

Linköping University Medical Dissertations No. 1042

Aspects on wall properties of the brachial artery in man

With special reference to SLE and
insulin-dependent diabetes mellitus

Niclas Bjarnegård

Division of Cardiovascular Medicine / Physiology
Department of Medical and Health Sciences
Linköping University, Sweden



Linköping University
FACULTY OF HEALTH SCIENCES

Linköping 2008

©Niclas Bjarnegård, 2008

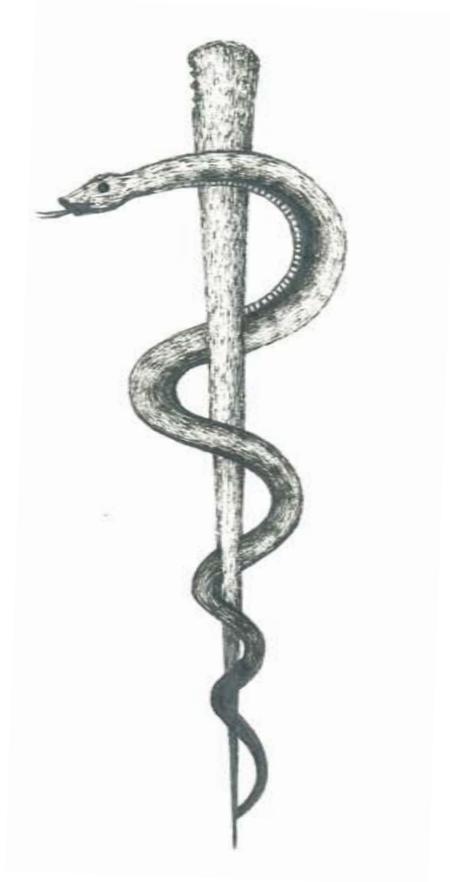
Cover picture: Original ultrasound B-mode (above) and M-mode image (below) of an artery together with a pasted tracing of the arterial diameter change.

Published articles have been reprinted with the permission of the copyright holder.

Printed in Sweden by LiU-Tryck, Linköping, Sweden, 2008

ISBN 978-91-7393-982-9

ISSN 0345-0082



Ignore any idea that is not initiated during outdoor activity, while also the muscles are filled with blood.

Nietzsche 1844-1900

CONTENTS

ABBREVIATIONS.....	1
ABSTRACT	2
LIST OF PAPERS	4
INTRODUCTION.....	6
AIMS	16
MATERIALS & METHODS	18
Subjects	18
Methods	21
RESULTS	32
DISCUSSION	44
CONCLUSIONS	53
SVENSK SAMMANFATTNING (SUMMARY IN SWEDISH)	54
ACKNOWLEDGEMENTS	57
REFERENCES	59
PAPER I-IV.....	

ABBREVIATIONS

Apo	apolipoprotein
AXA	axillary artery
BA	brachial artery
BMI	body mass index
BP	blood pressure
BSA	body surface area
C	control subjects
CRP	C-reactive protein
CC	cross-sectional compliance coefficient
DC	cross-sectional distensibility coefficient
DM	diabetes mellitus
FBF	forearm blood flow
FMD	flow mediated dilatation
FVR	forearm vascular resistance
GTN	glyceryl trinitrate
HR	heart rate
IGF	insulin-like growth factor
IGFBP	insulin-like growth factor binding protein
IL	interleukin
IMT	intima-media thickness
LD	lumen diameter
MAP	mean arterial pressure
MMP	matrix metalloproteinase
NO	nitric oxide
NMD	nitrate mediated dilatation
PWA	pulse wave analysis
PWV	pulse wave velocity
SLE	systemic lupus erythematosus
SMC	smooth muscle cell
TCC	terminal complement complex
TIMP	tissue inhibitor of MMP
TNF	tumor necrosis factor

ABSTRACT

The mechanical properties of the arterial wall are of great importance for blood pressure regulation and cardiac load. With increasing age, large arteries are affected by increased wall stiffness. Furthermore, atherosclerotic manifestations may increase the stiffness even further, both processes acting as independent cardiovascular risk factors affecting the arterial system in a heterogeneous way.

The aims of this thesis was to characterize the local mechanical properties of brachial artery (BA) with the aid of ultrasound technique and to evaluate the influence of 1) age, gender, sympathetic stimulation and examination site; 2) type 1 diabetes (DM) and its association to circulatory biomarkers; and 3) to evaluate the general properties of the arterial system with the aid of pulse wave velocity (PWV) as well as pulse wave analysis (PWA) in systemic lupus erythematosus (SLE) and correlate the arterial properties to disease activity and circulatory biomarkers.

In the most proximal arterial segment of the upper arm a pronounced age-related decrease in wall distensibility, increase in intima-media thickness (IMT), and a slight increase in diameter were seen. Sympathetic stimulation had no influence on wall mechanics. More distally in BA, no change in diameter, and only minor increase in IMT and decrease in distensibility were seen. No gender differences were found. These findings suggest that the principle transit zone between elastic and muscular artery behaviour is located in the proximal part of the upper arm.

Women with uncomplicated insulin-dependent DM had similar diameter, IMT and distensibility in their distal BA as controls, whereas flow-mediated dilatation (FMD) was slightly, and nitrate mediated dilatation (NMD) markedly reduced. NMD was negatively correlated with higher HbA_{1c} levels. Vascular smooth muscle cell function seems to be an early manifestation of vascular disease in women with DM, influenced by long-term hyperglycaemia.

Women with SLE had increased aortic PWV compared to controls, a finding positively associated with increased levels of complement factor 3 (C3), but not with disease activity. The increased stiffness of central arteries may be one factor contributing to the increased cardiovascular risk seen in SLE.

LIST OF PAPERS

This thesis is mainly based on the following papers referred to in the text by Roman numerals. The results from an additional study will also be presented.

I Bjarnegård N, Rydén Ahlgren Å, Sandgren T, Sonesson B, Länne T.

Age affects proximal brachial artery stiffness; differential behaviour within the length of the brachial artery?

Ultrasound Med Biol 2003; 29(8):1115-1121.

II Bjarnegård N, Rydén Ahlgren Å, Sonesson B, Länne T.

The effect of sympathetic stimulation on proximal brachial artery mechanics in humans - differential behaviour within the length of the brachial artery?

Acta Physiol Scand 2004; 182(1):21-27.

III Bjarnegård N, Bengtsson C, Brodzki J, Sturfelt G, Nived O, Länne T.

Increased aortic pulse wave velocity in middle-aged women with systemic lupus erythematosus.

Lupus 2006;15(10): 644-650.

IV Bjarnegård N, Arnqvist HJ, Lindström T, Jonasson L, Jönsson A, Länne T.

Impaired endothelial independent vasodilatation in women with type 1 diabetes.

Submitted

INTRODUCTION

The cardiovascular system

The cardiovascular system may be divided into four major components: the heart, the macro- and microcirculation and the lymph vascular system. In a human adult, about 5 litres of oxygenated blood is delivered to the microcirculation by the arterial system which has two interrelated functions: first, a conduit function that maintain an adequate blood supply to tissue and second, a cushioning function that dampens the intermittent ventricular ejections to a more continuous peripheral blood flow. The buffering of the stroke volume due to distension of preferentially large arteries with a concomitant volume increase (Windkessel effect), enhances cardiac performance (Nichols and O'Rourke 2005). The forward pressure and flow waveforms of ascending aorta are identical during early ejection phase. The speed of the pressure wave is however much higher and its reflection at bifurcations and peripheral resistance sites is summed to the outgoing wave (Figure 1), in sharp contrast to the reflected flow wave that is inverted and causes a reduction of the measured flow wave.

Blood pressure slowly rises in response to normal ageing, along with a reduction in pressure pulse amplification from central to peripheral arteries, which means that central and peripheral systolic pressure is almost equal in the elderly, in whom an isolated systolic hypertension is often found, resulting in increased pulse pressure, the pressure parameter most strongly associated with increased cardiovascular risk (Domanski et al. 1999). Elevated pulse pressure increases ventricular load, over time leading to target organ damage, such as left ventricular hypertrophy (Khattar et al. 1997) which together with a reduced diastolic pressure makes the myocardium more susceptible to ischemia. Systolic pressure is greatly influenced by the arterial wall distensibility, whereas end-diastolic pressure is determined by diastolic duration and rate of pressure fall, the latter in turn affected by peripheral resistance together with the mechanical properties of the arterial wall.

Structure of the arterial wall

The predominant elastic material found in the arterial wall is collagens and elastic fibres, which together with smooth muscle cells, proteoglycans, fibronectin and fibrillin contribute to the mechanical properties of the arterial wall (Nichols and O'Rourke 2005). The wall is organized into three concentric zones; the tunica intima, media and adventitia (Figure 2). The inner layer of intima consists of the endothelium, followed by a thin layer of connective tissue, and finally the internal elastic lamina, which is the demarcation between intima and media.

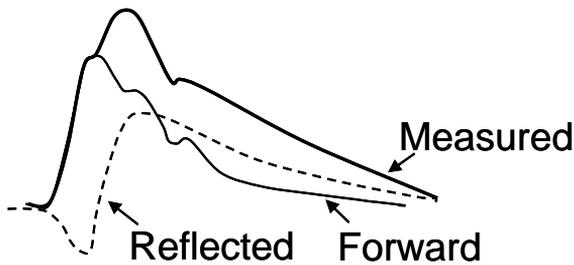


Figure 1 Schematic drawing of the aortic pressure wave in an elderly subject. The configuration of the measured wave is augmented by the reflected wave is added to the forward pressure wave during late systole and diastole.

The media forms the dominating part of the wall and is also the determinant of mechanical properties. It consists of collagen fibres that run spirally between circularly arranged layers of smooth muscle cells and elastic lamellae, which are linked to each other by fibrillin-1 and type-VI collagen containing bundles of microfibrils. The rest of the extracellular matrix volume is to a large part filled up with highly viscous proteoglycans and glycoproteins, which cushion smooth muscle cells (SMC) within the media. The outer elastic lamina demarks the border zone to the adventitia, the outer shelf of the arterial wall that blends with the connective tissue consisting of predominantly collagen, nerves and small blood vessels. The dry weight of the arterial wall consists of about 50 percent elastin and collagen, whereas the rest consists of smooth muscle cells and non-fibrous matrix. There are variations in the arterial wall structures within the arterial systems. Central elastic arteries like aorta and common carotid artery has a thicker intima and a media layer with

much more lamellae of elastic fibres than muscular arteries, such as the femoral and radial artery where numerous layers of smooth muscle cells are the dominating component of the media.

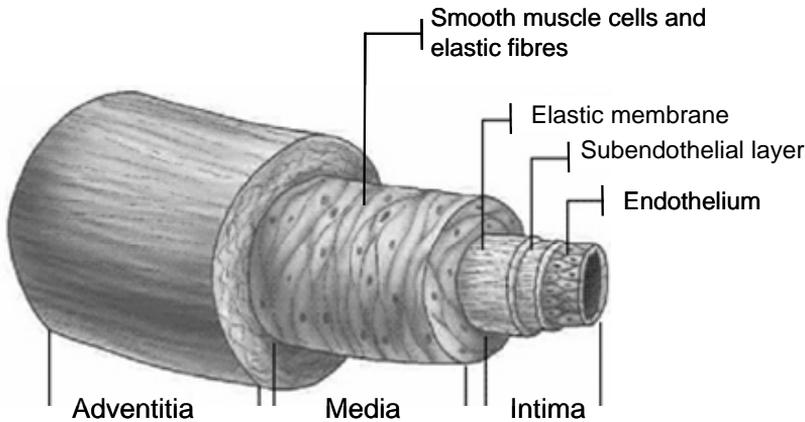


Figure 2 Schematic illustration of the arterial wall layers.

Mechanical properties of arteries

Young (1773-1829), but also other physicians and scientists like Moens and Kortweg explored much of the basic concepts about the relationship between elastic properties of arteries and the velocity of the pulse wave. More direct studies of the arterial wall properties were performed on excised isolated arteries first by Fuchs (1900), and later Bergel (1960), who found that the relative degree of arterial wall retraction differ within the arterial tree. The relation between the speed of the pressure wave, i.e. pulse wave velocity (PWV), and arterial wall elasticity was in 1878 described with the Moens-Kortweg equation. It was later modified (Bramwell and Hill 1922) and may be written as:

$$PWV = \sqrt{\frac{1}{\rho D}},$$

where ρ is the density of the blood, and D =distensibility. This means that the pulse wave travels faster in proportion to the decreasing distensibility of the vessel wall.

The arterial wall is able to distend under force and retract when the force is removed. The force per unit of area is named stress, which causes deformation of the wall material (Nichols and O'Rourke 2005). The relative degree of deformation from the "unstressed" state is called strain. The ratio between

stress and strain is used to calculate the elastic modulus (i.e. the stretch force per unit of cross-sectional area required to elongate a strip of vessel 100%), which describes the stiffness in materials with linear stress-strain relationships. The arterial wall in man shows a non-linear relation between stress (pressure) and strain, making the calculation of incremental elastic modulus a better option than Young's elastic modulus, as it is difficult to define the unstressed state in vivo (Figure 3). Because of the difficulty to measure the whole arterial wall thickness in vivo, Peterson et al. (1960) established the pressure strain elastic modulus (E_p), which relates pulse pressure to relative diameter change, but neglects wall thickness in the equation. The radial movement during the pulse wave propagation has been extensively studied (Figure 4), whereas the longitudinal movement of the arterial wall has been considered to be negligible until recently, when technical improvement has revealed a considerable longitudinal arterial wall motion in vivo (Cinthio et al 2006).

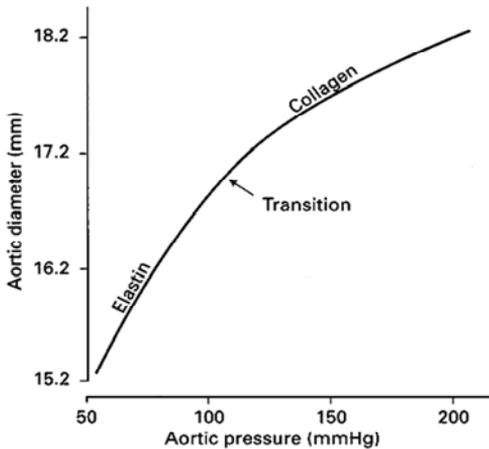


Figure 3 Pressure-diameter curve from abdominal aorta. The wall stiffens as the distending pressure increases (Länne et al. 1992).

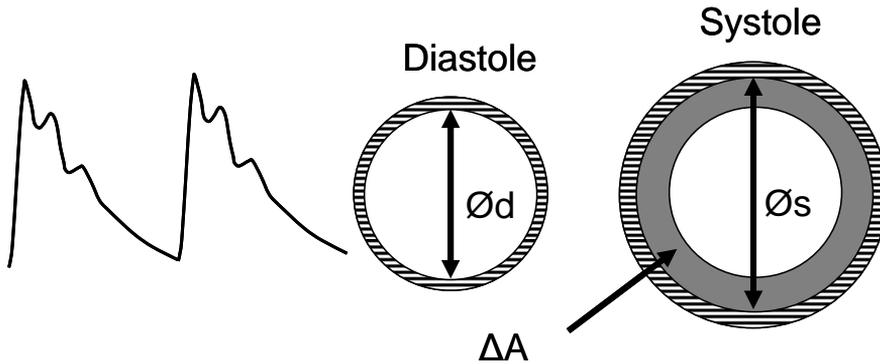


Figure 4 The arterial radial distension curve is obtained by recording the diameter change during the cardiac cycle. ΔA is the increase in cross-section area in response to the pressure pulse.

An important determinant of the passive mechanical properties of large arteries is the extra-cellular matrix, especially the relative amount of elastin and collagen (Nichols and O'Rourke 2005). In the proximal aorta, elastin is the dominant component, whereas collagen and smooth muscle cells predominate at peripheral sites. As collagen is about 100 to 1000 times stiffer than elastin, a considerable stiffening of the arterial wall is seen in young subjects from proximal aorta to the peripheral muscular arteries. Several papers have found that increased arterial stiffness independently predictive the risk of future cardiovascular events or all-cause mortality. A selection of such studies is compiled in Table 1.

Table 1 Longitudinal studies reporting an independent predictive value of arterial stiffness.

Method, site	Reference, year	Events	Cohort
PWV, Aorta	Blacher 1999	CV mortality	ESRD
	Laurent 2001	CV mortality	Hypertension
	Cruickshank 2002	All cause mortality	IGT, DM-2
	Willum-Hansen 2006	CV mortality	Gen population
Local CA dist	Blacher 1998	All cause mortality	ESRD
AP, Aorta	Chirinos 2005	CV event, mortality	CAD
PWA, Alx	Weber 2005	CV event, mortality	CAD

Alx, synthesized aortic augmentation index; AP, invasive aortic augmentation pressure; local CA dist, carotid artery distensibility; CAD, coronary artery disease; CV, cardiovascular; DM-2, type 2 diabetes mellitus; ESDR, end stage renal disease; IGT, impaired glucose tolerance.

Functional regulation of arterial size and mechanics

Distending pressure is the most important factor that influences the mechanical wall properties since it functionally decreases wall distensibility, because of the non-linear configuration of the pressure-diameter curve. The functional role heart rate *per se* has on arterial distensibility is less clear. In experimental studies in humans using pacing, central as well as peripheral distensibility has been found to decrease as heart rate exceeds 80 beats per minute (Giannattasio et al. 2003, Millaseau et al. 2005). This implicates that a significant shortening of the diastolic time interval probably decreases “operating” arterial distensibility.

Several vasoregulatory substances are synthesized by the endothelium, among them NO, that is considered to be the most important vasorelaxing factor with ability to regulate lumen diameter of arterioles and muscular arteries. Impairment of endothelium dependent NO-mediated vasodilatation is an early marker of endothelial dysfunction that accompanies vascular diseases such as atherosclerosis (Bonetti et al. 2003). Administration of an exogenous NO-donor such as nitroglycerin, increases arterial lumen, tensing the parallel collagen and elastin fibres, concomitantly as the reduced tension in smooth muscle has the opposite effect on distensibility (Bank et al. 1996, Bank & Kaiser 1998). The net effect alteration of smooth muscle tone has on arterial distensibility, differ between earlier studies, showing either increased (Bank et al. 1999), unchanged (Bank et al. 1995), or decreased (Peterson et al. 1960) distensibility in response to smooth muscle relaxation. Nevertheless, several studies have shown that sympathetic activation by different kinds of stimuli as well as vasoconstrictor drugs reduce arterial distensibility in human muscular arteries (Boutouyrie et al. 1994, Failla et al. 1997, Kelly et al. 2001), whereas sympathetic plexus anaesthesia increases distensibility (Failla et al. 1999). A similar response is not found in elastic arteries, where sympathetic activation or administration vasoactive drugs cause no pressure independent alteration of distensibility (Sonesson et al. 1997, Steward et al. 2003). Whether basal endogenous NO production regulates arterial distensibility in humans is still not proven, even if an experimental study suggests that local NO production is of importance (Schmitt et al. 2005).

Ageing of arteries

Ageing exerts a marked effect on the cardiovascular system in many ways. Nevertheless, it can be difficult to separate the effect of true normal vascular ageing from disease-related changes in Western populations. Cross-sectional studies have shown an age-related increase in arterial diameter, length, and wall thickness. This pattern has been most clearly demonstrated in elastic arteries and peripheral arteries within the lower extremities (Nichols and O'Rourke 2005). In elastic arteries, elastin become fragmented and degraded because of repeated mechanical loading and oxidative stress, and therefore replaced by much stiffer collagen with age, giving rise to decreasing wall distensibility. In addition, chemical degradation and calcification may stiffen elastic tissue. Increased metalloproteases activity and formation of advanced glycation end (AGE) -products are factors that have been suggested to further decrease elastin content and cause cross-links between collagen molecules within the matrix (Yasmin et al. 2005, Aronson et al. 2003). As a consequence of the age-related decrease in arterial distensibility, systolic pressure increases, causing a rise in pulse pressure, which is the most important blood pressure parameter for prediction of cardiovascular events in elderly subjects (Domanski et al. 1999). The augmented late systolic aortic pressure may enhance the effect of the normal age process that changes the passive diastolic properties of the left ventricle, and predisposes to left ventricular hypertrophy and interstitial fibrosis. Moreover, it may contribute to the increased incidence of diastolic dysfunction seen even in the absence of left ventricular hypertrophy in the elderly (Aronson et al. 2003). With a variety of techniques, it has been demonstrated that the aortic wall stiffens in a linear, or in elderly, even accelerated manner with age (Avolio et al. 1983, Sonesson et al. 1993, Rogers et al. 2001). In contrast to the marked age-related decrease of distensibility that is seen in elastic arteries, muscular arteries are much less affected. PWV along the arm and leg increases slowly with age in most population studies (Nichols and O'Rourke 2005), whereas local peripheral artery distensibility has been found to be either unaffected (Rydén-Ahlgren et al. 2001, Van der Heijden-Spek et al. 2000) or decreased in elderly subjects (Debasso et al. 2004). As a consequence of the altered arterial properties, pulse pressure amplification decreases, whereas augmentation index increases in response to ageing. Interestingly, central augmentation index seems to reach a plateau at about sixty years of age without increasing further in elderly subjects (Mitchell et al. 2004a, McEniery et al. 2005), suggesting that increases in central arterial stiffness and forward wave amplitude, rather than reflected

wave amplitude causes the increasing systolic pressure and pulse pressure seen at higher age. Also endothelial dependent vascular reactivity is affected by ageing. This is seen as a reduction in flow mediated dilatation (FMD) or less prominent forearm blood flow response to acetylcholine in middle aged and elderly subjects (Benjamin et al. 2004, Celermajer et al. 1994, DeSouza et al. 2002). In contrast to endothelial dependent dilatation, the response to nitroglycerin, which causes an endothelial independent dilatation, is rather well preserved in elderly subjects.

Diabetes and arterial disease

An increasing number of people worldwide are classified as having diabetes mellitus (DM), and the number of affected people is predicted to be 210 million in 2010 (Zimmet et al. 2001). The incidence of microvascular complications, seen as retinopathy, nephropathy and neuropathy is higher in DM with severe long-term hyperglycaemia, but also the classical cardiovascular risk factors are of importance for prognosis. In addition to microvascular complications, decreased arterial distensibility and vascular reactivity have been reported in DM, compared to age-matched healthy controls. A higher aortic PWV is usually present in DM, whereas central augmentation index is either unaffected (Lacy et al. 2004, Aoun et al. 2001), or increased (Wilkinson et al. 2000). Local mechanical wall properties is less studied in DM, showing either unchanged or decreased distensibility at different arterial locations (Kool et al 1995, Giannattasio et al. 1999, Henry et al. 2003), whereas impairment of endothelial function or increased carotid artery intima-media thickness (IMT) are usually seen in DM with microvascular complications, but not always in the absence of complications (Dogra et al. 2001, Ravikumar et al. 2002, Shivalkar et al. 2006). An important factor for the divergent results in earlier studies is probably the wide heterogeneity in inclusion criteria's. In most studies, data from males and females with DM are compiled, others mix type 1-and 2 DM, or include subjects with large differences regarding disease duration and complications, which makes it more difficult to analyse the interaction between different factors involved in their cardiovascular pathophysiology. Diabetes in general is an independent risk factor for cardiovascular disease, adding a 2-to-4-fold risk for cardiovascular events in an adult population, corresponding to the equivalent risk of ageing 10-15 years (Booth et al. 2006). A higher relative cardiovascular mortality-and morbidity risk is found in women than men with

type 1 as well as type 2 DM (Laing et al. 2003, Soedeman-Muthu et al. 2006, Juutilainen et al. 2004). In the study by Laing et al. (2003), the standardized mortality risk due to heart disease was 15 times higher in the cohort of type 1 DM women below 60 years of age. When the effect of gender was evaluated by Rydén-Ahlgren (1995), only females, but not males with type 1 DM, showed a decreased distensibility of elastic abdominal aorta and carotid artery, which speculatively link the decreased arterial distensibility to the higher cardiovascular risk seen in DM women. The pathogenesis of arterial arteriosclerosis and atherosclerosis is in many ways the same as in non-diabetic subjects. An important distinction is however the altered metabolic control with higher circulating levels of glucose and insulin that are usually seen in DM in general, and abnormalities within the IGF-system in type 1 DM (Hedman et al. 2004).

Arterial properties in connective tissue disease

An increased incidence of cardiovascular mortality has been reported in patients with autoimmune connective tissue such as rheumatoid arthritis (RA). Coronary atherosclerosis represents the main course of increased cardiovascular risk seen in RA patients (Solomon et al. 2003, Turesson et al. 2004), in whom lifespan is estimated to be shortened by 3-18 years (Van Dornum et al. 2002). Decreased arterial distensibility of elastic arteries (Turesson et al. 2005, Mäki-Petäjä et al. 2006), increased carotid IMT (Kumeda et al. 2002) and reduced FMD (Arosio et al. 2007) are usually found in RA patients. A less well-known systemic inflammatory connective tissue disease is systemic lupus erythematosus (SLE). Epidemiological studies suggest ethnics- and geographical differences in SLE prevalence. In Sweden about 50 per 100 000 individuals, predominantly women, are affected (Manzi 2001, Ståhl-Hallengren et al. 2000). High disease activity was earlier the main cause of premature death but improved treatment has reduced early mortality during the recent decades. Instead high rates of cardiovascular disease have come to light. Overall, patients with SLE have 5-10 times increased risk of coronary events than the general population (Jonsson et al. 1989, Sturfelt et al. 1992). Manzi et al. (1997) found a 50 times higher risk of myocardial infarction in 35-44 year old women with SLE in comparison to healthy women in the Framingham Offspring Study cohort. In a limited number of studies, large artery structure-and function of SLE patients has been compared with healthy controls, and increased IMT, higher prevalence of carotid plaques, and

impaired FMD have been reported (Roman et al. 2003, Colombo et al. 2007, Lima et al. 2002, El-Magadmi et al. 2004). As the reason for the accelerated atherosclerosis in SLE can not only be explained by traditional risk factors, it is possible that the disease *per se* or its treatment also could affect the mechanical properties of large arteries.

AIMS

1. To outline the local brachial arterial wall properties in healthy individuals and evaluate the effect of
 - age, gender and examination site along the upper arm
 - sympathetic stimulation

2. To study middle aged women with SLE and compare them with a control group without connective tissue disease in order to evaluate
 - regional arterial distensibility as well as timing and size of reflected waves
 - associations between arterial properties, disease activity and serological variables

3. To study young type 1 DM women and compare them with a healthy control group in order to evaluate
 - local mechanical and functional properties of the distal brachial artery
 - the relation between arterial properties and laboratory variables of specific interest in DM and cardiovascular disease

MATERIALS & METHODS

Subjects

Healthy subjects

Paper I

136 healthy non-smoking Caucasian volunteers, between 9 and 82 years old (52 males and 84 females) were examined. They were recruited among hospital staff, friends or through advertisement in the community. All were free from cardiovascular medication and none had a history of cardiovascular- or renal disease, diabetes or hypertension. Their systolic ankle / brachial pressure index were > 0.9 .

27 of the 136 subjects (11 females, range 22-70 years and 16 males, range 23-71 years) were also included in a sub-study where the accuracy of auscultatory blood pressure measurement was evaluated. Details regarding demographics and clinical data are given in the paper.

Paper II

18 healthy non-smoking Caucasian volunteers were studied. They were later compiled into either a young (n=9), mean age 25 years (range 23-30, five males and four females) or an elderly (n=9), mean age 69 years (range 67-72, four males and five females) group. None had a history of diabetes, cardiovascular- or renal disease, and all subjects were free from regular medication.

Paper III

27 Caucasian females, mean age 60 years (range 55-68), was recruited as control subjects from a cohort of registered volunteers at the Clinic of Obstetrics and Gynaecology, Lund University Hospital. None had a history of diabetes, cardiovascular- or connective tissue disease. Eight were smokers and two were under medical treatment with a β -blocker. In all subjects, the ratio between ankle and brachial systolic pressure was > 0.9 . Details regarding demographics and clinical data are given in the paper.

Paper IV

53 Caucasian female control subjects, mean age 34 years (range 22-45), were recruited among hospital staff and students. All were non-smokers, free from cardiovascular medication, with systolic ankle / brachial pressure index of at least 1.0. HbA_{1c} and fasting plasma glucose concentration were within the reference range. Details regarding demographics and clinical data are given in the paper.

Additional study

60 healthy non-smoking volunteers of both genders, 30 males (range 22-86 years) and 30 females (range 21-82 years), were studied. None of them had a history of diabetes, symptomatic cardiovascular-or renal disease, and their random capillary plasma glucose levels were <10 mmol/l. As blood pressure parameters and BA distensibility were similar in males and females, all subjects were compiled into three different age-categories, young (Y), middle aged (M) and elderly (E), which are presented in table 2.

Table 2 Demographics and clinical data of the healthy subjects included in the additional study.

Parameter	Y (n=20)	M (n=20)	E (n=20)
Male/female	10/10	10/10	10/10
Age, years	29±6	49±5	76±7
BMI, kg/m ²	23.6±2.3	25.0±2.4	23.6±2.9
Weight, kg	71±10	76±10	69±12
Height, cm	173±8	175±8	170±12
Heart rate, b/min	61±11	59±8	63±12
Systolic BP, mmHg	112±13	120±13	124±13
Diastolic BP, mmHg	61±7	71±9	64±8
Pulse pressure, mmHg	51±9	49±9	59±11
MAP, mmHg	78±8	88±10	84±8

Mean ± SD. Age categories; Y < 40; M 40-59; E > 60 years.

Subjects with systemic lupus erythematosus (SLE)

In paper III, 27 female Caucasian patients with SLE, mean age 60 years (range 52-68), median disease duration 15 years (range 3-47) were included and examined. All subjects were registered at the Department of Rheumatology, Lund University Hospital, four had a history of cardiovascular disease and eight were smokers. In all subjects, the ratio between ankle and brachial systolic pressure was > 0.9 . The cumulative organ damage of the SLE subjects was assessed by the Systemic Lupus International Collaborating Clinics/American Collage of Rheumatology Damage Index, SLICC/ACR-DI and ongoing disease activity was evaluated with the SLE Disease Activity Index, SLEDAI. All, except one subject, fulfilled four or more of the ACR criteria for SLE classification. Details regarding demographics and clinical data are given in the paper.

Subjects with type 1 diabetes mellitus

In paper IV, 37 type 1 DM women, mean age 34 years (range 21-45) were recruited from the Department of Endocrinology, Linköping University Hospital or Department of Medicine, City County Hospital Ryhov, Jönköping. All were non-smokers with median diabetes duration 18 years (range 8-39), without evident diabetic complications (except slight background retinopathy in 24 subjects). The ratio between ankle and brachial systolic pressure was at least 1.0 in all individuals. Details regarding demographics and clinical data are given in the paper.

Methods

All examinations were performed with the subjects in supine position after at least 10 minutes rest in a room, where the temperature was 22-24°C.

Non-invasive blood pressure

In paper I-II, auscultatory upper arm blood pressure was obtained with a sphygmomanometer. MAP was taken as the diastolic pressure plus one third of pulse pressure. In paper III-IV and the additional study, upper arm blood pressure was recorded with an oscillometric method (Dinamap PRO 200 Monitor, Critikon, Tampa, FL, USA). In order to calculate systolic ankle-brachial pressure index, a cuff placed around one ankle at the time was inflated simultaneously as the arterial pulsations on the foot were registered with a pen-Doppler.

Invasive brachial artery pressure

Using the Seldinger technique, the pressure catheter was inserted in the right brachial artery and the tip was placed in the middle portion of the artery. The invasive pressure was measured with a 3 F (SPC 330A) or 4 F (SPC 340) microtip catheter (Millar Instruments, Houston, Texas, USA), or with a fluid-filled catheter system (pressure monitoring kit DTX + with R:O:S:E, Viggo Spectramed, Oxnard, CA, USA). The frequency response of the Millar catheter (flat range 10 kHz) was higher than in the fluid-filled system (flat range 35 Hz). However, the amplitude was identical when the curves of one cardiac cycle from each pressure system, created by a blood systems calibrator (Bio Tech model 601A, Old Mill Street, Burlington, VT, USA), were superimposed on each other. In paper I, invasive and corresponding auscultatory blood pressure was measured consecutively three times in the right upper arm. In order to achieve pressure and diameter curves simultaneously (paper II), a data acquisition system, containing a personal computer (Express, Tokyo, Japan) and a 12-bit analogue-to digital converter (Analogue Devices, Norwood, MA, USA) was used.

Forearm blood flow

Venous occlusion pletysmography (Hokanson, EC-4, D.E. Hokanson Inc., Bellevue, WA, USA) was used to determine forearm blood flow with a strain-gauge placed around the left forearm, which rested comfortably slightly above the level of the heart. Occlusion of hand blood flow was accomplished by a wrist cuff inflated to 100 mmHg above systolic arterial pressure one minute before measurements, whereas venous occlusion was achieved by a blood pressure cuff applied proximal to the elbow and inflated to 50 mmHg for 8-10 seconds. Determination of forearm blood flow was made by the mean of two consecutive recordings. Forearm vascular resistance was calculated as mean arterial pressure (mmHg) divided by forearm blood flow ($\text{ml}/100\text{ml min}^{-1}$).

Lower body negative pressure (LBNP)

The lower body of each individual (to the level of mid-abdomen) was enclosed in an air-tight box connected to a vacuum source which permitted, within less than 5-10 seconds, a stable and defined LBNP of 60 cmH_2O (≈ 45 mmHg) to be produced (Taylor et al. 1992). The applied LBNP was continuously monitored with a manometer. LBNP, induce a rapid pooling of blood in the lower part of the body, with a decreased central blood volume as a consequence (Taylor et al. 1992). This leads to deactivation of central baro-receptors and sympathetic activation, shown as increased heart rate and forearm vascular resistance.

Measurement of local diameter change or wall thickness

Methodological background

The radial pulsatile arterial distension can be measured non-invasively. This was first described forty years ago followed by the introduction of the first phase-locked echo-tracking system (Arndt et al. 1968, Hokanson et al. 1972). Others have later modified the technique, using an echo-tracking device integrated with a modified ultrasound system, making it possible to detect arterial wall motion with very high resolution (Lindström et al. 1987, Bethin et al. 1991). Presently, several other types of techniques are available to detect arterial wall motion, such as Tissue Doppler Imaging (Schmidt-Trucksäss et al. 1998), M-mode registration with the use of automatic vessel wall algorithm

(Gamble et al. 1994), or wall tracking systems that uses radio frequency signal along a M-mode line to detect vessel wall movements or wall thickness (Hoeks et al. 1997).

Method

The Diamove that was used in paper I-II is an ultrasound echo-tracking system (Diamove, Teltec AB, Lund, Sweden), interfaced with a 5 MHz B-mode real time scanner (EUB 240, Hitachi, Tokyo, Japan). As the instrument is equipped with dual echo-tracking loops, it is possible to simultaneously track two separate echoes from opposite vessel walls with electronic markers that automatically lock to the luminal interface. The smallest detectable movement is 8 μm and the time resolution is approximately 1.2 ms. (Lindström et al. 1987, Bethin et al. 1991). The intra-observer measurement variability, assessed as the coefficient of variation, was found to be 14% in paper I when first and second registration of the pulsatile diameter change in the proximal region of the upper arm was compared (Bjarnegård et al. 2003).

In paper IV and the additional study, measurements were performed with an ultrasound scanner (Esaote AU5, Esaote Biomedica, Florence, Italy) equipped with a 7.5 MHz linear transducer. The scanner is connected to a PC, where the Wall Track System (WTS2, Pie Medical, Maastricht, The Netherlands) is installed. Details of the study technique have earlier been described (Kool et al. 1994, Hoeks et al. 1997). In short, ECG leads are connected to the subject. After visualisation of the artery in a longitudinal section, the scanner is switched to M-mode, and the M-mode line is positioned perpendicular to the anterior and posterior wall. A window of sufficient width to include the envelope from both anterior and posterior wall is chosen, and the radio frequency (RF) signal is transferred to the PC, where it is stored. Automatically, a sample volume is positioned on both the anterior and posterior wall. Manual adjustment of the sample volume can be made, before arterial distension waveforms are finally calculated and presented on the screen. As the Wall Track System measure the transition of the media-adventitia interface, the obtained diastolic vessel diameter will usually be higher than the measured lumen diameter from B-mode ultrasound (Segers et al. 2004.). In order to calculate intima-media thickness, the RF signal of the far vessel wall is stored separately with the adventitia-intima amplitude ratio set to 1.0. The averaged envelope for each heartbeat is presented off-line, and the mean far wall intima-media thickness is automatically calculated.

The coefficient of variation for absolute diameter change was 12% when first and second registration was compared in the distal segment at the upper arm.

B-mode ultrasound

A digital ultrasound system (HDI 5000, Philips Medical Systems, ATL Ultrasound, Bothell, WA, USA) equipped with a broadband linear transducer (L12-5) was used for scanning the left distal BA in longitudinal section. ECG leads are connected.

IMT and LD

In order to measure LD and far wall IMT, frozen end-diastolic images with special focus on lumen-intima echo and media-adventitia echo of the far arterial wall are saved for later analysis.

FMD and NMD

The transducer position is fixed by a stereotactic clamp after visualising the echoes of the anterior and posterior vessel wall, and care is taken not to compress the soft tissue of the arm. For FMD, B-mode images and flow velocity are first recorded at rest, followed by five minutes 200 mmHg inflation of the cuff placed on the proximal left forearm (Figure 5). In addition, the subject is squeezing a rubber ball 20 times between the third and fourth minute of ischemia in order to further augment post-occlusive hyperaemia (Betik et al. 2004). After cuff deflation, frozen B-mode images and flow velocities are stored again. After recovery, B-mode images of the artery are stored at baseline and after administration of 0.4 mg GTN sublingually.

Off line analysis

The digital B-mode images were calibrated and analysed with manually tracing of a cursor along the leading edge of the intima-lumen echo of the near wall, leading edge of the lumen-intima echo of the far wall, and for IMT, media-adventitia echo of the far wall (Artery Measurement System II, Image and Data Analysis, Gothenburg, Sweden). For calculation of FMD and NMD, care is taken to identify the same anatomical landmark in all subsequent images along a 5 mm long segment of the artery. During analysis, measurement window is hidden for the reader.



Figure 5. Experimental set-up during the flow-mediated dilatation (FMD) test. The inflated cuff obstructs forearm arterial inflow during 5 minutes. Release of occlusion pressure will induce hyperaemia, which enhance brachial artery shear stress and stimulate endothelial NO-release.

Measurement of regional and general arterial properties

Methodological background

The arterial pulse was recognized as the most fundamental sign of life already in early history. In the 19th century various types of sphygmographs were developed to analyse radial artery pressure waveform (Nichols and O'Rourke 2005). By simultaneous sampling of intra-arterial pressure waves from a peripheral artery and ascending aorta, a mathematical model can be created that predict aortic waveform from the peripheral waveform with the aid of an individual or generalized transfer function (Karamanoglu et al. 1996, Chen et al. 1997, Hope et al. 2007). Apart from aortic pulse pressure calculation, waveform analysis is a sensitive method that provides additional information about the complex coupling between cardiac performance and the geometric and mechanical properties of the arterial system.

For calculation of PWV, pulse transit time and distance between two arterial sites needs to be determined. This can be done by recording the same pressure wave simultaneous with two mechano-transducers (Asmar et al. 1995), or by sequentially measuring the duration from R-wave on the ECG to the foot of the pressure- or velocity wave from a proximal and distal site (Figure 6). Since higher PWV along the carotid-femoral segment (aortic PWV) has been convincingly shown to be an independent cardiovascular risk factor, PWV is

considered to be the “gold- standard” for measurement of arterial distensibility (Table 1). As there are at least three different ways to estimate the distance between carotid and femoral sites, and different algorithms are used for definition of the wave foot, universal reference values for aortic PWV are still missing (Laurent et al. 2006). PWV is also measured at other sites, such as along the carotid-radial and femoral-tibial segments, giving valuable data in pathophysiological and pharmacological studies, whereas its predictive value still needs to be proven.

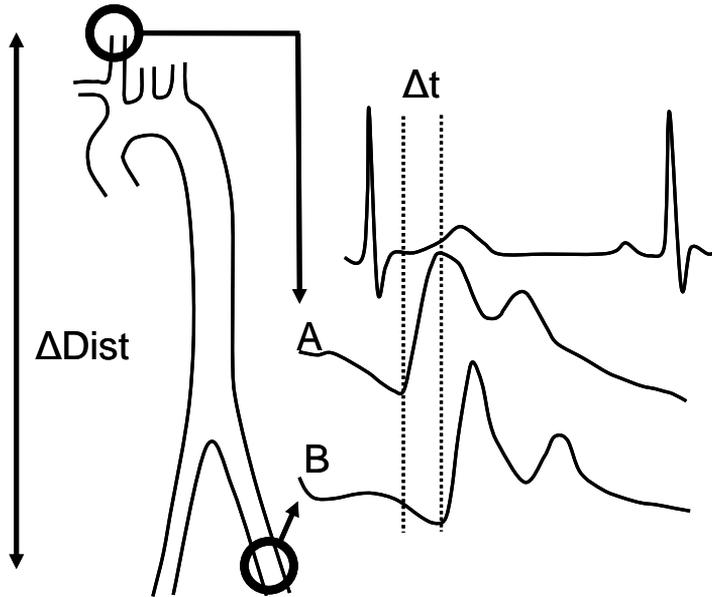


Figure 6. Schematic drawing of the pulse wave velocity method. A single tonometer is used to obtain the time delay (Δt) from R-wave on ECG to pulse wave arrival at the distal (B) and proximal site (A). Segmental length ($\Delta Dist$) is measured on the body surface.

PWA and PWV method

The SphygmoCor system (Model MM3, AtCor Medical, Sydney, Australia) equipped with a Millar pressure tonometer was used in order to derive non-invasively registered pulse waves, which were transferred on-line to a PC with software (SphygmoCor version 7.0) installed. For pulse wave analysis (PWA), the central pressure waveform was obtained by a generalized transfer function, calculated from a 10 seconds recording of the radial artery pressure waveform, which was calibrated with BA diastolic and systolic pressure from non-invasive oscillometric registration. Carotid artery pressure waveform was calibrated by taking mean arterial pressure (MAP) from the integrated radial

artery pressure curve in combination with diastolic brachial pressure (DBP). By connecting ECG to the SphygmoCor system, calculation of pulse wave velocity (PWV) is possible. Pulse wave transit time was achieved by recording duration from R-wave to intersection tangent of pulse wave arrival to proximal or distal sites during 10 seconds, whereas PWV distance was estimated by body surface measurements from the suprasternal notch to each pulse-recording site. Carotid → femoral (aortic PWV) and Carotid → radial (arm PWV) were automatically calculated (distance / time).

Calculations and data analysis

The local mechanical wall properties can be calculated from the absolute and relative arterial radial movement in combination with blood pressure recordings.

Absolute diameter change ($\Delta\emptyset$), fractional diameter change (strain) was defined as:

$$\Delta\emptyset = \emptyset_s - \emptyset_d$$

$$\text{Strain} = \frac{\emptyset_s - \emptyset_d}{\emptyset_d}$$

where \emptyset_s is the maximum systolic diameter, \emptyset_d is the minimum diastolic diameter and $\Delta\emptyset$ is pulsatile diameter change (all in mm).

The stiffness index (β) is an index of arterial stiffness, less influenced by distending pressure. It was used in vitro by Hayashi et al. (1980), and after modification, in vivo by Kawasaki et al. (1987):

$$\text{Stiffness } (\beta) = \frac{\ln(P_s - P_d)}{(\emptyset_s - \emptyset_d) / \emptyset_d}$$

where P_s and P_d are the systolic and diastolic blood pressure levels (mmHg).

The distensibility coefficient (DC) is the relative increase of arterial cross-section area for a given increase in pressure (Van der Heijden-Spek et al. 2000):

$$\text{DC} = \frac{2\emptyset_d\Delta\emptyset + \Delta\emptyset^2}{\Delta P \emptyset_d^2}$$

The unit for DC is $10^{-3}/\text{kPa}$.

In addition, the compliance coefficient (CC) was calculated. The compliance coefficient (CC) is the absolute increase in cross-section area for a given increase in arterial pressure, with the assumption that the length of the vessel is unaffected by the pulse wave. Consequently, measured change in cross-section area is supposed to correspond to the volume change per unit of length:

$$CC = \frac{\pi(2\Delta\varnothing d\Delta\varnothing + \Delta\varnothing^2)}{4\Delta P}$$

CC is expressed in mm²/kPa. $\Delta\varnothing^2$ is the square of the pulsatile diameter change (mm) and ΔP is pulse pressure (kPa).

In paper II individual pressure-diameter (P-D) curves were compiled and superimposed on each other by using the LOWESS (locally weighted regression scatter plot smoothing) method (Chambers et al. 1983) to form one typical P-D curve for each age group at rest and during LBNP.

Diastolic diameter of the BA in response to increased blood flow (FMD) or administration of 0.4 mg glyceryl trinitrate (NMD) was defined as:

$$FMD \% = 100 \left(\frac{(\varnothing_{45s} + \varnothing_{75s}) / 2}{\varnothing_{baseline}} - 1 \right)$$

where \varnothing_{45s} and \varnothing_{75s} is BA diameter (mm), 45 and 75 seconds after release of forearm arterial occlusion pressure.

$$NMD \% = 100 \left(\frac{\varnothing_{5min}}{\varnothing_{baseline}} - 1 \right)$$

where \varnothing_{5min} is BA diameter (mm) measured 5 minutes after the exogenous NO-donor is given.

Post velocity was defined as the sum of peak systolic and end-diastolic velocity (m/s) in BA during hyperaemia.

Velocity response (%) was defined as post velocity, divided by peak systolic velocity at baseline x 100.

PWA of the timing and amplitude of the returning reflection wave in central aorta during systole was defined according to Figure 7.

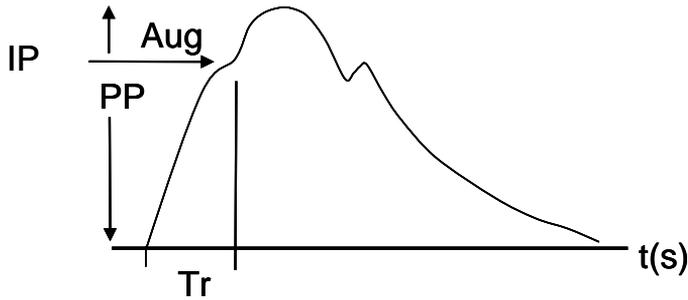


Figure 7 Aortic pressure waveform with late systolic pressure augmentation (Aug) from inflection point (IP) to peak systole (mmHg). Augmentation index (Alx%) was defined as Aug in relation to pulse pressure (PP), $Aug / PP \times 100$, whereas time to reflection (Tr) is the travel time (s) of the pressure wave to and from major reflection sites.

Analysis of the relative carotid pressure augmentation:

$$\text{Carotid Alx \%} = 100 \left(\frac{P_2}{P_1} - 1 \right)$$

P denotes the amplitude (mmHg) from the foot to the first (P1) and second (P2) systolic peak.

Laboratory analysis

Venous blood was drawn from the cubital vein in a fasting state.

In paper III routine analysis as well as CRP, IL-6 and TNF- α were measured in all subjects, whereas also complement C3, C4, C1q, C3dg, TCC and anti C1q were analysed in the SLE patients. All analysis were performed according to routine methods and assays used at the Department of Chemistry and Chemical Immunology, University Hospital of Lund.

The standard analysis in paper IV included lipids, haemoglobin, creatinine, glucose and HbA_{1c} from serum or plasma, analysed at Department of Clinical Chemistry, City County Hospital Ryhov, Jönköping or University Hospital of Linköping. Additionally, serum and plasma was stored at -70°C for later analysis. CRP, Apo A1 and Apo B were measured at the Department of Clinical Chemistry, Linköping, whereas serum concentration of MMP-3, MMP-9 and TIMP-1, C-peptide, Iso-insulin, Adiponectin, IGF-1, and IGFBP-3 were analysed at research laboratories at University Hospital of Linköping.

Statistics

Data are presented as mean \pm SD, unless otherwise stated. Differences of continuous variables between groups were tested with unpaired Student t-test or analysis of variance (ANOVA), unless otherwise stated. Adjustment for differences regarding confounding covariates between groups was made with analysis of covariance (ANCOVA). Post hoc tests according to Scheffe were used when the three age categories and measurement sites were compared in the additional study. In paper I and II Mann-Whitney U-test was used to evaluate the difference between invasive and non-invasive blood pressure or between the young and elderly group, whereas Wilcoxon's signed rank test was used to evaluate the effect of LBNP within each group.

Pearson's product moment correlation was used to test correlation between continuous variables, whereas chi-square test was used for categorical data. Multiple stepwise linear regression models were built to test how individual independent variables influence the dependent variable. For unevenly distributed variables, log transformation of the data was done before the parametric test. $P < 0.05$ was considered significant.

RESULTS

Healthy subjects (paper I-II)

Brachial artery properties – influence of age and gender

In the most proximal arterial segment of the upper arm, mean BA diameter was significantly larger in males 6.1 ± 1.0 mm, than in female subjects, 5.3 ± 0.8 mm ($p < 0.001$). When vessel diameter was related to body size, the gender difference was no longer seen. A slight but non-significant positive correlation between diameter and age ($r = 0.25$, $p = 0.10$) was seen in males, whereas no diameter increase could be detected in females with age.

Figure 8 shows DC in the proximal BA in relation to age in healthy males and females. Younger subjects had a higher DC, i.e. their wall was more distensible. DC decreased exponentially with age in both males and females without differences between genders. DC was mainly influenced by age (75-80%) and only to a small extent by MAP, in males by 8%, ($p < 0.001$) and females by 4% ($p < 0.001$), whereas BMI had no influence.

The stiffness (β) increased exponentially with age, approximately four times from 20 to 70 years of age in both males and females. No difference between genders was seen. Age was the dominating factor that influenced stiffness (75-80%), MAP was of minor importance in males (4%, $p < 0.01$), whereas MAP in females and BMI in both genders had no influence at all. An age-related decrease in CC was seen in both genders, but male subjects had higher compliance coefficient than females ($p < 0.001$). After adjustments for age, MAP and BSA, CC were still higher in males ($p = 0.04$). CC was negatively influenced by age (~50%) and MAP (~5%, $p < 0.05$). BMI had no influence at all.

Blood pressure parameters – influence of age and gender

In males, systolic pressure ($r = 0.54$), diastolic pressure ($r = 0.51$) and MAP ($r = 0.46$) increased with age ($p < 0.001$). No clear correlation between pulse pressure and age was noted in males ($r = 0.22$, $p = 0.12$). In female subjects, systolic pressure ($r = 0.55$), MAP ($r = 0.46$) and pulse pressure ($r = 0.55$) increased clearly with age ($p < 0.001$), while the increase in diastolic pressure was less important ($r = 0.26$, $p < 0.05$). No significant gender difference in blood pressure levels was seen.

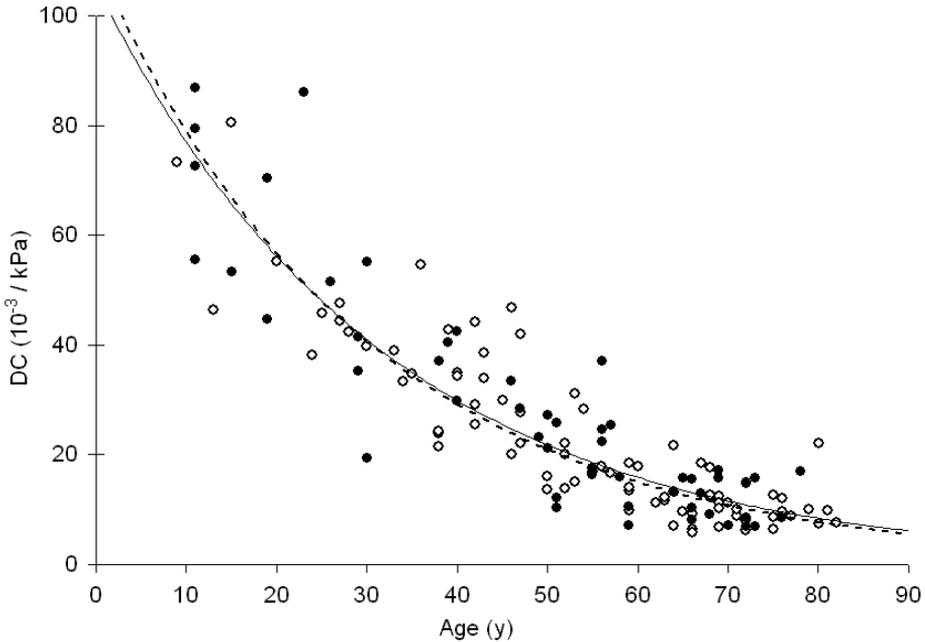


Figure 8. The distensibility coefficient (DC) of the proximal brachial artery in relation to age in 52 healthy males (•,—) and 84 females (○,---) Age was associated with a continuous reduction of arterial wall distensibility in both genders.

Comparison between invasive and non-invasive blood pressure measurements

There was a good agreement between invasive and non-invasive systolic pressure ($r=0.95$, $p<0.001$), with the non-invasive pressure being somewhat higher (on average 3 mmHg, 2%). These differences increased slightly with age ($r=-0.48$, $p=0.01$), without differences between genders. In the subgroup of young individuals ($n=8$, age 22-29 years) no difference between invasive- and non-invasive systolic blood pressures were found, whereas non-invasively measured systolic pressure was on average 6 mmHg (4 %) higher in the elderly subgroup ($n=10$, ages 66-71 years).

The diastolic pressure was lower when measured invasively in the brachial artery than when measured non-invasively in the upper arm at all ages (difference 12 mmHg on the average, 19%). In the younger group of individuals, non-invasively measured diastolic pressure was mean 8 mmHg higher, whereas this difference had increased to an average 14 mmHg in the group of older individuals. Thus, the difference between invasively-and non-

invasively measured diastolic blood pressure increased slightly with age ($r = -0.4$, $p = 0.02$) without differences between genders.

The resulting pulse pressure was systematically higher when calculated from invasively measured data, instead of using non-invasive measured data (an average difference of 10 mmHg, 16%). The difference was not influenced by either age or gender.

Table 3. Data on invasive blood pressure, heart rate, proximal brachial artery diameter and stiffness indices, forearm blood flow and vascular resistance, at rest and during LBNP in young and elderly individuals.

Variable	Young group (n=9)		Elderly group (n=9)	
	Rest	LBNP	Rest	LBNP
SBP, mmHg	115±12	109±13**	122±13	118±15
DBP, mmHg	59±7	62±6	60±8	65±9**
PP, mmHg	57±7	47±11*	62±8	53±10*
MAP, mmHg	79±8	79±6	86±11	88±11
HR, beats/min	55±8	71±18***	64±10	72±10***
BA Ø, mm	4.9±0.5	5.0±0.8	6.2±1.1§	6.3±1.1
DC, 10 ⁻³ /kPa	38.4±8.3	37.4±9.8	14.7±4.9§§§	12.0±4.1
Stiffness (β)	5.2±0.9	5.5±1.3	13.6±4.6§§§	16.1±4.7
CC, mm ² /kPa	0.65±0.13	0.66±0.22	0.40±0.14§§	0.35±0.12
FBF, %/min	3.29±1.81	1.83±1.22***	3.21±1.26	2.15±1.10***
FVR, mmHg ml ⁻¹ 100 g ⁻¹	31.2±17.5	62.4±41.7***	31.1±13.0	53.4±32.0***

Statistical significant difference between rest and LBNP * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and between young and elderly at rest § $P < 0.05$, §§ $P < 0.01$, §§§ $P < 0.001$.

Brachial artery properties in response to LBNP – influence of age

Table 3 shows heart rate, mean brachial diameter, blood pressure parameters, mechanical properties of the brachial artery, forearm blood flow and vascular resistance, at rest and during LBNP. MAP or brachial artery diameter were not affected. Heart rate increased ($p < 0.001$) during LBNP, 29% in the young and 13% in the elderly (NS). Further, systolic blood pressure decreased in the young ($p < 0.01$), but not significantly in elderly subjects, whereas pulse pressure decreased in both age groups ($p < 0.05$). Resting values of DC, CC and β were not different from values obtained during LBNP, neither in the young

or the elderly subjects, although a tendency to decrease in DC and increase in β ($p=0.11$) were seen in the elderly. The degree of response was however not different in the two age groups (NS).

Forearm vascular resistance increased markedly with 100 % and 72%, during LBNP in young and elderly subjects ($p<0.001$), without significant differences between the groups. The concomitant blood flow decreased in both groups ($p<0.001$), 44 % in the young and 33% in the elderly (NS difference between groups). Resting heart rate or blood pressure parameters were not different between the young and elderly group, whereas DC ($p<0.001$) and CC ($p<0.01$) were higher and β ($p<0.001$) lower in the young subjects.

Figure 9 shows the mean pressure-diameter (P-D) curves of the proximal brachial artery in young and elderly subjects at rest. With increasing age, the P-D curve became less steep, i.e. the proximal brachial artery wall becomes less distensible ($P<0.001$). The curves were essentially unaltered during LBNP in comparison to configuration at rest, regardless of age group.

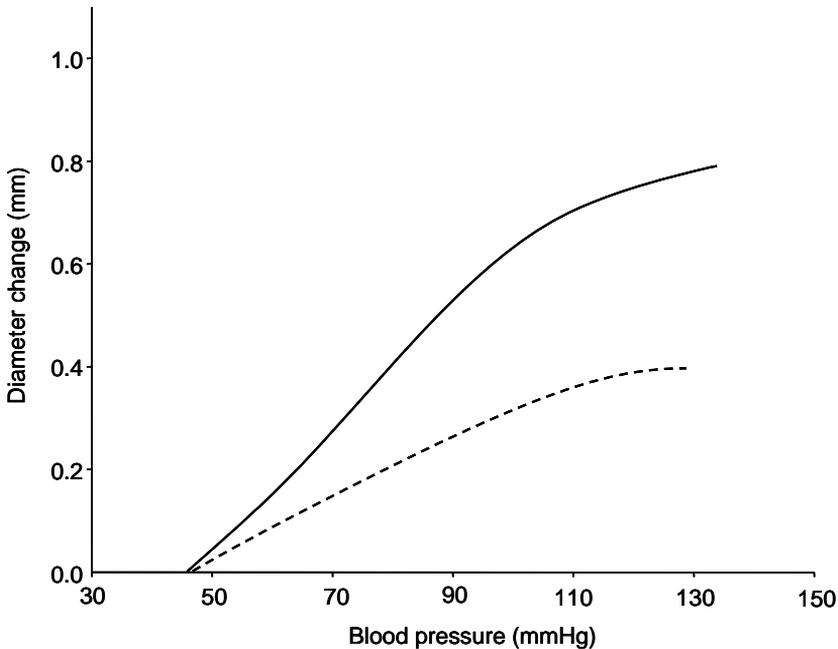


Figure 9. Pressure-diameter curve at rest in young ($n=9$, —) and elderly ($n=9$, - -) subjects.

Brachial artery wall properties along the upper arm – influence of age (additional study)

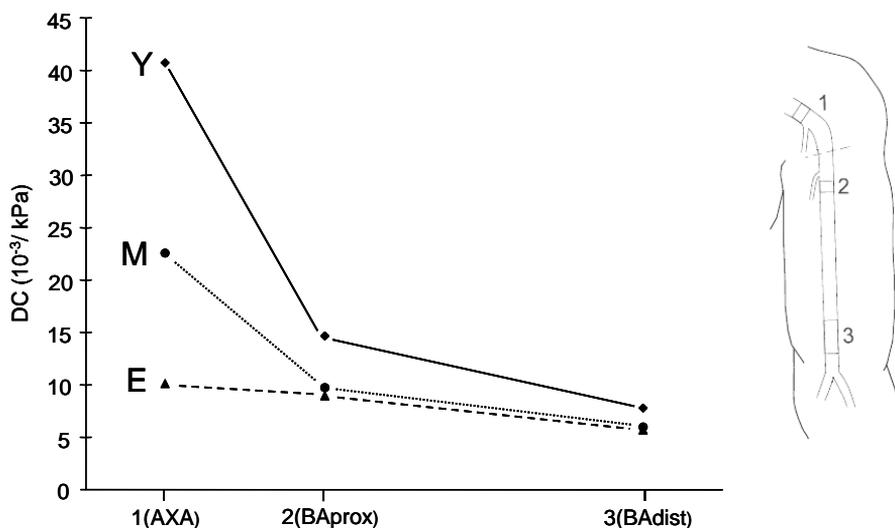


Figure 10 The three examination sites along the upper arm (**right**), defined as, 1 (AXA) 5-15 mm proximally from the origin of the subscapular artery, 2 (BAprox) 5-15 mm distal to the origin of deep brachial artery, 3 (BADist) 0-50 mm proximal to antecubital crease. Distensibility coefficient (DC) (**left**) decreases markedly between site 1 and 2 in age category young (Y) and middle aged (M) ($p < 0.001$), i.e. in subjects < 60 years. An additional drop in DC was seen from site 2 to 3 in all age categories ($p < 0.01$). Plots show mean values.

Table 4 shows the local wall properties along the upper arm in the healthy subjects.

DC at AXA decreased markedly, from 41 ± 10 in the young to 10 ± 4 $10^{-3}/\text{kPa}$ in the elderly ($p < 0.001$). DC was similar at BAprox and BADist in middle aged and elderly subjects, but slightly higher in the young than in the elderly, ($p < 0.05$) for BAprox and ($p < 0.01$) for BADist. The significant difference at BAprox disappeared after adjustment for MAP and BMI.

Figure 10 shows mean DC along the upper arm. DC dropped from AXA to BAprox and further to BADist in the young and middle aged, whereas in the E, DC were similar at AXA and BAprox, but dropped from 9.0 ± 3.4 to 5.7 ± 2.0 $10^{-3}/\text{kPa}$ ($p < 0.01$) between BAprox and BADist.

The diameter of AXA increased with age from 5.5 ± 1.0 mm in the young to 6.9 ± 1.5 mm in the elderly ($p < 0.01$), concomitantly as IMT increased from 0.40 ± 0.04 mm to 0.65 ± 0.15 mm ($p < 0.001$). Distally, BAprox and BADist had similar diameter in all age-categories, whereas IMT at AXA was higher in the middle aged than in the young, 0.49 ± 0.08 versus 0.40 ± 0.04 mm ($p < 0.01$), and in the elderly, 0.65 ± 0.15 mm, in comparison to the middle aged ($p < 0.001$).

Distally, IMT was higher in the elderly than in the young, both at BAprox 0.46 ± 0.13 versus 0.36 ± 0.02 mm ($p<0.001$), and BADist 0.43 ± 0.06 vs 0.36 ± 0.01 mm ($p<0.001$).

Figure 11 shows mean IMT and diameter along the upper arm. IMT as well as diameter decreased between distal AXA and BAprox, regardless of age-category ($p<0.001$). In contrast, no further change of diameter or IMT was found when travelling distally from BAprox to BADist within any age-category.

Table 4 Local geometrical and mechanical wall properties in the healthy subjects at three examination sites along the upper arm.

Variable	Y (n=20)	M (n=20)	E N(=20)
AXA Ø, mm	5.5±1.0	6.0±1.0	6.9±1.5¶¶
BAprox Ø, mm	4.1±0.8	4.2±0.7	4.2±0.9
BADist Ø, mm	3.9±0.7	4.1±0.6	3.8±0.5
AXA IMT, mm	0.40±0.04	0.49±0.08‡‡	0.65±0.15###
BAprox IMT, mm	0.36±0.02	0.39±0.03	0.46±0.13¶¶¶¶
BADist IMT,	0.36±0.01	0.40±0.05‡	0.43±0.06¶¶¶¶
AXA DC, 10^{-3} /kPa	40.7±10.0	22.5±6.8	10.1±3.8***
BAprox DC, 10^{-3} /kPa	14.7±8.8	9.7±4.5	9.0±3.4¶
BADist DC, 10^{-3} /kPa	7.7±2.0	5.9±2.0	5.7±2.0¶¶

Prox, proximal; dist, distal; Age categories, Y<40; M 40-59; E >60 years. Significant difference between age groups Y vs M †<0.05 ‡‡<0.01; Y vs E: ¶<0.05 ¶¶<0.01 ¶¶¶<0.001; M vs E: #<0.05 ##<0.01 ###<0.001; In between all groups ***<0.001.

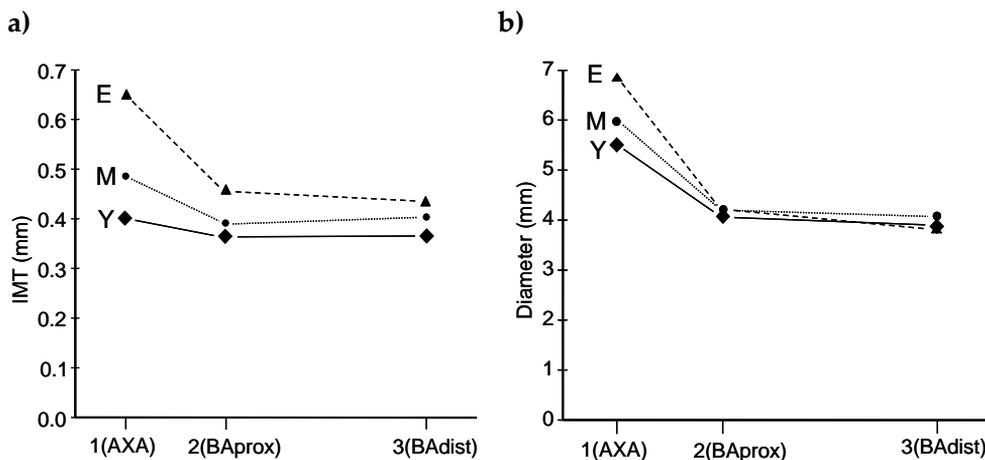


Figure 11 a) Intima-media thickness (IMT) and **b)** Diameter at the three examination sites. The arterial wall thickness and size decreased between site 1 and 2 in all age categories ($p < 0.001$). Plots show mean values.

Women with SLE (paper III)

Blood pressure data and pulse wave analysis (PWA)

There was no significant difference between SLE and control (C) subjects in blood pressure or pulse wave analysis data. Time to reflection (Tr) was 132 versus 134 ms, augmentation index (Alx) was 34 versus 33% and late systolic pressure augmentation (Aug) 16 versus 15 mmHg in SLE and controls, respectively (NS). Statistical adjustment for more frequent usage of blood pressure lowering drugs in SLE group (10 of 27 subjects) in comparison to C (2 of 27 subjects) did not change the results. Measured Alx in the carotid waveform was on average 41 % and 42 %, in the SLE and C, respectively (NS).

Pulse wave velocity (PWV)

Figure 12 shows aortic-and arm PWV in the SLE women and C.

Aortic PWV was higher in the SLE group than in C, unadjusted 9.8 m/s versus 8.2 m/s ($p < 0.01$), after adjustment for MAP and body mass index (BMI), 9.5 and 8.5 m/s ($p < 0.05$). Arm PWV did not differ between the groups, 8.4 and 8.5 m/s in SLE and C, respectively (NS).

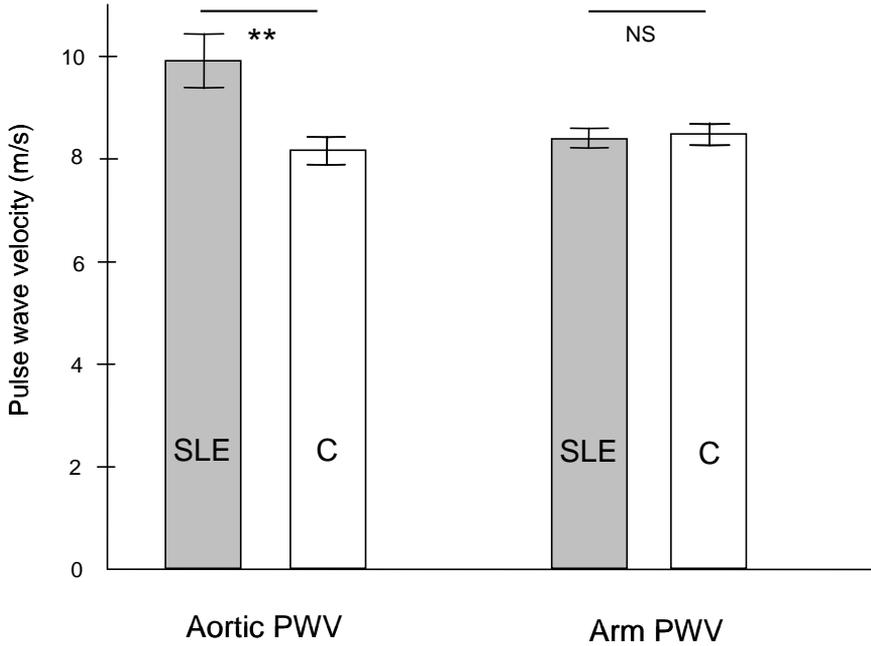


Figure 12 Aortic PWV in women with system lupus erythematosus (SLE) and controls (C). The aortic velocity is higher women with SLE than in controls ($p<0.01$). Mean \pm SE.

Serological parameters and relation to pulse wave data

CRP was higher in SLE than C; mean 8.8 versus 2.2 mg/l ($p<0.05$). After adjustment for BMI and MAP, the difference became less marked ($p=0.06$). After compiling data from all subjects, higher lnCRP was correlated with higher aortic lnPWV ($r=0.44$, $p<0.01$) and lower Tr ($r=0.39$, $p<0.01$).

The level of interleukin-6 in serum exceeded the lower threshold for detection, 2 pg/l in 73% versus 17% of subjects in SLE and C, respectively ($p<0.001$). No associations were found between arm PWV and calculated aortic pulse wave parameters in relation to serum levels of complement components C3, C4, C1q, C3dg, SLEDAI or SLICC/ACR-DI within the SLE group. There was a significant relation between serum levels of C3 and aortic lnPWV within the SLE cohort ($r=0.42$, $p<0.05$, Figure 13). No association was seen between aortic PWV and C4, C1q, C3dg and SLEDAI.

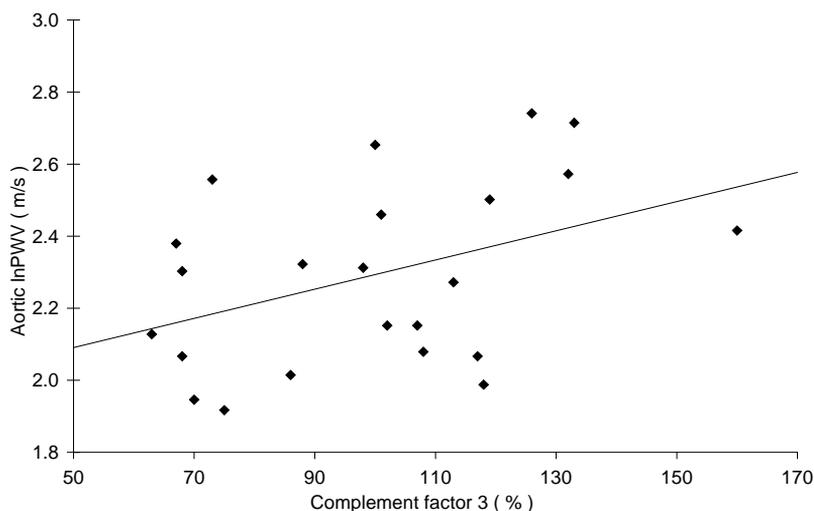


Figure 13 Correlation between complement factor 3 (C3) and aortic PWV in SLE women ($r=0.42$, $p<0.05$).

Women with type 1 DM (paper IV)

Table 6 shows heart rate, blood pressure and brachial artery data in women with type 1 DM and healthy controls (C). Resting HR, systolic and diastolic blood pressures were higher in DM than C ($P<0.05$). The size and IMT of the BA was similar in both groups, as well as the mechanical properties assessed as DC and CC.

Table 6 Heart rate, blood pressure and brachial artery data in the studied women.

Variable	C (n=53)	DM (n=37)	P
Heart rate (bpm)	63±8	67±8	<0.05
Systolic BP (mmHg)	106±11	111±11	<0.05
Diastolic BP (mmHg)	65±8	69±8	<0.05
Mean arterial pressure (mmHg)	79±9	83±9	<0.05
Pulse Pressure (mmHg)	41±7	42±5	NS
Lumen diameter (mm)	2.96±0.32	2.96±0.33	NS
Intima-media thickness (mm)	0.27±0.02	0.28±0.03	NS
Baseline velocity (m/s)	0.70±0.15	0.56±0.10	<0.001
Post velocity (m/s)	1.98±0.36	1.71±0.33	<0.001
Velocity response (%)	291±53	311±65	NS
Distensibility coefficient ($10^{-3}/\text{kPa}$)	8.2±4.4	7.2±4.0	NS
Compliance coefficient (mm^2/kPa)	0.57±0.30	0.58±0.26	NS

Figure 14 shows distribution of FMD and NMD in DM and C. Both FMD and NMD was lower in DM than C, $8.1\pm 4.3\%$ versus $10.3\pm 4.9\%$ for FMD ($p<0.05$), and $21.7\pm 6.6\%$ versus $31.4\pm 5.7\%$ for NMD ($p<0.001$). FMD was still significantly lower in DM after adjustment for BA velocity response, but not after adjustment for absolute flow velocity during peak hyperaemia.

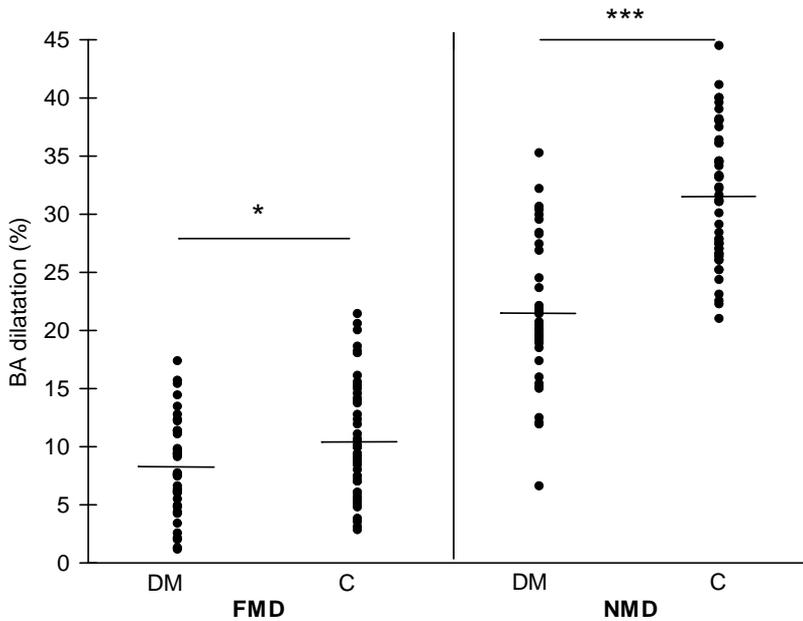


Figure 14 Flow mediated dilatation (FMD) and nitrate mediated dilatation (NMD) in women with type 1 diabetes (DM) and controls (C). The dilatation is expressed as percentage increase in brachial artery lumen diameter from baseline. Horizontal lines show mean value for each group.* $p<0.05$; *** $p<0.001$.

The DM had higher CRP (2.30 ± 2.7 (median 1.10) versus 1.45 ± 2.3 mg/ml (median 0.35), $p<0.05$), adiponectin (12.9 ± 4.3 versus 10.2 ± 4.3 $\mu\text{g/ml}$, $p<0.01$) and TIMP-1 (151 ± 25 versus 140 ± 21 ng/ml, $p<0.05$) than C. MMP-3 and MM-9 were similar in DM and C, 10.8 ± 6.1 versus 9.0 ± 2.8 ng/ml (NS) and 225 ± 95 versus 213 ± 117 ng/ml (NS), respectively. DM had lower IGF-1 (231 ± 65 versus 349 ± 68 ng/ml, $p<0.001$) and higher IGFBP-1 66 ± 34 and 26 ± 12 ng/ml ($p<0.001$) than C.

HbA_{1c} was positively associated to HR in both groups, $r = 0.34$ ($p<0.05$). In C, lnCRP were associated to lnBMI ($r=0.36$, $p<0.01$), lnMMP-9 ($r=0.42$, $p<0.01$) and TIMP-1 ($r=0.37$, $p<0.01$). FMD was negatively related to lnDC, $r = -0.36$ ($p<0.05$), $r = -0.27$ ($p<0.05$) in DM and C, respectively. There was a negative

association between $\ln\text{HbA}_{1c}$ and NMD, $r=-0.44$ ($p<0.01$, Figure 15) and $r=-0.30$ ($p<0.05$), in DM and C, respectively.

In a multiple stepwise regression model, $\ln\text{HbA}_{1c}$ (R^2 17%, $p<0.01$), LD (R^2 14%, $p<0.01$) and $\ln\text{MMP}$ (R^2 8%, $p<0.05$), were found to be negative independent predictors of NMD in the DM, whereas MAP and FMD were excluded from the model.

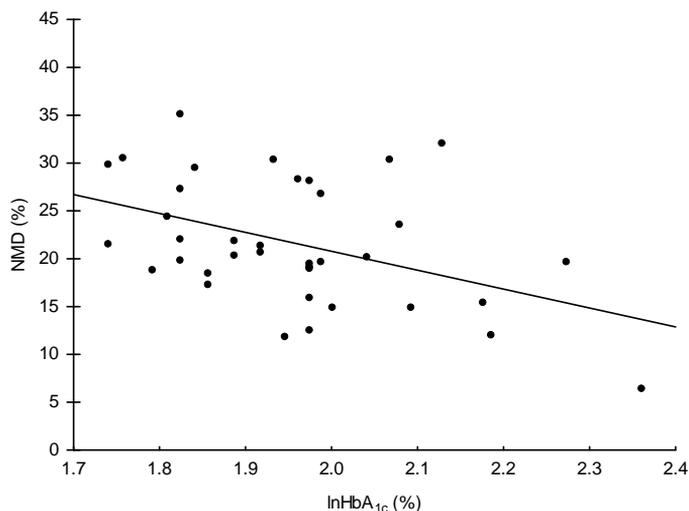


Figure 15 Correlation between glycosylated hemoglobin (HbA_{1c}) and nitrate mediated dilatation (NMD) of the brachial artery in women with type 1 diabetes $r=-0.44$ ($p<0.01$)

DISCUSSION

Brachial artery wall properties – influence of age and gender

It is well known that ageing exerts a marked effect on the cardiovascular system, although it can be difficult to differentiate manifestations of ageing *per se* from disease in elderly subjects. Several studies have demonstrated an age-related decrease of regional as well as local arterial distensibility of elastic arteries regardless of gender, concomitantly as arterial diameter and intima-media thickness increases (Sonesson et al. 1993, Hansen et al. 1995, Rogers et al. 2001, Åstrand et al. 2005). The peripheral arteries are less examined, but increasing diameter and wall thickness of the popliteal and common femoral artery have been reported with age, whereas ageing influence their mechanical properties in different ways. Strangely, a marked age-related decline of distensibility is seen only in the popliteal artery, in contrast to the common femoral artery where no obvious alteration is demonstrated. (Debasso et al. 2004, Rydén Ahlgren et al. 2001). In contrast to the arteries along the lower extremities, focal atherosclerosis or aneurysm formation are rarely seen in the arteries along the arm, making the muscular brachial artery, suitable as a model in experimental studies of vascular reactivity. The vascular endothelium is of special interest, since dysfunction seems to disturb vascular homeostasis, a condition that may precede atherogenesis (Bonetti et al. 2003). Results from earlier studies implicate that ageing has no or only minor influence on carotid-radial velocity and local radial artery distensibility (Boutouyrie et al. 1992, Bortolotto et al. 1999, Nichols and O'Rourke 2005), in sharp contrast to the age-related decline seen in elastic arteries. In the present study of healthy subjects, the distensibility within the most proximal part of the brachial artery decreased in an exponential manner with age in both genders (Figure 8). This finding differs from earlier data obtained more distally in the BA, where ageing seemed to have no distinct influence on local distensibility (Van der Heijden-Spek et al. 2000). In muscular arteries a reduction of sympathetic discharge by brachial plexus anaesthesia, increases distensibility (Failla et al. 1999), whereas sympathetic stimulation by cold pressor test, mental stress or smoking reduces arterial wall distensibility (Boutouyrie et al. 1994, Failla et al. 1997, LaFleche et al. 1998). Thus, sympathetic nerve activity modulates mechanical wall properties of muscular arteries. Sympathetic stimulation caused by LBNP did however not affect the

mechanical properties in the proximal part of BA. This finding together with the pronounced reduction in distensibility with age fits well with a more elastic than muscular artery behaviour (Sonesson et al. 1997). Since differences in sympathetic discharge have been shown between young and old subjects, this might influence arterial distensibility (Ng et al. 1993, Dinneno et al. 2000). No differences in resting FVR between young and elderly subjects was however seen, and LBNP-induced sympathetic stimulation caused no change in the pressure-diameter relation either in young or old subjects, despite a significant elevation of heart rate and FVR. Thus, it seems unlikely that smooth muscle tone should have any major impact on the age-related drop in local proximal BA distensibility (Figure 8). As our data differed from those obtained more distally in the BA by Van der Heijden-Spek et al. (2000), we extend our study on healthy volunteers in order to characterise the artery at three well-defined sites along the upper arm (Figure 10). The results confirmed earlier data and showed that ageing has only minor influence on distal BA wall mechanics (Van der Heijden-Spek et al. 2000, Figures 10-11).

Apart from the fatiguing effect of cyclic stress and changed wall composition (Nichols and O'Rourke 2005), ageing may lead to accumulation of smooth muscle cells and atherosclerosis, indicated as increasing IMT, which was more obvious in distal AXA than distal BA (Figure 11). In theory, wall thickness influence the stiffness of the wall, but in the present study, the IMT variations that were present within the healthy cohort did not restrict wall distensibility at any of the three examination sites (data not shown), which is agreement with earlier findings at other arterial locations (Riley et al. 1997, Jogestrand et al. 2003). Data from the carotid artery, an area more susceptible to atherosclerosis than the upper arm suggest that IMT must exceed a threshold thickness that represent an atherosclerotic manifestation before distensibility is affected (Bots et al. 1997, Giannattasio et al. 2001, Popele et al. 2001). The mechanical properties of the proximal BA (paper I and II) and distal AXA (Figure 10) are closely related to the behaviour seen in the adjacent elastic carotid artery (Pannier et al. 1995, Hansen et al 1995), suggesting that the principal transit zone between elastic to predominantly muscular artery behaviour is located in the proximal BA, close to the origin of the deep brachial artery.

Arterial wall properties in SLE

Inflammation-and immune system activation is regarded to be important components involved in the development of atherosclerosis (Libby et al 2002). A relation between low grade inflammation (e.g. assessed as circulating C-

reactive protein), and aortic stiffness has been found in a population sample by determining PWV (Yasmin et al. 2004). Rheumatoid arthritis (RA), the most common inflammatory connective tissue disease, is characterized by an increased incidence of cardiovascular disease. An even higher relative risk is seen in SLE, a less common autoimmune connective tissue disease, where patients have 5-10 times higher risk of coronary events than the general population, which can not only be explained by classic risk factors (Jonsson et al. 1989, Bruce et al. 2000). Arterial distensibility has earlier been reported to be decreased in RA and systemic vasculitis (Turesson et al. 2005, Booth et al. 2004). Our present finding of higher aortic PWV implies decreased aortic wall distensibility in the SLE cohort, strengthening the opinion that inflammatory connective disease is characterized by premature arterial stiffening. It may seem surprising however, that aortic pressure and pressure waveform were similar in SLE patients and controls, since a correlation between aortic Alx and PWV has been reported earlier (Yasmin et al. 1999). However, Alx is apart from PWV also affected by reflected wave velocity in peripheral arteries as well as peripheral resistance and location of reflection sites. Kelly et al. (2001) showed that vasoactive drugs influenced Alx and arm PWV more markedly than aortic PWV. We can not rule out that the higher consumption of antihypertensive drugs among the SLE patients could have decreased their aortic pressure augmentation, and covered a true difference between groups. Correction for this potential confounder did not change the result however, but the limited number of participants makes such analysis doubtful. Moreover, an age-related increase in aortic PWV is found without an accompanying decrease in time to reflection or increase of Alx after middle age, suggesting that the main reflecting sites move distally (McEniery et al. 2005). The increased aortic PWV in SLE might thus be accompanied by a change in reflective sites, explaining the mismatch between PWV and PWA.

There are several possible mechanisms for the increased aortic PWV in SLE. One is immune complex formation with activation of complement, attracting leukocytes with activation of Fc-receptors, inducing elastase production, which would result in elastin degradation and possibly increased aortic stiffness (Yasmin et al 2005). Decreased anti-tropo and anti- α -elastin serum antibodies have been found in SLE, suggesting a diminished production and enhanced degradation of elastin (Colburn et al. 2003). These observations are in line with our finding of an increased aortic PWV.

Earlier studies have found a diminished BA FMD response in SLE patients, indicating an impairment of endothelial function (Lima et al. 2002, El-Magadmi et al. 2004). Endothelin-1 (ET-1) stimulates proliferation and

contraction of vascular smooth muscle cells and is released in greater amounts from endothelial cells in contact with serum from SLE subject than healthy controls (Yashio et al. 1995). In experimental studies ET-1 has been shown to increase PWV (McEniery et al. 2003). Data on ET-1 production and its ability to modulate the arterial wall in SLE is however unknown at present. It is still not clarified whether basal NO production directly influences arterial distensibility in humans, even if some experimental studies suggest that NO inhibition causes a pressure independent decrease of arterial distensibility (Stewart et al. 2003, Schmitt et al. 2005). Since arm PWV as well as blood pressure were similar in SLE and control subjects, it is not likely that differences in NO or ET-1 synthesis can explain the higher aortic PWV in SLE.

An extensively studied marker of inflammation is CRP that rapidly increases in the liver in response to cytokine release from the immune system at sites of tissue injury. One main physiological function of CRP is the activation of the complement system, where component 3 (C3) plays central role. Our observation of a strong positive correlation between serum complement factor 3 (C3) and CRP in the SLE cohort was thus expected. In accordance with Selzer et al. (2001) we found a positive relation between C3 and aortic PWV, which may seem surprising since SLE disease activity is usually associated with low levels of C3, and moreover, rarely elevated CRP levels (Barnes et al. 2005). However, in a multivariate analysis, BMI and MAP, but not C3 were independently related to aortic PWV, suggesting that there is no causative relation between C3 and aortic PWV. Since earlier studies have shown that higher levels of C3 and CRP are related to several classical cardiovascular factors as well as established atherosclerosis, we believe that such risk factors have greater influence on the positive relation between aortic PWV and C3 than SLE *per se* (Ridker et al. 2004, Onat et al. 2005, Wärnberg et al. 2006). Moreover, no other complement components or cytokines were related to aortic PWV, ongoing disease-or cumulative organ damage score.

Higher prevalence of carotid plaques, a factor that might reduce local arterial wall distensibility, has earlier been reported in women with SLE (Roman et al. 2001, Giannattasio et al 2001). Only a few SLE subjects had a history of cardiovascular disease. Even if major peripheral arterial occlusive disease were excluded by ankle- and arm blood pressure measurements, we can not rule out a possible existing difference between groups regarding atherosclerosis.

Several individuals in the SLE group were taking glucocorticoids- and antimalarial drugs to suppress disease activity. Glucocorticoids may have

negative influence on risk factors for cardiovascular disease and prolonged treatment may be linked to subclinical atherosclerosis and cardiovascular mortality (Petri et al. 1994, Jonsson et al. 1989, Manzi et al. 1999). Whether these drugs indirectly influence arterial stiffness and pressure wave reflection remains unknown.

We conclude that post-menopausal women with SLE have higher aortic PWV, even after correction for blood pressure and BMI, indicating decreased distensibility of their central elastic arteries. This may contribute to the increased cardiovascular risk seen in SLE.

BA wall properties in insulin-dependent DM

Female patients with type 1 DM have a higher increase in cardiovascular risk than males, the reason being unclear at present (Laing et al. 2003, Soedamah-Muthu et al. 2006). Microcirculatory disturbances as well as premature atherosclerosis may contribute to the increased prevalence of myocardial infarction, stroke and amputation. In addition, large artery stiffness may be of importance since it seems to be increased in central elastic arteries of diabetes patients in general (Aoun et al. 2001, Lacy et al. 2004), and more interestingly, selectively in diabetes patients of female, but not male gender (Rydén Ahlgren et al. 1995, De Angelis et al. 2004). In order to further evaluate the effect of female gender in type 1 DM, we studied the macrovascular function in the brachial artery (BA) on females without clinical evidence of vascular complications or other cardiovascular risk factors except diabetes. We found a preserved BA distensibility, which may be explained by 1) the selection of subjects, and 2) the fact that the distensibility of distal BA is little affected by ageing, in contrast to central arteries (Van der Heijden-Spek et al. 2000), possibly making the BA less sensitive to accelerated vascular aging, which is proposed to occur in DM (Cameron et al. 2003). Our finding is in agreement with data from Kool et al. (1995), who analysed data where both genders were compiled. Thus, the mechanical properties of the distal BA seem to be well preserved in DM patients without evident complications, women not excepted.

FMD was reduced in the DM women (Figure 14), implicating an impaired endothelial function (Coretti et al. 2002). The FMD impairment was however rather subtle. Experimental studies indicate that acute hyperglycaemia may impair FMD, also in non-diabetic subjects (Kawano et al. 1999), whereas normalization of glycaemia in combination with antioxidant treatment

improves FMD (Ceriello et al. 2007). Thus hyperglycaemia may induce oxidative stress that affects endothelial function negatively. The long-term consequence of poor glucose control can result in accumulation of AGE-product that forms cross-links between matrix proteins (Schalkwijk et al. 2005). The question is whether the slightly lower FMD response we observed is a feature of the DM per se, or a consequence of hyperglycaemia. No correlation between plasma glucose level and FMD was however found, but HbA_{1c} was negatively associated to FMD. We can thereby not exclude that the divergent FMD response in the DM and C was influenced by differences in glucose and insulin levels, even if high p-glucose levels during the experiments were an exclusion criteria. Confounding factors that may have influenced the FMD response are resting BA diameter and relative flow velocity response during hyperaemia. These variables were however similar between groups, but correction for the lower absolute flow velocity in DM during hyperaemia reduced the difference in FMD response between the DM and the C to a non-significant level (Mitchell et al. 2004b).

There was a striking decrease in responsiveness to exogenous NO-donor in the BA of DM women (Figure 14). Instead of a lower FMD/NMD ratio, we found a trend to increased ratio in the DM women, suggesting that the main impairment is related to structural or functional factors at the smooth muscle cell level rather than reduced production, or bioavailability of endogenous NO. Earlier studies have shown a dominating endothelial dysfunction in DM, but none, or only minor reduction in vascular smooth muscle cell responsiveness (Meeking et al. 1999, Dogra et al. 2001). All these studies have however compiled cohorts of DM men and women. Thus, this may be a specific finding for the female DM. As IMT and DC were unchanged, concomitantly the impaired dilatory response may reflect decreased smooth muscle cell responsiveness to NO, or attenuated biotransformation of GTN to NO metabolites within the vascular smooth muscle cell. HbA_{1c} was independently correlated with NMD in a negative manner (Figure 15). A negative correlation has earlier been reported between NMD and short-term glycaemic control in patients with type 1 diabetes (Sörensen et al. 2005), but to our knowledge this is the first study to find a correlation between long-term glycaemic control and NMD in DM (Figure 15). The finding may be of significance since a reduced NMD response is associated with cardiovascular risk factors as well as higher incidence of future cardiovascular events in patients with coronary artery disease (Adams et al. 1998, Schächinger et al. 2000, Jarvisalo et al. 2004). The decreased NMD response seen in the DM patients should be interpreted with some caution, since GTN was

administered sublingually. The response to sublingual GTN seems however to be consistent between three to nine minutes after administration in DM subjects (McVeigh et al. 2002).

A higher resting HR has convincingly been shown to predict cardiovascular risk in men, but seems to be a risk factor also in women (King et al. 2006). The higher HR in the female patients with DM, is in accordance with some earlier studies (Ittersum et al. 1998). Spectral analysis of heart rate have demonstrated a reduction in high frequency power and correlation between resting heart rate and decreased response in autonomic function tests in DM patients without clinical evidence of autonomic dysfunction, suggest that parasympathetic dysfunction might contribute to the elevated resting HR seen in the DM patients (Ittersum et al. 1998, Weston et al. 1998). Moreover, parasympathetic activity is influenced by cardiovascular fitness, and we can not rule out the possibility that the increase in HR in DM women may reflect a difference in aerobic capacity between groups rather than incipient parasympathetic dysfunction, but the difference in resting HR were still present after correction for aerobic capacity on bicycle (data not shown). Finally, baseline sympathetic tone may be of importance for resting HR. Even if data on sympathetic tone is missing in the present study, it has previously not been found to be higher in uncomplicated type 1 diabetes (Fagius et al. 2000).

IGF-1 is a mitogenic peptide with endocrine effects, stimulated by growth hormone secretion. Only a small portion of the circulatory IGF-1 is bioactive but the measured total IGF-1 correlates to the concentration of free bioactive IGF-1. A reduced IGF-1 has been suggested to be involved in the pathogenesis of atherosclerotic events (Juul et al. 2002). The female patients with DM had decreased serum levels of IGF-1. It is conceivable that low total IGF-I and high IGFBP-1 negatively affects the vascular smooth muscle cells function, even if we failed to find any association between NMD and IGF-1 in the present study, when the DM and C cohort was analysed separately. MMP-9 in serum, a zinc dependent endopeptidase degrading extra-cellular matrix proteins, has been found to positively correlate with aortic PWV (Yasmin et al. 2005), MMP-9 was found to negatively affect NMD in the DM although similar MMP-9 levels were seen in DM and C. Higher serum levels of TIMP-1 have been shown to be independently associated with the presence of carotid atherosclerosis, and the increase in TIMP-1 in the women with DM, suggests that TIMP-1 may constitute an early marker of vascular remodelling (Zureik et al. 2005). The higher circulating levels of adiponectin and CRP in the DM are in agreement with earlier findings (Schalkwijk et al. 1999, Lindström et al.

2006). When the DM and the C were analysed separately, no association was however seen between these two markers and BA wall properties.

To summarize, BA responsiveness to exogenous NO-donor was markedly reduced in patients with type 1 diabetes without evident complications, despite only limited reduction in endothelial dependent dilatation, unaltered arterial size, stiffness and wall thickness. A negative association between the GTN response and HbA_{1c} suggests that poor long-term glycaemic control in DM impair vascular smooth muscle cell function.

Methodological considerations and limitations

Correct measurement of the local blood pressure is crucial for accurate calculation of the local vessel wall distensibility and invasive pressure is gold standard. Intra-arterial BA pulse pressure (PP) was about 10 mmHg higher than auscultatory PP obtained with a sphygmomanometer. This was not affected by age and BA distensibility was thus systematically underestimated when based on auscultatory pressure determination (paper I). To increase accuracy in the pressure measurements and eliminate observer bias, upper arm blood pressure was later recorded with an oscillometric device (paper III-IV and additional study) that uses an algorithm tested against intra-arterial pressure, which means that data obtained with these two non-invasive methods are therefore not comparative (Lehmann et al. 1998, Ni et al. 2006).

Ascending aortic pressure was synthesised by sampling the radial artery pressure wave with an external tonometer and apply a generalized transfer function (Sphygmocor). This technique predicts central pressure with acceptable accuracy when calibrated against invasive radial pressure (Pauca et al. 2001, Sharman et al. 2006). When the tonometer derived radial pulse pressure wave is calibrated from systolic- and diastolic non-invasive upper arm pressure, an underestimation of central aortic pulse pressure is often seen, most apparent with the auscultatory technique (Cloud et al. 2003, Hope et al. 2004), but partly also because of the minor systolic pressure augmentation from brachial to radial artery (Verbeke et al. 2005). Further, the use of a generalized transfer function may be questioned since it assumes that amplification of pressure waves as a function of frequency is independent of age, gender or disease state (Hope et al. 2007). Since PWA of the carotid pressure wave as well as the synthesised aortic pressure wave were similar in SLE and control subjects, the result was not biased by use of the transfer function (paper III). Furthermore, the influence of HR (due to changes in

ejection duration), and height (due to changes in distance to point of reflection) must be taken into consideration (McGrath et al. 2001, Wilkinson et al. 2002). Since these variables were similar, they had no major influence on the comparison of PWA data between the groups (paper III).

For calculation of PWV, pulse transit time and distance between two arterial sites need to be determined. In order to record the arrival of the pressure wave at two arterial sites simultaneously, two transducers are needed. Another option is to record pressure waves, distension- or Doppler waveform sequentially from different arterial sites by measuring the duration from R-wave on the ECG to the foot of the pressure wave at the proximal and distal site in order to calculate the time delay between the two sites. High quality recordings and clear definition of the foot of the wave is needed for reliable calculation of the pulse transit time, and the second derivative and intersecting tangent methods show best reproducibility (Chiu et al. 1991), but occasionally also these algorithms fail. Further, the distance between the two arterial sites must be defined correctly. The distance is measured at the surface of the body with a measuring tape or stick. This is less accurate if the vessels are tortuous or the subject obese (Wenn et al. 1990, Van Bortel et al. 2002). Especially in obese subjects, it is important to measure the straight horizontal distance between the two sites without following the body surface to avoid overestimation of the segmental length. Despite these potential confounders, PWV measurement is considered to be the “gold standard” for measurement of regional arterial distensibility (Laurent et al. 2006).

FMD may be calculated by relating baseline BA diameter to either peak BA diameter during hyperaemia, or the BA diameter at a certain time-point after cuff-release. In this study the average BA diameter at 45 and 75 seconds post-occlusion were taken as the subject’s post-occlusion diameter since diameter increase usually peaks within this time-interval. To increase hyperaemia, a short-term dynamic hand exercise was added during the ischemic period to further stimulate blood flow increase after cuff release. The ischemic hand-grip exercise diverges from the standard FMD procedure and may have induced a dilatation that is not completely NO-dependent (Agewall et al. 2002).

CONCLUSIONS

1. Local proximal brachial artery (BA) wall distensibility is similar in males and females, without differences in the rate of age-related decline. Ageing appears to influence the arterial wall much more proximally than distally along the upper arm, seen as a pronounced decrease in distensibility, and thickening of the intima-media complex in the distal axillary artery, while the BA, distally to the branching of deep brachial artery are only modestly affected by the process of ageing.

Sympathetic stimulation, obtained by LBNP, causes no significant alteration in the mechanical wall of proximal BA.

2. Middle aged women with SLE have higher aortic PWV than controls, in contrast to arm PWV, blood pressure, amplitude and timing of the systolic reflection wave that are similar, implicating that women with SLE have less distensible central elastic arteries.

Ongoing SLE disease activity is not correlated with arterial properties or serological variables. Higher aortic PWV is however associated with higher serum C3 level, which has a central role in the complement activation system.

3. Type 1 diabetic women have a marked reduction in the distal BA dilation response to exogenous NO (NMD), despite only limited reduction in FMD, similar arterial size and distensibility as healthy controls.

Higher HbA_{1c} is negatively related to NMD. This finding suggests that poor long-term glucose control directly or indirectly induces vascular smooth muscle cell dysfunction.

SVENSK SAMMANFATTNING

(SUMMARY IN SWEDISH)

Från hjärtats vänstra kammare pumpas det syrerika blodet ut i stora kroppspulsådern för att härifrån distribueras vidare inom ett nätverk av allt mindre blodkärl som slutligen levererar syre och näring till kroppens olika organ och vävnader. De elastiska egenskaperna hos de större pulsådernas väggar gör att de kan buffra delar av vänster hjärtkammarens stötvis utkastade blodvolym och härigenom underlätta hjärtats arbete samtidigt som blodflödet i kroppen flyter fram mjukare. Med stigande ålder ökar förekomsten av högt blodtryck, en viktig riskfaktor för hjärtinfarkt eller stroke. Orsaken till högt blodtryck är hos äldre personer ofta stela pulsådror, vilket leder till att pulsvågen flyttar sig snabbare i kärlen varvid utgående och studsande tryckvågen möts och förstärker varandra under senare delen av vänster hjärtkammarens pumpfas. Detta ökar storleken på det pulserande blodtrycket i kroppspulsådern och gör att hjärtmuskeln på längre sikt kan överbelastas och försvagas samtidigt som dess genomblödning försämras. Vid vissa kroniska tillstånd som t.ex diabetes (sockersjuka) och SLE (speciell inflammatorisk sjukdom) ses ökad förekomst av kärlsjukdom, vilket inte enbart kan förklaras av de vanliga riskfaktorerna, vilket talar för att dessa sjukdomar i sig kan få blodkärlen att åldras i förtid.

Avsikten med avhandlingen har varit att: 1) utvärdera hur överarmspulsåderns lokala elastiska egenskaper påverkas av ålder, kön och cirkulatorisk stress 2) belysa tidiga störningar i pulsåderns egenskaper på överarmen hos yngre kvinnor med insulin behandlad diabetes och även relatera dessa fynd till blod analyser 3) utvärdera den allmänna kärlkonditionen hos medelålders kvinnor med SLE för att hitta eventuella defekter som kan kopplas till deras inflammatoriska sjukdom.

I delarbete ett undersöktes 136 friska försökspersoner (52 män och 84 kvinnor) i varierande åldrar, med en speciell ultraljudsteknik som gör det möjligt att med stor noggrannhet mäta kärldiameter förändringen varje gång pulsvågen passerar. Genom att även registrera blodtrycket med manschett kunde pulsåderns lokala väggstyvhet högt upp på överarmen beräknas. Pulsåderns styvhet var likartad hos båda könen, samtidigt som stigande ålder var associerat med en markant styvhetsökning utan att kärlets dimension påverkades nämnvärt. Metoden att mäta blodtryck med manschett och

stetoskop jämfördes även mot direkt tryckmätning hos 27 individer, vilket visade att det diastoliska trycket (undre trycket) överskattas med i genomsnitt ca 10 mmHg, då det mäts manuellt med manschett.

I delarbete två undersöktes 9 yngre och 9 äldre friska försökspersoner med samma ultraljudsteknik som i föregående delarbete, med skillnaden att försökspersonens underkropp nu placerats i en låda i vilken lufttrycket kan regleras. Under samma pulsslåg registrerades kärldiameterförändring med ultraljud och tryckförändring med kateter högt upp i överarmspulsådern såväl i vila som i samband med standardiserat undertryck i lådan. Undertrycket simulerar en akut blodförlust genom att mera blod sugts ner i försökspersonens ben, samtidigt som pulsen stiger och blodkärlen i armarna drar sig samman för att minska blodtrycksfallet i överkroppen. Sambandet mellan tryck och diameter förändring plottades för respektive åldersgrupp såväl i vila som i samband med cirkulatorisk stress. Kurvans utseende förblev oförändrade i samband med stress, d.v.s gruppen med de äldre individerna uppvisade hela tiden en mera flack kurva som tecken på högre lokal pulsåderstyvhet. Det icke-viljestyrda nervsystemet har således ingen uppenbar förmåga att reglera kärlväggens egenskaper i det undersökta området högt upp på överarmen.

I en uppföljande studie till tidigare delarbeten undersöktes 60 friska försökspersoner av båda könen med likartad ultraljudsteknik för att bestämma kärldiameterförändring och vägg tjocklek på tre olika nivåer längs överarmen, där blodtryck registrerades automatiskt med manschett. Individerna delades in i tre ålderskategorier, <40 år, 40-59 år och > 60 år. I övergången mellan bål och överarm sågs en markant högre styvhet, vägg tjocklek och även större kärldiameter hos den äldre åldersgruppen, medan den åldersrelaterade ökningen i artärstyvhet och vägg tjocklek var betydligt mindre längre ner på överarmen, där diametern inte påverkades alls. Således finns det en zon högt upp på överarmen där pulsåderns väggegenskaper förändras från elastisk karaktär till en mera muskulär sådan, då avståndet till hjärtat ökar.

I delarbete tre bestämdes pulsvågshastighet och pulsvågsform hos 27 medelålders kvinnor med SLE i jämförelse mot 27 kontroller. Blodtrycket registrerades på överarmen med manschett och pulsvågen avbildades med hjälp av en sensor som trycktes mot huden på platsen där pulsen kan kännas i ljumsken, på halsen och vid handleden. Med hjälp av speciell mjukvara beräknades automatiskt kroppspulsåderns tryck och pulsvågsform i bröstkorgen från den registrerade pulsvågens utseende vid handleden. Den regionala hastigheten beräknades genom att mäta tidsskillnad i ankomsttid för

tryckvågen samt uppskattad distans mellan registreringspunkterna. Kvinnorna med SLE hade samma blodtryck och pulsvågsutseende som kontrollerna medan pulsvågshastigheten var högre i bålen, vilket talar för ökad styvhet i kroppspulsådern hos medelålders kvinnor med SLE. Högre pulsvågshastighet i bålen var emellertid inte kopplat till den aktuella graden av SLE aktivitet, men däremot till högre nivå av komplementfaktor 3, en markör som nyligen kopplats till ökad förekomst av kranskärlsjukdom och det metabola syndromet.

I delarbete fyra studerades överarmspulsådern egenskaper hos 37 komplikationsfria yngre kvinnor med insulin behandlad diabetes och 53 friska kontroller med hjälp av ultraljudsteknik och automatisk blodtrycksregistrering med manschett. Blodprover togs för att analysera koncentrationen av specifika markörer som tidigare setts avvika hos grupper med diabetes eller hjärtkärlsjukdom. Blodtryck och puls var något högre hos diabetikerna. Däremot sågs ingen skillnad avseende pulsåderns diameter, vägg tjocklek och styvhet längst ner på överarmen i jämförelse med kontrollerna. Förmågan att kunna öka överarmspulsåderns diameter i samband med flödesstimuli var inte tydligt nedsatt hos diabetikerna, vilken däremot reaktionen på nitroglycerin var. Hos diabetikerna var reducerad diameter ökning efter nitroglycerin relaterat till högre HbA_{1c} nivå även efter justering för andra betydelsefulla faktorer, talande för att högre blodsocker nivåer försämrar nedbrytningen av nitroglycerin eller gör muskelcellerna i kärlväggen mindre känsliga.

ACKNOWLEDGEMENTS

I would like to express my sincerely gratitude to everyone who have contributed and supported me through the work with this thesis and life in general. Especially I would like to mention the following:

Professor Toste Länne, my tutor, for building up the vascular research laboratories and introducing me into the area of cardiovascular research. Your never ending joy and enthusiasm promote essential energy for me and the research group.

Associated Professor Åsa Rydén-Ahlgren, co-author at the Department of Clinical Physiology, Malmö, for your expertise advice and support in the beginning of my work.

All co-authors, for contributing to the papers by skilful experiment work, recruitment of volunteers, or invaluable advices during the manuscript writing process.

Anita Eriksson, Ann-Kristin Jönsson and Ingbritt Vicktor at the Department of Clinical Physiology, Malmö, for skilful Diamove examinations, support and hospitality during my practical training.

Ulrica Axelsson-Franke, Department of Surgery, Jönköping, for excellent secretarial support and co-operation.

Christina Andersson, IMH, Linköping, for being helpful with all kinds of paperwork and correspondence with the institution.

Nora Östrup, former administrator, IMV, Linköping, for skilful linguistic revision of the thesis.

Professor Jan Åke Nilsson, Malmö, for expertise advice regarding statistics.

Christina Svensson and Elisabet Kindberg, my colleagues at the twin-vascular research laboratory in Linköping, who are my invaluable discussion partners regarding methodology and equipment.

Hasse Skoglund, colleague and inventor, for fruitful discussions and improvement of the ergonomics at the laboratories in Jönköping and Linköping.

The Management team at the Department of Clinical Physiology, Jönköping, especially Peter Blomstrand, Elisabet Hresan and Christina Rogemark, for

being tolerant and giving me the opportunity to adjust my work schedule. It's a privilege to have a vascular research laboratory within the clinic.

PhD student fellows within our research group and at the Department of Clinical Physiology for necessary exchange of experiences.

To all subjects who have volunteered in the studies, making this work possible.

Finally, not to be forgotten

My parents, Lars and Karin for accepting my choices in life and always being supportive, and my sisters Åsa and Sofia for being hospitable when I show up.

Ann-Louise, my partner, for patience and love, Sanna, Fanny and Findus for being helpful at home when I am busy elsewhere.

FUTURUM, the Academy of Health Care, Jönköping County Council and its predecessor, for invaluable financial support to me and our research group ever since the day it all began.

The studies, upon which this thesis is based, were also supported by grants from FORSS, the Research Council of South-East Sweden, the Swedish Research Council (no.12161), the funds of Linköping University Hospital and the Swedish Heart-Lung Foundation.

REFERENCES

- Adams M, Robinson J, McCredie R et al. Smooth Muscle Dysfunction Occurs Independent of Impaired Endothelium-Dependent Dilatation in Adults at Risk of Atherosclerosis. *J Am Coll Cardiol* 1998;32:123-127.
- Agewall S, Hulthe J, Fagerberg B, Gottfridsson B, Wikstrand J. Post-occlusion brachial artery vasodilation after ischemic handgrip exercise is nitric oxide mediated. *Clin Physiol & Func Im* 2002;22:18-23.
- Aoun S, Blacher J, Safar ME, Mourad JJ. Diabetes mellitus and renal failure: effects on large artery stiffness. *J Hum Hypertens* 2001;15:693-700.
- Arndt JO, Klauske J, Mersch F. The diameter of the intact carotid artery in man and its change with pulse pressure. *Pfluger Arch* 1968;301:230-240.
- Aronson D. Cross-linking of glycated collagen in the pathogenesis of arterial and myocardial stiffening of aging and diabetes. *J Hypertens* 2003;21:3-12.
- Arosio E, Marchi S, Rigoni A, Prior M, Delva P, Lechi A. Forearm haemodynamics, arterial stiffness and vascular reactivity in rheumatoid arthritis. *J Hypertens* 2007;25:1273-1278.
- Asmar R, Benetos A, Topouchian J et al. Assessment of arterial distensibility by automatic pulse wave velocity measurement. Validation and clinical application studies. *Hypertension* 1995;26:485-490.
- Avolio AP, Chen S, Wang R, Zhang C, Li M. Effects of aging on changing arterial compliance and left ventricular load in a northern Chinese urban community. *Circulation* 1983;68:50-58.
- Bank AJ, Wilson RF, Kubo SH, Holte JE, Dresing TJ, Wang H. Direct effects of smooth muscle relaxation and contraction on in vivo human brachial artery elastic properties. *Circ Res* 1995;77:1008-1016.
- Bank AJ, Wang H, Holte J, Mullen K, Shammass R, Kubo SH. Contribution of collagen, elastin, and smooth muscle to in vivo human brachial artery wall stress and elastic modulus. *Circulation* 1996;94:3263-3270.
- Bank A, Kaiser, D. Smooth muscles relaxation: effects on arterial compliance, distensibility, elastic modulus, and pulse wave velocity. *Hypertension* 1998; 32:356-359.

- Bank A, Kaiser R, Rajala S, Cheng A. In vivo human brachial artery elastic mechanics: Effects of smooth muscle relaxation. *Circulation* 1999;100:41-47.
- Barnes FV, Narain S, Naranjo A et al. High sensitivity C-reactive protein in systemic lupus erythematosus: relation to disease activity, clinical presentation and implications for cardiovascular disease. *Lupus* 2005;14:576-582.
- Benjamin E, Larson M, Keyes M et al. Clinical Correlates and Heritability of Flow-Mediated Dilatation in the Community. The Framingham Heart Study. *Circulation* 2004;109:613-619.
- Benthin M, Dahl P, Ruzicka R, Lindström K. Calculation of pulse-wave velocity using cross correlation-effects of reflexes in the arterial tree. *Ultrasound Med Biol* 1991;17:461-469.
- Betik A, Luckham V, Hughson R. Flow-mediated dilatation in human brachial artery after different circulatory occlusion conditions. *Am J Physiol Heart Circ Physiol* 2004;286:H442-H448.
- Bjarnegård N, Rydén Ahlgren Å, Sandgren T, Sonesson B, Länne T. Age affects proximal brachial artery stiffness: Differential behavior within the length of brachial artery? *Ultrasound Med Biol* 2003;29:1115-1121.
- Blacher J, Pannier B, Guerin A, Marchais A, Safar M, London G. Carotid Arterial Stiffness as a Predictor of Cardiovascular and All-Cause Mortality in End-Stage Renal Disease. *Hypertension* 1998;32:570-574.
- Blacher J, Guerin A, Pannier B, Marchais S, Safar M, London G. Impact of Aortic Stiffness on Survival in End-Stage Renal Disease. *Circulation* 1999;99:2434-2439.
- Bonetti P, Lerman L, Lerman A. Endothelial dysfunction: A Marker of Atherosclerotic Risk. *Arterioscler Thromb Biol* 2003;23:168-175.
- Booth AD, Wallace S, McEniery C et al. Inflammation and arterials stiffness in systemic vasculitis: A model of vascular inflammation. *Arthritis Rheum* 2004;50:581-588.
- Booth G, Kapral M, Fung K, Tu J. Relation between age and cardiovascular disease in men and women with diabetes compared with non-diabetic people: a population-based retrospective cohort study. *Lancet* 2006;368:29-36.
- Bortolotto LA, Hanon O, Franconi G, Boutouyrie P, Legrain S, Girerd X. The aging process modifies the distensibility of elastic but not muscular arteries. *Hypertension* 1999;34:889-892.

Bots M, Hofman A, Grobbee D. Increased common carotid intima-media thickness: Adaptive response or a reflection of atherosclerosis? Findings from the Rotterdam Study. *Stroke* 1997;28:2442-2447.

Boutouyrie P, Laurent S, Benetos A, Girerd X, Hoeks A, Safar M. Opposing effects of ageing on distal and proximal large arteries in hypertensives. *J Hypertens* 1992;10:S87-S91.

Boutouyrie P, Lacolley P, Girerd X, Beck L, Safar M, Laurent S. Sympathetic activation decreases medium-sized arterial compliance in humans. *Am J Physiol* 1994;267:1368-1376.

Bramwell JC, Hill AV. Velocity of transmission of the pulse wave and elasticity of arteries. *Lancet* 1922;1:891-892.

Bruce IN, Gladman DD, Urowitz MB. Premature atherosclerosis in systemic lupus erythematosus. *Rheum Dis Clin North Am* 2000;26:257-278.

Cameron J, Bulpitt C, Pinto E, Rajkumar C. The Aging of Elastic and Muscular Arteries. A comparison of diabetic and nondiabetic subjects. *Diabetes Care* 2003;26:2133-2138.

Celermajer D, Sørensen K, Spiegelhalter D, Georgakopoulos D, Robinson J, Deanfield J. Aging Is Associated With Endothelium Dependent Dysfunction in Healthy Men and Women. *J Am Coll Cardiol* 1994;24:471-476.

Cerillo A, Kumar S, Piconi L, Esposito K, Giugliano D. Simultaneous Control of Hyperglycemia and Oxidative Stress Normalizes Endothelial Function in type 1 Diabetes. *Diabetes Care* 2007;30:649-654.

Chambers JM, Cleveland WS, Kleiner B. *Graphical Methods for Data Analysis*. Belmont, CA, USA: Wadsworth International Group and Duxbury Press, 1983.

Chen CH, Nevo E, Fetis B et al. Estimation of central aortic pressure waveform by mathematical transformation of radial tonometry pressure: Validation of generalized transfer function. *Circulation* 1997;95:1827-1836.

Chirinos J, Zambrano J, Chakko S, Veerani A, Schob A, Willens H, Perez G, Mendez A. Aortic Pressure Augmentation Predicts Adverse Cardiovascular Events in Patients With Established Coronary Artery Disease. *Hypertension* 2005;45:980-985.

Chiu YC, Arand PW, Shroff SG, Feldman T, Carroll JD. Determination of pulse wave velocities with computerized algorithms. *Am Heart J* 1991;121:1460-1470.

- Cinthio M, Ryden Ahlgren Å, Bergkvist J, Jansson T, Persson HW, Lindström H. Longitudinal movements and resulting shear strain of the arterial wall. *Am J Physiol Heart Physiol* 2006;291:H394-H402.
- Cloud G, Rajkumar C, Kooner J, Cooke J, Bulpitt C. Estimation of central aortic pressure by Sphygmocor requires intra-arterial peripheral pressure. *Clin Sci* 2003;105:219-225.
- Colburn K, Langga-Shariffi E, Kelly T et al. Abnormalities in Serum Antielastin Antibodies in Connective Tissue Disease. *J Invest Med* 2003;51:104-109.
- Colombo BM, Murdaca G, Caiti M et al. Intima-Media Thickness: A Marker of Accelerated Atherosclerosis in Women with Systemic Lupus Erythematosus. *Ann N Y Acad Sci* 2007;1108:121-126.
- Corretti M, Anderson T, Benjamin E et al. Guidelines for the Ultrasound Assessment of Endothelial-Dependent Flow-Mediated Vasodilation of the Brachial Artery. *J Am Coll Cardiol* 2002;39:257-265.
- Cruickshank K, Riste L, Anderson S, Wright J, Dunn G, Gosling R. Aortic Pulse-Wave Velocity and Its Relationship to Mortality in Diabetes and Glucose Intolerance: A Integrated Index of Vascular Function? *Circulation* 2002;106:2085-2090.
- De Angelis L, Millasseau S, Smith A et al. Sex Differences in Age-Related Stiffening of the Aorta in Subjects With Type 2 Diabetes. *Hypertension* 2004;44:67-71.
- Debasso R, Åstrand H, Bjarnegård N, Ryden Ahlgren Å, Sandgren T, Länne T. The popliteal artery, an unusual muscular artery with wall properties similar to the aorta: Implications for susceptibility to aneurysm formation? *J Vasc Surg* 2004;39:836-842.
- DeSouza C, Clevenger C, Greiner J, Smith D, Hoetzer G, Shapiro L, Stauffer B. Evidence for agonist-specific endothelial vasodilator dysfunction with ageing in healthy humans. *J Physiol* 2002;541:255-262.
- Dinneno FA, Jones PP, Seals DR, Tanaka H. Age-associated arterial thickening is related to elevation in sympathetic activity in healthy humans. *Am J Physiol Heart Circ Physiol* 2000; 278:H1205-H1210.
- Dogra G, Rich L, Staton K, Watts GF. Endothelium-dependent and independent vasodilation studied at normoglycemia in type 1 diabetes mellitus with and without microalbuminuria. *Diabetologia* 2001;44:593-601.

Domanski M, Davis B, Pfeffer M, Kastantin M, Mitchell G. Isolated Systolic Hypertension. Prognostic Information Provided by Pulse Pressure. *Hypertension* 1999;34:375-380.

El-Magadmi M, Bodill H, Ahmed Y et al. Systemic Lupus Erythematosus: An Independent Risk Factor for Endothelial Dysfunction in Women. *Circulation* 2004;110:399-404.

Fagius J, Berne C. The increase in sympathetic nerve activity after glucose ingestion is reduced in type 1 diabetes. *Clin Sci* 2000;98:627-632.

Failla M, Grappiolo A, Carugo, S, Calchera I, Giannattasio C, Mancina G. Effects of cigarette smoking on carotid and radial artery distensibility. *J Hypertens* 1997;15:1659-1664.

Failla M, Grappiolo A, Emanuelli G et al. Sympathetic tone restrains arterial distensibility of healthy and atherosclerotic subjects. *J Hypertens* 1999;17:1117-1123.

Gamble G, Zorn J, Sanders S, MacMahon S, Sharpe N. "Estimation of arterial stiffness, compliance, and distensibility from M-mode ultrasound measurements of the common carotid artery". *Stroke* 1994;25:11-16.

Giannattasio C, Failla M, Piperno A et al. Early impairment of large artery structure and function in Type 1 diabetes mellitus. *Diabetologia* 1999;42:987-994.

Giannattasio C, Failla M, Emanuelli G et al. Local effects of atherosclerotic plaque on arterial distensibility. *Hypertension* 2001;38:1177-1180.

Giannattasio, C., Vincenti, A., Failla, M. et al. Effect of heart rate changes on arterial distensibility in humans. *Hypertension* 2003;42:253-256.

Hansen F, Mangell P, Sonesson B, Länne T. Diameter and compliance in the human common carotid artery – variations with age and sex. *Ultrasound Med Biol* 1995;21:1-9.

Hayashi K, Handa H, Nagasawa S, Okumura A, Moritahe K. Stiffness and elastic behavior of human intracranial and extracranial arteries. *J Biomech* 1980;13:175-184.

Hedman C, Frystyk J, Lindström T et al. Residual β -Cell Function More than Glycemic Control Determines Abnormalities of the Insulin-Like Growth Factor System in Type 1 Diabetes. *J Clin Endocrinol Metab* 2004;89:6305-6309.

- Henry R, Kostense P, Spijkerman A et al. Arterial Stiffness Increases With Deteriorating Glucose Tolerance Status: The Hoorn Study. *Circulation* 2003;107:2089-2095.
- Hoeks A, Willekes C, Boutouyrie P, Brands P, Willigers J, Reneman R. Automated detection of local artery wall thickness based on m-line signal processing. *Ultrasound Med Biol* 1997;23:1017-1023.
- Hokanson DE, Mozersky CJ, Sumner DJ, Strandness Jr DE. A phase-locked echo tracking system for recording arterial diameter changes in vivo. *J Appl Physiol* 1972;32:732-733.
- Hope S, Meredith I, Cameron J. Effects of non-invasive calibration of radial waveforms on error in transfer-function-derived central aortic waveform characteristics. *Clin Sci* 2004;107:205-211.
- Hope S, Meredith I, Tay D, Cameron J. 'Generalizability' of radial-aortic transfer function for the derivation of central aortic waveform parameters. *J Hypertens* 2007;25:1812-1820.
- Ittersum F, Spek J, Praet I et al. Ambulatory blood pressure and autonomic nervous function in normoalbuminuric type 1 diabetes. *Neuphol Dial Transplant* 1998;13:326-332.
- Jogestrand T, Eiken O, Nowak J. Relation between the elastic properties and thickness of the common carotid artery. *Clin Physiol & Func Im* 2003;23:134-137.
- Jonsson H, Nived O, Sturefelt G. Outcome in systemic lupus erythematosus: A prospective study of patients from a defined population. *Medicine* 1989;68:141-150.
- Juul A, Scheike T, Davidsen M, Gyllenborg J, Jørgensen T. Low Serum Insulin-Like Growth factor 1 Is Associated With Increased Risk of Ischemic Heart Disease. *Circulation* 2002;106:939-944.
- Juutilainen A, Kortelainen S, Lehto S, Rönnemaa T, Pyörälä K, Laakso M. Gender Differences in the Impact of Type 2 Diabetes on Coronary Heart Disease Risk. *Diabetes Care* 2004;27:2898-2904.
- Järvisalo M, Lehtimäki T, Raitikari O. Determinants of Arterial Nitrate-Mediated Dilatation in Children: Role of Oxidized Low-Density Lipoprotein, Endothelial Function, and Carotid Intima-Media Thickness. *Circulation* 2004;109:2885-2889.

Karamanoglu M, Feneley MP. Derivation of the ascending aortic-carotid pressure transfer function with an arterial model. *Am J Physiol Heart Circ Physiol* 1996;271:H2399-H2404.

Kawano H, Motoyama T, Hirashima O et al. Hyperglycemia Rapidly Suppresses Flow-mediated Endothelium-Dependent Vasodilatation of Brachial Artery. *J Am Coll Cardiol* 1999;34:146-154.

Kawasaki T, Sasayama S, Yagi S-I, Asakawa T, Hirai T. Non-invasive assessment of the age related changes in stiffness of major branches of the human arteries. *Cardiovasc Res* 1987;21:678-687.

Kelly RP, Millasseau SC, Ritter JM, Chowienczyk PJ. Vasoactive Drugs Influence Aortic Augmentation Index Independently of Pulse-Wave Velocity in Healthy Men. *Hypertension* 2001;37:1429-1433.

Khattar RS, Acharya DU, Kinsey C, Senior R, Lahiri A. Longitudinal association of ambulatory pulse pressure with left ventricular mass and vascular hypertrophy in essential. *Hypertension* 1997;15:737-743.

King D, Everett C, Mainous III A, Liszka H. Long-Term Prognostic Value of Resting Heart Rate in Subjects With Prehypertension. *Am J Hypertens* 2006;19:796-800.

Kool M, van Merode T, Reneman R, Hoeks A, Struijker Boudier H, Van Bortel L. Evaluation of reproducibility of a vessel detector system for assessment of large artery properties. *Cardiovasc Res* 1994;28:610-614.

Kool M, Lambert J, Stehouwer C, Hoeks A, Struijker Boudier H, Van Bortel L. Vessel wall properties of large arteries in uncomplicated IDDM. *Diabetes Care* 1995;18:618-624.

Kumeda Y, Inaba M, Gato H et al. Increased Thickness of the Arterial Intima-Media Detected by Ultrasonography in Patients With Rheumatoid Arthritis. *Arthritis Rheum* 2002;46:1489-1497.

Lacy P, O'Brien D, Stanley A, Dewar M, Swales P, Williams B. Increased pulse wave velocity is not associated with elevated augmentation index in patients with diabetes. *J Hypertens* 2004;22:1937-1944.

Lafleche A, Pannier B, Laloux B, Safar M. Arterial response during cold pressor test in borderline hypertension. *Am J Physiol Heart Circ Physiol* 1998;275:H409-H415.

Laing SP, Swerdlow AJ, Slater SD et al. Mortality from heart disease in a cohort of 23,000 patients with insulin-treated diabetes. *Diabetologia* 2003;46:760-765.

- Laurent S, Boutouyrie P, Asmar R et al. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. *Hypertension* 2001;37:1236-1241.
- Laurent S, Cockcroft J, Van Bortel L et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. *Eur Heart Jour* 2006;27:2588-2605.
- Lehmann K, Gelman J, Weber M, Lafrades A. Comparative Accuracy of Three Automated Techniques in the Noninvasive Estimation of Central Blood Pressure in Men. *Am J Cardiol* 1998;81:1004-1012.
- Libby P, Ridker P, Maseri A. Inflammation and atherosclerosis. *Circulation* 2002;105:1135-1143.
- Lima D, Sato E, Lima V, Miranda F, Hatta F. Brachial Endothelial Function Is Impaired in Patients with Systemic Lupus Erythematosus. *J Rheumatol* 2002;29:292-297.
- Lindström K, Gennser G, Sindberg Eriksen P, Benthin M, Dahl P. An improved echo-tracker for studies on pulse-wave in the fetal aorta. In: Rolfe P, ed. *Fetal physiological measurements*. London: Butterworths 1987:217-226.
- Lindström T, Frystyk J, Hedman CA, Flyvbjerg A, Arnqvist HJ. Elevated circulating adiponectin in type 1 diabetes is associated with long diabetes duration. *Clin Endocrinol (Oxf)* 2006;65:776-82.
- Länne T, Stahle H, Bengtsson H et al. Noninvasive measurement of diameter changes in the distal abdominal aorta in man. *Ultrasound Med Biol* 1992;18:451-457.
- Manzi S, Meilahn E, Rairie J et al. Age-specific Incidence Rate of myocardial Infarction and Angina in Women with Systemic Lupus Erythematosus: Comparison with the Framingham Study. *Am J Epidemiol* 1997;145: 408-415.
- Manzi S, Selzer F, Sutton-Tyrrell K et al. Prevalence and risk factors of carotid plaque in women with systemic lupus erythematosus. *Arthritis Rheum* 1999;42:51-60.
- Manzi S. Epidemiology of Systemic Lupus Erythematosus. *Am J Manag Care* 2001; 7:S474-S479.
- McEniery C, Qasem A, Schmitt M, Avolio A, Cockcroft J, Wilkinson I. Endothelin-1 regulates arterial pulse wave velocity in vivo. *J Am Coll Cardiol* 2003;42:1975-1981.

McEniery C, Yasmin, Hall I, Qasem A, Wilkinson I, Cockcroft J. Normal Vascular Aging: Differential Effects on Wave Reflection and Aortic Pulse Wave Velocity. *J Am Coll Cardiol* 2005;46:1753-1760.

McGrath BP, Liang Y-L, Kotsopoulos D, Cameron JD. Impact of Physical and physiological factors on arterial function. *Clin Exp Pharmacol Physiol* 2001;28:1104-1107.

McVeigh G, Morgan D, Allen P et al. Early vascular abnormalities and *de novo* nitrate tolerance in diabetes mellitus. *Diabetes Obes Metab* 2002;4:336-341.

Meeking DR, Cummings MH, Thorne S et al. Endothelial dysfunction in Type 1 diabetic subjects with and without microalbuminuria. *Diabet Med* 1999;16: 841-847.

Millasseau S, Stewart A, Patel S, Redwood S, Chowienczyk P. Evaluation of Carotid-Femoral Pulse Wave Velocity: Influence of Timing Algorithm and Heart Rate. *Hypertension* 2005;45:222-226.

Mitchell G, Parise H, Benjamin E et al. Changes in Arterial Stiffness and Wave reflection With Advancing Age in Healthy Men and Women. *Hypertension* 2004;43:1239-1245(a).

Mitchell G, Parise H, Vita J et al. Local Shear Stress and Brachial Artery Flow-Mediated Dilatation: The Framingham Heart Study. *Hypertension* 2004;44:134-139(b).

Mäki-Petäjä K, Hall F, Booth A et al. Rheumatoid Arthritis Is Associated With Increased Aortic Pulse-Wave Velocity, Which Is Reduced by Anti-Tumor Necrosis Factor- α Therapy. *Circulation* 2006;114:1185-1192.

Ni H, Wu C, Prineas R et al. Comparison of Dinamap PRO-100 and Mercury Sphygmomanometer Blood Pressure Measurements in a Population-Based Study. *Am J Hypertens* 2006;19:353-360.

Nichols WW, O'Rourke MF. McDonald's blood flow in arteries, 5:th ed London: Hodder Arnold, 2005.

Ng AV, Callister R, Johnson DG, Seals DR. Age and gender influence muscle sympathetic nerve activity at rest in healthy humans. *Hypertension* 1993;21:498-503.

Onat A, Uzunlar B, Hergenc G et al. Cross-sectional study of complement C3 as a coronary risk factor among men and women. *Clin Sci* 2005;108:129-135.

- Pannier B, Slama MA, London GM, Safar ME, Cuche JL. Carotid arterial hemodynamics in response to LBNP in normal subjects: methodological aspects. *J Appl Physiol* 1995;79:1546-1555.
- Pauca A, O'Rourke M, Kon N. Prospective Evaluation of a Method for Estimating Ascending Aortic Pressure from the Radial Artery Pressure Waveform. *Hypertension* 2001;38:932-937.
- Peterson LH, Jensen RE, Parnell J. Mechanical properties of arteries in vivo. *Circ Res* 1960;8:622-639.
- Petri M, Lakatta C, Magder L, Goldman D. Effects of prednisolone and hydroxychloroquine on coronary artery disease risk factors in systemic lupus erythematosus: a longitudinal data analysis. *Am J Med* 1994;96:254-259.
- Popele N, Grobbee D, Bots M et al. Association Between Arterial Stiffness and Atherosclerosis. The Rotterdam Study. *Stroke* 2001;32:454-460.
- Ravikumar R, Deepa R, Shanthirani S, Mohan V. Comparison of Carotid Intima-Media Thickness, Arterial Stiffness, and Brachial Artery Flow Mediated Dilatation in Diabetic and Non-diabetic Subjects. *Am J Cardiol* 2002;90:702-707.
- Ridker P. High-sensitivity C-reactive protein, inflammation, and cardiovascular risk: From concept to clinical practice to clinical benefit. *Am Heart J* 2004;148:S19-S26.
- Riley W, Evans G, Sharrett R, Burke G, Barnes R. Variation of common carotid artery elasticity with intima-medial thickness: The ARIC study. *Ultrasound Med Biol* 1997;23:157-164.
- Rogers W, Hu Y-L, Coast D et al. Age-Associated Changes in Regional Aortic Pulse Wave Velocity. *J Am Coll Cardiol* 2001;38:1123-1129.
- Roman M, Salmon J, Sobel R et al. Prevalence and relation to risk factors of carotid atherosclerosis and left ventricular hypertrophy in systemic lupus erythematosus and antiphospholipid antibody syndrome. *Am J Cardiol* 2001;87:663-666.
- Roman MJ, Shanker BA, Davis A et al. Prevalence and correlates of accelerated atherosclerosis in systemic lupus erythematosus. *N Engl J Med* 2003;349:2399-2406.
- Rydén Ahlgren Å, Länne T, Wollmer P, Sonesson B, Hansen F, Sundkvist G. Increased arterial stiffness in women, but not men, with IDDM. *Diabetologia* 1995;38:1082-1089.

Rydén Ahlgren Å, Åstrand H, Sandgren T, Vernersson E, Sonesson B, Länne T. Dynamic behaviour of the common femoral artery: Age and gender of minor importance. *Ultrasound Med Biol* 2001;27:181-188.

Schalkwijk CG, Poland DCW, Van Dijk W et al. Plasma concentration of C-reactive protein is increased in Type 1 diabetic patients without clinical macroangiopathy and correlated with markers of endothelial dysfunction: evidence for chronic inflammation. *Diabetologia* 1999;42:351-357.

Schalkwijk C, Stehouver C. Vascular complications in diabetes mellitus: the role of endothelial function. *Clin Sci* 2005;109:143-159.

Schmidt-Trucksäss A, Grathwohl D, Schmid A et al. Assessment of carotid artery wall motion with tissue Doppler imaging. *Ultrasound Med Biol* 1998;24:639-646.

Schmitt M, Avolio A, Quasem A et al. Basal NO Locally Modulates Human Iliac Artery Function In Vivo. *Hypertension* 2005;46:227-231.

Schächinger V, Britten M, Zeiher A. Prognostic Impact of Coronary Vasodilator Dysfunction on Adverse Long-Term Outcome of Coronary Heart Disease. *Circulation* 2000;101:1899-1906.

Segers P, Rabben I, DE Backer J et al. Functional analysis of the common carotid artery: relative distension differences over the vessel wall measured in vivo. *J Hypertens* 2004;22:973-981.

Selzer F, Sutton-Tyrrell K, Fitzgerald S, Tracy R, Kuller L, Manzi S. Vascular Stiffness in Women With Systemic Lupus Erythematosus. *Hypertension* 2001;37:1075-1082.

Sharman J, Lim R, Qasem A et al. Validation of a Generalized Transfer Function to Noninvasively Derive Central Blood Pressure During Exercise. *Hypertension* 2006;47:1203-1208.

Shivalkar B, Dhondt D, Goovaerts I et al. Flow Mediated Dilatation and Cardiac Function in Type 1 Diabetes Mellitus. *Am J Cardiol* 2006;97:77-82.

Soedamah-Muthu S, Fuller J, Mulnier H, Raleigh V, Lawrenson R, Colhoun H. High risk of Cardiovascular Disease in Patients With Type 1 Diabetes in U.K. *Diabetes Care* 2006;29:798-804.

Solomon D, Karlson E, Rimm E et al. Cardiovascular Morbidity and Mortality in Women Diagnosed With Rheumatoid Arthritis. *Circulation* 2003;107:1303-1307.

- Sonesson B, Hansen F, Stale H, Länne T. Compliance and diameter in the human abdominal aorta-the influence of age and sex. *Eur J Vasc Surg* 1993;7:690-697.
- Sonesson B, Vernersson E, Hansen F, Länne T. Influence of sympathetic properties of the aorta in humans. *Acts Physiol Scand* 1997;159:139-145.
- Steward A, Millasseau S, Kearney M, Ritter J, Chowienczyk P. Effects of Inhibition of Basal Oxide Synthesis on Carotid-Femoral Pulse Wave Velocity and Augmentation Index in Humans. *Hypertension* 2003;42:915-918.
- Sturfelt G, Eskilsson J, Nived O, Truedsson L, Valind S. Cardiovascular disease in systemic lupus erythematosus. *Medicine* 1992;4:216-223.
- Ståhl-Hallengren C, Jönsen A, Nived O, Sturfelt G. Incidence Studies of Systemic Lupus Erythematosus in southern Sweden: Increasing Age, Decreasing Frequency of Renal Manifestation and Good Prognosis. *Rheumatology* 2000;3:685-691.
- Sörensen VR, Mathiesen ER, Clausen P, Flyvbjerg A, Feldt-Rasmussen B. Impaired vascular function during short-term poor glycaemic control in Type 1 diabetic patients. *Diabet Med* 2005;22:871-876.
- Taylor JA., Hand GA, Johnson DG, Seals DR. Sympathoadrenal-circulatory regulation of arterial pressure during orthostatic stress in young and older men. *Am J Physiol* 1992;263:R1147-R1155.
- Tureson C, Jarenros A, Jacobsson LT. Increased incidence of cardiovascular disease in patients with rheumatoid arthritis – results from a community based study. *Ann Rheumat Dis* 2004;63:952-955.
- Tureson C, Jacobsson L, Rydén Ahlgen Å, Sturfelt G, Wollmer P, Länne T. Increased stiffness of the abdominal aorta in women with rheumatoid arthritis. *Rheumatology (Oxford)* 2005;44:896-901.
- Van Bortel L, Duprez D, Starmans-Kool M et al. Clinical Application of Arterial Stiffness, Task Force III: Recommendations for User Procedures. *Am J Hypertens* 2002;15:445-452.
- Van der Heijden-Spek JJ, Staessen JA, Fagard RH et al. Effect of age on brachial artery wall properties differs from aorta and is gender dependent. *Hypertension* 2000;35:637-642.
- Van Doornum S, McColl G, Wicks IP. Accelerated Atherosclerosis. An Extraarticular Feature of Rheumatoid Arthritis? *Arthritis Rheum* 2002;46:862-873.

Verbeke F, Segers P, Heireman S, Vanholder R, Verdonck P, Van Bortel L. Noninvasive Assessment of Local Pulse Pressure: Importance of Brachial-to-Radial Pressure Amplification. *Hypertension* 2005;46:244-248.

Weber T, Auer J, O'Rourke M et al. Increased arterial wave reflection predicts severe cardiovascular events in patients undergoing percutaneous coronary intervention. *Eur Heart J* 2005;26:2657-2663.

Wenn CM, Newman DL. Arterial tortuosity. *Australas Phys Eng Sci Med* 1990;13:67-70.

Weston P, James M, Panerai R, McNally P, Potter J, Thurston H. Evidence of defective cardiovascular regulation in insulin-dependent diabetic patients without clinical autonomic dysfunction. *Diabetes Res Clin Pract* 1998;42:141-148.

Wilkinson I, MacCallum H, Rooijmans DF et al. Increased augmentation index and systolic stress in type 1 diabetes mellitus. *Q J Med* 2000;93:441-448.

Wilkinson I, Mohammad N, Tyrell S et al. Heart Rate Dependence of Pulse Pressure Amplification and Arterial Stiffness. *Am J Hypertens* 2002;15:24-30.

Willum-Hansen T, Staessen JA, Torp-Pedersen C et al. Prognostic value of aortic pulse wave velocity as index of arterial stiffness in the general population. *Circulation* 2006;113:664-670.

Wärnberg J, Nova E, Moreno L et al. Inflammation proteins are related to total and abdominal adiposity in a healthy adolescent population: the AVENA Study. *Am J Clin Nutr* 2006;84:505-512.

Yashio T, Masuyama J, Mimori A, Takeda A, Minota S, Kano S. Endothelin-1 release from cultured endothelial cells induced by sera from patients with systemic lupus erythematosus. *Ann Rheum Dis* 1995;54:361-365.

Yasmin, Brown MJ. Similarities and differences between augmentation index and pulse wave velocity in the assessment of arterial stiffness. *Q J Med* 1999;92:595-600.

Yasmin, McEniery C, Wallance S, Mackenzie I, Cockcroft J, Wilkinson I. C-Reactive Protein Is Associated With Arterial Stiffness in Apparently Healthy Individuals. *Arterioscler Thromb Vasc Biol* 2004;24:969-974.

Yasmin, Wallace S, McEniery C et al. Matrix Metalloprotease-9 (MMP-9), MMP-2, and Serum Elastase Activity Are Associated With Systolic Hypertension and Arterial Stiffness. *Arterioscler Thromb Vasc Biol* 2005;25:372-378.

Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. *Nature* 2001;414:782-787.

Zureik M, Beaudoux J-L, Courbon D, Bénétos A, Ducimetière P. Serum tissue inhibitors of metalloproteinases 1 (TIMP-1) and carotid atherosclerosis and aortic arterial stiffness. *J Hypertens* 2005;23:2263-2268.

Åstrand H, Rydén Ahlgren Å, Sandgren T, Länne T. Age-related increase in wall stress of the human abdominal aorta: An in vivo study. *J Vasc Surg* 2005;42:926-931.