisms: increased NO production in the alveolar epithelium, decreased NO diffusion from alveolar air to capillaries, or increased NO release from the capillary walls. Previously, increased alveolar NO concentration has been found in diseases affecting pulmonary parenchyma, probably due to increased inducible NOS expression in the alveolar epithelium [34, 49]. Increased alveolar NO concentration has also been reported in subjects with liver cirrhosis [50], presumably as a result of increased endothelial NO production [51]. In addition, the administration of the ACE-inhibitor enalapril has increased NO level in exhaled air, which was thought to reflect enhanced NO release from the capillary endothelium [52]. Since inhaled salbutamol is not likely to influence NO production in the alveolar epithelium or change NO permeability of the alveolar wall, the present findings suggest that salbutamol inhalation increased NO release from the capillary endothelium. This supports the view that salbutamol stimulates the endothelial cells in the arterial tree.

The purpose of the present study was also to evaluate the use of three pharmacological tools in the haemodynamic screening of humans, and not directly to compare the effects of L-arginine, salbutamol and nitroglycerin. Therefore, a double-blind placebo infusion was not included in the study protocol. The crucial role of the endothelium in different cardiovascular disorders, and as a target of pharmacological interventions, underlies the importance of assessing endothelial function *in vivo*. The more pronounced cardiovascular effects of salbutamol than L-arginine, together with low-cost and easy administration via inhalation, imply that salbutamol provides a clinically more applicable tool for the assessment of the

role of endothelium in haemodynamic responsiveness. Finally, the present study underscores the need for comprehensive methods in the measurement of haemodynamics in humans.

Competing interests

None to declare.

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Reduced systemic vascular resistance in healthy volunteers with presyncopal symptoms during a nitrate-stimulated tilt-table test

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

• Nitrates may facilitate syncope through various pathways, but the precise mechanism of nitrate-induced syncope is still under debate. The purpose of the present study was to compare the underlying haemodynamic mechanisms in subjects without and with presyncopal symptoms during a nitroglycerin-stimulated tilt-table test.

WHAT THIS STUDY ADDS

• A major decrease in systemic vascular resistance was documented in subjects with presyncope during 0.25 mg nitroglycerin-stimulated tilt-table test, in the absence of changes in cardiac output. These findings indicated that even a small dose of nitroglycerin significantly decreased arterial resistance and cardiac afterload.

AIMS

The mechanism of nitrate-induced syncope remains controversial. We examined the haemodynamic changes in healthy volunteers during nitroglycerin-stimulated tilt-table test.

METHODS

Continuous radial pulse wave analysis, whole-body impedance cardiography and plethysmographic finger blood pressure were recorded in a supine position and during head-up tilt in 21 subjects with presyncopal symptoms (6 male/15 female, age 43 ± 3 years) after 0.25 mg sublingual nitroglycerin and 21 control subjects (6 male/15 female, age 43 ± 2 years). The drug was administered in the supine position and a passive head-up tilt followed 5 min later. Additionally, nitroglycerin was only administered during head-up tilt in 19 subjects and the haemodynamics were recorded.

RESULTS

Supine and upright haemodynamics were similar before nitroglycerin administration in the two groups. During the nitroglycerin-stimulated tilt test, aortic and radial mean blood pressure decreased significantly more in the presyncope group when compared with the controls ($P = 0.0006$ and $P = 0.0004$, respectively). The decreases in systemic vascular resistance ($P = 0.0008$) and heart rate ($P = 0.002$), and increase in aortic reflection time ($P = 0.0002$) were greater in the presyncope group, while the change in cardiac index was not different between the groups ($P = 0.14$). If nitroglycerin was administered during the upright tilt and not in supine position, the haemodynamic changes were quite corresponding.

CONCLUSIONS

Presyncopal symptoms during nitrate-stimulated tilt test were explained by decreased systemic vascular resistance and increased aortic reflection time, while cardiac output remained unchanged. These findings indicated reduced arterial resistance in nitroglycerin-induced presyncope.

Introduction

Nitroglycerin (NTG) challenge during head-up tilt-table testing is often utilized to determine the aetiology of unexplained vasovagal syncope [1, 2]. Nitrates may facilitate syncope through various pathways, but the precise mechanism of nitrate-induced syncope is still under debate [3]. Traditionally, venodilatation and venous pooling of blood into the lower extremities and splanchnic vasculature, and subsequently reduced left ventricular preload, have been regarded as major haemodynamic effects of nitroglycerin in humans, while arterial dilatation is thought to occur to a lesser extend [4, 5]. However, a reduction in wave reflection and central rather than peripheral blood pressure (BP) has been documented after sublingual NTG in patients undergoing cardiac catheterization [6]. This implies that small doses of NTG dilate arteries resulting in reduced left ventricular afterload.

Conflicting results concerning nitrate-induced changes in haemodynamics during tilt-table testing have been reported. In some studies, nitrate-induced presyncope has been considered to be cardiac output-mediated without a decrease in systemic vascular resistance [7, 8]. However, another report demonstrated a decrease in systemic vascular resistance without a marked change in heart rate (HR) or cardiac filling during NTG-induced presyncope [9]. The latter response would be classified as 'vasodepressive' according to the Vasovagal Syncope International Study, i.e. a positive tilt test is characterized by preserved cardiac function during presyncope [10]. Besides direct haemodynamic alterations, changes in autonomic tone, neurohormonal substances and central nervous system function may be involved in nitrate-induced syncope [3]. Animal models also support the view of a direct central sympatho-inhibitory effect of NTG [11].

The purpose of the present study was to compare the underlying haemodynamic mechanisms in subjects with and without presyncopal symptoms during NTG-stimulated tilt-table test using a protocol during which NTG was administered in the supine position and a passive head-up tilt followed 5 min later. To examine whether body position affects the NTG response, some of the subjects were invited for additional measurements during which NTG was only administered during the head-up tilt. The methods were continuous non-invasive pulse wave analysis combined with the measurement of cardiac output and systemic vascular resistance by whole-body impedance cardiography and continuous blood pressure (BP) recordings from a finger.

Methods

Study subjects

The study subjects participated as normal controls in an ongoing haemodynamic measurement study (DYNAMIC–

study), in which non-invasive haemodynamics of hypertensive and normotensive subjects were recorded in the supine position and during repeated head-up tilt in the absence and presence of 0.25 mg sublingual NTG. Thus far 211 subjects have been included in the study. The presyncope group consisted of all normotensive subjects ($n = 21$) who developed presyncopal symptoms and a progressive fall of BP during the NTG-stimulated tilt-table test. From the remaining normotensive subjects, 21 healthy, gender, age (± 3 years) and body mass index (BMI ± 4 kg m^{-2}) -matched subjects were included in the control group, and the absence of subjective presyncopal symptoms was the only haemodynamic information that was used for the selection. After the first measurements, 19 subjects (randomly selected, $n = 10$ from the presyncope group, $n = 9$ from the control group) were invited for additional measurements during which NTG was only administered in the upright position. The additional measurements were performed to test the hypothesis with an alternative protocol, and no matching of case-control pairs was performed. All of the subjects underwent a physical examination performed by a physician, and lifestyle habits, family history of cardiovascular disease and medical history were documented. None of the subjects had spontaneously reported unexplained syncope in their medical history. All subjects gave written informed consent. The study complied with the declaration of Helsinki and was approved by the ethics committee of Tampere University Hospital.

Laboratory analyses

After fasting for a minimum of 12 h blood and urine samples were obtained in the morning for laboratory analyses, and a standard 12-lead electrocardiogram was recorded. Plasma sodium, potassium, calcium, glucose, creatinine, triglyceride, and total, high-density and low-density lipoprotein cholesterol concentrations were determined by Cobas Integra 700/800 (F. Hoffmann-LaRoche Ltd, Basel, Switzerland), and blood cell count by ADVIA 120 or 2120 (Bayer Health Care, Tarrytown, NY, USA). Creatinine clearance was estimated using the Cockroft-Gault formula [12].

Heamodynamic measurement protocol

Haemodymamic 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 electrodes for impedance cardiography were placed on the body surface, a tonometric sensor for pulse wave analysis on the radial pulsation to the left wrist, an oscillometric brachial cuff for BP calibration to the right upper arm and a plethysmographic cuff for finger BP measurement to the right middle finger.

The measurement consisted of six consecutive 5 min intervals, and haemodynamic data were captured continu-

ously. At first, the subjects were resting supine on the tilt table (5 min) followed by head-up tilt to 60 degrees (5 min), after which the tilt table was returned to the horizontal position (5 min). Sublingual NTG 0.25 mg (Nitro resoriblet, Orion Pharma, Espoo, Finland) was administered, and the same protocol was repeated (5 min supine – 5 min head-up tilt – 5 min supine). If the subject reported presyncopal symptoms during the 5 min head-up tilt after NTG (dizziness, light-headedness, sweating, nausea), and the research nurse observed progressively falling BP, the tilt table was returned to the horizontal position before the intended 5 min of head-up tilt were fulfilled. The total measurement time in the control group was 30 min, and in the presyncope group 27–29 min. Due to the shorter measurement duration in the presyncope group, only the same amount of data were analyzed from the matched control subject so that the time scale would be comparable. The repeatability and reproducibility of the measurement protocol has been previously demonstrated [13].

To examine the NTG response during an alternative protocol, 19 study subjects were invited to additional measurements. In this protocol the subjects were resting supine on the tilt table (5 min) followed by a 15 min head-up tilt to 60 degrees, after which the tilt table was returned to the horizontal position (5 min). Then the subjects were tilted to 60 degrees again, and sublingual NTG 0.25 mg was administered after 5 min in the upright position, as a distinction from the previous protocol in which NTG was administered supine. The tilt test was continued for 20 min after NTG administration, or aborted if the subject reported presyncopal symptoms.

Pulse wave analysis

Radial BP and pulse wave form were continuously determined from the radial pulse by an automatic tonometric sensor (Colin BP-508T, Colin Medical Instruments Corp., USA), which was fixed on the radial pulse with a wrist band. Radial BP signal was calibrated every 2.5 min by a brachial BP measurement. Continuous aortic BP was derived with the SphygmoCor pulse wave monitoring system (Spygmo-Cor PWMx, AtCor Medical, Australia) using the previously validated generalized transfer function [14]. Ejection duration, aortic reflection time and augmentation index (AIx, augmented pressure/pulse pressure $\times$ 100) were determined.

Whole-body impedance cardiography

A whole-body impedance cardiography device (CircMon[R], JR Medical Ltd, Tallinn, Estonia), which records the continuous changes in body electrical impedance during a cardiac cycle, and plethysmographic BP recordings from a finger (Finapres, Ohmeda, Englewood, Colorado, USA) were used to determine beat-to-beat heart rate (HR), stroke index (stroke volume in proportion to body surface area, ml m^{-2}), cardiac index (cardiac output/body surface area, l min^{-1} m^{-2}), systemic vascular resistance index (SVRI,

systemic vascular resistance/body surface area, dyn s cm^{-5} m^{-2}) and pulse wave velocity (PWV) [15–18]. To calculate the PWV, the CircMon software measures the time difference between the onset of the decrease ('foot') in impedance in the whole-body impedance signal and the popliteal artery signal. From the time difference and the distance between the electrodes, the PWV can be determined. The whole-body impedance cardiography tends to overestimate the PWV when compared with Doppler ultrasound method, and therefore a validated equation was utilized to calculate values that corresponded to the ultrasound method (PWV = (PWV$_{impedance}$ $\times$ 0.696) + 0.864) [17]. The cardiac output values measured with CircMon[R] whole-body impedance cardiography are in good agreement with the values measured by the thermodilution method, both in supine position and during head-up tilt [15]. A detailed description of the method and electrode configuration has been previously reported [15–17]. PWV was not assessed during the head-up tilt due to less accurate timing of left ventricular ejection during reduced stroke volume.

Statistical analysis

The software package R 2.10.1 was used [19]. To address the matched pair design of the study, differences between controls and cases were analyzed. The focus was on the average of the last minute during head-up tilt in the absence of NTG (measurement point 4, Figure 1) and the corresponding last minute in the presence of NTG (measurement point 10, Figure 1). To adjust for possible differences between controls and cases already present in the absence of NTG, the differences of the two differences (measurement point 4 – measurement point 10, Figure 1) were used for statistical testing. The general difference between controls and cases was evaluated using a multivariate Wilcoxon signed-rank test based on marginal signed-ranks for the variables radial mean BP, aortic mean BP, HR, cardiac index, SVRI, augmentation index (AIx), aortic pulse pressure and aortic reflection time [20]. Due to the small sample size a permutation version of the test was applied using 1000 replications. For further interpretation the univariate sample quartiles (quartile 1 – median – quartile 3) were reported and exact marginal Wilcoxon signed-rank tests results are given. Average graphs with standard errors of the mean (SEM) are shown in Figure 1, but the statistical analyses were based on the differences between individual case-control pairs.

Results

Study population

The presyncope group consisted of 21 subjects (six male and 15 female) aged 21–61 years (43 (36–53) years), and the control group of 21 subjects (six male and 15 female) aged 23–59 years (43 (37–53) years). None of the subjects
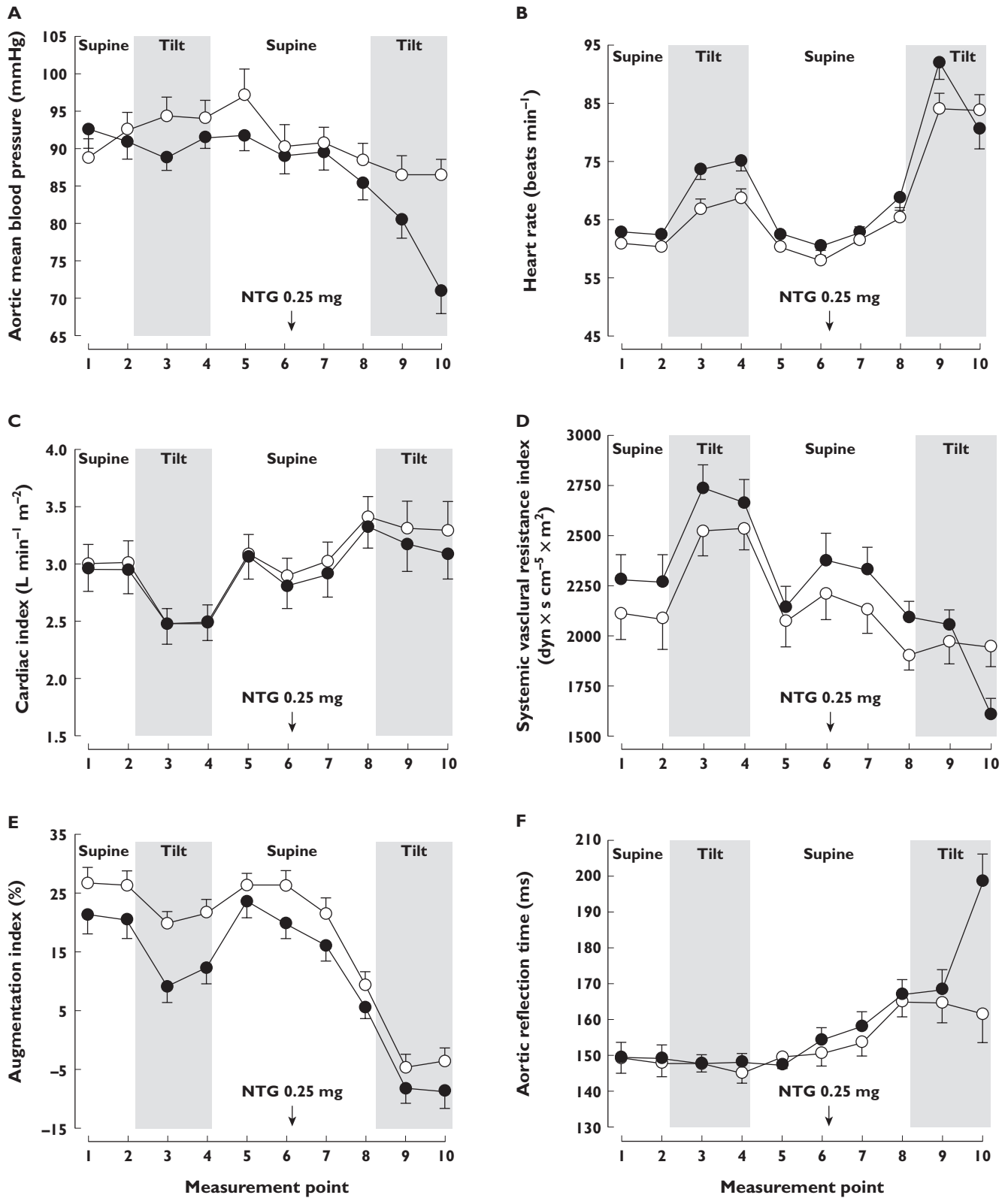
Figure 1

Aortic mean blood pressure (A), heart rate (B), cardiac index (C), systemic vascular resistance index (D), augmentation index (E) and aortic reflection time (F) in the presyncope (solid circles, $n = 21$) and control (open circles, $n = 21$) groups. Averages of the second and last minutes of each study phase are presented. A passive head-up tilt was performed during measurement points 3–4 and 9–10, and 0.25 mg sublingual nitroglycerin was administered at measurement point 6. Measurement point 10 is the last minute before tilt abortion. Mean ± SEM. Control (—○—); Presyncope (—●—)

had a medical history of cardiovascular disease or continuous medication. In the presyncope and control groups, there were one and two current smokers, and six and six subjects with a previous smoking history, respectively. BMI of the subjects in the presyncope group was 23.3 (22.0–27.0) kg m^{-2} and in the control group 23.8 (21.7–25.9) kg m^{-2}. Plasma lipid profile, fasting glucose, electrolytes, and kidney function were all within the normal range, and all electrocardiograms were normal. In the control group, the study protocol was carried out completely, as described above. In the presyncope group, the head-up tilt after NTG administration was aborted after 3.4 ± 0.2 min due to presyncopal symptoms and a progressive fall of BP. In spite of the security measures three subjects in the presyncope group lost consciousness after NTG, but consciousness was quickly restored when the tilt table was returned to the supine position. These subjects were excluded from the final analysis (the original number of subjects in the presyncope group was 24).

In addition, 19 subjects participated in measurements using an alternative protocol during which NTG was only given in the upright position. Ten subjects (six female, four male) experienced presyncopal symptoms, and the tilt test was aborted 5.9 ± 0.5 min after NTG administration. Nine subjects (five female, four male) completed the protocol so that the tilt test was continued for 20 min after NTG was given. The basic characteristics of these groups did not significantly differ. Two subjects classified to the presyncope group in the alternative protocol were originally in the non-syncope group (when NTG was administered in supine position), and *vice versa*. Thus, 79% of the subjects retained their classification whether NTG was given supine or during the head-up tilt.

Haemodynamics before NTG administration

Before NTG administration the recordings of the presyncope and control groups did not significantly differ ($P > 0.05$ for all variables presyncope *vs.* control, Figure 1, Table 1).

During the head-up tilt HR and SVRI increased both in the presyncope and control groups, while diastolic or systolic BP did not significantly change in either group when compared with supine values. The following variables decreased in response to head-up tilt: aortic pulse pressure, AIx, ejection duration, stroke index and cardiac index. The change in aortic reflection time was minor in both groups. All variables except diastolic and systolic BP and aortic reflection time changed statistically significantly in both groups during the head-up tilt ($P < 0.05$ supine *vs.* head-up tilt).

Haemodynamics after NTG administration

Supine position before head-up tilt During the 5 min in the supine position following NTG administration (recording from 15 to 20 min in the protocol), the changes in the haemodynamic variables were similar in both study groups ($P > 0.05$ presyncope *vs.* non-syncope, Figure 1). When compared with the supine values before NTG administration, aortic BP and radial BP decreased in both study groups. A decrease was also observed in aortic pulse pressure, AIx and SVRI. In the supine position, NTG increased cardiac index, HR and aortic reflection time. The change in stroke index was minor in both groups. All variables except stroke index changed statistically significantly after NTG administration when compared with the supine values before drug administration ($P < 0.05$).

Head-up tilt During the head-up tilt with NTG, the changes in haemodynamics were more pronounced than without NTG, and the haemodynamic patterns of many of the variables were completely changed when compared with the first head-up tilt (Figure 1). The general difference during the NTG-stimulated tilt-table test between the study groups for all variables of interest was significant at the 0.05 level ($P = 0.0020$). For individual variables this means that mean aortic BP decreased significantly more in

Table 1

Haemodynamics in the supine position and during head-up tilt in the absence of NTG, median (quartile 1 – quartile 3). No differences between cases and controls were observed

	Supine Control	Presyncope	Head-up tilt Control	Presyncope
Tonometry				
Blood pressure (mm Hg)				
Radial systolic	128 (124–134)	127 (116–137)	127 (119–136)	125 (118–130)
Radial diastolic	79 (71–85)	75 (73–83)	79 (73–87)	77 (74–85)
Finger systolic	110 (104–116)	112 (104–119)	105 (103–116)	110 (104–114)
Finger diastolic	59 (51–70)	64 (59–70)	62 (55–74)	64 (60–69)
Aortic pulse pressure	38 (34–43)	37 (32–43)	34 (29–37)*	28 (27–34)*
Ejection duration (ms)	337 (321–347)	339 (324–345)	291 (280–302)*	272 (258–279)*
Impedance cardiography				
Stroke index (ml m^{-2})	52 (45–55)	45 (38–56)	37 (33–39)*	33 (31–37)*
Pulse wave velocity (m s^{-1})	7.3 (6.8–8.1)	7.3 (6.6–8.8)	–	–

*$P < 0.05$ supine *vs.* head-up tilt.

Table 2

Haemodynamics in the presence of 0.25 mg NTG (administered supine) during the last minute prior to tilt-back, median (quartile 1 – quartile 3)

	Control	Presyncope	P value
Tonometry			
Blood pressure (mm Hg)			
Radial systolic	121 (113–129)	97 (89–108)*	<0.001
Radial diastolic	78 (71–82)	63 (55–70)*	<0.001
Finger systolic	102 (95–112)	77 (71–91)*	<0.001
Finger diastolic	67 (57–79)	52 (47–62)*	0.003
Aortic pulse pressure (mm Hg)	26 (24–30)	22 (18–28)	ns
Ejection duration (ms)	245 (240–265)	266 (244–302)*	0.043
Impedance cardiography			
Stroke index (ml m⁻²)	39 (37–43)	37 (35–43)	ns

*$P < 0.05$ non-syncope *vs.* presyncope.

the presyncope group ($P = 0.0006$) than in the control group, and the same was observed in mean radial BP ($P = 0.0004$). The decreases in SVRI ($P = 0.0008$), HR ($P = 0.002$) and increase in aortic reflection time ($P = 0.0002$) were also greater in the presyncope group than in the control group. The changes in aortic pulse pressure ($P = 0.43$), AIx ($P = 0.15$) and cardiac index ($P = 0.14$) were not different between the study groups.

The haemodynamic variables during the last minute before the tilt-back in both study groups are shown in Figure 1 and Table 2. In Figure 2, boxplots of the difference of the matched differences between controls and cases in the absence and presence of NTG are presented, and the value '0' indicates that the effect of NTG was not different between the groups. Table 3 gives the differences between controls and cases in the absence and presence of NTG, and a positive sign indicates that controls had larger values than cases (first and second columns). Statistical results are based on the differences of these two differences to adjust for possible dissimilarities in the absence of NTG (measurement point 4, third column). For all differences it was of interest if they differed significantly from 0.

Haemodynamics in three subjects with loss of consciousness

During the NTG-stimulated tilt test, three subjects rapidly lost consciousness after reporting presyncopal symptoms. The following immediate return of the tilt table to the horizontal position quickly restored consciousness in all subjects. The mean aortic BP, SVRI, cardiac index and HR of these subjects during the last 90 s before tilt-back are shown in Figure 3 as individual line-graphs. SVRI decreased markedly during 90–60 s prior tilt-back (from 2030 to 1520 dyn s cm⁻⁵ m⁻², Figure 3B) with a simultaneous decrease in BP (from 76 to 60 mmHg, Figure 3A). During the last 60 s before tilt-back, SVRI showed a moderate further decrease, and the values were lower than in the whole presyncope group (1280 *vs.* 2100 dyn s cm⁻⁵ m⁻², $P < 0.05$). BP remained constant during 60–30 s and cardiac
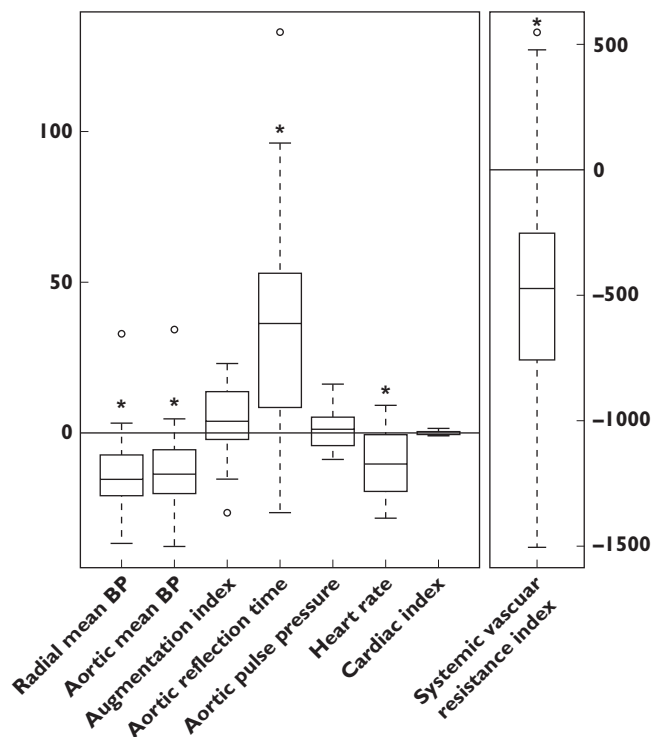

Figure 2

Box plots of the difference of the matched differences between controls and cases in the absence and presence of NTG. The value 0 indicates that NTG administration had no effect. The general difference between controls and cases for all eight variables of interest is significant at the 0.05 level ($P = 0.0020$). *$P < 0.05$ presyncope *vs.* control for individual variables.

index during 90–30 s before the tilt-back (Figure 3A and C). Just before loss of consciousness the recordings showed a clear decrease in cardiac index, HR and BP (30–0 s before tilt-back, Figure 3A, C and D).

Alternative measurement protocol: NTG administration during the head-up tilt

In 19 subjects NTG was administered in the upright position and the tilt-table test was continued for up to 20 min, if possible. Aortic mean BP, SVRI and cardiac index of presyncope and non-syncope groups are shown in Figure 4. After the administration of NTG in the upright position, both presyncope and non-syncope groups showed an initial decrease in SVRI and an increase in cardiac index. During presyncope, SVRI and BP (radial and aortic mean BP, aortic pulse pressure) further decreased and aortic reflection time increased in the presyncope group, while cardiac index was preserved at a higher level than it was before NTG administration (Figure 4). During the last minute in the upright position, SVRI was significantly lower ($P = 0.001$) and aortic reflection time longer ($P = 0.049$) in the presyncope group when compared with the non-syncope group, while there were no statistically significant differences in other variables. The results

Table 3

Differences between the control and presyncope groups in the absence and presence of NTG (first and second columns), and the difference of these two differences (third column). For all differences it was of interest whether they differed significantly from 0

	Head-up tilt without NTG (measurement point 4, Figure 1) Median (quartile 1 – quartile 3)	Head-up tilt with NGT (measurement point 10, Figure 1) Median (quartile 1 – quartile 3)	Difference in the response in the absence and presence of NTG Median (quartile 1 – quartile 3)
Mean radial blood pressure (mm Hg)	2.6 (−6.4 to 10.2)	17.4 (7.0 to 23.7)	−15.5 (−20.9 to −7.5)*
Mean aortic blood pressure (mm Hg)	−0.3 (−6.7 to 9.8)	15.0 (7.3 to 21.0)	−13.6 (−20.5 to −5.5)*
Aortic pulse pressure (mm Hg)	4.0 (−0.3 to 7.2)	4.1 (−1.2 to 8.3)	1.1 (−4.2 to 5.0)
Heart rate (beats min^{-1})	−8.8 (−14.9 to −0.85)	4.0 (−9.1 to 14.4)	−10.4 (−19.5 to −0.6)*
Cardiac index (l min^{-1} m^{-2})	−0.02 (−0.34 to 0.26)	0.47 (−0.46 to 0.84)	−0.53 (−0.75 to 0.24)
SVRI (dyn s cm^{-5} m^{-2})	−40 (−778 to 281)	373 (−25 to 817)	−474 (−757 to −253)*
Augmentation index (%)	7.8 (−0.6 to 19.2)	3.0 (−6.3 to 12.0)	3.7 (−2.3 to 13.6)
Aortic reflection time (ms)	−3.0 (−13.9 to 6.9)	−40.1 (−61.6 to −9.1)	36.2 (8.5 to 51.3)*

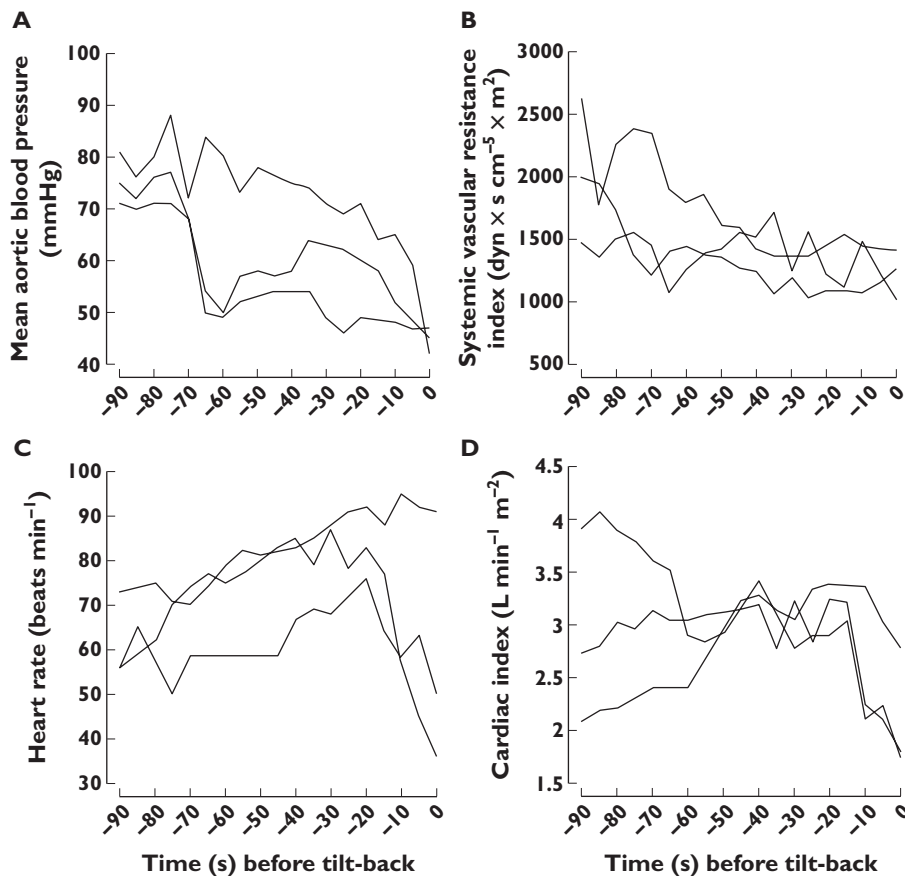*$P < 0.05$, presyncope *vs.* control.

Figure 3

Individual graphs of aortic blood pressure (A), systemic vascular resistance index (B), heart rate (C) and cardiac index (D) during the last 90 s of nitroglycerin-stimulated tilt-table test in three subjects with short-lasting total loss of consciousness.

of the alternative measurement protocol strengthen the view of decreased peripheral arterial resistance as a cause for presyncopal symptoms during NTG-stimulated tilt test.

Discussion

Since the precise mechanism of nitrate-induced syncope is still unclear, the present study examined the
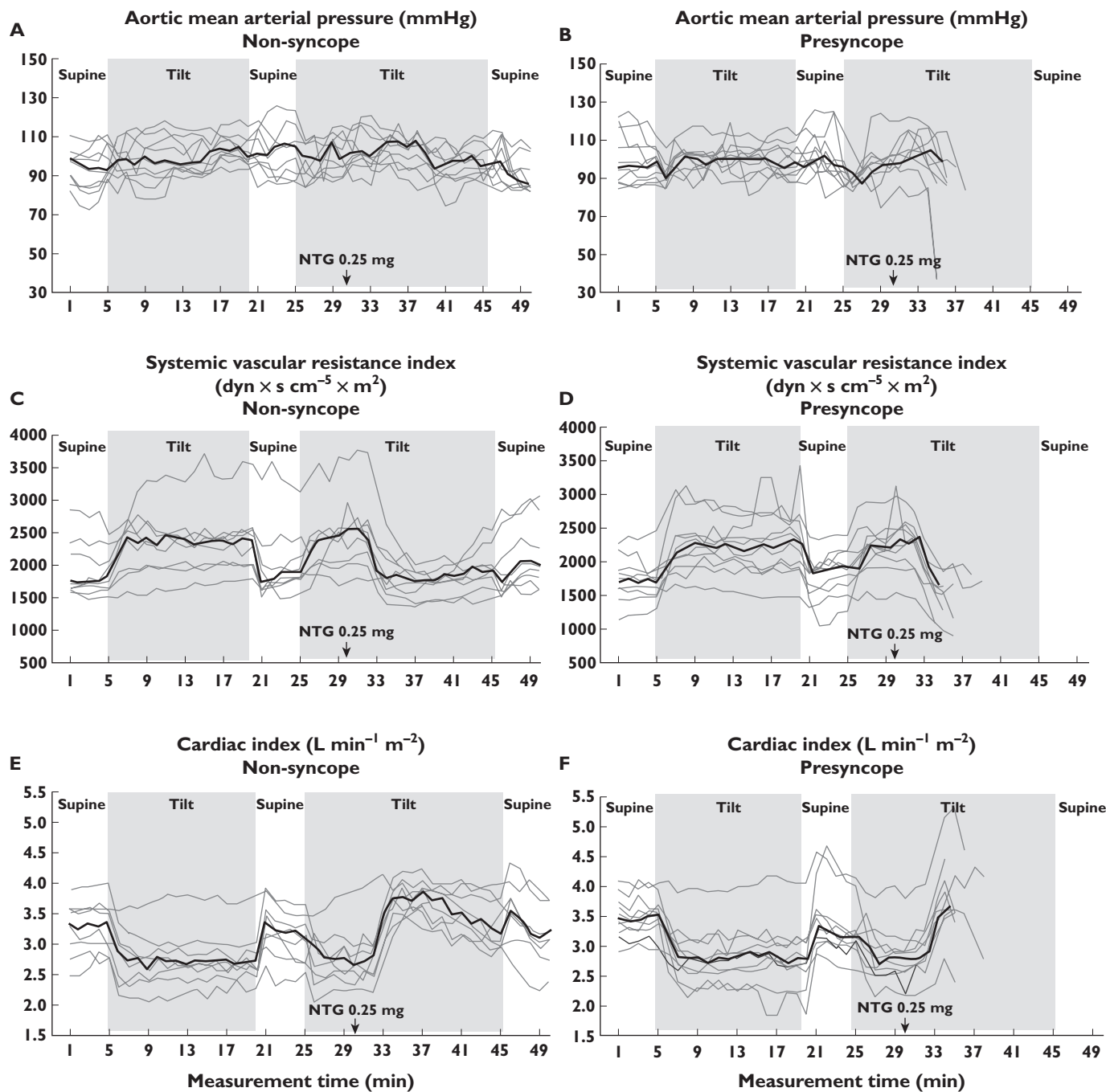
Figure 4

Additional measurements using the alternative protocol during which NTG was administered in the upright position: Medians (bold line) and individual graphs of each subject (grey lines) for mean aortic blood pressure, systemic vascular resistance index and cardiac index in non-syncope (A, C, E) and presyncope (B, D, F) groups.

haemodynamic alterations in subjects with presyncopal symptoms during NTG-stimulated tilt-table test. The findings demonstrated that the presyncopal symptoms were caused by a decrease in arterial resistance, in the absence of a compensatory increase in cardiac output.

Small doses of nitrates have been traditionally considered as potent venodilatators, leading to diminished ven-

tricular preload and cardiac output, with a lesser effect on arterial resistance [4, 21]. Supporting this view, two studies have suggested a decrease in cardiac output during presyncope in the nitrate-stimulated tilt test without a simultaneous decrease in systemic vascular resistance, as evaluated from the pulsations of indirect finger arterial pressures [7, 8]. However, the results remain controversial,

and, for example, Koole *et al*. found no evidence of increased venous pooling during nitrate-stimulated tilt test in patients with a history of vasovagal syncope, as studied using isotope techniques [22]. In addition, two reports utilizing either thoracic bioimpedance or echocardiography did not support the view of decreased cardiac filling after NTG administration [9, 23].

In agreement with the present findings, decreased systemic vascular resistance has been previously suggested as an explanation for the nitrate-stimulated presyncope, as estimated from the pulsations of finger arterial pressures or by means of thoracic bioimpedance [9, 24]. Here we administered NGT in the supine position, followed by the tilt test 5 min later, while an unmedicated tilt-phase had preceded the administration of NTG in the previous investigations [8, 21, 25]. In the first head-up tilt without NTG, we observed a major increase in SVRI (>20%, $P < 0.05$ compared with supine values) as a result of activated vasopressor mechanisms in the upright position [26], and administration of NTG in the supine position appeared to sensitize to the decrease in SVRI during the following head-up tilt. Autonomic nervous tone was not assessed in the present study, but in addition to its direct vasodilatory effect NTG can also sensitize the baroreflex arc, thus leading to reduced sympathetic tone and decreased SVRI [11]. Reduced SVRI and increased HR after NTG administration explain why cardiac index was not reduced during the subsequent head-up tilt.

As in the majority of previous reports, the tilt test in the present study was aborted in the presyncope group, except for the three subjects who developed rapid loss of consciousness just prior to the tilt-back. In these subjects the decrease of SVRI to a very low level was documented first, while the final vasovagal reaction and the subsequent total syncope were accompanied by a decrease in HR and cardiac index (Figure 3). Also in the whole presyncope group, HR decreased during the last minute after NTG, which may be a sign of incipient vasovagal reaction (Figure 1). However, the HR during presyncope was still relatively high (~80 beats min^{-1}, Figure 1) and did not explain the onset of presyncopal symptoms. These findings also imply a possible central sympatho-inhibitory effect of NTG in the final phase of the tilt test [11].

After the first measurements the possibility remained that methodological differences between the present and the previous studies (NTG administered supine *vs*. upright) could explain the divergent findings. Therefore, we performed additional measurements with 19 study subjects to examine the influence of body position on the NTG response. We found that in those subjects with presyncopal symptoms SVRI was reduced clearly more than in the non-syncope group, while cardiac index increased similarly in both groups. In addition, 15/19 (79%) subjects responded similarly to NTG whether the drug was administered before or during head-up tilt. Altogether, the haemodynamic pattern during presyncope remained very

similar whether NTG was administered in the supine or upright position.

In addition to decreased SVRI, in the present study NTG increased aortic reflection time and decreased pulse pressure, further supporting the view of decreased arterial resistance [27, 28]. A decrease in AIx was also observed in both study groups, probably due to increased compliance and reduced SVRI subsequently shifting the reflected wave towards diastole [29]. However, increased HR decreases the duration of systole and also shifts the reflected wave towards diastole, thus diminishing the AIx. We observed that AIx remained decreased during the last 5 min of the protocol when compared with values before NTG administration (14 ± 3% before and −11 ± 2 after NTG), during which period HR had already returned to the level preceding NTG (not shown). Thus, decreased AIx was not solely explained by increased HR, but rather reflected the effects of NTG on wave reflection, large arterial compliance and SVRI. Since the AIx, PWV or aortic reflection time did not differ between the study groups at baseline, increased arterial stiffness or different mechanical properties of the large arteries were clearly not explanations for increased sensitivity to NTG.

In the literature, a wide scale of methodological protocols in nitrate-stimulated tilt tests has been presented [30]. NTG doses ranging from 0.3 to 0.8 mg have been used, but this range of doses has not markedly affected the specificity or sensitivity of the tilt test when used as a diagnostic test for unexplained syncope [30]. Commonly, a passive upright tilt phase of 20–60 min has preceded the administration of NTG, which has been given in the upright position, and the duration of the stimulated tilt phase has varied from 10 to 30 min [25, 30, 31]. Although NTG administration has shortened the protocol when compared with the unstimulated tilt test, the test duration has remained rather long. Recently, a shorter 30 min tilt test protocol without a preceding unstimulated tilt phase has been presented [32].

The present continuous measurement protocol provides comprehensive haemodynamic information, and the cardiac output values measured with CircMon^R whole-body impedance cardiography are in good agreement with the values measured by the thermodilution method, both in the supine position and during head-up tilt [15]. By simultaneously determining peripheral and central BP, AIx, PWV, cardiac function and systemic vascular resistance we could continuously monitor haemodynamics during both unmedicated and NTG-stimulated tilt tests, in contrast to the approach where only the changes in NTG-stimulated tilt test would have been documented [7, 33]. Indeed, already a short (5 min) tilt-table test in the absence of NTG clearly increased SVRI and HR, and decreased cardiac index, stroke index, pulse pressure and AIx, while all of these changes were significantly influenced by NTG. Corresponding to previous results, BP measured from a finger (plethysmographic cuff) and BP from the radial artery

(pulse wave analysis, Tables 2 and 3) showed a systematic difference due to the different methodology and anatomical site of the measurements [34]. Arteries progressively narrow towards the periphery, and in general mean finger arterial pressure is lower than BP measured more proximally as a result of the pressure gradient in the arterial tree caused by flow [34]. In the present study the changes in both finger and radial artery BP values in response to head-up tilt and NTG administration were corresponded well.

Most of the previous studies have been designed to evaluate the test outcome, i.e. to uncover whether the subjects develop syncope or not, while the purpose of the present study was to examine the NTG-induced haemodynamic changes in presyncope. The aim was not to study the sensitivity or specificity of the protocol, and the subjects in the presyncope group had not spontaneously reported unexplained syncope in their medical history. Indeed, the diagnostic use of the present protocol in syncopal patients should be evaluated in the future. However, in previous reports control subjects with no history of syncope have shown parallel haemodynamic changes with the study subjects, but the changes were more pronounced in subjects with a history of syncope [7]. Finally, age may influence the haemodynamic pattern observed during NTG-induced presyncope, with younger subjects showing a more sudden fall of BP when compared with the older ones [8]. In the present analysis, age was used as a matching variable in control subject selection and its effect on the results has thus been eliminated. Although the study population was relatively small, the present number of subjects was similar to those in the majority of the previous studies [25, 35].

In conclusion, a major decrease in SVRI and an increase in aortic reflection time were documented in subjects with presyncope during NTG-stimulated tilt test, in the absence of changes in cardiac index. These findings do not support the view of decreased cardiac filling and preload as plausible mechanisms for the NTG-induced presyncope but indicate that a small dose of NTG significantly decreases arterial resistance and cardiac afterload.

Competing Interests

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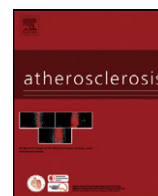
Ageing and cardiovascular responses to head-up tilt in healthy subjects

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ABSTRACT

Objective: Ageing is associated with increased postural blood pressure changes and attenuated cardiovascular reactivity. As the background of these changes remains uncertain, we examined arterial stiffness, cardiac function and vascular resistance in healthy subjects in supine position and during orthostatic challenge.
Methods: Haemodynamics of 179 normotensive subjects (109 female and 70 male, 21–59 years) were examined using continuous radial pulse wave analysis, whole-body impedance cardiography, and plethysmographic finger blood pressures.
Results: Both in supine position and during head-up tilt central and peripheral blood pressure and augmentation index (amplitude of the reflected pressure wave divided by pulse pressure) increased, and time of pulse wave reflection decreased with age. Supine pulse wave velocity progressively increased with age. There were only minor differences in supine and upright systemic vascular resistance, cardiac index, stroke index, and heart rate that were not systematically related to age. In regression analysis, the explanatory factors for a more pronounced decrease in central systolic blood pressure during the head-up tilt were in the age of 50–59 years, higher baseline pulse wave velocity and central systolic BP. None of the changes in other haemodynamic variables during tilt were related to age.
Conclusion: As no systematic age-related differences were observed in cardiac function or vascular resistance, these results support the view that progressive reduction of large arterial compliance contributes to the exaggerated age-related decrease in central systolic blood pressure in response to head-up tilt.

1. Introduction

Ageing is associated with progressive structural and functional changes in the cardiovascular system. With increasing age large arteries dilate and stiffen as a result of thickening of the intima, reduced elastin content and vascular smooth muscle cell number, and increased collagen composition in the vessel wall [1–3]. Stiffening of the arteries leads to increased pulse wave velocity (PWV) and earlier wave reflection, thus increasing systolic and pulse pressure and decreasing perfusion of the myocardium during the diastole [3,4]. Young, compliant arteries cushion the arterial pulsations so that the flow in the capillary level remains continuous [3]. Reduced arterial compliance transfers pulsatile flow further to the periphery, which has harmful influences in the microcirculation [5]. Ageing is

also associated with impairments in nitric oxide and prostanoid pathways, resulting in endothelial dysfunction [6]. Today arterial stiffening is recognized as an independent risk factor for cardiovascular morbidity and mortality [7].

Circulatory regulation becomes attenuated with increasing age. For example, the prevalence of orthostatic hypotension is greater among elderly when compared with younger subjects, but the determinants of postural blood pressure (BP) changes are not completely understood [8]. Changes in baroreflex sensitivity, arterial stiffness, humoral regulation or blood volume distribution may play a role [9–11]. Only few studies have examined the head-up tilt induced simultaneous changes in central haemodynamics, arterial stiffness, systemic vascular resistance and cardiac function.

Here we tested the hypothesis whether reduced large arterial compliance with increasing age could explain putative differences in the cardiovascular responses induced by passive orthostatic challenge. We examined haemodynamics in 179 healthy, normotensive in young to middle-aged adults utilizing continuous whole-body

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impedance cardiography, radial pulse wave analysis and BP recording from fingers.

2. Methods

2.1. Study subjects

The study population was 179 healthy individuals, who were divided into four age groups: 20–29 ($n = 34$), 30–39 ($n = 40$), 40–49 ($n = 53$) and 50–59 ($n = 52$) years. All subjects underwent a physical examination, and lifestyle habits, family history for cardiovascular disease, and medical history were documented. All subjects gave a written informed consent. The study complies with the declaration of Helsinki, and was approved by the ethics committee of the Tampere University Hospital.

2.2. Laboratory analyses

Blood and urine samples were obtained after a minimum of 12-h fast, and a standard 12-lead electrocardiogram was recorded. Blood cell count was determined by ADVIA 120 or 2120 (Bayer Health Care, Tarrytown, NY, USA), and other laboratory values by Cobas Integra 700/800 (F. Hoffmann-LaRoche Ltd., Basel, Switzerland). Glomerular filtration rate was estimated using the Cockroft–Gault formula [12].

2.3. Heamodynamic measurement protocol

Measurements were performed by a trained nurse, and the subjects had refrained from caffeine, smoking and heavy meal for at least 4 h and from alcohol for at least 24 h prior to the investigation. The electrodes for impedance cardiography were placed on the body surface, a tonometric sensor for pulse wave analysis on the radial pulsation to the left wrist, a brachial cuff for BP calibration (every 2.5 min) to the right upper arm, and a plethysmographic cuff for finger BP measurement to the right middle finger.

The subjects were first resting supine on the tilt table (5 min) followed by head-up tilt to 60° (5 min), after which tilt table was returned to the horizontal position (5 min), and data was captured continuously. The good repeatability and reproducibility of the measurement protocol both at rest and during head-up tilt have been demonstrated [13].

2.4. Pulse wave analysis

Radial BP and pulse waveform were continuously determined by a tonometric sensor (Colin BP-508T, Colin Medical Instruments Corp., USA). Aortic BP was derived with the pulse wave monitoring system (SpygmoCor PWMx, AtCor Medical, Australia) using a validated transfer function [14]. Ejection duration (ms), time to pulse wave reflection (time to return of the reflection wave of the aortic waveform, ms) and augmentation index (%, augmented pressure/pulse pressure × 100) were determined.

2.5. Impedance cardiography

A whole-body impedance cardiography device (CircMon, JR Medical Ltd., Tallinn, Estonia), and plethysmographic BP recordings from fingers (Finapres, Ohmeda, Englewood, CO, USA) were used to determine beat-to-beat heart rate, stroke index (stroke volume/body surface area, ml/m^2), cardiac index (cardiac output/body surface area, l/min/m^2), systemic vascular resistance index (SVRI, systemic vascular resistance/body surface area, dyn s/(cm^5 m^2)) and PWV (m/s) [15,16]. The description and the repeatability of the method have been previously reported [15,16]. PWV was not assessed during the head-up tilt due to less accurate timing of left ventricular ejection during reduced stroke volume [15,16].

2.6. Statistical analysis

The software package R 2.8.1 was used [17]. The data contained artifacts for example due to movements of the subjects during the experiment or re-calibration of the machines. Therefore, robust and nonparametric methods were applied instead of the classical methods to allow for outliers and heavy tails in the data.

As descriptive summary the three sample quartiles, denoted as Q1, Q2 and Q3, were reported (Table 1). Univariate one-way ANOVA was performed using the Kruskal–Wallis test and independence in two-way contingency tables was evaluated using Fisher's exact test. As information about alcohol consumption or smoking was not available from all subjects, division to fewer subgroups was performed to increase the power of the multivariate analysis. All participants with the exception of two subjects consumed none to moderate amounts of alcohol, and for the statistical analysis the subjects were divided into two subgroups: ≤3 alcohol doses/week and >3 alcohol doses/week. Smoking status was divided into two groups: present or previous smoker versus subjects who never smoked.

To evaluate haemodynamic differences between the four age groups, stratified multivariate c-sample tests were performed. The test applied here was the affine equivariant version of the spatial sign test and as corresponding point estimate the affine equivariant

Table 1
Characteristics of the study population.

	20–29 years	30–39 years	40–49 years	50–59 years
Number of subjects	34	40	53	52
Female/male	21/13	24/16	30/23	34/18
Body mass index (kg/m^2)	23.1 (22.0–25.8)	23.8 (22.0–27.5)	25.9 (22.8–28.7)[*]	26.8 (23.5–31.5)[*,#]
Waist circumference (cm)	84 (75–85)	81 (73–95)	90 (80–96)[*]	95 (83–102)[*,#]
Smoking status (present or previous/never)	11/23	12/20	12/26	10/13
Alcohol consumption, doses/week (≤3/>3)	13/21	7/26	14/23	11/11
Cornell voltage product (ms × mm)	1558 (1227–1744)	1423 (1067–1805)	1360 (1104–1710)	1562 (1242–1924)
Radial systolic BP (mmHg)	125 (115–129)	130 (121–137)[*]	133 (123–139)[*]	136 (129–140)[*,#]
Radial diastolic BP (mmHg)	74 (70–78)	80 (73–84)[*]	83 (76–89)[*,#]	84 (79–88)[*,#]
Pulse wave velocity (m/s)	8.40 (7.62–8.82)	9.14 (8.14–10.37)[*]	10.22 (8.82–11.95)[*]	11.34 (10.10–13.30)[*,#,†]
C-reactive protein (mmol/l)	0.7 (0.5–1.1)	0.9 (0.5–2.2)	0.7 (05–1.1)	1.0 (0.7–1.8)
HDL cholesterol (mmol/l)	1.7 (1.5–2.0)	1.5 (1.2–1.9)	1.5 (1.3–1.8)	1.6 (1.3–1.9)
LDL-cholesterol (mmol/l)	2.0 (1.6–2.4)	2.2 (2.1–3.0)[*]	2.8 (2.2–3.2)[*]	3.2 (2.6–3.6)[*,#,†]
Triglycerides (mmol/l)	0.8 (0.6–1.1)	0.8 (0.6–1.4)	1.0 (0.7–1.4)	1.1 (0.7–1.6)
Creatinine (μmol/l)	74 (65–82)	71 (66–81)	74 (67–83)	72 (62–78)

For smoking status and alcohol consumption the numbers do not add up to 179 due to missing values.

[*] $p < 0.05$ compared with 20–29-year-old subjects.
[#] $p < 0.05$ compared with 30–39-year-old subjects.
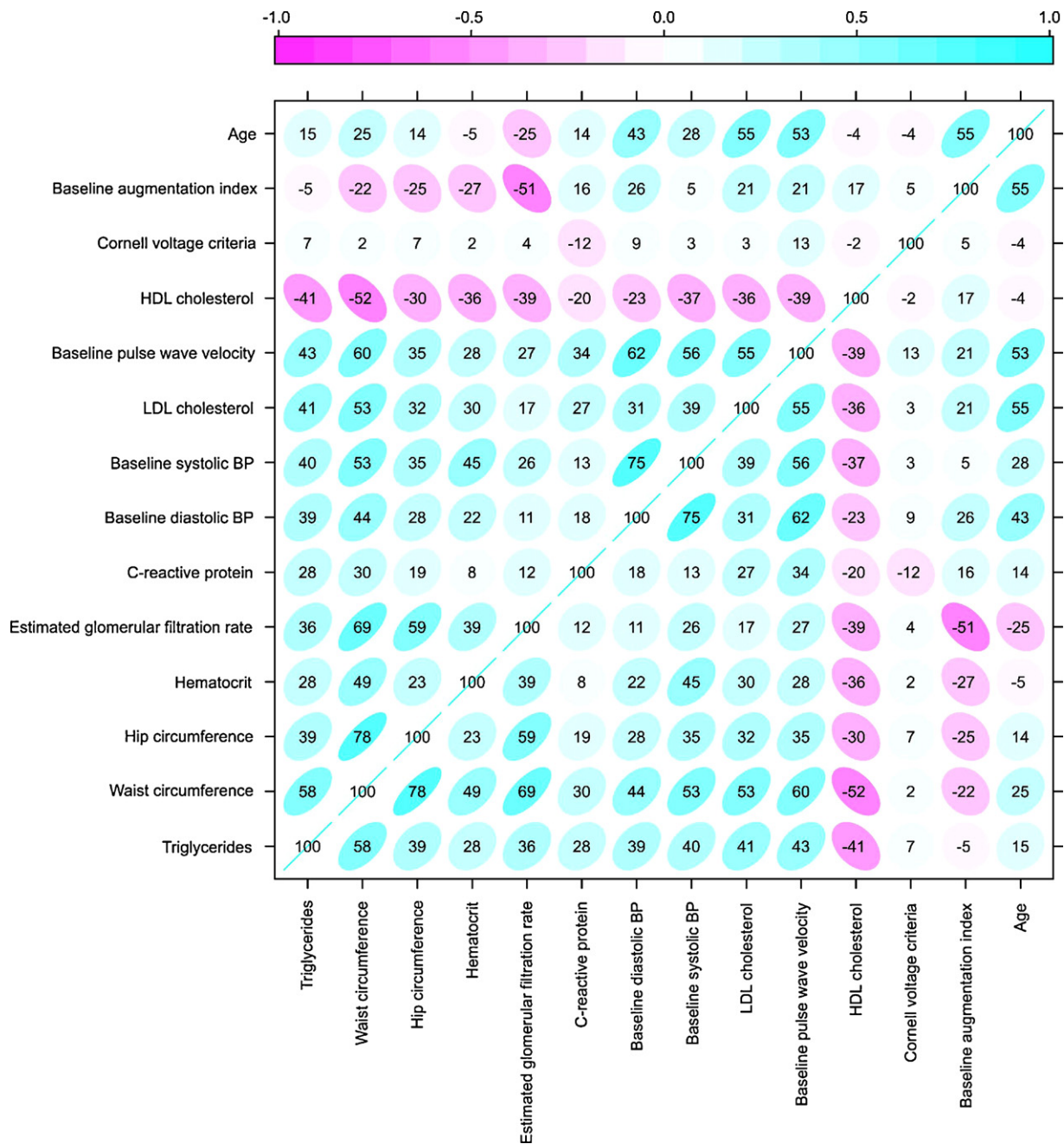[†] $p < 0.05$ compared with 40–49-year-old subjects.

Fig. 1. Correlogram representing the correlation matrix of subject age and the numerical explanatory variables used in the regression analysis.

version of the spatial median was reported [18]. Stratification was done separately for sex, smoking, alcohol consumption level and for a binary indicator based on a canonical analysis between other confounding variables (baseline BP, augmentation index, PWV, aortic reflection time, waist and hip circumference, cholesterol status, hematocrit, C-reactive protein, estimated glomerular filtration rate, Cornell voltage product) and the response.

A robust linear MM-regression [19] was used to model the reaction of a subject to the tilt-table test. The model selection was performed using the smallest complete subset and successively removing the explanatory variable with the highest p-value (>0.05), while always keeping age as the variable of interest in the model. All explanatory variables entered the model linearly and no interactions were considered. The final model was refitted using the largest possible subset of the data and the model assumptions graphically evaluated. A difficulty of the model fitting was the correlation

between several explanatory variables (multicollinearity problem, i.e. an explanatory variable in the final model might rather stand for a group of variables). The correlation structure of the variables can be evaluated by the correlogram in Fig. 1, which is based on the correlation matrix of the MCD [19].

3. Results

3.1. Study population

The demographic data is shown in Table 1. The 179 healthy subjects (109 females and 70 males) aged 21–59 years were divided into four groups according to age (20–29, 30–39, 40–49 and 50–59 years). The proportion of females and males did not significantly differ between the groups. None of the subjects had a medical his-

tory for cardiovascular disease or continuous medication. Smoking habits or alcohol consumption did not differ between the study groups. Blood cell count, plasma lipid profile, fasting glucose, electrolytes, and kidney function were all within the normal range, and all electrocardiograms were normal.

BMI and waist circumference of subjects aged 20–29 years were lower than in 40–49- and 50–59-year-old subjects, and those of 30–39-year-old subjects were lower than in 50–59-year-old subjects ($p < 0.05$, Table 1). Estimated glomerular filtration rate was higher in 20–29-year-old subjects when compared with 40–49- and 50–59-year-old subjects, and in 30–39-year-old subjects when

compared with 50–59-year-old subjects ($p < 0.05$, Table 1). Plasma LDL-cholesterol increased with age ($p < 0.05$), and the only comparison without statistical significance was between 30–39- and 40–49-year-old subjects ($p = 0.07$) (Table 1). Other laboratory values did not differ between the study groups.

3.2. Haemodynamics

Data captured during the 15-min protocol is shown in Figs. 2 and 3. There was a significant age-dependent difference in aortic (Fig. 2A) and radial systolic BP and aortic pulse pressure
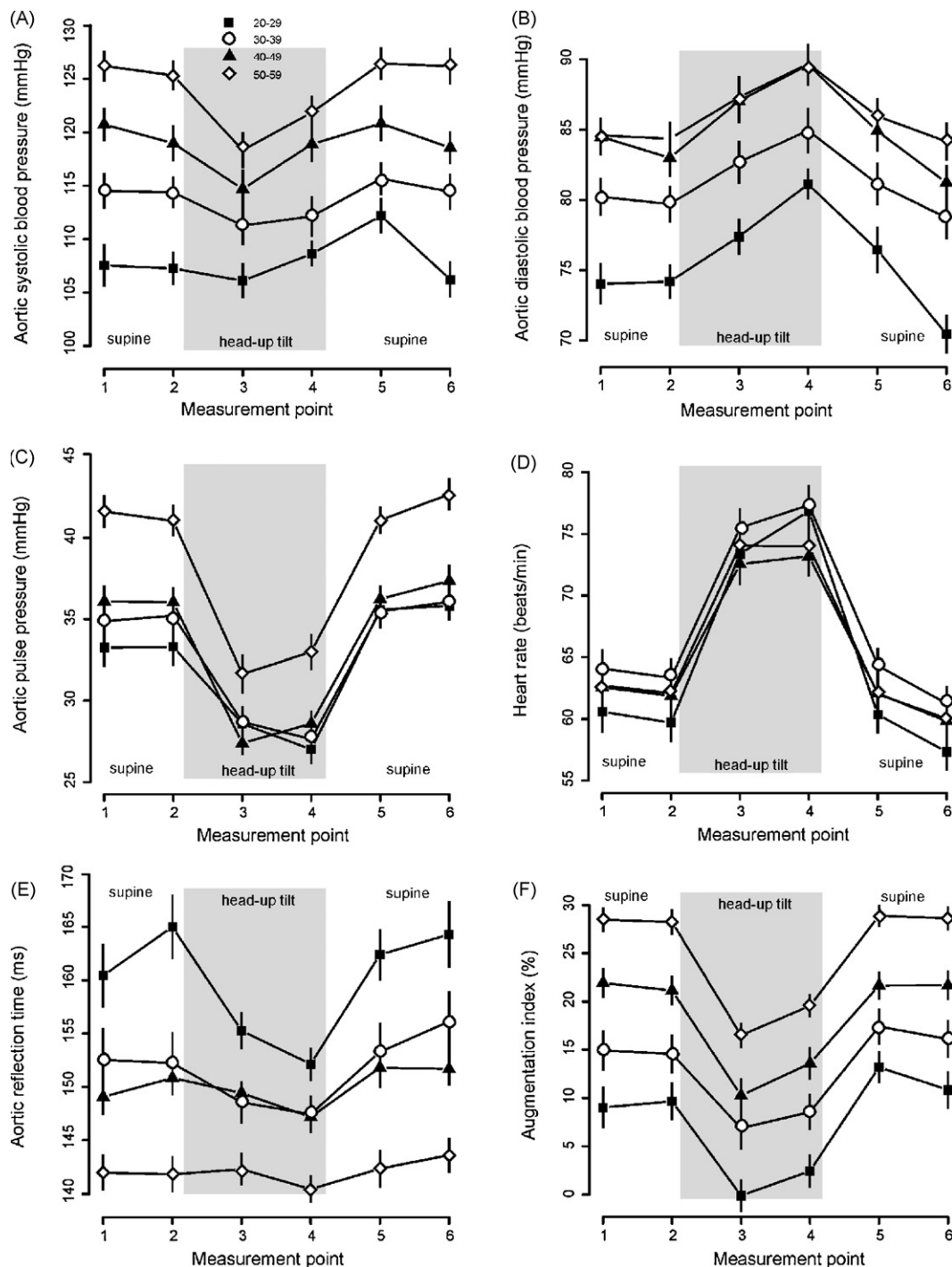

Fig. 2. Spatial medians and standard errors for aortic systolic* (A) and diastolic blood pressure† (B), aortic pulse pressure* (C), heart rate* (D), aortic reflection time* (E) and augmentation index* (F) of study subjects aged 20–29, 30–39, 40–49 and 50–59 years. A passive head-up tilt was performed from 5 to 10 min of the protocol. *A significant age-related difference before and after adjustment for sex or other confounding variables ($p < 0.05$). †A significant age-related difference after adjustment for sex ($p < 0.05$).

(Fig. 2C) with older subjects showing higher values ($p < 0.05$). These differences remained significant after adjustment for sex or other confounding variables as presented in methods ($p < 0.05$). There was also a significant age-dependent difference in aortic and radial diastolic BP (Fig. 2B) after adjustment for sex ($p < 0.05$), but the difference was no more significant after adjusting for other confounding variables. Smoking status or alcohol consumption did not have an influence on BP.

Heart rate of male and female subjects differed by age in the statistical analysis, but the spatial median values did not show a systematic increase or decrease with age (Fig. 2D). The difference in heart rate was also significant after adjustment for confounding factors. Heart rate differed also by the smoking status and the level of alcohol consumption ($p < 0.05$), so that the smokers and subjects consuming more alcohol had a higher heart rate during the head-up tilt but not in the supine position.

Aortic reflection time decreased and augmentation index increased in both sexes with age, and there was a significant age-related difference before and after adjustment for confounding variables ($p < 0.05$, Fig. 2E). Smoking status did not have an influence on the level of augmentation or the time of pulse wave reflection, but aortic reflection time differed by the level of alcohol consumption ($p < 0.05$). The difference was only observed during the tilt, so that lower alcohol consumption corresponded to a lesser decrease in aortic reflection time.

There was no age-related difference in cardiac index or in stroke index (Fig. 3A and B) ($p > 0.05$, Fig. 3). There was a general age-related difference in SVRI, but after adjustment for sex or other confounding variables the difference was no more statistically significant (Fig. 3C). PWV increased significantly with age in both sexes before and after adjustment for confounders ($p < 0.05$, Table 1). Cardiac index, stroke index, SVRI or PWV did not differ by smoking status or the level of alcohol consumption.

3.3. Haemodynamic responses to head-up tilt

All haemodynamic variables changed significantly during tilt (illustrated in Figs. 2 and 3). A robust linear MM-regression was applied to model whether the magnitudes of the changes differed by age. The age of 50–59 years was a significant explanatory factor for the change in aortic systolic BP during the head-up tilt ($p = 0.007$), with older subjects showing more pronounced decrease when compared with younger ones. In addition, baseline PWV ($p = 0.001$) and central systolic BP ($p < 0.001$) remained significant explanatory factors in the final regression model for the change. However, age was not an explanatory factor for the observed changes in the other variables in the regression analysis, but the baseline level of each variable (i.e. the value in the first measurement point) remained in all final models. For example, the change in aortic reflection time (Fig. 2E) clearly differed between the different age groups. However, in the regression analysis age was not but the baseline value of the reflection time and PWV were significant explanatory factors for the change ($p < 0.05$). As stated in the methods, an explanatory variable in the final model might stand for a group of variables due to multicollinearity (Fig. 1).

4. Discussion

The present study showed that large arterial stiffness – reflected as higher augmentation index, aortic pulse pressure, aortic reflection time, and PWV – increased with age, while only minor differences were observed in cardiac function and vascular resistance in the normotensive subjects of different ages. In response to head-up tilt, the decrease in aortic systolic BP was greatest in the highest age group, and higher baseline PWV and central systolic BP were significant explanatory factors for a more pronounced
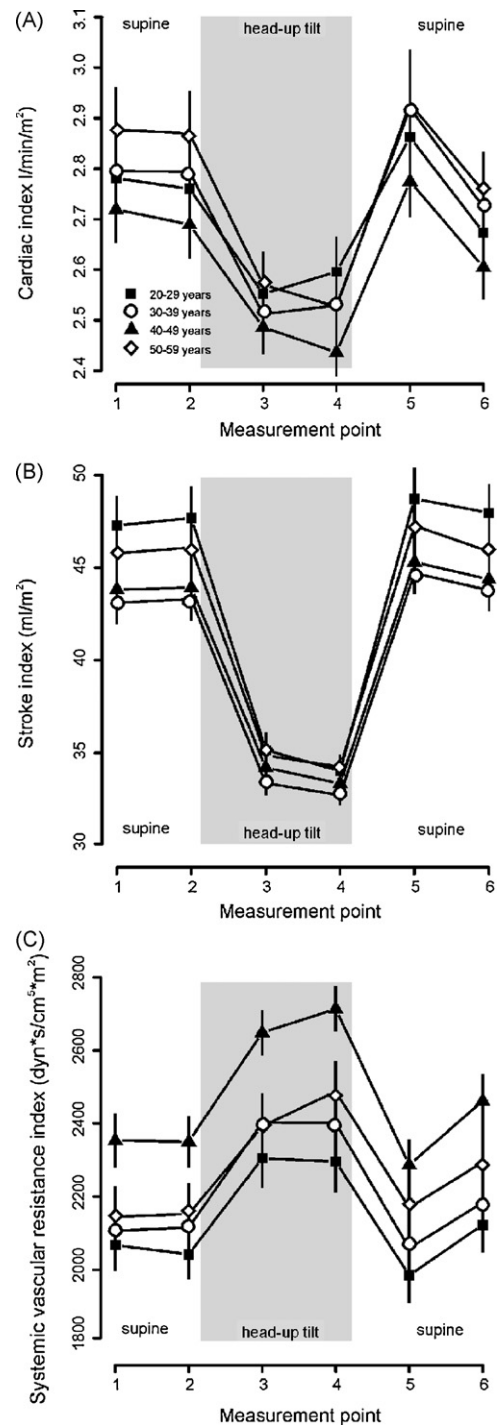

Fig. 3. Spatial medians and standard errors of cardiac index (A), stroke index (B) and systemic vascular resistance index* (C) of study subjects aged 20–29, 30–39, 40–49 and 50–59 years. A passive head-up tilt was performed from 5 to 10 min of the protocol. *A significant age-related difference before but not after the adjustment for confounding variables.

decrease. The present findings suggest that reduced arterial compliance contributes to this age-related change during the tilt-table test.

Increased arterial stiffness is an independent risk factor for cardiovascular morbidity and mortality [7], and the close association between ageing and large arterial stiffness has been well demonstrated [4]. However, ageing does not seem to affect the mechanical properties of smaller, muscular arteries to the same extent [20]. As

cushioning of the pulsations generated by the heart is impaired due to decreased arterial compliance, blood flow in the microcirculation becomes pulsatile, which may damage the circulation especially in the brain and the kidney [5,21]. In the present study, augmentation index, PWV, and aortic pulse pressure increased and aortic reflection time decreased with age, indicating increased arterial stiffness [4]. It should be noted that augmentation index and PWV differed significantly already in younger age groups, while aortic pulse pressure increased only after the age of 50 years (Fig. 2C). This suggests that aortic pulse pressure is not a sensitive marker of arterial stiffness. As cardiac output, stroke volume, heart rate and SVRI did not systematically differ between the age groups, both cardiac and small artery function appeared to be maintained with increasing age in the present study.

Reduced arterial compliance and ageing have been linked to impaired haemodynamic regulation, for example to increased incidence of orthostatic hypotension [10,22], but the mechanisms of different postural BP changes at different ages are not clear. As one explanation, decreased baroreflex sensitivity and attenuated heart rate response to head-up tilt have been observed with increasing age [9], and arterial stiffness at sites rich of baroreceptors may further decrease baroreflex sensitivity [22]. In the present study, the heart rate and SVRI responses to head-up tilt were preserved in the 50–59-year-old subjects, indicating that baroreflex sensitivity was not impaired, in spite of several haemodynamic markers of increased arterial stiffness. In previous studies differences in humoral regulation, for example decreased plasma renin activity in older subjects, have also been suggested to impair upright haemodynamics [11]. In addition, changes in blood volume distribution may play a role in the different haemodynamic responses to postural changes, with older people showing evidence of increased venous pooling and decreased cardiac output in the upright posture [23].

We performed a regression analysis to examine whether the haemodynamic changes during the head-up tilt differed by age. Aortic systolic BP decreased more with advancing age during the tilt, and in the age of 50–59 years, baseline PWV, and central systolic BP remained as explanatory factors in the final regression model. However, the increases in heart rate and SVRI were well preserved, while augmentation index, stroke index and cardiac index showed a similar decrease in all age groups. Thus, there were no signs of attenuated heart rate response or increased venous pooling during tilt in up to 59-year-old normotensive subjects. In the younger subjects aortic reflection time decreased during the head-up tilt, while it hardly changed in the older age groups (Fig. 2E). Decreased pulse wave reflection time during the tilt in younger subjects shifts the reflected wave towards systole and prevents a reduction in systolic BP (Fig. 2A). As the time of pulse wave reflection does not change during the tilt in older subjects, the simultaneous decrease in cardiac index results in a decreased systolic BP. Baseline aortic reflection time and PWV were significant explanatory factors also for the change in reflection time during the tilt. Taken together, these findings indicate that increased large arterial stiffness largely contributes to the observed age-related haemodynamic changes in response to head-up tilt. However, the cross-sectional design could be considered as a study limitation. The present study addresses whether the responses differ in subjects of different ages, but a longitudinal study is needed to examine how these responses change in an individual with ageing.

The demographics of the study groups were well corresponding except for higher BMI and LDL-cholesterol levels in the older age groups, and higher estimated glomerular filtration rate in the younger age groups. Previously, an association between cholesterol level and arterial stiffness has been demonstrated in hypercholesterolemic patients in some [24] but not all studies [25], and it has been shown that even in a population without atherosclerosis arterial compliance decreases with increasing age [26]. It is unlikely that the differences in subject characteristics were the primary explanations for the observed haemodynamic differences with increasing age, since they were included as confounding factors in the multivariate and regression analyses.

In conclusion, the present results demonstrated that arterial stiffness increased with age in 21–59-year-old subjects, while only minor differences were observed in the regulation of vascular resistance or cardiac function that were not systematically related to age. Altogether, the observed differences in cardiovascular responses to passive head-up tilt were largely explained by reduced large arterial compliance with increasing age.

Acknowledgements

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