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Regulation of aortic wall mechanics and stress

An experimental study in man

Håkan Åstrand

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

Linköping University
FACULTY OF HEALTH SCIENCES

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Cover picture: Arrows indicate the directions of stresses acting on an artery.

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To my family!

Always laugh when you can. It is cheap medicine.
Lord Byron

CONTENTS

ABSTRACT

The abdominal aorta (AA) in man is a vulnerable artery prone to atherosclerosis as well as aneurysmatic dilation. The underlying aortic composition, mechanical properties as well as the mechanisms responsible for age-related changes and vascular disease are however largely unknown. The aims of this study were 1) to characterize the age- and gender-related changes of the aortic wall components in vivo, using a mechanical model based on ultrasound measurements of pulsatile aortic diameter changes combined with intra-arterial pressure; 2) to validate ultrasound measurements of diameter and intima-media thickness (IMT) of the AA in order to calculate wall stress; 3) to study the stress driven remodeling response of the aortic wall in healthy individuals and the influence of age and gender; and 4) to study wall stress and remodeling of the AA in diabetic patients in order to elucidate the protective influence of diabetes on abdominal aortic aneurysm formation.

The stiffness of the isotropic material (mainly elastin) increased in males despite the known decrease in elastin content with age. Further, an exponential increase in stiffness of the anisotropic material (mainly collagen) in males at high physiological pressure was found. This might be due to changed isoforms of collagen and increased glycation with age. Females were less affected than males.

The reproducibility of the ultrasound measurements of diameter and IMT in the AA was acceptable (CV; 4% and 11% respectively), making it possible to calculate circumferential aortic wall stress in vivo. The age-related remodeling of the arterial wall led to increased diameter, and compensatory thickening of the wall preventing the circumferential wall stress from increasing in the common carotid artery of males and females, and the AA of females. However, the compensatory increase in wall thickness was defect in the male AA, where stress increased with age. Pulsatile stress influenced the material parameters of the AA, leading to increased stiffness of anisotropic material (mainly collagen), whereas stiffness of isotropic material (mainly elastin) was unaffected.

Patients with diabetes mellitus had increased aortic wall thickness than controls, generating less circumferential stress. This coincides with the known reduction of abdominal aortic aneurysms in diabetic patients and may act as a protective factor.

LIST OF PAPERS

This thesis is based on the following Papers, which will be referred to in the text by their Roman numerals. The results from complimentary studies will also be presented.

I. **Åstrand H, Sandgren T, Ahlgren ÅR, Länne T.** Noninvasive ultrasound measurements of aortic intima-media thickness: Implications for in vivo study of aortic wall stress. *J Vasc Surg* 2003;37:1270-6.

II. **Åstrand H, Ahlgren ÅR, Sandgren T, Länne T.** Age-related increase in wall stress of the human abdominal aorta: An in vivo study. *J Vasc Surg* 2005;42:926-31

III. **Åstrand H, Stålhand J, Sonesson B, Karlsson M, Länne T.** In vivo estimation of the contribution of elastin and collagen on the mechanical properties in the abdominal aorta of man - effect of age and gender. Submitted.

IV. **Åstrand H, Ahlgren ÅR, Sundkvist G, Sandgren T, Länne T.** Reduced aortic wall stress in diabetes mellitus**.** *Eur J Vasc Endovasc Surg* 2007;33:592-98.

ABBREVIATIONS

AA	Abdominal aorta (below renal arteries)
AAA	Abdominal aortic aneurysm
AGEs	Advanced glycosylation end-products
BMI	Body mass index
BSA	Body surface area
CCA	Common carotid artery
CFA	Common femoral artery
$Collagen_{ani}$	Anisotropic material
CTGF	Connective tissue growth factor
CV	Coefficient of variation
DBP	Diastolic blood pressure
EC	Endothelial cell
$Elastin_{iso}$	Isotropic material
IMA	Intima-media area
IMT	Intima-media thickness
LD	Lumen diameter
MAPK	Mitogen activated protein kinase
MMP	Matrix metalloproteinase
PA	Popliteal artery
PP	Pulse pressure
PS	Pulsatile stress
PWV	Pulse wave velocity
SBP	Systolic blood pressure
TIMP	Tissue inhibitor of metalloproteinase
VSMC	Vascular smooth muscle cell
YRS	Years

INTRODUCTION

Structure of the arterial wall

The structure of the arterial wall varies with location along the vascular tree, age, gender and disease. Nonetheless the arteries can be categorized according to two general types: *elastic arteries,* such as the aorta, common carotid artery, common iliac artery and main pulmonary artery; and *muscular arteries*, such as the coronary artery, femoral artery, renal artery, popliteal artery and cerebral artery. Elastic arteries tend to have larger diameter and be located closer to the heart. Furthermore the structures of the vessels differ, most strikingly in the central part of the vessel, the tunica media.

All arteries consist of three different layers, the tunica intima, the tunica media and the tunica adventitia (Fig. 1). In healthy and young individuals the innermost layer, *the tunica intima*, constitutes a single layer of endothelial cells which align according to the flow, resting on a sub-endothelial layer of elastin and collagen which anchors to the internal elastic lamina. The sub-endothelial layer is usually present only in larger elastic arteries such as the human aorta and develops gradually to become fibrous and cellular with age (Rhodin 1979). Towards the intima-media border the elastic fibers of the subendothelial layer have a longitudinal arrangement (Seifert 1962). The internal elastic lamina is a basement membrane that separates the intima from the media and consists of collagen type IV, fibronectin and laminin. It acts as a cushion that allows bending and changes in diameter associated with changes in blood pressure (Silver et al. 1989).

The tunica media is the part that differs the most between elastic and muscular arteries. In muscular arteries vascular smooth muscle cells (VSMC) dominates whereas in the elastic arteries concentric layers of elastic lamellae, bundles of collagenous fibrils, and VSMCs build up the media. In mammals the numbers of aortic lamellae are proportionate to the radius of the aorta. However, the abdominal aorta in man deviates from the usual pattern and a smaller number of lamellar elastic units have been found in the human abdominal aorta than

other mammalian aortas. Furthermore, the major part of the human abdominal aortic media lacks vasa vasorum, in contrast to the media of thoracic aorta (Wolinsky and Glagov 1969). The organization of the main structural proteins, collagen and elastin, differs in the aortic wall. The elastic lamellaes in the media consists of fenestrated sheets of elastin. Between the lamellae, collagen forms fibrillar thick bundles (mainly collagen type I and III), which have helical arrangement of various degrees in the wall (Fig. 1). The adventitial bundles have a more longitudinal arrangement than the medial fibers (Holzapfel 2006). VSMCs tend to align in the same direction in the aortic media as the collagen bundles and are associated with the lamellae through fibrillin-1 and collagen type VI microfibrils (Dingemans et al. 2000). Of the dry weight of the media, VSMCs account for about 20%, and collagen together with elastin account for about 60%. The relation between collagen and elastin changes along the aorta and with increasing distance from the heart the amount of collagen increases (Fischer and Llaurado 1966). The remaining 20% of the media consists of proteoglycans (chondroitin sulphate, dermatan sulfate, heparin sulphate), fibronectin (protein that binds to collagen and cell surface integrins to connect VSMCs with the extracellular matrix), fibrillin (microfibrils surrounding elastin) and to a lesser extent hyaluronic acid (polysaccharide that confers the ability to resist compression upon tissues by providing a counteracting turgor force by absorbing a lot of water, Dingemans et al. 2000).

The external elastic lamina separates the media from the outermost layer, *the tunica adventitia*. It consists of fibroblasts, collagen fibrils, some elastin and associated proteoglycans, nerves, vasa vasorum and function as the vessels' attachment to surrounding tissue.

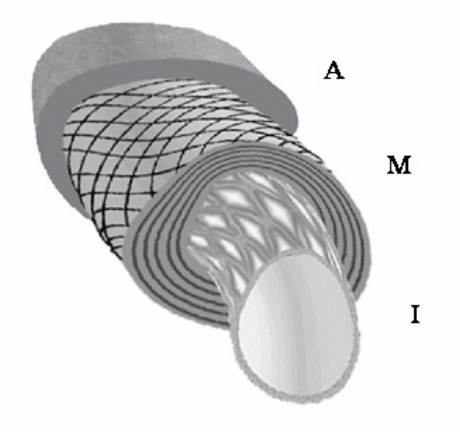

Figure 1. A schematic picture of the three different wall layers in the aortic wall. Note the alignment of the endothelial cells in the intima (I), the lamellar structure of elastin as well as the helical structure of the collagen bundles in the media (M). Outermost is the adventitia (A). With permission from Stålhand.

Aging

The age-related remodeling of the abdominal aorta (AA), results in increased aortic diameter and stiffness (Länne et al. 1994). The cardiovascular system is affected by aortic function in several ways, e.g. increased aortic stiffness with age induce increased pulse-wave velocity causing premature return of reflected pulse waves in late systole increasing central pulse load, myocardial oxygen demand, and workload of the left ventricle (Laurent et al. 2006). Thus, associations between aortic stiffness and left ventricular hypertrophy as well as cardiovascular morbidity/mortality have been found (Laurent et al. 2006).

Due to lack of vasa vasorum in the media and increasing thickness with fewer elastic lamellae than expected, the nutrition of the aortic wall might deteriorate with age. Old elastin seems more susceptible to proteolysis and increased levels of metalloproteinase-2 (a proteolytic matrix enzyme) have been found, resulting in decreased levels of elastin and thinning, splitting and fraying of the medial elastic lamellae (Faber and Oller-Hou 1952, Schlatmann and Becker 1977, Sell and Monnier 1995, Wang et al. 2003b). The fracture of elastin fibers could also be a fatiguing effect of repetitive pulsations since the synthesis of new elastin with age is scarce. Total collagen levels increase with age (Faber and Oller-Hou 1952), and a shift to more type 1 and less type 3 collagen has been described (Silver et al. 2001). Furthermore, both collagen and elastin are subjected to enzymatically and non-enzymatically cross-linkings during maturation and growth of matrix. With age cross-linkings increase mainly due to non-enzymatic glycation (Konova et al. 2004, Reiser et al. 1992). The age-related change in composition of the aortic wall means a transfer of load bearing to collagenous structures.

Remodeling and mechanical stimuli

Arteries are subjected to mechanical stimuli in the form of tensile stress and shear stress. Stress is defined as force per area unit. Blood pressure and arterial geometry of the arteries are the main determinants of tensile stress, creating radial, longitudinal and circumferential components affecting all cells in the vessel wall (Fig. 2).

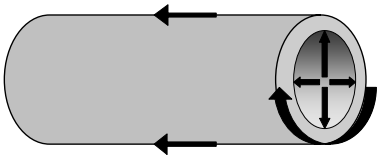

Figure 2. Blood pressure and arterial geometry determines wall stress in arteries. Arrows show the circumferential, axial and radial directions of tensile stress.

The law of Laplace states that circumferential wall stress can be expressed:

$$Stress = \frac{pressure \cdot radius}{wall\ thickness} \quad (1)$$

On the other hand shear stress, resulting from the friction of blood against the luminal side of the vessel wall, affects mainly the endothelial cells which cover and are aligned along the inside of the vessel wall. This frictional force is determined by blood flow, vessel geometry and fluid viscosity.

Endothelial (ECs) and vascular smooth muscle cells (VSMCs) detect mechanical stimuli via focal adhesion sites, integrins, cellular junctions and the extracellular matrix (Lehoux et al. 2006). The ECs are covered on the luminal side with glycocalix (membrane-bound macromolecules), that sense shear stress exerted by the luminal flow and transmits it to the intracellular structure. The extracellular matrix contains glycoproteins that are displaced by stretch or shear forces and interact with integrins which can result in increased cell binding to the extra-cellular matrix (Jalali et al. 2001). Furthermore, stretch has been shown to affect the VSMC's production and secretion of collagen to the extra cellular matrix (Leung et al. 1976). This effect may be mediated through tyrosine kinase-dependant mechanisms inducing transforming growth factor beta (TGF-β) mRNA expression and extra cellular matrix mRNA expression (Joki et al. 2000; Li et al. 1998). Increased shear stress has also been shown to affect the VSMCs from a contractile to a more synthesizing phenotype (Wang et al. 2003a) as well as decreasing the proliferation of VSMCs (Papadaki et al. 1996, Ueba et al. 1997), mediated by TGF-$\beta 1$ in an autocrine manner.

There seem to be differential pathways in response to tensile and shear stress as shown by studies on gene expression and MAPK (mitogen activated protein kinase) activation in endothelial cell (Andersson et al. 2005, Azuma et al. 2000, Carosi et al. 1992, Malek and Izumo 1992). The differentiated remodeling

response has further been shown in histological studies (Ben Driss et al. 1997), where high tensile stress seems to induce medial thickening whereas low shear stress seem to induce intimal thickening (Dobrin 1995). Thus both tensile stress and shear stress seems to be of importance in the remodeling response and the interplay between the two stresses determines the geometry of the vessel (Scuteri et al. 2001). The arterial wall stress hypothesis postulates that an increase in diameter or blood pressure, leads to increased wall stress, which in turn activates smooth muscle cells with an increase in wall matrix and wall thickness. This means that the circumferential wall stress is restored according to the law of Laplace. Pulse pressure may be more important in elastic arteries and mean blood pressure in muscular arteries as remodeling stimulus (Boutouyrie et al. 2000).

The remodeling in arteries depends not only on secretion of newly synthesized, but also breakdown of old matrix. Matrix metalloproteinases (MMPs) are digestive enzymes capable of degrading extracellular matrix throughout the body and involved in both normal and diseased tissue remodeling. VSMCs, ECs, fibroblasts and inflammatory cells have all been linked to the secretion of MMPs. In vascular diseases such as abdominal aortic aneurysms (AAA) MMPs seem to play a major role.

Abdominal aortic aneurysm

An AAA is defined as a more than 50% larger aortic diameter than expected, but most often physicians consider an aortic diameter of 3 cm or more to be an aneurysm. In a necropsy study prevalences of 5% in males and 1-2% in females were found (Bengtsson et al. 1992). However, due to differences in diagnostic criteria the prevalence may vary (Wanhainen et al. 2001). With increasing size of an AAA the risk for rupture increases and in case of a rupture the mortality average 80%. Historically AAA has been considered an effect of atherosclerosis, in part due to the shared risk factors such as old age, smoking, male gender, hypertension and hypercholesterolemia. However, studies on MMPs, genetic predisposition for AAA and the negative correlation to diabetes mellitus have challenged the historical view and AAA is at present described as a degenerative disease of the aortic wall with dilatation of the fragile vessel as a consequence (Wassef et al. 2007). The histopathological features of AAAs are characterized by chronic transmural inflammation, destructive remodeling of the elastic media and depletion of medial smooth

muscle cells. MMP-2 and MMP-9 have been shown to be involved in the degradation of the vessel wall during development of AAA, and the levels of MMPs are related to the AAA enlargement, suggesting that the enzymatic activity varies with aortic diameter (Freestone et al. 1995, McMillan et al. 1997). Increased pressure and strain activates MMP-2 and MMP-9 (Chesler et al. 1999, O'Callaghan and Williams 2000). Furthermore, the possible importance of wall stress in remodeling and degeneration of the aortic wall is emphasized by the fact that there is a relation between blood pressure and increasing aortic aneurysm diameter, as well as aneurysm diameter and risk of rupture (Cronenwett et al. 1990, Szilagyi et al. 1972). As the artery dilates, the circumferential wall stress increase according to Laplace's law and may further accentuate the stress, and a direct relation between wall stress and risk of aneurysm rupture has been proposed (Fillinger et al. 2003, Hall et al. 2000). Atherosclerosis could affect the development of an AAA since the medial lamellar architecture in the abdominal aorta with fewer elastic lamellae and reduced supply by vasa vasorum seems to be important. Due to lack of blood supply when the wall thickness increase with age or in case of an intimal plaque obstructing diffusion, ischemic damage to the media may cause reduced number of elastic lamellae and increased stress on the remaining lamellae, and aneurysms may develop (Zatina et al. 1984).

Diabetes mellitus

Diabetic patients suffer from macro- and micro-vascular disease to a larger extent than non-diabetic individuals. The macrovascular disease also has a more severe course with greater prevalence of multiple-vessel coronary artery disease and more diffuse elongated atheromas in affected blood vessels. Despite the preponderance of atherosclerotic manifestations, diabetic patients exhibit a low prevalence of AAA (LaMorte et al. 1995, Lederle et al. 2000, Lederle et al. 1997). If diabetic patients develop AAAs, the expansion rate of those AAAs is only 30% compared to non-diabetic patients (Brady et al. 2004). The low prevalence of AAAs in the diabetic population has caused limited attention, and the cause for the reduced frequency is unknown. A recent study has shown that diabetes duration is inversely related to the risk of developing an AAA and that aortic diameter has an inverse relationship with fasting glucose concentration in healthy individuals (Le et al. 2007). Patients with diabetes manifest increased arterial stiffness at a younger age than healthy individuals, due to increased depositions of collagen and cross-linkings of

proteins (Aronson 2003). Glucose form glycosylated products with proteins (Shiff bases), which is a fast and reversible reaction, but over a period of days this unstable product rearrange to form stable Amadori-type products (Hemoglobin A_{1c} for example), a process called glycation. These products can then undergo further chemical rearrangements to form very stable, virtually irreversible cross-linkings known as AGEs (advanced glycosylation end-products), which is determined by glucose concentration and time of exposure (Aronson 2003). These cross-links between collagen molecules are believed to yield stiff vessels and high resistance to enzymatic breakdown, and also upregulate TIMP-1 (a MMP inhibitor) via stimulation by connective tissue growth factor (CTGF) (McLennan et al. 2004, Twigg et al. 2001). The carotid intima-media thickness (IMT) is increased in diabetic patients, and there is an association between carotid IMT in non-diabetic patients and the level of postprandial hyperglycemia (Giannattasio et al. 1999, Hanefeld et al. 1999, Jarvisalo et al. 2004). Thus, the breakdown of vessel matrix seems to be altered in diabetes mellitus.

Gender differences

The gender differences in cardiovascular disease are well established with higher cardiovascular morbidity in males until late in life. However, once affected by ischaemic heart disease females actually do worse than their male counterparts (Greenland et al. 1991). This is especially true for diabetic patients who suffer from cardiac disease. One reason could be the found gender differences in aortic stiffness among diabetics (Ryden Ahlgren et al. 1995). Vascular disease such as aortic aneurysm are more common among males, however in a likewise fashion, once females are affected they do worse than males, since the risk of aneurysm expansion and rupture in females have been reported to be higher (Brown and Powell 1999, Mofidi et al. 2007). It has only recently been appreciated that significant gender differences also exist in cardiovascular function. The diameter of the aorta is larger in males mostly due to a larger body surface area (BSA), although the diameter continues to increase after the termination of growth. The age related increase in stiffness that occurs in the AA is more pronounced in males (Sonesson et al. 1994), most obvious before menopause (Laogun and Gosling 1982). Sex hormones affect arterial wall properties (Westendorp et al. 1999), and postmenopausal hormone replacement therapy in females seems to reduce arterial stiffness (Rajkumar et al. 1997). Both estradiol and progesterone decrease the

collagen/elastin ratio in the aortic wall (Fischer and Swain 1980). Furthermore, testosterone increases the activity of MMP-3, an enzyme degrading aortic wall elastin and fibrillin-1, supporting the fact that female gender seems protective from elastolysis (Natoli et al. 2005, Sinha et al. 2006).

The study of arterial wall mechanics

When the artery is subjected to stress (force/area unit) in the form of a blood pressure, it responds by deforming. A measure of this deformation is the strain, defined as the ratio of deformation from its original form. The deformation from diastole to systole is greatest in the circumferential direction resulting in increased diameter and decreased wall thickness (Fig. 3).

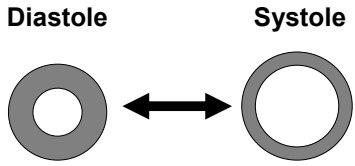

Figure 3. Illustrates the change in diameter and wall thickness in an artery during the cardiac cycle.

The stiffness of a material can be expressed as Young's modulus (E):

$$E = \frac{Force\ per\ unit\ area}{Extension\ per\ unit\ length} \qquad (2)$$

However, due to the components in the elastic artery the vessel deformation is nonlinear, i.e. with increasing pressure the increase in diameter is reduced. In other words, the more distended, the stiffer the artery gets. Matrix degrading experiments have shown that elastin and collagen are the main determinants of the mechanical characteristics of the human aorta (Hoffman et al. 1977). The first part of the stress-strain curve in an elastic artery depends mainly on elastin and at higher stress the influence of collagen increases (Fig. 4, Roach and Burton 1957).

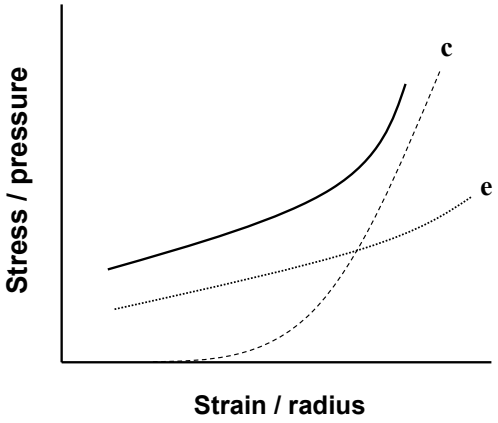

Figure 4. Schematic drawing describing a typical stress-strain curve of an elastic artery under distension (solid line) based on data from Roach and Burton (1957). Note the biphasic appearance, which depends on the contribution of collagen (dashed line) and elastin (dotted line) to the global response (solid line).

Elastin can experience uniaxial extension of 150% when being straight, without breaking and return to its original configuration when unloaded. For that reason elastin is believed to store and then return mechanical energy. This is in sharp contrast to collagen that can extend less than 10% when straight and have a 250 times greater elastic modulus than elastin (Armentano et al. 1991, Humphrey 2002). In the aortic wall however, collagen bundles have a wavy form in the low-pressure region and the fibers are not straight until the waviness of the bundles have unfolded. Thus, collagen seems to have a protecting effect to prevent overextension of the vessel. Furthermore, the arrangement of the fibers makes collagen an anisotropic material. This means that if the same force is applied in different directions, the resulting strain will differ between the directions. This is in contrast to the elastin fibers, which due to the mesh-like arrangement of elastin molecules behave as an isotropic material, i.e. the same strain will be observed irrespectively of the direction in which the force is applied (Dobrin et al. 1990).

In order to measure the stiffness of an artery, in vitro methods as well as in vivo methods exists. With in vitro methods, besides the obvious problem of having to excise the artery from the body, the information is confounded by post mortem changes in the vessel, lack of sympathetic innervation and circulating hormones affecting the wall, loss of peri-adventitial tissue, and vessel attachment to surrounding tissue that changes the mechanical properties (Liu et al. 2007, Nichols and O'Rourke 2005a). In vivo techniques are thus preferable. Since the pulse wave travels faster along a stiff artery, pulse wave velocity measurements (PWV) has been widely used to study regional aortic stiffness, although local stiffness in the aorta is better assessed

with echo-tracking ultrasound, which detects diameter change locally during the cardiac cycle (Pannier et al. 2002). Together with simultaneous pressure registrations, stress-strain curves (σ_θ) can be obtained. Using this technique, only global stiffness of arteries has been evaluated. Since the stress-strain curve (σ_θ) is composed of an elastin-dominated part (isotropic) and a collagen-dominated part (anisotropic), see Figure 4, an equation can be proposed:

$$\sigma_\theta = \sigma_\theta^{iso} + \sigma_\theta^{ani}, \tag{3}$$

where σ_θ^{iso} is the isotropic part, σ_θ^{ani} is the anisotropic part and the index θ denotes the circumferential direction. Note the exponential appearance of σ_θ^{ani} (Fig. 4).

So far, the behavior of collagen and elastin separately, has not been studied in vivo in man, although in vitro experiments have been performed.

AIMS

- To validate non-invasive ultrasound measurements of intima-media thickness and diameter in the abdominal aorta in order to calculate circumferential wall stress, and evaluate the effect of gender.

- To study aortic remodeling and circumferential stress in the aorta and the impact of age and gender.

- To study the age and gender related changes in aortic material parameters in vivo with the aid of a newly developed mechanical model.

- To study the impact of aortic wall stress on the remodeling response in the aorta.

- To study aortic wall stress, in diabetic patients compared to healthy individuals.

MATERIALS

Ethics

The studies were approved by the Ethics Committee at Lund University.
All healthy individuals and all diabetic patients gave informed consent according to the Helsinki declaration.

Healthy individuals (Paper I-IV)

A total of 111 healthy Caucasian subjects were investigated (52 males and 59 females, range 10-83 yrs, mean 47 ± 22 yrs) in Paper I, II and IV. They were recruited among friends, medical staff and from advertising. All were non-smokers, without hereditary factors regarding aneurysmal disease. They had no history of cardio-pulmonary or cerebro-vascular disease and were all normotensive. All had an ankle brachial index ≥1. There was no regular treatment with pharmacological substances. The females had no hormonal replacement therapy. In Paper I, 25 males and 25 females were investigated. In Paper II, another 27 males and 34 females were included to study age related phenomena. In Paper IV we used 46 age- and sex-matched subjects (17 males and 29 females, range 28-69 yrs, mean 44.4 ± 10.5 yrs) out of the 111 subjects and compared with diabetic patients.

In Paper III we investigated another 30 healthy Caucasian subjects (15 males and 15 females, age 23-72 yrs), of whom five men and five women were recruited in each three age categories; young (23-30 yrs), middle-aged (41-54 yrs) and elderly (67-72 yrs). They were recruited from advertising. All were non-smokers, without hereditary factors regarding aneurysmal disease. None had a history of cardio-pulmonary or cerebro-vascular disease. All had an ankle brachial index ≥1. None were taking any regular medication. The females had no hormonal replacement therapy.

Diabetic patients (Paper IV)

We studied 39 patients with diabetes mellitus type 1 (17 males and 22 females, range 27-69 yrs, mean 43.3 ± 10.6 yrs). All were Caucasian. They were recruited from a University hospital setting. None of the patients had any history of acute myocardial infarction, cerebro-vascular events nor intermittent claudication. The ankle brachial index was ≥1 in all diabetic patients. A high incidence of retinopathy was found among the diabetic patients, 18 had background retinopathy and eight had proliferative retinopathy. Smoking was reported in 14 diabetic patients. Two diabetic patients suffered from albuminuria (>0.5g/24h) and eight from microalbuminuria (30-300 mg/24h). Five were treated for hypertension, four with ACE-inhibitors and one with β-blocker. All diabetic patients were treated with insulin. The diabetic patients had mean diabetes duration of 26 ± 8 years, range 15-45 years. Their mean HbA1c was 7,4 ± 1,3% and their mean creatinine level was 76 ± 36 micromol/l.

METHODS

Intima-media thickness and diameter measurement with ultrasound (Paper I-II, IV)

For measuring the intima-media thickness (IMT) and the lumen diameter (LD) of the arteries we used a Philips P700 ultrasound device (Philips Ultrasound, Santa Ana, California, United States) with a 7.5 MHz linear transducer for scanning of the superficial vessels. For aortic imaging either a 5 MHz or a 3.5 MHz transducer was used. A longitudinal perpendicular image of the vessel was insonated and recorded on a video monitor (Panasonic Ag-7350l, Matsushita Electric Industrial Co., Osaka, Japan). The analyzing system was built up by a PC (Intel 486, Santa Clara, CA, USA), a video monitor (Panasonic H1450, Matsushita Electric Industrial Co., Osaka, Japan) and a video recorder (Panasonic NV-HS1000, Matsushita Electric Industrial Co., Osaka, Japan), linked to a text monitor and a digitizer (Summagraphics Summa Sketch III, GTCO CalComp, Scottsdale, USA). The longitudinal image was frozen in diastole, according to the prevailing standard of IMT measurement (Fig. 5). Although tilting the transducer away from the transverse axis will falsely decrease the measured diameter and increase the IMT, this error is minimized because the echoes representing the IMT will not be clear enough unless the transducer is positioned in the midline of the vessel. The image was recorded on videotape, then measured manually by tracing a cursor along the echo edges on a 10 mm section with the aid of the digitizer (Wendelhag et al. 1991). The 10 mm longitudinal image provides approximately 100 boundary points between the echo edges where the IMT is measured and the mean value of IMT were then automatically calculated with a computerized system (VAP version 2.0). The software was written in Microsoft Pascal under MS-DOS operating system. The analyzing system was developed by the Department of Applied Electronics, Chalmers University of Technology, Gothenburg, Sweden.

This system's actual resolution, which represents the minimum distance at which two distinct echoes can be separated, is about 0.3-0.4 mm, which means that vessels walls thinner than that size cannot be evaluated.

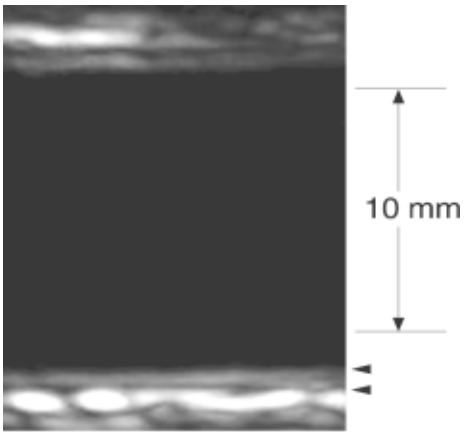

Figure 5. Ultrasonic image of the abdominal aorta imaged with a 5 MHz transducer. Upper arrowhead, luminal-intimal interface of the aortic wall; lower arrowhead, corresponding medial-adventitial interface. Intima-media thickness was defined as measured distance between these interfaces.

IMT was measured on the far wall, from the interface between blood and the intima, to the interface between the media and the adventitia (Pignoli et al. 1986, Wendelhag et al. 1991). LD was measured from the leading edge of the second echo in the near wall to the leading edge of the first echo of the far wall. This corresponds to the actual interfaces between intima and lumen of the near and far walls respectively (Wendelhag et al. 1991). The means of the IMT and the LD were calculated from the first two good quality images. The cross-sectional intima-media area (IMA) was also calculated according to the formula:

$$IMA = \pi\left(\frac{LD}{2} + IMT\right)^2 - \pi\left(\frac{LD}{2}\right)^2, \qquad (4)$$

where IMT is the intima-media thickness (mm) and LD the lumen diameter (mm).

Wall stress was calculated according to the law of Laplace. The wall thickness is not included in its classical form; $Wall\ tension = pressure \cdot radius$. We used the

extended formula, sometimes also named Lamé (Nichols and O'Rourke 2005b) or Frank (Bader 1967), to calculate the circumferential wall stress (dyne/cm²):

$$Wall\ stress = \left(MAP \cdot \frac{LD}{2} \right) \Big/ IMT \qquad (5)$$

Mean arterial blood pressure (MAP) was calculated as diastolic pressure + 1/3 of the pulse pressure (dyne/cm²). 1 mm Hg equals 1333 dyne/cm², 1 dyne/cm² equals 0.1 Pa. LD, the lumen diameter (cm). IMT, intima-media thickness (cm).

The abdominal aorta (AA) was examined at the midpoint between the renal arteries and the aortic bifurcation. The right common carotid artery (CCA) was examined 1 cm proximal to the bifurcation. The right common femoral artery was examined at the site of the inguinal fossa with the hip joint as a landmark. The right popliteal artery was examined at the site of the popliteal fossa with the patient in prone position and the patella as a landmark. All examinations were performed after at least 15 minutes rest, with the subjects in a supine position, except for the ones on the popliteal artery.

In Paper II we examined 111 healthy individuals. In 12 subjects, it was not possible to evaluate IMT of the AA due to bowel gas, plaque formation at the site of interest, obesity or other problems in visualizing the vessel. Also, in three of those 12 subjects it was not possible to visualize the IMT of the CCA, due to plaque formation at the site of interest. Thus, in 48 males and 51 females the IMT of the AA was visualized and in 50 males and 58 females the IMT of the CCA was visualized.

In Paper IV we examined 39 diabetic patients and it was possible to measure the carotid IMT in all diabetic patients. However, in 12 of the diabetic patients it was not possible to obtain high enough sonographic image quality to measure aortic IMT. The 12 did not differ significantly from the successfully examined diabetic patients regarding blood pressure, carotid wall stress, carotid IMT, carotid LD, BMI (body mass index), BSA (body surface area), diabetes duration, HbA1c, smoking habits, degree of albuminuria or retinopathy.

Non-invasive blood pressure measurements (Paper I-II, IV)

All examinations were performed after at least 15 minutes rest, with the subjects in a supine position. When examining the popliteal artery the examinations were performed in prone position.

At the beginning of the investigation, pressure was measured in the upper arm bilaterally non-invasively with a cuff and a sphygmomanometer. No significant difference in pressure between the arms was found and the right arm was used in the pressure measurements. Non-invasive brachial pressure has been shown to generate a slight overestimation of the aortic diastolic pressure, but without sex or age-related differences (Sonesson et al. 1994).

Invasive blood pressure measurements (Paper III)

Invasive blood pressure was obtained in the abdominal aorta with a 3F (SPC 330A) or 4F (SPC 340) micromanometer tip 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). When compared, the two systems showed no difference in amplitude (Blood Systems Calibrator, Bio Tech Model 601 A, Old Mill Street, Burlington, VT 05401, USA). With local anesthesia in the groin, the pressure catheters were inserted with Seldinger technique through the right femoral artery. The catheters were positioned with ultrasonic guidance in the infrarenal abdominal aorta just distal to the selected point of pulsatile diameter measurement, i.e. at the midpoint between the renal arteries and the aortic bifurcation.

A data acquisition system containing a PC type 386 (Express, Tokyo, Japan) and a 12-bit analogue to digital converter (Analogue Devices, Norwood, USA) was included for the simultaneous monitoring of the arterial blood pressure and vessel diameter (Fig. 6, left). Pressure was sampled at the same rate as the diameter. The curves could be registered for a maximum of time of 11 seconds. The sampling frequency was 870 Hz.

Non-invasive monitoring of diameter changes (Paper III)

The method for non-invasive monitoring of pulsatile diameter changes in the distal abdominal aorta has been described previously (Länne et al. 1992b). Briefly, we used an electronic echo-tracking instrument (Diamove, Teltec AB, Lund, Sweden), interfaced with a real-time ultrasound scanner (EUB-240, Hitachi, Tokyo, Japan) and fitted with a 3.5 MHz linear array transducer. An echo-tracking phase locked loop circuit restores the position of an electronic gate relative to the moving echo. The discrete compensatory steps of the gate yield the echo movement per unit time. The instrument is equipped with dual echo-tracking loops which makes it possible to track two separate echoes from opposite vessel walls simultaneously. The differential signals between them instantaneously indicate any change in vessel diameter. The smallest detectable movement is 7.8 μm and the repetition frequency 870 Hz, the consequent time resolution approximately 1.2 ms. The CV for static diameter is 5% and for pulsatile diameter change 16% (Hansen et al. 1993). The abdominal aorta between the renal arteries and the bifurcation was visualized in a longitudinal section on the real time image of the ultrasound scanner and the measuring point for pulsatile diameter change was selected 3-4 cm proximal to the aortic bifurcation.

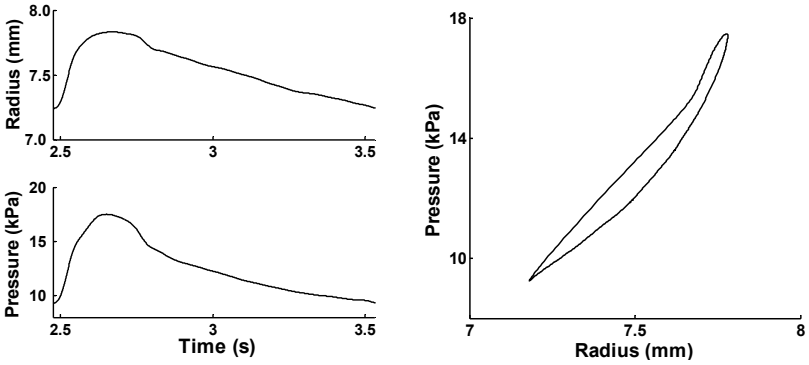

Figure 6. Simultaneously recorded pressure and radius during a cardiac cycle in the abdominal aorta of a 54 year old female (left). Pressure - radius curve based on a mean of 10 consecutive cardiac cycles in the abdominal aorta of a 54 year old female (right).

Mechanical model for characterization of material parameters of the aorta (Paper III)

To compute the material parameters, we use a novel method presented in Stålhand (2008). The method comprises of a signal processing routine and a mechanical model. The signal processing routine removes noise and computes an average pressure-radius loop from the registered signals (Fig. 6). The result is subsequently fed into the mechanical model and material parameters describing the geometry and the material properties for the arterial wall are computed by a nonlinear curve-fitting. For a detailed description see Stålhand (2008) and Appendix of Paper III. The mechanical model can be summarized in the following steps (Fig. 7).

1. The mechanical model first determines the average stresses in the arterial wall using the pressure-radius loop and an estimation of the cross-sectional area (A) of the aortic wall together with Laplace's law. The cross-sectional area A (mm) is determined by recalculating the data from Paper II, resulting in the expressions, $A = 19.60 + 0.80 \cdot age\,(years)$ in males ($p<0.0001$), and $A = 20.52 + 0.58 \cdot age\,(years)$ in females ($p<0.0001$). To account for the thickness contribution from the adventitia, the cross-sectional area is corrected by assuming the intima-media complex to comprise 2/3 of the wall (Holzapfel et al. 2000).

2. The mechanical model then computes a second set of stresses using continuum mechanics and the pressurized radius as in-data (Holzapfel et al. 2000). These stresses, however, become dependent on the parameters c, k_1, k_2, β, R_0 and λ_z described below.

3. Values for the parameters are, finally, obtained in a parameter identification process by tuning the second set of stresses to the average stresses computed using Laplace's law. The parameter identification can be done using standard nonlinear minimization procedures (Holzapfel et al. 2000), for instance the function `fmincon` in Matlab (The MathWorks, Natick, MA, USA).

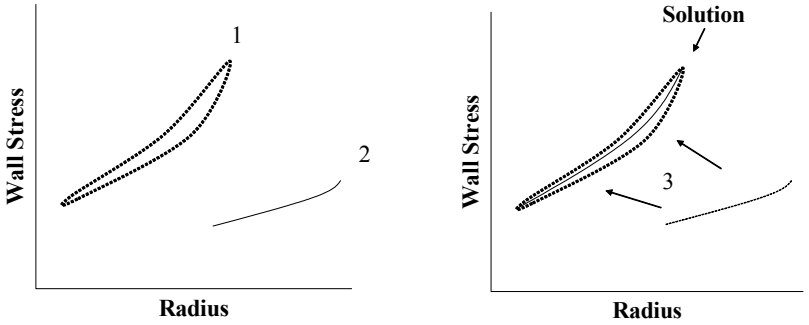

Figure 7. An illustration of the 3 different steps of the mechanical model. 1: average stresses are calculated 2: second stresses calculated using continuum mechanics 3: tuning parameters results in a calibration of stresses (Stålhand, 2008).

The following parameters are determined with the mechanical model:

c: A material parameter relating to the stiffness of the isotropic materials ($elastin_{iso}$), mainly elastin but also proteoglycans, fibronectin, fibrillin, and hyaloronan. Note that *c* is not equal to the slope of the stress-strain curve; the slope also depends on the strain since this is a nonlinear material. However, *c* is a constant and thus pressure independent.

k_1: A material parameter for the anisotropic material ($collagen_{ani}$), mainly collagen. This parameter is the principle determinant of $collagen_{ani}$ stiffness in the small stretch region of the pressure-radius response (pressure below physiological level) where the crimped collagen molecules are primarily straightened and the tissue is resilient. As for *c*, the parameter k_1 is not equal to the slope of the stress-strain curve, since it also depends on the strain.

k_2: A parameter related to $collagen_{ani}$. An increasing value for k_2 results in a leftward shift of the transition region between the resilient (elastin dominated) and the stiff (collagen dominated) parts of the pressure-radius response, thus indicating earlier collagen recruitment.

β: A parameter describing the angle of $collagen_{ani}$ relative to the circumferential direction. Since the direction of collagen varies through the arterial wall, β has no histological interpretation; it is simply a (phenomenological) fiber angle resulting in the correct anisotropy.

R_0: The radius in the unloaded reference state of the artery.

λ_z: The axial stretch of the vessel in vivo relative to the unloaded reference state.

The signals from the simultaneously recorded pressure and radius in the abdominal aorta yield a stress-strain curve (σ_θ), which can be described with the formula:

$$\sigma_\theta = 2c\left(\lambda_\theta^2 - \frac{1}{(\lambda_\theta\lambda_z)^2}\right) + 4k_1(I-1)e^{k_2(I-1)^2}\lambda_\theta^2(\cos\beta)^2, \quad (6)$$

where, $I = \lambda_\theta^2(\cos\beta)^2 + \lambda_z^2(\sin\beta)^2$, and $\lambda_\theta = \frac{R_0}{r_0}\frac{4\pi r_0^2 + A}{4\pi R_0^2 + \lambda_z A}$.

The indices θ and z denote the circumferential and axial directions, respectively. λ_θ denotes circumferential stretch of the vessel in vivo relative to the unloaded reference state and r_0 the inner radius of the vessel in its physiological state. Note that the stress-strain equation is composed of a part describing the isotropic materials, mainly elastin:

$$\sigma_\theta^{iso} = 2c\left(\lambda_\theta^2 - \frac{1}{(\lambda_\theta\lambda_z)^2}\right), \quad (7)$$

and an exponential part describing the anisotropic material, mainly collagen:

$$\sigma_\theta^{ani} = 4k_1(I-1)e^{k_2(I-1)^2}\lambda_\theta^2(\cos\beta)^2. \quad (8)$$

In a complimentary study we also analyzed wall stresses calculated with the formulas found in Stålhand (2008) and Paper III (Appendix). Furthermore we separated the isotropic part (S_{SBP}^{iso}) and the anisotropic part (S_{SBP}^{ani}) of S_{SBP}, the global stiffness in circumferential direction at systolic blood pressure. S_{SBP}^{iso} can be calculated by substituting Eq. (7) for σ_θ in the following equation for the stiffness, see also Paper III (Appendix):

$$S = \frac{\partial\sigma_\theta}{\partial\lambda_\theta}, \quad (9)$$

where right handed side of Eq. 9 denotes the derivative of σ_θ with respect to λ_θ. An anisotropic part, S_{SBP}^{ani}, can be obtained in a similar way by substituting Eq. (8) for σ_θ in the formula above.

With the aid of the mechanical model above proposed by Stålhand (2008), see also Appendix of Paper III, the following parameters can be calculated with the equations in those two papers.

S_{SBP}**:** Total wall stiffness in the circumferential direction, i.e. the slope of the stress-strain curve at systolic blood pressure.
S_{SBP}^{iso}: The wall stiffness in the circumferential direction due to the isotropic materials, mainly elastin, at systolic pressure.
S_{SBP}^{ani}: The wall stiffness in the circumferential direction due to the anisotropic material, mainly collagen, at systolic pressure.
SC_{SBP}: The stiffness of the anisotropic material, mainly collagen, in the fiber direction, at systolic blood pressure.
ϕ_{DBP} The fraction of load bearing attributed to the anisotropic material at diastolic blood pressure.
ϕ_{SBP} The fraction of load bearing attributed to the anisotropic material at systolic blood pressure.

Statistics

Statistical evaluation of data was carried out using STATISTICA 8.0 (StatSoft Inc., Tulsa, USA).

Data are presented as mean value ± SD in Paper I, II and IV. In Paper III data are presented as mean ± SE. In the reproducibility study (Paper I) means and SDs for differences between the two observers or examinations were calculated. Inter and intra-observer error (s) was then calculated according to $s = SD/\sqrt{2}$. The coefficient of variation (CV) was calculated according to Bland and Altman (1986). Comparisons in Paper I, II and IV between gender and between arterial regions were made with unpaired and paired Student *t*-test respectively. The comparisons between groups in Paper III were performed with unpaired Student *t*-test.

In Paper II-IV, linear regression and forward stepwise multiple regression models was used. For comparing the gender differences of the slopes of regression curves we included an interaction term (gender·age). In Paper IV differences in IMT, LD, wall stress, age, height, weight, BSA, BMI, and blood pressures between diabetic patients and controls, was evaluated using

unpaired students t-test. For calculating differences in smoking habits, albuminuria and retinopathy between diabetic patients and controls we used Chi-square test with Yates correction and Fishers exact test. For analyzing differences between the group of diabetic patients in whom it was not possible to examine the aorta, and the group possible to examine the aorta, we used Mann-Whitney-U test, Chi-square test with Yates correction and Fishers exact test.

RESULTS

Reproducibility of measurements (Paper I)

54 subjects were examined twice consecutively by one experienced ultrasonographer. In four of the subjects, it was not possible to evaluate intima-media thickness (IMT) in the infrarenal abdominal aorta (AA) due to bowel gas, plaque formation at the site of interest or other problems in visualizing the vessel. Thus in 25 males (age 47.6 +/- 12.0) and 25 females (age 47.0 +/- 9.9) the AA, common carotid artery (CCA), common femoral artery (CFA) and popliteal artery (PA) were studied. The IMT of the CCA was visualized in all the subjects. In one male and one female the IMT of the PA, and in four males and one female, the IMT of the CFA could not be visualized due to plaque formation at the site of interest.

Figure 8 shows the intra-individual reproducibility of the IMT measurements in the AA. The CV (coefficient of variation) was 11%. The CV's of the CCA, CFA and PA were 6, 8, and 10% respectively. The intima-media area (IMA) showed similar results with CV's: AA, CCA, CFA and PA, 12, 6, 8, and 10% respectively. When measuring the lumen diameter (LD) CV was lower: 4, 2, 2, and 2% in AA, CCA, CFA and PA respectively.

Eleven healthy subjects were included in an interobserver variability study performed by two different sonographers (eleven subjects for AA examinations, and in five we analyzed both the right and left CCA). Each examiner performed three consecutive recordings of the eleven AA and the ten CCA. The first recording of each examiner was compared, as well as the second and the third. This resulted in three different CVs from the aorta and CCA with the mean calculated. The inter-individual variability in the aorta was 10% regarding IMT. The CV of the IMA was 14%, and the CV of the LD was 6%. The CCA had a CV of 8% regarding IMT and IMA, and a CV of 3% regarding LD.

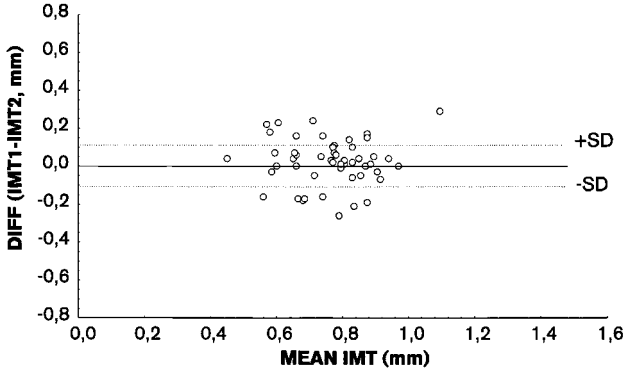

Figure 8. The intra-observer variability of IMT measurements in the infrarenal abdominal aorta according to Bland and Altman (1986). The difference between measurement 1 and 2 is denoted on the vertical axis and the mean of measurement 1 and 2 on the horizontal axis. Coefficient of variation (CV), 11%.

Wall stress in healthy individuals (Paper I-II)

The IMT, IMA, LD and circumferential diastolic wall stress were investigated in the AA, CCA, CFA and the PA of healthy subjects. The IMTs in the four arterial regions are shown in Figure 9. The aorta had greater IMT than the other vessels (P<.001). Men had greater IMT than women in both the CFA (P<.05) and PA (P<.01). In the CCA there was a tendency for men to have greater IMT than women, but this failed to reach significance. In the AA no difference between men and women was seen.

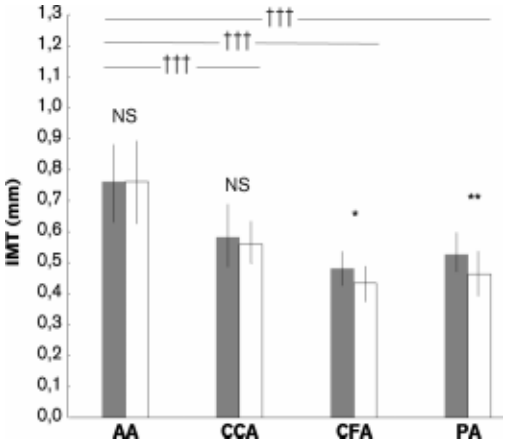

Figure 9. Regional and gender differences in IMT of abdominal aorta (AA), common carotid artery (CCA), common femoral artery (CFA) and popliteal artery (PA). Bars, means ± SD. ††† significant differences between arterial regions, p<.001. ***** Significant differences between males (filled bars) and females (empty bars), p<.05.
****** Significant differences between gender, p<.01.

The circumferential diastolic wall stresses, IMA and LD in the four arteries studied are shown in Table 1. The AA had larger wall stress than CCA and PA, but not significantly larger then CFA. There was a gender difference in all the vessels with men having larger circumferential wall stress in all regions.

Table 1. Wall stress, Intima-media area and Lumen diameter in four vascular regions.

	AA	CCA	CFA	PA
Wall Stress (kPa)				
Males	107 ± 18 ***	60 ± 10 ††† **	101 ± 22 **	67 ± 11 ††† ***
Females	87 ± 16	53 ± 7 †††	85 ± 15	56 ± 8 †††
IMA (mm^2)				
Males	38.4 ± 7.8	13.1 ± 3.5 ††† *	14.5 ± 2.1††† ***	12.0 ± 2.7 ††† ***
Females	33.8 ± 9.5	11.4 ± 1.7 †††	10.6 ± 2.2 †††	8.2 ± 1.8 †††
LD (mm)				
Males	15.2 ± 1.6 ***	6.5 ± 0.7 ††† **	9.1 ± 1.2 ††† ***	6.7 ± 0.9 ††† ***
Females	13.1 ± 2.2	5.9 ± 0.4 †††	7.3 ± 0.8 †††	5.1 ± 0.7 †††

IMA, intima-media area; LD, lumen diameter; AA, abdominal aorta; CCA, common carotid artery; CFA, common femoral artery PA; popliteal artery. Values represent means ± SD. ††† *denotes significant difference between marked vessel and aorta, p<.001.* * *denotes significant differences between gender, p<.05.* ** *denotes significant differences between gender, p<.01.* *** *denotes significant differences between gender, p<.001.*

Age related changes (Paper II)

A total of 111 healthy Caucasian subjects, 52 males and 59 females were investigated in Paper II. In 48 males and 51 females the IMT of the AA was visualized and in 50 males and 58 females the IMT of the CCA was visualized.

Figure 10 shows the changes in diameter with age in the AA (left) and the CCA (right). LD increased with increasing age in both the AA and CCA in both males and females (p<.001). The diameter was larger in males than in females in both the AA and the CCA (both p<.001). The dilatation was larger in males than in females in both the AA and the CCA (both p<.01). In adults the AA diameter between the ages of 25 and 70 years increased from 13.3 mm to 17.3 mm (30%) in males, and from 11.4 mm to 14.3 mm (25%) in females. When adjusted for body surface area however, the gender difference disappeared. Male aortic LD was not correlated with blood pressure (SBP,

DBP, MAP, and PP respectively) or IMT, only age and body surface area. The female aortic LD showed correlation with IMT, as well as age and body surface area.

In adults the CCA diameter increased from 5.9 mm to 7.1 mm (19%) in males and from 5.6 mm to 6.3 mm (12%) in females. Males had larger diameters ($p<.001$) and a more pronounced diameter increase with age, $p<0.05$. There was no correlation between LD and IMT, body surface area and blood pressure (SBP, DBP, MAP and PP respectively), but a correlation with age. Males and females were analyzed separately. Since there was no correlation between body surface area and LD, we did not correct the age-related diameter increase for body surface area in the CCA.

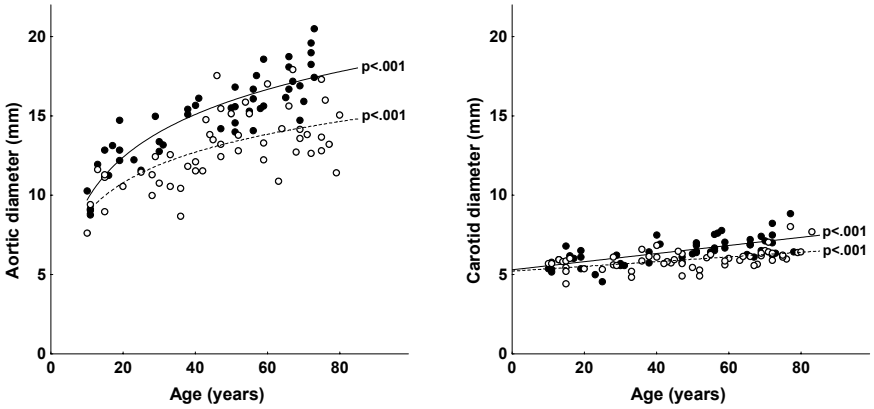

Figure 10. The relation between diameter and age in the abdominal aorta (AA, left) and common carotid artery (CCA, right). The diameter increased with age in the AA in both males (filled circles, r=0.88) and females (open circles, r=0.66), as well as in the CCA in both males (filled circles, r=0.67) and females (open circles, r=0.52).

Figure 11 shows the IMT in relation to age in the AA (left) and the CCA (right). There was an age-related increase in both males and females ($p<.001$). In adults between the ages of 25 and 70 years the aortic IMT increased 41% and 38% in males and females respectively. In the CCA the IMT increased by 46% and 40% in males and females respectively. There were no gender differences regarding the mean value of IMT or the change in IMT with age neither in the AA nor in the CCA.

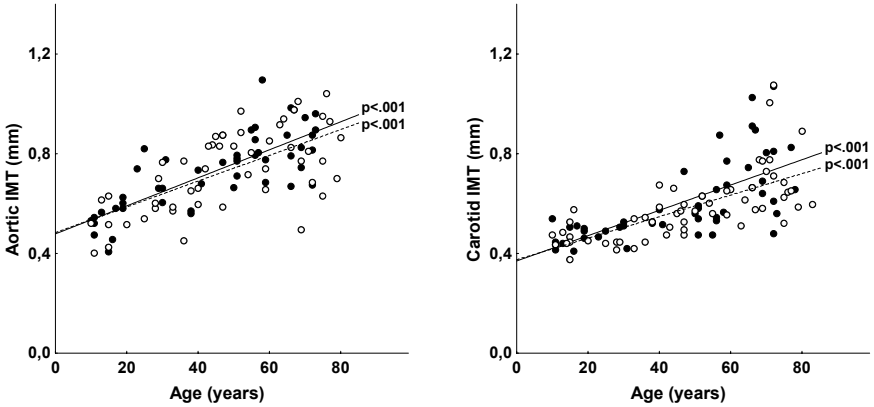

Figure 11. The relation between intima-media thickness (IMT) and age in the abdominal aorta (AA, left) and common carotid artery (CCA, right). The IMT increased with age in the AA in both males (filled circles, r=0.78) and females (open circles, r=0.65), as well as in the CCA in both males (filled circles, r=0.69) and females (open circles, r=0.68).

In both males and females there was a similar age-related increase in diastolic pressure ($p<.001$). Above the age of 50-60 years however, the increase in diastolic pressure diminished and a slight reduction was seen. There was no gender difference in the age-related changes in diastolic pressure. Mean arterial pressure increased similarly in both genders with age ($p<.001$). Pulse pressure increased in females ($p<.001$), the same tendency was found in males, but did not reach statistical significance ($r=.26$, $p=0.067$). Systolic pressure increased in both genders ($p<.001$). The increase in systolic pressure was larger in females ($p<.05$).

Figure 12 shows the changes in circumferential diastolic wall stress with age in the AA (left) and the CCA (right). Wall stress was larger in the AA than in the CCA in both genders ($p<.001$). Furthermore, males had larger wall stress, in both the AA and CCA ($p<.001$ and $p<.05$ respectively, see also Table 1). Despite this female aortic wall stress was significantly larger than male carotid wall stress (9.0 ± 1.8 vs. $5.7 \pm 1.3 * 10^5$ dynes/cm^2, $p<.001$). Aortic wall stress in males increased between the ages of 25 years and 70 years by 14% ($r=.40$, $r^2=.16$, $p=.005$). No such increase was found in the female AA. In the CCA no age-related change in wall stress was found neither in males or females.

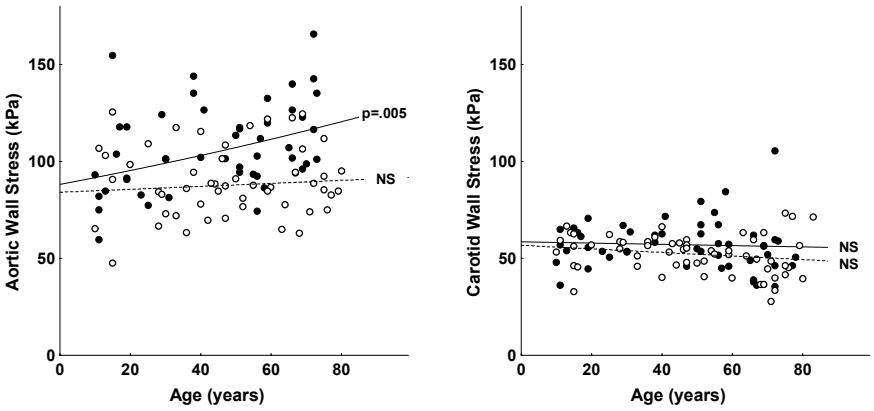

Figure 12. The relation between circumferential diastolic wall stress and age in the abdominal aorta (AA, left) and the common carotid artery (CCA, right). The wall stress increased with age in males in the AA (filled circles, r=0.40), but not in females (open circles, r=0.06). There was no correlation between wall stress and age the CCA in males (filled circles, r=-0.06) nor in females (open circles, r=-0.20).

Aortic material parameters in healthy individuals (Paper III)

In Paper III we investigated 15 males and 15 females of different ages. ***c***, relating to the stiffness of the isotropic materials ($elastin_{iso}$), mainly elastin, of the aorta in relation to age in males and females is shown in Figure 13. ***c*** increased with age in males, 40.8 ± 6.6 and 192.3 ± 24.4 kPa in the young and elderly group respectively (p<.001), and a high correlation between age and the increase in ***c*** was found, with a 394% increase from 25 to 70 years of age (r^2=0.74, p<.001). *c* did not increase with age in females, 50.5 ± 17.0 and 113.8 ± 37.5 kPa in the young and elderly group respectively (p=.16), and no correlation between age and ***c*** was found (p=.15). The increase with age was larger in males than females (p<.05), although the mean value of ***c*** did not differ (118 ± 20.1 vs. 87.4 ± 16.3 kPa, p=.25).

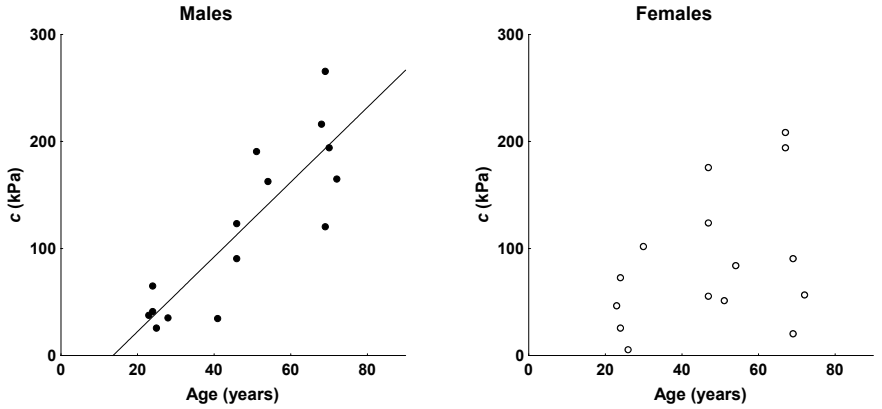

Figure 13. Stiffness relating to elastin$_{iso}$ (***c***) in the aorta in relation to age in males (filled circles) and females (open circles). ***c*** increased with age in males, r^2=0.74, p<.001, but not in females.

The stiffness k_1, related to the anisotropic material (collagen$_{ani}$), mainly collagen, at aortic pressures below physiological level decreased with age in males, 16.6 ± 3.6 and 1.8 ± 1.4 kPa in the young and elderly group respectively (p<.01), and a high correlation between age and k_1 was found, with a 96% decrease from 25 to 70 years of age (r^2=0.42, p<.01). k_1 did not decrease with age in females, 8.1 ± 4.1 and 7.3 ± 3.9 kPa in the young and elderly group respectively (p=.81), and no correlation between age and k_1 was found (p=.81). The decrease with age was larger in males than in females (p<.05), although the mean value of k_1 did not differ (9.2 ± 4.4 vs. 7.5 ± 4.0 kPa, p=.63).

The constant k_2 is coupled to the stress-strain curve of collagen$_{ani}$ and a high value indicates earlier collagen recruitment during the cardiac cycle. k_2 increased with age in males, 7.3 ± 1.8 and 471.1 ± 93.2 in the young and elderly group respectively (p<.01), and a high correlation between age and k_2 was found, with a 7487% exponential increase from 25 to 70 years of age (r^2=0.85, p<.001). k_2 increased with age in females, 20.4 ± 11.4 and 170.6 ± 57.8 in the young and elderly group respectively (p<.05), and a high correlation between age and k_2 was found, with a 1297% exponential increase from 25 to 70 years of age (r^2=0.42, p<.01). The group of young males did not differ from the group of young females (7.3 ± 1.8 vs. 20.4 ± 11.4, p=.28). The increase with age was more pronounced in males, with a larger k_2 in the group of elderly males than the group of elderly females (471.1 ± 93.2 vs. 170.6 ± 57.8, p<.05). The mean value of k_2 did not differ between males and females however (202.9 ± 63.5 vs. 98.4 ± 29.4, p=.15).

The load bearing fractions attributed collagen$_{ani}$ in the aorta at high (ϕ_{SBP}) and low (ϕ_{DBP}) physiological pressures in males and females are shown in Figure 14. There was a significant larger load bearing fraction of collagen$_{ani}$ at higher than lower physiological pressure in both males (30.5 ± 3.9% vs. 11.8 ± 3.1%, p<.001) and females (32.9 ± 5.6% vs. 15.6 ± 6.0%, p<.05).

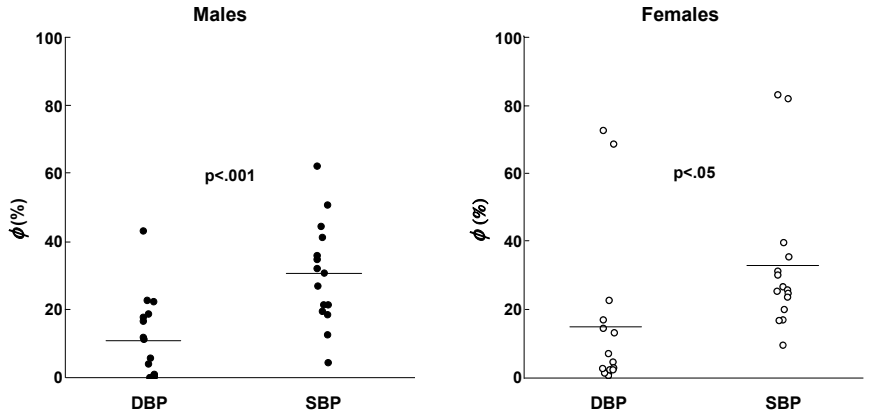

Figure 14. Load bearing fraction attributed to collagen$_{ani}$ in the aorta at high (ϕ_{SBP}) and low (ϕ_{DBP}) physiological pressures in males (filled circles) and females (open circles). Each circle represents an individual. Solid lines represent means. There was a significant larger fraction of collagen$_{ani}$ load bearing at systolic than diastolic pressure in both males, p<.001, and females, p<.05.

The fraction of the total load bearing in the wall attributed to collagen$_{ani}$ at low physiological aortic pressures (ϕ_{DBP}) decreased 84% with age in males from 25 to 70 years of age (r^2=0.33, p<.05), although the difference between the young and old group failed to reach significance, 20.2 ± 5.9% and 4.9 ± 4.4% respectively (p=.07). ϕ_{DBP} did not decrease with age in females, 21.7 ± 13.1% and 18.3 ± 12.8% in the young and elderly group respectively (p=.86), and no correlation between age and ϕ_{DBP} was found (p=.83). The decrease with age was not significantly different between genders (p=0.45) and the mean value of ϕ_{DBP} did not differ between males and females (11.8 ± 3.1% vs. 15.6 ± 6.0%, p=.58).

The fraction of the total load bearing in the aortic wall attributed to collagen$_{ani}$ at high physiological aortic pressures (ϕ_{SBP}) did not change with age in males, 38.4 ± 6.2% and 21.6 ± 7.8% in the young and elderly group respectively (p=.13), and no correlation between age and ϕ_{SBP} was found (p=.06). ϕ_{SBP} did

not change with age in females either, 37.5 ± 11.8% and 38.5 ± 11.1% in the young and elderly group respectively (p=.95), and no correlation between age and ϕ_{SBP} was found (p=.96). The mean value of ϕ_{SBP} did not differ between males and females (30.5 ± 3.9% vs. 32.9 ± 5.6%, p=.73).

The stiffness related to collagen$_{ani}$ in its fiber direction at high physiological aortic pressures (SC_{SBP}), in relation to age in males and females is shown in Figure 15. SC_{SBP} increased exponentially with age in males. Due to large variation, no difference between the young and the elderly group was seen, $65.7 * 10^3 \pm 26.9 * 10^3$ kPa and $37.0 * 10^6 \pm 26.8 * 10^6$ kPa, respectively (p=.21). However, a high correlation between age and SC_{SBP} was found, with a 16500% increase from 25 to 70 years of age (r^2=0.46, p<.01). SC_{SBP} did not increase with age in females, $3.05 * 10^6 \pm 1.9 * 10^6$ kPa and $0.34 * 10^6 \pm 0.19 * 10^6$ kPa in the young and elderly group respectively (p=.20), and no correlation between age and SC_{SBP} was found (p=.73). The increase with age was larger in males than females (p<.05), although the mean value of SC_{SBP} did not differ between males and females ($49.9 \pm 37.8 * 10^6$ vs. $2.0 \pm 0.8 * 10^6$ kPa, p=.22).

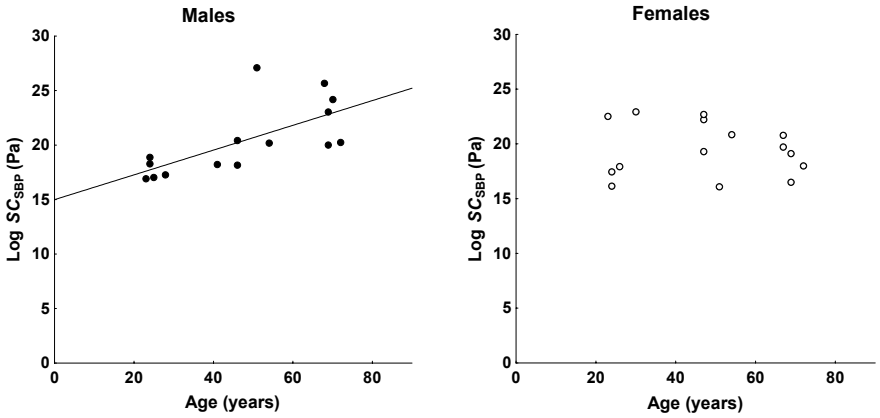

Figure 15. Collagen$_{ani}$ stiffness in fiber direction at high physiological aortic pressures (SC_{SBP}), in relation to age in males (filled circles) and females (open circles). SC_{SBP} increased with age in males, r^2=.46, p<.01, but not in females.

The global aortic wall stiffness (combined effect of elastin$_{iso}$ and collagen$_{ani}$) in the circumferential direction at high physiological aortic pressures (S_{SBP}), in relation to age in males and females are shown in Figure 16. S_{SBP} increased

with age in males, 0.99 ± 0.18 and 5.69 ± 1.09 MPa in the young and elderly group respectively (p<.01), and a high correlation between age and S_{SBP} was found, with a 427% increase from 25 to 70 years of age (r^2=0.55, p<.01). S_{SBP} increased with age in females, 1.27 ± 0.26 and 3.99 ± 0.82 MPa in the young and elderly group respectively (p<.05), and a high correlation between age and S_{SBP} was found, with a 204% increase from 25 to 70 years of age (r^2=0.40, p<.05). Although S_{SBP} seemed to increase more in males with age (427 vs. 204%) this failed to reach significance (p=.13). However if the middle-aged and elderly groups were compiled there was a tendency for males having greater S_{SBP} than females (4.91 ± 0.92 vs. 2.95 ± 0.54 MPa, p=.06), while young males did not differ from young females (0.99 ± 0.10 vs. 1.27 ± 0.26 MPa, p=.41).

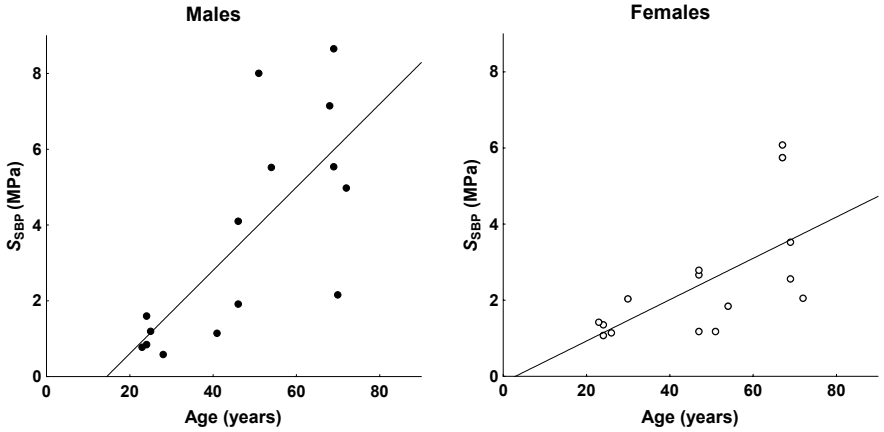

Figure 16. Global aortic wall stiffness (combined effect of $elastin_{iso}$ and $collagen_{ani}$) in the circumferential direction at high physiological aortic pressures (S_{SBP}), in relation to age in males (filled circles) and females (open circles). S_{SBP} increased with age in males, r^2=0.55, p<.01, and females, r^2=0.40, p<.05.

The angle (β) between the circumferential direction and the $collagen_{ani}$ helices of the aorta decreased with age in males, 47.1 ± 0.9° and 39.0 ± 1.3° in the young and elderly group respectively (p<.01). A high correlation between age and β was found, with an 18% decrease from 25 to 70 years of age (r^2=0.67, p<.001). β decreased also in females, 46.5 ± 1.2° and 37.7 ± 0.2° in the young and elderly group respectively (p<.001). A high correlation between age and β was found, with an 18% decrease from 25 to 70 years of age (r^2=0.82, p<.001). The decrease with age was similar between genders and the mean value of β did not differ between males and females (42.4 ± 1.1° vs. 42.3 ± 1.1°, p=.96).

The unloaded radius of the aorta (R_0), in relation to age increased with age in males, 5.75 ± 0.23 mm and 9.19 ± 0.47 mm in the young and elderly group respectively (p<.001), and a high correlation between age and R_0 was found, with a 66% increase from 25 to 70 years of age (r^2=0.77, p<.001). R_0 increased also in females, 5.22 ± 0.50 mm and 7.63 ± 0.57 mm in the young and elderly group respectively (p<.05), and a high correlation between age and R_0 was found, with a 46% increase from 25 to 70 years of age (r^2=0.49, p<.01). The increase was similar between genders (p=0.10) and the mean value of R_0 did not differ between males and females, although the difference was of borderline significance (7.69 ± 0.45 vs. 6.55 ± 0.37 mm, p=.06).

The axial stretch (λ_z) of the aorta, in relation to age in males and females are shown in Figure 17. λ_z decreased with age in males, 1.053 ± 0.003 and 1.013 ± 0.003 in the young and elderly group respectively (p<.001). A high correlation between age and λ_z was found, with a 4% decrease from 25 to 70 years of age (r^2=0.67, p<.001). λ_z did not decrease with age in females, 1.097 ± 0.042 and 1.032 ± 0.013 in the young and elderly group respectively (p=.18). No correlation between age and λ_z was found (p=.09). The decrease with age did not differ significantly between genders, and the mean value of λ_z did not differ between males and females (1.033 ± 0.010 vs. 1.052 ± 0.016, p=.27).

Table 2 shows the cumulative data of age-related changes in material parameters, as well as the differences in change with age between genders.

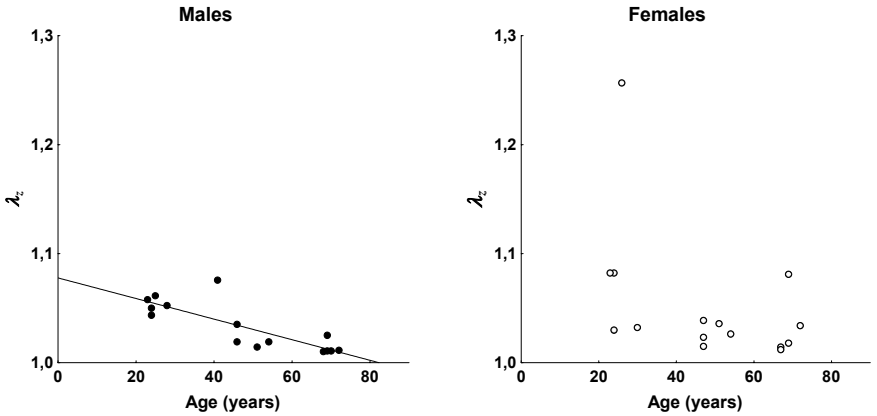

Figure 17. Axial stretch (λ_z) of the aorta, in relation to age in males and females. λ_z decreased with age in males (filled circles), p<.001, but not in females (open circles).

Table 2. Cumulative data on material parameters of the aorta.

	Males	Females	Gender differences in age-related changes, p-value
c	↑↑↑	→	<.05
k_1	↓↓	→	<.05
k_2	↑↑↑	↑↑	<.05
ϕ_{DBP}	↓	→	NS
ϕ_{SBP}	→	→	NS
SC_{SBP}	↑↑	→	<.05
S_{SBP}	↑↑	↑	=.06
β	↓↓↓	↓↓↓	NS
R_0	↑↑↑	↑↑	NS
λ_z	↓↓↓	→	NS

→ denotes no change with age. ↓ or ↑ denotes change with age (p<.05), ↑↑ or ↓↓ denotes p<.01, ↑↑↑ or ↓↓↓ denotes p<.001. c*, stiffness of elastin*$_{iso}$*.* k_1*, collagen*$_{ani}$ *stiffness at sub-physiological pressure.* k_2*, constant coupled to the inflection point of the stress-strain curve of collagen*$_{ani}$*.* ϕ*, load bearing fraction of collagen*$_{ani}$*.* SC_{SBP}*, collagen*$_{ani}$ *stiffness in fiber direction.* S_{SBP}*, global stiffness in circumferential direction.* β*, mean collagen*$_{ani}$ *fiber angle.* R_0*, unloaded radius.* λ_z*, axial stretch.*

Arterial circumferential wall stress in diabetes mellitus (Paper IV)

39 patients with type 1 diabetes (17 males and 22 females) and 46 healthy age- and sex-matched controls (17 males and 29 females) were studied. For baseline characteristics of the study population, see Paper IV. It was possible to measure the carotid IMT in all 39 diabetic patients. However, in 12 of the diabetic patients it was not possible to obtain high enough sonographic image quality to measure IMT of the AA. The 12 did not differ significantly from the successfully examined diabetic patients regarding blood pressure, carotid wall stress, IMT, LD, BMI, BSA, diabetes duration, HbA1c, smoking habits, degree

of albuminuria or retinopathy. The intraobserver variability was 6% for carotid IMT and 10% for aortic IMT in the diabetic patients.

There was no difference between diabetic patients and controls in aortic LD (13.29 ± 2.20 vs. 13.73 ± 2.10 mm), nor in carotid LD (6.17 ± 0.96 vs. 6.02 ± 0.61 mm).

The IMTs of AA and CCA in diabetic patients and controls are shown in Figure 18. The aortic IMT was 22% larger in the diabetic patients (0.89 ± 0.17 vs. 0.73 ± 0.11 mm), p<.001. The carotid IMT was 11% larger in the diabetic patients (0.61 ± 0.11 vs. 0.55 ± 0.09 mm), p<.05.

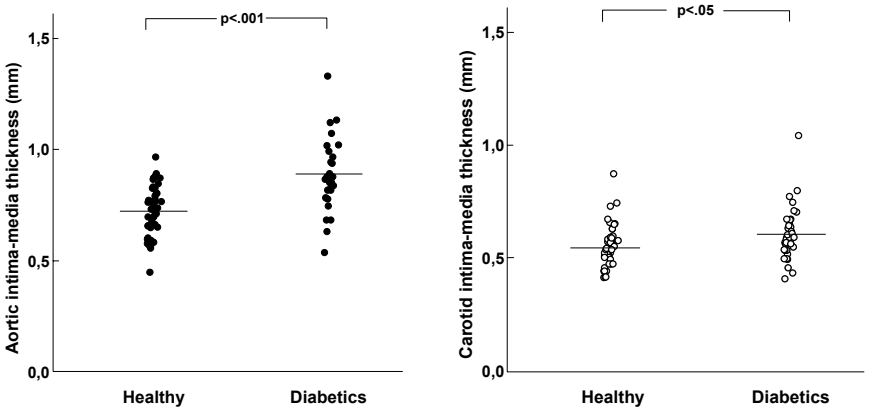

Figure 18. IMT in the AA (left) in the CCA (right). Each circle represents an individual. Solid lines represent mean IMT. Increased IMT in diabetic patients was found in both AA (p<.001) and CCA (p<.05).

The calculated aortic and carotid circumferential diastolic wall stresses in diabetic patients and controls are shown in Figure 19. The aortic wall stress was 20% lower in the diabetic patients (7.8 ± 1.7 * 10^5 vs. 9.7 ± 1.9 * 10^5 dynes/cm^2), p<.001. No difference between diabetic patients and controls was found (5.3 ± 1.2 * 10^5 vs. 5.6 ± 0.9 * 10^5 dynes/cm^2).

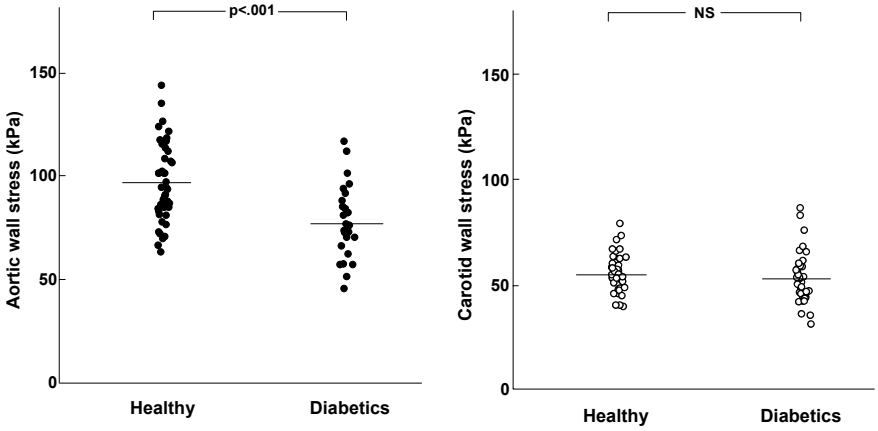

Figure 19. Circumferential diastolic wall stress in the AA (left) and the CCA (right). Each circle represents an individual. Solid lines represent mean wall stress. Reduced wall stress in diabetic patients was found in the AA (p<.001), but not in the CCA. 10^5 dyne/cm^2=10 kPa.

The smoking diabetic patients did not differ from non-smoking regarding aortic and carotid wall stress, IMT, LD and blood pressure.

There was a correlation between aortic IMT and diabetes duration (r=0.57, p<.01), age (r=0.43, p<.05), SBP (r=0.40, p<.05), but not with aortic LD, DBP, BMI, BSA and HbA1c in diabetic patients. Multiple regression was performed including pulse pressure (PP), aortic LD, age and sex. Only PP was found to correlate with aortic IMT (p<.05). If also diabetes duration was included in the model no significant correlations were found, but the p-values were 0.07 and 0.11 for diabetes duration and PP respectively. Only 27 diabetic patients were included in the model however. The annual growth rate of IMT was 0.007 mm/y in diabetic patients.

There was a correlation between aortic IMT and age (r=0.55, p<.001), aortic LD (r=0.45, p<.01), SBP (r=0.36, p<.05), DBP (r=0.30, p<.05), but not with BMI and BSA in healthy matched controls. When performing multiple regression, only age stayed significantly correlated with aortic IMT in the healthy controls (r=0.36, p<.05). The annual growth rate of IMT was 0.006 mm/y.

Stress-driven response in mechanical parameters

In Paper II the factors affecting IMT in the AA and CCA were analyzed. In the male AA, there was no correlation between IMT, LD and blood pressure. Age accounted for 60% of the increase in IMT. In the female AA, LD accounted for 43% and age for 9% of the increase in IMT, but no correlation with blood pressure was found. In the male CCA, age accounted for 47% and PP for 10% of the increase in IMT, but no correlation with LD was found. In the female CCA, age accounted for 47% and PP for 7% of the increase in IMT, but no correlation with LD was found.

In the additional study the pressure was measured locally in the AA (the individuals from Paper III, 15 males and 15 females) and the systo-diastolic pressure changes during the cardiac cycle were recorded. Accordingly diastolic, systolic and pulsatile wall stresses could be evaluated. Furthermore, with the aid of the mechanical model stresses in both circumferential and axial directions were analyzed. The gender difference reported in Paper II, with males having greater circumferential diastolic wall stress than females, was confirmed (Fig. 20, $p<.05$). Furthermore, males had greater circumferential systolic wall stress than females (Fig. 20, $p<.05$). No gender differences in pulsatile circumferential ($p=.05$) or in axial diastolic ($p=.11$), axial systolic ($p=.08$), and axial pulsatile ($p=.11$) wall stresses were found (Fig. 20).

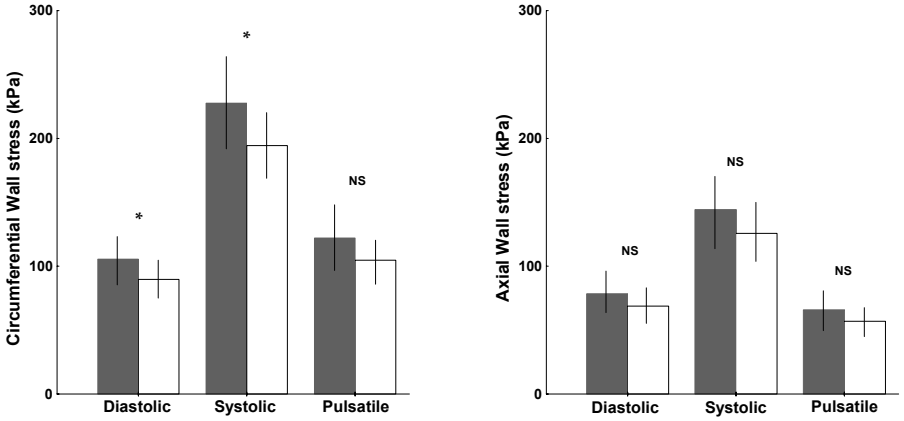

Figure 20. Gender differences in circumferential (left) and axial (right) wall stresses of the abdominal aorta. Males (filled bars) and females (empty bars), * p<.05. Mean ± SD

In Paper II an age-related increase in circumferential wall stress was found selectively in the male population. In the present study, no significant correlations between wall stresses (circumferential and axial diastolic, systolic and pulsatile wall stress) and age was found irrespective of gender. This was probably due to the small number of individuals. When analyzing the young group no gender difference in stresses was found (n=10). However when analyzing the group of middle-aged and elderly (n=20), a gender difference was found with greater wall stress in males in circumferential diastolic and systolic wall stress, both p<.05, and axial diastolic and systolic wall stress, both p<.05. Thus, the findings are in accordance with Paper II with older males having larger wall stress than older females.

The correlations between stresses and aortic mechanical parameters found in this additional study were examined. Circumferential diastolic wall stress correlated with S_{SBP} (r^2=.17, p<.05) and R_0 (r^2=.18, p<.05). Circumferential pulsatile wall stress correlated with k_1 (r^2=.15, p<.05). Axial diastolic wall stress correlated with S_{SBP} (r^2=.21, p<.05) and R_0 (r^2=.21, p<.05). Axial systolic wall stress correlated with S_{SBP} (r^2=.17, p<.05).

The correlations between wall stresses and mechanical parameters were tested with multiple regression including age, gender and SBP. Circumferential diastolic wall stress and gender did not seem to influence S_{SBP} (global stiffness in circumferential direction at SBP), while age and SBP did (p<.001 and p<.05, respectively). Circumferential systolic wall stress and age seemed to influence

S_{SBP} (p<.01 and p<.001, respectively), while SBP and gender did not. Circumferential pulsatile wall stress and age seemed to influence S_{SBP} (p<.05 and p<.001, respectively), while SBP and gender did not.

Furthermore, S_{SBP} was separated in S_{SBP}^{ani} (stiffness of collagen$_{ani}$ in circumferential direction at SBP) and S_{SBP}^{iso} (stiffness of elastin$_{iso}$ in circumferential direction at SBP), then tested against the circumferential wall stresses in multiple regression including age, gender and SBP. Circumferential diastolic wall stress and gender did not seem to influence S_{SBP}^{ani}, while age and SBP did (p<.01 and p<.05, respectively). Circumferential systolic wall stress and age seemed to influence S_{SBP}^{ani} (p<.05 and p<.001, respectively), while SBP and gender did not. Circumferential pulsatile wall stress and age seemed to influence S_{SBP}^{ani} (p<.05 and p<.001, respectively), while SBP and gender did not. However, none of the wall stresses influenced S_{SBP}^{iso} (although circumferential systolic wall stress was of borderline significance, p=.065), neither did SBP or gender, only age (p<.05).

Figure 21 shows the value of S_{SBP}^{ani} in relation to circumferential pulsatile stress in a partial residual plot for pulsatile stress, where stiffness values are adjusted for age, gender and SBP.

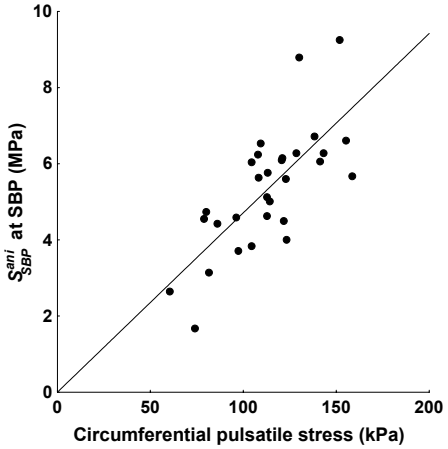

Figure 21. Partial residual plot for pulsatile stress and S_{SBP}^{ani} when variables adjusted for age, gender and SBP, r^2=.53, p<.001.

DISCUSSION

Previous in vivo studies of arterial remodeling in humans have mainly focused on changes in arterial diameter and intima-media thickness (IMT). Furthermore, stiffness estimations have described only global conditions of the vessels. This thesis makes an attempt on a more detailed analysis of the different wall components in the abdominal aorta (AA), as well as the stress driven remodeling response of the aortic wall.

Material parameters of the abdominal aortic wall in man

The mechanical behavior of the abdominal aorta has previously been characterized non-invasively, with findings of increased stiffness with age and more extensive changes in males (Sonesson et al. 1994). In order to gain further insight into the composition of the aortic wall and the influence of age and gender, a novel mechanical model was used (Stålhand 2008).

The stress-strain curve used for the analysis has been shown to depend mainly on elastin for lower stress and collagen for higher stress (Roach and Burton 1957). Although smooth muscle cells, proteoglycans, fibronectin, fibrillin and hyaloronan contribute to wall mechanics, matrix degrading experiments have shown that elastin and collagen are the main determinants of the mechanical characteristics of the human aorta (Hoffman et al. 1977). The fibers of collagen have certain directions in the aortic wall and are not randomly distributed, which makes collagen an anisotropic material. This means that if the same force is applied in two directions (i.e. axial and circumferential), the resulting strain will be different in the two directions. This is in contrast to the elastin fibers, which due to the mesh-like arrangement of elastin molecules behave as an isotropic material, i.e. the same strain will be observed irrespectively of the direction in which the force is applied (Dobrin et al. 1990, Holzapfel 2006). The model describes the isotropic material (mainly elastin), defined as $\text{elastin}_{\text{iso}}$ and the anisotropic material (mainly collagen) defined as $\text{collagen}_{\text{ani}}$ of the aortic wall.

The magnitude of c, a stiffness parameter of elastin$_{iso}$, was in agreement with earlier reported values for Young's modulus of elastin in the aorta (Wuyts et al. 1995). Further, elastin has a low turnover rate ($T_{½}$=70 yrs) and with increasing age becomes glycated, which might contribute to the increased c that was found with age in males (Fig. 13), despite the decrease in elastin content due to elastolysis (Faber and Oller-Hou 1952, Hornebeck et al. 1978, Konova et al. 2004, Rucker and Tinker 1977). This finding is in contrast to a recent in vitro study of the aorta by Zulliger et al (2007) that did not detect any age-related changes in the elastic modulus of elastin. However, they studied the thoracic instead of abdominal aorta with differing medial structure, and used a pressure dependent stiffness measure of elastin neglecting in vivo pressures (Wolinsky and Glagov 1969).

The magnitude of the material parameter k_1 relating to stiffness of collagen$_{ani}$ at low stretch (pressure below physiological level) was in reasonable agreement with earlier in vitro results for coronary arteries (Holzapfel et al. 2005). k_1, decreased somewhat with age in males. The reduction in stiffness of collagen$_{ani}$ at sub-physiological pressures may be due to isotropic expansion of the collagen network, since accumulation of glucosaminoglycans with a high content of carbohydrate groups makes the folded collagen molecules easier to spread out and bind large volumes of water (Dingemans et al. 2000, Sell and Monnier 1995). Thus, dry weight of the aortic wall decrease with age despite an increase in wall thickness, indicating a more humid environment (Cattell et al. 1996). The entanglement between the collagen molecules is reduced with a subsequent softening in analogy with the process of swelling in rubber (rubber is an organic polymer, similar to soft tissues, Treloar 1975). The contribution of collagen to arterial stiffness is quite small however during low pressures where elastin may be the important determinant (Armentano et al. 1991, Marsh et al. 2004, Nichols and O'Rourke 2005a). The total load bearing fraction in the aortic wall attributed to collagen$_{ani}$ (ϕ) oscillated from 12 to 31% between diastolic and systolic pressures during the cardiac cycle in males (Fig. 14), in line with in vitro animal models that have found elastin to be the main load bearing component within physiological pressures in the carotid artery (Dobrin and Canfield 1984). During aging, the recruitment of collagen$_{ani}$ began at lower pressures, probably due to increased cross-linking during maturation and glycation of the collagen fibers (Table 2, Bailey et al. 1998, Samila and Carter 1981).

At high pressures, the stiffness of the unfolded collagen$_{ani}$ in its fiber direction (SC_{SBP}) increased exponentially with age in males (Fig. 15). This may be due to increased glycation as well as changed isoforms of collagen within the aortic wall (Qiu et al. 2007). Furthermore, a marked increase in circumferential global stiffness (S_{SBP}) with age was observed in line with the exponential increase in global stiffness of the abdominal aorta earlier reported (Fig. 16, Sonesson et al. 1993)

β, the mean collagen$_{ani}$ fiber angle, was about 40° in accordance with earlier in vitro findings (Results section, Holzapfel et al. 2000, Holzapfel et al. 2002). In the cardiac cycle the aortic diameter increases during systole, and the collagen fibers become more circumferentially oriented due to the stretch of the network of helices (Table 2, Fig. 6, Bigi et al. 1981). The unloaded radius (R_0) of the aorta increased with age in males corresponding to earlier in vivo studies (Results section, Länne et al. 1992a). The remodeling of the aortic wall with increased radius with age seems to have a similar effect on the orientation of the fiber angle of the collagen$_{ani}$ (Table 2).

The stretch (λ_z) of the aortic wall in longitudinal direction was about 5% from the unloaded state (Fig. 17). Earlier in vitro studies on human arteries have shown a stretch of 7-10% in medium sized arteries (Schulze-Bauer et al. 2003). Learoyd and Taylor (1966) found a 25% stretch of the aorta, but the experimental design in vitro might have led to confounding results. Since elastin seems to provide the major longitudinal retractile force in human iliac arteries and collagen bears load mainly in the circumferential direction, the age-related degradation of elastin would explain the earlier finding of increased longitudinal arterial stiffness as well as our finding of a decreased axial stretch (λ_z) with age in males (Fig. 17, Dobrin and Canfield 1984, Dobrin et al. 1990, Learoyd and Taylor 1966, Schulze-Bauer et al. 2003, Sherebrin et al. 1989).

Effect of gender

Earlier studies have indicated a less accelerated aging process in the aorta of females than males. Both the age related increase in diameter as well as stiffness are less accentuated in females and studies imply that the gender differences in vascular stiffness that develop with aging are programmed at an early age (Achimastos et al. 2007, Laogun and Gosling 1982, Sonesson et al. 1993). The stiffness parameters relating to elastin$_{iso}$ and collagen$_{ani}$ were less

affected in females. c (stiffness parameter of elastin$_{iso}$), k_1 (relating to stiffness of collagen$_{ani}$ at pressure below physiological level) as well as SC_{SBP} (stiffness of collagen$_{ani}$ in the fiber direction at high physiological pressures) was unchanged with age in females in contrast to males where a highly significant age-related effect was seen (Table 2, Figs. 13 and 15). The fact that the female aortic wall seems much less affected by age could be due to a number of factors. Sex hormones affect arterial wall properties (Westendorp et al. 1999), and postmenopausal hormone replacement therapy in females seems to reduce arterial stiffness (Rajkumar et al. 1997). Both estradiol and progesterone decrease the collagen/elastin ratio in the aortic wall (Fischer and Swain 1980). Furthermore, testosterone increases MMP-3 activity in the aortic wall degrading elastin and fibrillin-1, supporting the fact that female gender seems protective from elastolysis (Natoli et al. 2005, Sinha et al. 2006). Based on data from a large animal model, Qui et al. (2007) recently suggested that the larger increase in arterial stiffness of males is attributed to both decreased elastin content, and changed collagen isoforms from type 3 to type 8 (Qiu et al. 2007). This fits well with the gender-specific differences in mechanical properties of the aorta found in our study. Another factor of importance might be a gender difference with increased cross-linkings, such as the formation of AGEs, in males.

Stress-driven remodeling response of large arteries

The diameter and IMT of healthy males and females were studied in AA and common carotid artery (CCA). As expected males had larger diameters in both AA and CCA (Table 1, Hansen et al. 1995, Sonesson et al. 1993). With age the diameters in the AA and the CCA increased and was larger in males (Fig. 10), when adjusted for BSA (body surface area) the gender differences disappeared however (Hansen et al. 1995, Sonesson et al. 1993). The IMT was larger in the AA than in the CCA (Fig. 9). In the aorta, no gender difference in IMT was found (Fig. 9). Since arterial wall thickness is a factor determining pulsatile wall motion in response to changes in arterial pressure, this finding may be somewhat surprising because a significant gender difference in aortic wall stiffness has been reported (Sonesson et al. 1994). Thus, the composition of the aortic wall must differ, possibly due to a different collagen/elastin ratio but isoforms of the structural proteins may diverge. The lack of gender difference in IMT that was found in the AA, is in contrast with the CCA as well as most

studies on superficial arteries, where male gender and age seems to be a risk factor for IMT thickening (Fig. 9, Handa et al. 1990, O'Leary et al. 1992). Accordingly, with age an increase in IMT was found in both males and females (Fig. 11).

Mechanical stimuli such as stress or strain yield a biological response in the arterial wall. The arterial wall stress hypothesis postulates that an increase in diameter or blood pressure, leads to increased wall stress, which in turn activates cells in the arterial wall with an increase in wall matrix and wall thickness (Ben Driss et al. 1997). The coexistence of a larger diameter and IMT in the AA compared to the CCA supports the "vessel protection" theory, with increasing IMT to reduce wall stress according to the law of Laplace (Table 1, Fig. 9). This is in accordance with Girerd et al (1994) who showed that the IMT of the radial artery in hypertensive patients increases to a thickness where the wall stress is stabilized. It is obvious however that the wall stress in the AA was not fully compensated or set at a level higher than the other studied arteries (Table 1).

With age diastolic pressure increased. Further, the diameter of the CCA increased with age but wall stress was unchanged due to a compensatory increase in IMT (Figs. 10-12). Thus, arterial wall stress seems to be restored and stabilized on a predetermined level and may be an important determinant for vessel wall remodeling during aging in man. In animal models increase in circumferential stress has been shown to generate medial hypertrophy and thickening (Ben Driss et al. 1997, Dobrin 1995). To our knowledge, there are no earlier studies regarding the age-related changes in circumferential wall stress of the human AA. Bader (1967) studied the circumferential wall stress in the thoracic aorta in vitro, and found that the aortic wall stress decreased with age, there are however several differences between his study and ours. The adventitia was included in his calculation of wall stress. The thoracic instead of the infrarenal AA was studied, which might be of importance since there is a higher frequency of aneurysmal dilatation in the AA (Bengtsson et al. 1992, Svensjo et al. 1996). More importantly, his study was performed in vitro, without recognizing the fact that blood pressure increases with age (Results section). Also, no separation of gender was performed, which seems to be of fundamental importance (Fig. 12). The AA is a vulnerable artery since the major part of the human abdominal aortic media lacks vasa vasorum in contrast to the media of thoracic aorta (Wolinsky and Glagov 1969). Further, a smaller number of lamellar elastic units have been found in the human AA

compared to other mammals of similar size, indicating that mean stress per lamella is higher. The high wall stress found in the AA, as well as the increase in stress with age that was found in males probably enhances the vulnerability even further (Fig. 12). Finally, the main load bearing media may increase in thickness to a lesser extent than the intima during ageing, in contrast to other mammals (Cliff 1970, Movat et al. 1958, Virmani et al. 1991). This indicates greater wall stress within the aortic media than calculated in our study, although the intima might bear greater load with age and thickening (Holzapfel et al. 2000, Masawa et al. 1994).

In the female subjects, no age-related increase in aortic circumferential wall stress was found due to a sufficient compensatory increase in IMT in contrast to the males, where apart from greater mean wall stress also an age related increase in wall stress was found (Table 1, Figs. 11-12). An analysis of the underlying factors showed that only age affected wall thickness in the male AA, while in the female AA arterial diameter and in the CCA (both males and females) both arterial diameter and blood pressure affected the increase in wall thickness, keeping wall stress unchanged with age. Thus the male aorta seemed to have a defect wall stress auto-regulation. Although we have shown that the IMT is not regulated by pressure in the abdominal aorta in healthy subjects, IMT is not equal to the quality of the wall, thus a remodeling within the aortic wall still occurs resulting in increased stiffness with age.

Pulsatile stress as well as diastolic and systolic stress in the AA correlated with the stiffness in circumferential direction at SBP (S_{SBP}). When separating collagen$_{ani}$ from elastin$_{iso}$, the highest correlation was found between pulsatile stress and the stiffness of collagen$_{ani}$ (S_{SBP}^{ani}, Fig. 21), while the different stresses did not correlate to the stiffness of elastin$_{iso}$ (S_{SBP}^{iso}). This is in accordance with findings in the CCA where pulse pressure seems more important for remodeling than mean blood pressure (Boutouyrie et al. 2000). In vitro studies have shown cyclic stretching of aortic smooth muscle cells to stimulate synthesis of collagen, and remodeling of collagen seems to be stress-driven, where fiber direction aligns according to the direction of applied stresses (Hariton et al. 2007). Tyrosine kinase-dependant mechanisms may mediate this effect through inducing transforming growth factor beta (TGF-β) mRNA expression, and extra cellular matrix mRNA expression (Joki et al. 2000, Li et al. 1998). Thus pulsatile stress seems important for collagen remodeling in the abdominal aorta of man.

Abdominal aortic aneurysms (AAAs) are more frequently found in males and aortic wall stress may be of importance for aneurysm growth and rupture (Cronenwett et al. 1990, Hall et al. 2000, Szilagyi et al. 1972). Furthermore, female gender has been found protective from elastase-induced AAA in a rodent model (Sinha et al. 2006). Since MMPs in the arterial wall are activated by wall stress, it may be hypothesized that a more proteolytic profile is found in the male abdominal aorta compared to other arteries, and possibly forms a background to the increased risk for aneurysm development (Chesler et al. 1999, O'Callaghan and Williams 2000). Increased proteolytic profile has recently been described in the ascending aorta compared to the internal mammary artery in humans (McNulty et al. 2005).

A link between diabetes and the protection from abdominal aortic aneurysm formation

Abdominal aortic aneurysms (AAAs) are often described as atherosclerotic (Zarins et al. 2001), despite the fact that aneurysms may develop in aortas free of atherosclerosis. Abdominal aortic aneurysms and atherosclerosis share, however, several risk factors, such as high age, male gender, smoking, hyperlipidemia, inflammation and hypertension. Despite this, patients suffering from diabetes, a disease with a preponderance of atherosclerotic manifestations, seldom develop AAA (LaMorte et al. 1995, Lederle et al. 2000, Lederle et al. 1997). The reason for this discrepancy has caused limited attention, and the cause for the reduced frequency of AAA in the diabetic population is unknown. Furthermore, if diabetic patients develop AAAs, the expansion rate of those AAAs is only 30% compared to non-diabetic patients (Brady et al. 2004). Wall stress has been implied as a pathological factor in the development of AAA and it was hypothesized that diabetic patients might be affected by a changed remodeling response leading to a protection from aneurysmal disease.

The AA was studied in diabetic patients with diabetes duration of varying length (15-45 years) and compared to an age- and sex-matched group of non-diabetic individuals. Apart from a high incidence of smoking, the diabetic patients were relatively healthy. No differences in aortic diameters or blood pressures were found between the two groups. However, aortic IMT was larger in the diabetic patients with a concomitant decrease in circumferential stress of the aortic wall (Figs. 18-19). Smoking diabetic patients did not differ

from non-smoking diabetic patients regarding IMT, LD or blood pressure. Thus, smoking did not seem to be a confounder, although smoking has been associated with increased carotid IMT (Liang et al. 2001).

High glucose-levels induce altered MMP expression in cell cultures (Death et al. 2003) and a down regulation of MMP seems to be the reason for the matrix accumulation in diabetic nephropathy (Singh et al. 2001). Furthermore, carotid IMT is increased in diabetic patients, and there is an association between IMT in non-diabetic patients and the level of postprandial hyperglycemia (Giannattasio et al. 1999, Hanefeld et al. 1999). A down regulation of MMP activity has been shown in the internal mammary and tibial arteries of diabetic patients as well as the coronary arteries of diabetic rats (Jesmin et al. 2003, Mondy et al. 2002, Portik-Dobos et al. 2002), and a reduced MMP activity would fit well with our findings of markedly increased aortic wall thickness in the diabetic patients (Fig. 18). Thus high glucose levels inactivating metalloproteinases responsible for aortic wall degradation might be the mechanism behind reduced aortic wall stress, decreased aneurysm prevalence and reduced rate of increasing aneurysmal size found in the diabetic population (Brady et al. 2004, LaMorte et al. 1995, Lederle et al. 2000, Lederle et al. 1997).

An interesting finding in the diabetic patients was that pulse pressure (PP) influenced wall thickness (IMT) in the AA, in contrast to the healthy individuals where pressure had no influence. It might be speculated that the autoregulation of wall stress in the AA, that has been shown to be defect in healthy subjects (Fig. 12), is more effective in patients with diabetes. Collagen synthesis is stimulated by cyclic stretching of VSMCs mediated through TGF-β via an autocrine/paracrine mechanism that may be potentiated by high glucose levels (Joki et al. 2000, Li et al. 1998, Lindschau et al. 2003, Asbun et al. 2005, Asbun and Villarreal 2006). The effects of glucose and PP on aortic wall remodeling reducing wall stress might be the mechanism behind decreased aneurysm prevalence, and reduced rate of increasing aneurysmal size found in the diabetic population.

Methodological considerations and limitations

The IMT of the AA is considered to be more difficult to study with aid of ultrasound than superficial arteries since it lies deep, and is more difficult to image due to bowel gas and abdominal fat. The resolution is less than in superficial arteries, since transducers of lower frequency must be used. The IMT measurements in the abdominal aorta showed a coefficient of variation (CV) of 10-11% (Fig. 8), which is comparable with the early results on superficial arteries by Wendelhag et al (1991). However, the CVs of CCA and CFA in our study were 6 and 8% respectively, which is comparable to later studies (Schmidt and Wendelhag 1999). The calculations of the intima-media area (IMA) showed similar results as the IMT measurements, with an intra-observer variability of 12% in the aorta. Thus, the IMT of the AA can be measured with satisfactory reproducibility, although it is evident that the AA is somewhat more difficult to examine than superficial arteries.

With knowledge of arterial diameter, blood pressure and wall thickness, wall stress can be calculated according to the law of Laplace. IMT has during recent years been used as a surrogate to arterial wall thickness in the calculation of wall stress (Carallo et al. 1999, Dinenno et al. 2001), although the adventitial layer is excluded in the measurements. However, it is evident that the major part of the total wall thickness is included with the adventitia being approximately one third of the total wall thickness in the abdominal aorta (Gamble et al. 1993, Holzapfel 2006, Holzapfel et al. 2000) as well as in the iliac and the coronary arteries (Holzapfel et al. 2000, Holzapfel et al. 2005). Further, the relation between adventitial thickness and the IMT does not seem to be affected by gender or age (Hodges et al. 1994), making this approximation acceptable.

When evaluating circumferential wall stress (Paper I, II and IV), the measurements of IMT were performed in diastole according to prevailing standards. The diastolic blood pressure was measured auscultatory in the brachial artery and not locally in the aorta. This means a slight overestimation of the aortic diastolic pressure, but without sex or age-related differences (Sonesson et al. 1994). In Paper III however, we measured pressure invasively in the AA and were therefore able to evaluate diastolic, circumferential and pulsatile wall stresses. When comparing circumferential stress values obtained in Paper I and Paper III (note conversion factor, 10^5 dyne/cm^2 = 10 kPa), no differences were found in either males or females. Thus, the use of brachial

diastolic pressure to calculate circumferential diastolic wall stress in the abdominal aorta seems acceptable.

It was only possible to examine 69% of the diabetic patients regarding aortic IMT. The reasons for this are not known. A factor of importance might be the amount of intra-abdominal fat influencing the possibility to perform ultrasound scans. However, BMI and BSA did not differ between the diabetic patients that were possible to study and the rest. Neither did diabetes duration, blood pressure, carotid IMT, carotid LD, wall stress, HbA1c, smoking habits, degree of albuminuria or retinopathy indicating that the two groups were comparable. The vessels in diabetic patients are commonly known to be more difficult to examine with ultrasound, although the reasons are unknown. An alternative technique would have been CT or MRI. However, these techniques offer less resolution. Furthermore, it could be argued that patients with diabetes mellitus type II instead of type I should have been investigated, since they are affected by diabetic disease at a similar time in life as patients with abdominal aortic aneurysms. However, we chose type I diabetic patients since they are affected earlier in life, and more profound glucose-altered changes in their aortic walls with time thus would be expected.

A novel method was used in Paper III to examine different mechanical parameters in vivo, with aid of simultaneously recorded pressure and radius curves from the aorta (Fig. 6, Stålhand 2008). The arterial wall is assumed to be a membrane, which implies that the wall thickness is negligible in comparison with the radius. This assumption is questionable for the abdominal aorta where the wall thickness to radius ratio is approximately 0.1-0.2. In addition, the abdominal aortic wall has three distinct layers with separate mechanical properties that should be accounted for in a truly correct model (Holzapfel et al. 2005). From a parameter identification point of view, however, this high resolution is likely to introduce dependencies among the parameters (Stålhand et al. 2004). Furthermore, the parameters from a membrane model can be thought of as averages describing the global response of the three layers. The model does not include the residual stress present in arteries (Fung 1997, Holzapfel et al. 2000). The major role of the residual stress is to redistribute the stress field in such a manner that it becomes transmurally uniform (Holzapfel et al. 2000). In a membrane model, the transmural variation in both the stress and stretch fields are neglected, and the inclusion of a residual stress would only shift the stress level upwards. Therefore, it is assumed that the levels of

the membrane stresses are close to the stresses in the arterial wall in situ. This is also motivated by the fact that the model is tuned to the stress field obtained by a global stress balance. Furthermore, the orientation of collagen fibers seems to be helical within the media, and more longitudinal towards the adventitia, but the variation of fiber orientation is not accounted for since it is a membrane model (Clark and Glagov 1985, Holzapfel et al. 2002, Raspanti et al. 2006).

CONCLUSIONS

- It was possible, with the aid of non-invasive ultrasound, to study the diameter and intima-media thickness (IMT) with acceptable reproducibility in the abdominal aorta (CV 4% and 11%, respectively). This made it possible to calculate circumferential wall stress in vivo. In middle-aged individuals, aortic wall stress was larger than in the common carotid artery. Further, males had larger arterial wall stress than females.

- The diameters of the abdominal aorta and common carotid arteries increased with age. A compensatory increase in IMT prevented circumferential wall stress from increasing. However, this response was insufficient in the male aorta, where the stress increased with age.

- With a new mechanical model the contribution of isotropic material (mainly elastin, $elastin_{iso}$) and anisotropic material (mainly collagen, $collagen_{ani}$) to the mechanical behavior of the abdominal aorta were characterized in vivo. With age, stiffness of $elastin_{iso}$ increased despite the decrease in elastin content that has been found due to elastolysis. Furthermore, an exponential increase in stiffness of $collagen_{ani}$ at high physiological pressure was found. A marked gender difference was observed, with a much less age-related effect both on the stiffness of $elastin_{iso}$ and $collagen_{ani}$ in females.

- Pulsatile stress influenced $collagen_{ani}$ but not $elastin_{iso}$ remodeling, leading to increased stiffness of the abdominal aortic wall.

- Patients with diabetes mellitus had increased aortic IMT than controls, generating less circumferential stress. This coincides with the known reduction of abdominal aortic aneurysms in diabetic patients and may act as a protective factor.

POPULÄRVETENSKAPLIG SAMMANFATTNING

(Summary in Swedish)

Artärers (pulsådror) mekaniska egenskaper är av stor betydelse för hur pulsvågens breder ut sig i kärlträdet. Sammansättningen i kärlväggen bestämmer tänjbarheten, och vid åldrande och olika sjukdomstillstånd förändras sammansättningen vilket kan leda till styvare artärer. En elastisk aorta (stora kroppspulsådern) ger en buffrande effekt av pulsvågen och blodets framfart blir mjukare och genomblödningen effektivare. Om aortaväggen blir styvare leder det till att pulsvågen breder ut sig snabbare och den reflekterade pulsvågen tar sig snabbare tillbaka till hjärtat, vilket i sin tur gör att hjärtats egna blodkärl (som skall fyllas i relaxationsfasen av hjärtcykeln) får svårare att fyllas. Vidare blir pulstrycket större och hjärtat får arbeta mot ett högre motstånd. Kärlväggens egenskaper bestäms framförallt av två proteiner, elastin och kollagen, där elastin bildar elastiska lameller som karakteriseras av att kunna tänjas ut i stor utsträckning. Kollagenet å andra sidan är uppbyggt i spiralformationer runt längsaxeln av kärlväggen. Vid låga tryck är kollagenet hopvecklat. När kollagenet vecklas ut, vid högre blodtryck och större sträckning av kärlväggen, är det mycket styvt och har hög hållfasthet. En stor del av aortans buffrande effekt beror på elastinets struktur och egenskaper, emedan kollagenet verkar skyddande mot översträckning av kärlet. Om elastinet, och i viss mån kollagenet, bryts ned kan vidgning av aortan ske (pulsåderbråck). Ju större bråck, desto större risk för ytterligare tillväxt och att det spricker. Detta kan beskrivas med Laplace lag som beräknar väggspänningen, vilken är proportionell mot blodtrycket och kärlets radie och omvänt proportionell mot kärlväggens tjocklek. Hög spänning i aortaväggen är en riskfaktor för tillväxt samt sprickbildning av pulsåderbråck. Huruvida väggspänningen även påverkar utvecklandet av ett pulsåderbråck är ofullständigt känt. Det är också till stora delar okänt varför kroppspulsådern i bukhålan har störst risk av alla pulsådror att drabbas av bråckbildning och varför män drabbas betydligt oftare än kvinnor. Riskfaktorer för utvecklande av bråckbildning är t.ex. rökning, manligt kön, hög ålder, högt blodtryck, höga nivåer av blodfetter, d.v.s. snarlikt de riskfaktorer som gäller för ateroskleros

(åderförkalkning). Diabetes mellitus (sockersjuka) är en stark riskfaktor för ateroskleros, men ter sig skyddande mot pulsåderbråcksutveckling i bukaorta. De bakomliggande orsaker är emellertid okända. För att kunna förstå utvecklingen av åldrande och artärsjukdomar, behöver artärväggens komponenter studeras, vilket kan göras genom att ta ut artären ur kroppen och studera väggens innehåll genom mikroskopi, kemiska analyser, och mekaniska experiment. Det leder till att artärväggen förändrar sina egenskaper då den berövas den omgivning den är uppfäst vid, hormonell påverkan försvinner, och i avsaknad av syre och näring förändras väggens egenskaper. En stor nackdel är förstås att det inte är möjligt att plocka ut stora artärer på friska individer, och att den påverkan som föreligger i levande vävnad förblir okänd. Det finns alltså ett stort behov att kunna studera artärväggars uppbyggnad på levande individer, så kallade ”in vivo” undersökningar.

Målen med avhandlingen har varit att 1) utveckla ett in vivo system som ger möjlighet att studera de elastiska komponenterna av betydelse för kärlväggsmekaniken i aorta, 2) karakterisera reglermekanismerna för aortaväggens ombyggnad vid åldrande och diabetessjukdom, samt 3) öka förståelsen för bakgrunden till aneurysmsjukdom.

I det **första delarbetet** värderades om intima media tjockleken (IMT), ett surrogat mått för total väggtjocklek, i bukaorta kan studeras med icke invasiv ultraljuds teknik för att kunna beräkna den cirkumferentiella väggspänningen i aorta. 65 friska försökspersoner undersöktes. Variabiliteten i mätningarna var tillfredställande. Vidare noterades att IMT var tjockare i aorta än i knäpulsådern, halspulsådern och lårpulsådern. Trots det, var väggspänningen högre i aorta. Män hade högre väggspänning än kvinnor.

I det **andra delarbetet** studerades den ålders- och könsrelaterade regleringen av bukaortas väggspänning, med halspulsådern som jämförelse på 111 friska försökspersoner med den tidigare beskrivna ultraljuds-tekniken. Väggspänningen var högre i aorta än i halspulsådern, och män hade högre kärlväggspänning än kvinnor. Väggspänningen var välreglerad, konstant och oberoende av ålder i halspulsådern både hos män och hos kvinnor, samt i aorta hos kvinnor, medan spänningen ökade i mäns aorta med stigande ålder. Fynden kan bidra att förklara varför just mäns aorta är speciellt utsatt för pulsåderbråck.

I det **tredje delarbetet** studerades aortaväggens komponenter med hjälp av en nyutvecklad mekanisk modell. 30 stycken friska försökspersoner, 10 unga, 10 medelålders och 10 äldre individer undersöktes, hälften män och hälften kvinnor. Aortaväggens pulsativa rörlighet studerades icke invasivt med ultraljud echotracking teknik kombinerat med intraarteriell tryckmätning (blodtrycksmätning inuti kärlet) i bukaorta. Storleken på de uppmätta materialparametrarna stämde väl överens med tidigare in vitro studier (studier på kärl hos avlidna människor och djur). Kollagenets lastbärande fraktion varierade från 10 till 30% mellan diastoliskt och systoliskt tryck. Med ökande ålder ökade styvheten både avseende elastinet och kollagenet i aortaväggen, sannolikt beroende på en ökande glykocylering (sockerinlagring) och/eller en förändring av kollagen-typen i aortaväggen. En påtaglig könsskillnad observerades med en mycket mindre effekt av ålder bland kvinnor både på styvheten hos elastin och hos kollagen. Orsaken till det kan vara en effekt av könshormoner och/eller skilda kollagen typer mellan könen.

I en **kompletterande studie** studerades väggspänningen i både längs- och circumferentiella riktningen av bukaorta på de försökspersoner som ingår i delarbete tre. Med hjälp av den mekaniska modellen samt Laplace lag kunde spänningen bestämmas över hela hjärtcykeln. Den pulsatila spänningen visade sig påverka ombyggnaden av kollagen komponenten i bukaorta, emedan elastin komponenten inte påverkades av väggspänning.

I det **fjärde arbetet** studerades spänningen i aortaväggen hos patienter med diabetes mellitus (sockersjuka) – en sjukdom som är känd för att ha en skyddande effekt avseende utveckling av pulsåderbråck. 41 diabetiker jämfördes med 46 friska kontroller med den tidigare beskrivna ultraljudstekniken. Väggstressen var lägre i aorta hos diabetikerna beroende på en tjockare vägg än hos friska kontroller. Den reducerade väggstressen skulle kunna bidra till den låga förekomsten och tillväxthastigheten av pulsåderbråck som man finner hos patienter med diabetes.

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