Chapter 1

Introduction

Life as we know it has self-sustaining and self-organizing processes. It is organized in units called cells that have a membrane that separates the interior of a cell from the outside environment. In multicellular organisms the cells need signaling to regulate growth and tissue morphology. Further, for sufficient exchange of nutrients and gases with the outside environment a transport system is needed. Humans for example have a respiratory and a circulatory system.

The cardiovascular system is organized in a systemic circulation and a pulmonary circulation and collaborates with the lymphatic system. The main components of the cardiovascular system are the heart, blood vessels and blood. The heart pumps blood through the blood vessels. Healthy blood vessels adjust their diameter to meet flow demands of the organs they perfuse. This is of importance during development from fetus to adult.

In pathological states the remodeling of blood vessels might be affected (section 1.1). Large arteries might progressively dilate as can be seen in abdominal aortic aneurysms and in pulmonary arterial hypertension or display lumen narrowing as can be seen in advanced stages of atherosclerosis. What happens to arterial remodeling due to pathological states? I describe briefly what is known in section 1.2. In section 1.3 I will explain gaps in our knowledge of arterial remodeling in pathological states. The study of these topics form the main objective of this thesis. An overview of the thesis is given in
section 1.4.

1.1 Large arteries and disease

Atherosclerosis is the disease with the highest mortality in the western world. Despite its large socio-economical impact, the underlying mechanisms are only partially known (Fig. 1.1). It has been accepted for decades that atherosclerosis is a lipid-driven disease, despite the fact that risk factors related to lipid metabolism only partially explain atherogenesis. Furthermore, new therapies specially focused upon lipid metabolism only partially reduce plaque size. Inflammation and wall shear stress have gained a lot of interest as complementary explanations for plaque formation [249, 83, 75, 223]. Atherosclerosis is nowadays considered a lipid-driven inflammatory disease. Inflammation has been associated with plaque progression, plaque rupture, thrombosis, and subsequent myocardial infarction [32, 213, 259, 176].

Pulmonary hypertension is a physiological state defined by chronically elevated mean pulmonary artery pressure that exceeds 25 mmHg at rest [157]. Pulmonary arterial hypertension is a clinical syndrome characterized by an increase in pulmonary vascular resistance leading to right-sided heart failure and, ultimately, death [157]. Diagnosis early in the course of the disease is difficult because of its nonspecific nature and symptoms, such as dyspnea, exercise intolerance, and fatigue.

Abdominal aortic aneurysm is a disease occurring in 5–7% of people over 60 years [52]. The largest problem of this disease is the chance of rupture, which increases with aneurysm diameter (Fig. 1.2). In current clinical practice, the chance of rupture of abdominal aortic aneurysm is estimated based on maximum aortic diameter only [52], with 55 mm or more being the generally accepted cut-off point for consideration of elective repair. In a study by Lederle et al. however, a significant number of patients with an aneurysm larger than 55 mm never experienced a rupture [124], whereas, in another study, rupture did occur in aneurysms smaller than 55 mm [14].

Due to these observations, several new criteria for the decision of elective surgery have been proposed [49, 109]. These newer criteria are derived from the
Figure 1.1: Atherosclerosis. Drawn are cross sections of an artery developing atherosclerosis. A healthy artery has three main layers (left). The most inner layer is called the intima. It is a thin layer consisting of mainly endothelial cells. In the middle is the media. A thick layer with a lot of smooth muscle cells. The outer layer is the adventitia. It consist of mainly connective tissue. The intima protects the vessel wall. Atherosclerosis is a chronic disease in which fatty materials build up between intima and media. The vessel wall remodels outward keeping lumen diameter constant (middle). In later stages of the disease, remodeling is not sufficient to keep the correct diameter and inward remodeling occurs. Lumen narrowing can cause inadequate perfusion of the tissues the artery supplies. There is also risk of plaque rupture and blood clotting. This can suddenly stop blood flow to the tissues the artery supplies.
Figure 1.2: Aneurysm expansion. The diameter of a healthy abdominal aorta is approximately 2 cm (left). If the diameter of the abdominal aorta is dilated and exceeds normal diameter by more than 50% then the diagnosis is abdominal aortic aneurysm (center). The chance of rupture increases with aneurysm diameter. An aneurysm will be considered for elective repair if the diameter is at least 55 mm (right).
assumption that rupture occurs when tissue stress exceeds a critical level [77]. Hence, several laboratories have developed methods to estimate the chance of rupture on basis of wall stress distribution and failure stress [261, 267]. Recent studies indicate that even peak wall stress alone is a better predictor of rupture than maximal diameter [49, 109].

1.2 Stress and vascular remodeling

Blood vessels possess the capacity to adjust their diameter to meet flow demands of the organs they perfuse [140]. This is of importance during development from fetus to adult, during exercise and after surgical intervention. Large arteries cannot directly sense the flow. Instead, the endothelial layer is detecting the wall shear stress by an as yet unknown mechanism [33]. Wall shear stress, the frictional force per unit area between blood and endothelium, is an important determinant of endothelial cell function, gene expression, and structure [201]. Luminal diameter is adapted in such ways that mean wall shear stress is kept within small limits [69, 104, 71].

Currently, researchers in the field often assume mean wall shear stress levels of $\sim 15 \text{ dyn/cm}^2$ as acceptable, because it represents the average wall shear stress values over the cardiac cycle of the large straight arteries with steady laminar flow (Fig. 1.3). This is based on studies in patients and animal models, which provide evidence that wall shear stress actively influences vessel wall remodeling [149, 119, 69, 104]. This compensatory response mediated by the endothelium aims at the maintenance of a wall shear stress of approximately 15–20 dyn/cm$^2$. Partially based on this notion, it is also commonly assumed that this acceptable range of wall shear stress is rather constant throughout the vascular system [206, 71, 100, 101, 241, 240].

Another argument for a constant wall shear stress value of $\sim 15 \text{ dyn/cm}^2$ at different locations in the arterial system is derived from the principle of minimal work for the cardiovascular system as proposed by Murray [173]. He stated that the total energy to drive the blood and to maintain blood volume is minimized in the arterial system. Deducted from this principle is Murray’s law [172], which states that the cube of the radius of the mother vessel equals
Figure 1.3: Wall shear stress in a straight artery. Drawn is a cross section of an artery in axial direction. The velocity field is illustrated with velocity vectors and an idealized parabolic velocity profile. This velocity profile can only develop if there is a no slip condition at the wall and the viscosity of the liquid is sufficiently large. Due to those conditions the resistance between the laminar velocity layers results in a shear stress. A larger velocity difference corresponds with a larger shear stress. In the illustration the largest velocity difference is at the wall. The shear stress at the wall is called the wall shear stress.
1.2. STRESS AND VASCULAR REMODELING

Figure 1.4: Murray’s law. Illustration of an arterial bifurcation cut in axial direction. Murray’s law states that the cube of the radius of the mother vessel equals the sum of cubes of the radii of the daughter vessels. In this example the radii of the daughter vessels are equally large. All blood that flows through the mother vessel is split and both daughter vessels receive half of the total flow. Because of the cube law, both the radius and the peak velocity scale in proportion. Therefore, the flow profile looks exactly the same in mother and daughter vessels. This also means that the wall shear stress is equal in mother and daughter vessel.

The effect of blood flow in atherosclerosis is based upon the observation that plaques are not evenly distributed over the arterial system [223, 228, 34]. These predilection sites are at or near side branches, where blood flow is nonuniform, or at the inner curve of vessels, where blood velocity is relatively low. The wall shear stress affects the phenotype of the endothelial cells and thereby the inflammatory component, plaque progression and plaque composition. Several studies indicated that plaque inflammation is unevenly distributed over its length, with a predominant upstream presence of inflammatory cells [259, 48, 146, 42, 251, 216].

Low-density lipoprotein (LDL) accumulates in the vessel wall where it may become oxidized (oxLDL). OxLDL is known to be involved in many processes
related to atherosclerosis, including stimulation of macrophage infiltration and foam cell formation, stimulation of vascular smooth muscle cell migration and proliferation, and endothelial cell apoptosis [158, 4, 37, 94, 180]. A recent study indicated that oxLDL is associated with plaque instability [180]. This observation might be explained by the modulation of activation of some matrix metalloproteinase family members by oxLDL [178].

Given all these changes it is remarkable that only when the lesion occupies forty percent of the internal elastic lamina area, outward remodeling seems hampered and lumen narrowing is initiated [70]. Lumen narrowing causes a reduction of oxygen delivery to e.g. the heart muscle. This conversion from outward to inward remodeling is an essential switch in vessel adaptation. During the development of atherosclerosis, many processes are coupled and depend on each other. A simulation model can offer great help for understanding the individual processes and the role of atherosclerosis therein.

In children with only an effective left ventricle a surgical procedure can divert the systemic venous return to the pulmonary arteries without passing through the right ventricle (Fig. 1.5). This procedure was named after Fontan who described the procedure in 1971 [53]. Fontan completion results in substantial or total loss of pulsatile flow into the lung [196, 118, 198, 110]. The long-term effects of this abnormal flow pattern on growth and function of the pulmonary artery are a matter of concern. Several groups have studied the effects of a bidirectional Glenn or Fontan pathway on pulmonary artery growth and diameters [197, 15, 245], but have shown equivocal results.

After Fontan operation, increased wall shear stress was found in one study [168] and endothelial dysfunction has been reported that may interfere with the normal remodeling process of the pulmonary artery [118, 107, 129].

Various studies have shown that in patients with pulmonary hypertension the flow pattern in the main pulmonary artery differs from that seen in healthy people [108, 182, 112, 171, 200]. One of the main findings is the appearance of retrograde flow at the right dorsal side of the main pulmonary artery. Okamoto et al. suggested that this indicates the appearance of a vortex [182].

Secondary effects of abnormally elevated pulmonary blood pressure on right-sided structures, such as pulmonary artery dilatation and right ven-
Figure 1.5: Fontan procedure. On the left side is shown an illustration of the heart and large blood vessels before Fontan procedure. On the right side is shown the new configuration after Fontan completion. The vena cava superior is anastomosed to the distal end of the right pulmonary artery. The proximal end of the right pulmonary artery is anastomosed to the right atrium by means of an aortic valve homograft. The atrial septal defect is closed and the main pulmonary artery is ligated. At the level of the vena cava inferior with the right atrium a pulmonary valve is inserted. The right atrium directs blood from the vena cava inferior to the left lung. Blood from the vena cava superior is directed to the right lung.
tricular hypertrophy, may be helpful in the diagnosis of pulmonary arterial hypertension. The diameter of the pulmonary artery can be obtained noninvasively and is, therefore, one of the parameters in pulmonary arterial hypertension that has been studied throughout the years. Early investigators found through chest radiography reasonable correlations between the diameter of the right descending pulmonary artery and the mean pulmonary artery pressure [246]. After the introduction of helical CT imaging, several studies have been performed to measure the diameter of the main pulmonary artery and showed that an increased diameter is a reliable indicator of pulmonary arterial hypertension, especially the diameter in proportion to the diameter of the ascending aorta [80, 117, 179, 215, 87, 166, 78].

Wall stress also controls vascular remodeling (Fig. 1.6) [55, 232]. Remodeling, i.e. a structural change of the vessel wall occurs when local wall stress exceeds a reference value [76]. This stress related remodeling has been studied extensively in hypertensive conditions [55, 76].

Several studies provided a mechanical explanation for rupture of the abdominal aorta as it has been shown that the stress at which the aneurysm ruptures is close to the stresses present in advanced aneurysm formation [50, 77, 265]. The vessel wall in a long standing aneurysm consists mainly of acellular material interspersed in abundant extracellular matrix [247]. Consequently, some studies have indicated that the local strength of the aneurysm tissue is determined by the amount of extracellular matrix, which again is affected by the balance between synthesis and breakdown of this matrix [133]. On a cellular level this balance is regulated by the activity of smooth muscle cells, which synthesize collagen, and by macrophages, which synthesize proteases, including matrix metalloproteinases which breakdown extracellular matrix. Numerous studies indicate that the activity of both cell types is affected by wall stress or wall stretch [133, 153, 242]. Areas of large stretch or stress leads to a changed balance of extracellular matrix turnover (“remodeling”) resulting in expansion of the aneurysm.

The rate of aneurysm expansion has been studied often [13], because this factor determines surveillance interval and time to intervention. If aneurysmal expansion in a 6 month to 12 month period is much greater than expected,
Figure 1.6: Wall stress. If we imaginary cut a blood vessel in axial direction, blood pressure will push away both halves. This pressure multiplied with the diameter and a unit length is a force. According to Newton this force should be balanced with a counter force if the blood vessel is not split in reality. Thus both sides bear half the force. The wall stress can be calculated by dividing this force by the wall thickness and the unit length. Thus wall stress is simply equal to the pressure multiplied by the radius and divided by the wall thickness. This is also known as law of Laplace.
the risk of rupture may also be higher. The expansion rate depends on several modulating factors including smoking, diabetes and gender [13, 164].

1.3 Scope of the thesis

At present, it remains unclear whether the endothelium throughout the arterial system is primed with the same range of wall shear stress values. Although Murray’s law implies a constant wall shear stress throughout the vascular system [279], a number of publications show a broad range in the actual mean wall shear stress levels that could be measured in the different types of arteries in humans [72, 27, 113, 275]. Flow measurements in animal models also show differences in wall shear stress levels between species [105, 150, 205, 137]. These data indicate that wall shear stress varies with the location across the cardiovascular system within one species, and that there are cross-species differences. In spite of this, a paucity of data exists which compare wall shear stress at different anatomical locations [275, 30] or between species in one type of vessel. Until now, no reviews are available which summarize the separate wall shear stress values found in literature to provide an adequate overview about this subject.

Atherosclerotic plaque heterogeneity indicates a spatially oriented mechanism, which to date has not received much attention. To study the underlying mechanism of such a highly spatially localizing mechanism, there is a need for a precise, quantitative technique enabling the study of plaque heterogeneity in experimental atherosclerosis.

Most of the oxLDL-related studies have been conducted on isolated cells in vitro, which are devoid of the complex environment of the atherosclerotic vessel wall, thereby identifying the need to study the role of oxLDL in vivo. It is presently unknown whether oxLDL is distributed heterogeneously within the plaque or if it is associated locally with gelatinolytic activity in plaques in vivo.

Wall shear stress not only influence plaque formation, it has been proposed that it is also of importance in remodeling of atherosclerotic vessels, but data on this topic are sparse and contra dictionary. During the development of
1.4 Overview of the thesis

In chapter 2 we present evidence from literature that supports the concept that wall shear stress levels are not identical throughout the vascular tree. We also provide evidence that wall shear stress levels differ between species. The effect of shear stress on vascular inflammation and plaque development is described in chapter 3.

In chapter 4 we present a three-dimensional histological technique that permits the study of plaque heterogeneity in more detail than before. A new
technique has been introduced to measure gelatinolytic activity in histological cross sections with a high spatial resolution [165, 278]. We have adopted this method for vascular tissue and incorporated it into three-dimensional histological reconstructions. Further we evaluated, using a combination of the above techniques, whether matrix metalloproteinases are active very locally or multiple cell types are involved in experimental atherosclerosis in vivo. Therefore, we evaluated whether oxLDL might be the cause of the spatially restricted accumulation of activated macrophages in plaques in vivo.

The aim of chapter 5 was to study diameter changes of atherosclerotic arteries after a sudden change in flow. We increased blood flow in five atherosclerotic rabbits and recorded time series of the aortic luminal diameter by magnetic resonance imaging. We tested the action of wall shear stress normalization.

Remodeling of large arteries due to a change in flow was also studied in patients (chapter 6). The objectives were: 1) to assess the size of a branch of the pulmonary artery, its local flow pattern, and the local wall shear stress after Fontan operation performed at a young age using phase contrast velocity-encoded cardiovascular MRI; and 2) to simulate the effects of exercise on pulmonary arteries with low-dose dobutamine stress.

The question in chapter 7 is whether early onset of retrograde flow indicates the presence of pulmonary hypertension and what cutoff value should be used. Therefore, we conducted a study in patients with pulmonary arterial hypertension and subjects suspected of having pulmonary hypertension, in whom both right heart catheterization and MRI are performed. In chapter 8 we investigated whether change in the diameter of the pulmonary artery over time reflects changes in pressure, cardiac output, or both in patients with pulmonary arterial hypertension.

In order to provide evidence for the feasibility of the concept that a wall stress induced change in the stiffness of the aneurysm may induce expansion we developed a finite element model that incorporates this concept (chapter 9). The aim of chapter 10 is to extend the above-mentioned wall stress concept by including aneurysmal expansion. Towards this end, a hybrid model consisting of both a wall stress remodeling rule and risk factors is proposed and tested.
1.4. **OVERVIEW OF THE THESIS**

to predict patientspecific aneurysm expansion.
Chapter 2

Large variations in absolute wall shear stress levels within one species and between species


2.1 Abstract

Wall shear stress (WSS), the frictional force between blood and endothelium, is an important determinant of vascular function. It is generally assumed that WSS remains constant at a reference value of 15 dyn/cm². In a study of small
rodents, we realized that this assumption could not be valid. This review presents an overview of recent studies in large and small animals where shear stress was measured, derived from velocity measurements or otherwise, in large vessels. The data show that large variations exist within a single species (human: variation of 2–16 N/m²). Moreover, when we compared different species at the same location within the arterial tree, an inverse relationship between animal size and wall shear stress was noted. When we related WSS to diameter, a unique relationship was derived for all species studied. This relationship could not be described by the well-known $r^3$ law of Murray, but by the $r^2$ law introduced by Zamir et al. in 1992. In summary, by comparing data from the literature, we have shown that: (i) the assumption of a physiological WSS level of $\sim 15$ dyn/cm² for all straight vessels in the arterial tree is incorrect; (ii) WSS is not constant throughout the vascular tree; (iii) WSS varies between species; (iv) WSS is inversely related to the vessel diameter. These data support an “$r^2$ law” rather than Murray’s $r^3$ law for the larger vessels in the arterial tree.

### 2.2 Introduction

Wall shear stress (WSS), the frictional force between blood and endothelium, is an important determinant of endothelial cell function, gene expression, and structure. Indeed, a variety of studies provided evidence that WSS has to be maintained between certain limits in order to maintain vascular haemostasis. WSS is actively maintained within limits during intrauterine growth, during the neonatal period and early childhood, and during exercise in the adult. Inappropriate values of WSS have been associated with maladaptive growth, patent ductus arteriosus, congenital malformations of the heart and atherosclerosis [90, 92, 67]. Indeed, when WSS is reduced by 30% in vivo in ApoE mice, the expression of several atherogenic genes is induced, which triggers the development of large atherosclerotic lesions [25]. To avoid these conditions, the endothelium in the arterial system should be responsive to WSS within a narrow range of values that are considered “normal”.

At present, it remains unclear whether the endothelium throughout the arterial system is primed with the same range of WSS values. Currently,
researchers in the field often assume mean WSS levels of $\sim 15$ dyn/cm$^2$ ($1$ dyn/cm$^2 = 0.1$ N/m$^2$) as acceptable, because it represents the average WSS values over the cardiac cycle of the large straight arteries experiencing steady laminar flow. This is based on studies in patients and animal models, which provide evidence that WSS actively influences vessel wall remodeling [149, 119, 69, 104]. This compensatory response mediated by the endothelium aims at the maintenance of a WSS magnitude of approximately 15–20 dyn/cm$^2$. Partially based on this notion, it is also commonly assumed that this acceptable range of WSS is rather constant throughout the vascular system [206, 71, 100, 101, 241, 240].

Another argument for a constant WSS value of $\sim 15$ dyn/cm$^2$ at different locations in the arterial system is derived from the principle of minimal work for the cardiovascular system as proposed by Murray [173]. He stated that the total energy to drive the blood and to maintain blood volume is minimized in the arterial system. Deduced from this principle is Murray’s law [172], which states that the cube of the radius of the mother vessel equals the sum of cubes of the radii of the daughter vessels. While this optimization principle predicts a constant WSS throughout the vascular system [279], a number of recent publications show a broad range in the actual mean WSS levels that could be measured in the different types of arteries in humans [72, 27, 113, 275]. Flow measurements in animal models also show differences in WSS levels between species [105, 150, 205, 137]. These data therefore indicate that WSS varies with the location across the cardiovascular system within one species, and that there are cross-species differences. In spite of this, a paucity of data exists which compare WSS at different anatomical locations [275, 30] or between species in one type of vessel. Until now, no reviews are available which summarize the separate WSS values found in literature to provide an adequate overview about this subject.

In this review, we present evidence from literature that supports the concept that WSS levels are not identical throughout the vascular tree. We also provide evidence that WSS levels differ between species. The interpretation of these data will be discussed in relation to a modification of Murray’s law. Acceptance of this concept would have significant implications for further WSS
research, as the importance of the effects of anatomical localization of the studied endothelial cells and the species from which they are derived is often overlooked in current studies.

2.3 Variations in mean WSS in the arterial system

2.3.1 Human individuals

Vascular disease affects the vascular remodeling capacities of arteries, which could result in an alteration of the mean WSS level. Accordingly, only studies performed in healthy human subjects are included in this review.

In Table 2.1, the WSS values in different types of arteries derived from experimental data of several investigators are summarized [72, 27, 113, 275, 30, 185, 186, 209, 208, 184, 193, 244, 58, 229, 161, 154, 57, 46]. All WSS values are derived from in vivo measurements in conscious human subjects, by applying either ultrasound or MRI techniques. All values are stationary WSS values or recalculated to become stationary WSS values, and therefore the time dependence of the WSS measurements are not taken into account. Excellent studies and reviews are present on this topic [141, 31, 143]. For more information on the studies that we have included in Table 2.1 concerning background, and details in methods, we refer to Table 2.2. It should be mentioned that the WSS values in Table 2.1 are obtained from studies testing different hypotheses (e.g. the effect of smoking or exercise on blood flow), with different study designs testing specific parameters. However, only WSS values obtained from the control (non-treatment) groups were included in this review. Selection criteria for the incorporated studies are as followed: subjects of the selected control group are younger than 40 years old, do not show clinical manifestation of cardiovascular disease, are non-smokers and do not receive any prescribed medication. An exception to this rule is the study performed by the group of Mitchell et al. that provides values of WSS for the brachial artery (Table 2.1). This study includes a large population of subjects (N = 2045), with an average age of 61 ± 10 years. One has to take into account that from this
particular population, 13% had prevalent cardiovascular disease, 12.5% had diabetes, 45% was diagnosed with hypertension, 32.5% took antihypertensive medication, 20.6% took lipidlowering medications, 29.5% took aspirin daily, and 13.5% were smokers.

WSS levels range from 9.5 dyn/cm$^2$ to 15.0 dyn/cm$^2$ in the common carotid artery, from 6.2 to 9.3 dyn/cm$^2$ in the suprarenal aorta, from 3.5 to 8.4 dyn/cm$^2$ in the supracleiliac aorta, from 1.3 to 5.4 dyn/cm$^2$ in the infrarenal aorta, from 2.9 to 6.5 dyn/cm$^2$ in the common femoral artery, from 3.9 to 4.9 dyn/cm$^2$ in the superficial femoral artery, from 4.3 to 7.8 dyn/cm$^2$ in the brachial artery, and from 10.9 to 15.7 dyn/cm$^2$ in the left anterior descending artery. When calculating the average of these WSS values per type of artery, it becomes obvious that there are clear differences in WSS values between the anatomical locations (Fig. 2.1), which seem dependent on the distance from the aortic root. The more downstream that the vessel is located, the lower is its WSS value. The highest average values are found in the left anterior descending artery (12.7 dyn/cm$^2$), and the common carotid artery (11.6 dyn/cm$^2$). The values in the aorta are dependent on the location, and seem to decrease downstream from the branchpoints of the renal arteries (7.3 dyn/cm$^2$ in the suprarenal aorta to 4.2 dyn/cm$^2$ in the supracleiliac/infrarenal aorta).

In addition, estimation of the WSS in the fetal descending aorta during the second half of pregnancy demonstrated a mean value of 22 dyn/cm$^2$ [233]. This exceeded level of WSS may provide a stimulus for outward remodeling of the fetus. Further downstream in the arterial tree, the average of the WSS values remains around the level of the suprarenal aorta in the common femoral artery (4.3 dyn/cm$^2$) and in the superficial femoral artery (4.4 dyn/cm$^2$). In the brachial artery, a comparable value for the WSS can be calculated (5.6 dyn/cm$^2$) (Fig. 2.1).

These data clearly indicate that there is non-uniformity in mean WSS levels in the human vascular system. The differences in WSS values could be the result of the application of different techniques to measure flow and diameter in the separate studies. Recently, two groups became aware of this phenomenon and conducted excellent experiments in which the mean WSS and mean shear rate (mean WSS = mean shear rate × viscosity) were compared
Table 2.1: Mean wall shear stress levels in non-atherosclerotic humans. The table shows data for different blood vessels: The common carotid artery (CCA), the suprarenal aorta (SRA), the supraceiliac aorta (SCRA), the infrarenal aorta (IRA), the common femoral artery (CFA), the superficial femoral artery (SFA) and the brachial artery (BA). The lumen diameter is not given if the concerning data was not available (N.A.).

<table>
<thead>
<tr>
<th>Reference</th>
<th>Structure</th>
<th>Mean wall shear stress (dyn/cm²) (^a)</th>
<th>Lumen diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gnasso et al. [72]</td>
<td>CCA</td>
<td>12.1 ± 3.1</td>
<td>5.5 ± 0.7</td>
</tr>
<tr>
<td>Oyre et al. [186]</td>
<td>CCA</td>
<td>9.5</td>
<td>7.2</td>
</tr>
<tr>
<td>Samijo et al. [209]</td>
<td>CCA</td>
<td>10.5-4.5</td>
<td>5.9-6.5</td>
</tr>
<tr>
<td>Samijo et al. [208]</td>
<td>CCA</td>
<td>12.4 ± 2.0</td>
<td>6.3 ± 0.5</td>
</tr>
<tr>
<td>Dammers et al. [30]</td>
<td>CCA</td>
<td>11.5 ± 2.1</td>
<td>6.7 ± 0.5</td>
</tr>
<tr>
<td>Wu et al. [275]</td>
<td>CCA</td>
<td>10.0 ± 1.8</td>
<td>6.3 ± 0.6</td>
</tr>
<tr>
<td>Oshinski et al. [184]</td>
<td>SRA</td>
<td>9.3</td>
<td>N.A.</td>
</tr>
<tr>
<td>Tang [244]</td>
<td>SCRA</td>
<td>8.4 ± 1.8</td>
<td>N.A.</td>
</tr>
<tr>
<td>Cheng et al. [27]</td>
<td>SCRA</td>
<td>3.5 ± 0.8</td>
<td>N.A.</td>
</tr>
<tr>
<td>Oyre et al. [185]</td>
<td>SRA</td>
<td>6.3</td>
<td>N.A.</td>
</tr>
<tr>
<td>Pedersen et al. [193]</td>
<td>SRA</td>
<td>6.2</td>
<td>N.A.</td>
</tr>
<tr>
<td>Tang [244]</td>
<td>IRA</td>
<td>5.1 ± 1.3</td>
<td>N.A.</td>
</tr>
<tr>
<td>Pedersen et al. [193]</td>
<td>IRA</td>
<td>2.7</td>
<td>N.A.</td>
</tr>
<tr>
<td>Oyre et al. [185]</td>
<td>IRA</td>
<td>2.8</td>
<td>N.A.</td>
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<tr>
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<td>IRA</td>
<td>1.3 ± 0.6</td>
<td>16.0 ± 2.0</td>
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<td>IRA</td>
<td>5.4</td>
<td>N.A.</td>
</tr>
<tr>
<td>Kornet et al. [113]</td>
<td>CFA</td>
<td>3.5 ± 1.8</td>
<td>N.A.</td>
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<tr>
<td>Gaenzer et al. [58]</td>
<td>CFA</td>
<td>2.9</td>
<td>7.8 ± 0.7</td>
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<tr>
<td>Silber et al. [229]</td>
<td>CFA</td>
<td>6.5</td>
<td>7.1 ± 0.8</td>
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<td>Kornet et al. [113]</td>
<td>SFA</td>
<td>4.9 ± 1.5</td>
<td>N.A.</td>
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<td>Wu et al. [275]</td>
<td>SFA</td>
<td>3.9</td>
<td>6.9 ± 0.9</td>
</tr>
<tr>
<td>Dammers et al. [30]</td>
<td>BA</td>
<td>4.8 ± 1.5</td>
<td>3.7 ± 0.7</td>
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<td>Gaenzer et al. [58]</td>
<td>BA</td>
<td>7.8</td>
<td>4.2 ± 0.6</td>
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<td>Wu et al. [275]</td>
<td>BA</td>
<td>5.8</td>
<td>3.7 ± 0.8</td>
</tr>
<tr>
<td>Mitchell et al. [161]</td>
<td>BA</td>
<td>4.3 (female group)</td>
<td>4.9 ± 0.6</td>
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<tr>
<td>Mitchell et al. [161]</td>
<td>BA</td>
<td>4.7 (male group)</td>
<td>3.7 ± 0.6</td>
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<tr>
<td>Silber et al. [229]</td>
<td>BA</td>
<td>6</td>
<td>7.0 ± 0.8</td>
</tr>
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</table>

\(^a\)1 dyn/cm² = 0.1 N/m².
Table 2.2: Details on study populations and methods. Blood velocities were measured with different techniques: High resolution echo Doppler (HRED), MRI phase contrast velocity mapping (MRI PCVM) and Ultrasound shear rate estimation (USRE).

<table>
<thead>
<tr>
<th>Reference</th>
<th>Age (years)</th>
<th>Number of subjects</th>
<th>Technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gnasso et al. [72]</td>
<td>35.9 ± 12.9</td>
<td>21 males</td>
<td>HRED(^a)</td>
</tr>
<tr>
<td>Oyre et al. [186]</td>
<td>26.4</td>
<td>7 males</td>
<td>MRI PCVM(^b)</td>
</tr>
<tr>
<td>Samijo et al. [209]</td>
<td>10-60</td>
<td>55 females, 56 males</td>
<td>USRE</td>
</tr>
<tr>
<td>Samijo et al. [208]</td>
<td>44 (34-58)</td>
<td>4 females, 9 males</td>
<td>USRE</td>
</tr>
<tr>
<td>Dammers et al. [30]</td>
<td>23.7 ± 3.4</td>
<td>3 females, 7 males</td>
<td>USRE</td>
</tr>
<tr>
<td>Wu et al. [275]</td>
<td>23.9 ± 1.9</td>
<td>10 females, 10 males</td>
<td>MRI PCVM(^c)</td>
</tr>
<tr>
<td>Oshinski et al. [184]</td>
<td>N.A.</td>
<td>8 males</td>
<td>MRI PCVM</td>
</tr>
<tr>
<td>Tang [244]</td>
<td>20-30</td>
<td>2 females, 3 males</td>
<td>MRI PCVM(^d)</td>
</tr>
<tr>
<td>Cheng et al. [27]</td>
<td>40.4</td>
<td>1 female, 7 males</td>
<td>MRI PCVM</td>
</tr>
<tr>
<td>Oyre et al. [185]</td>
<td>N.A.</td>
<td>6 volunteers</td>
<td>MRI PCVM</td>
</tr>
<tr>
<td>Pedersen et al. [193]</td>
<td>24.4</td>
<td>8 volunteers</td>
<td>MRI PCVM</td>
</tr>
<tr>
<td>Kornet et al. [113]</td>
<td>20-59</td>
<td>23 females, 16 males</td>
<td>USRE(^e)</td>
</tr>
<tr>
<td>Gaenzer et al. [58]</td>
<td>39.6 ± 5.8</td>
<td>10 males</td>
<td>HRED</td>
</tr>
<tr>
<td>Silber et al. [229]</td>
<td>27.6</td>
<td>15 females, 9 males</td>
<td>MRI PCVM</td>
</tr>
<tr>
<td>Mitchell et al. [161]</td>
<td>61 ± 10</td>
<td>1107 females, 938 males</td>
<td>HRED</td>
</tr>
</tbody>
</table>

\(^a\)Blood flow at measure point was shown to be parabolic, Reynolds number was < 1000.

\(^b\)The method used for estimating the mean wall shear stress value applies fundamental hemodynamic theory using a three-dimensional paraboloid model for fitting of blood velocity data in the boundary layer. The data were obtained by standard MRI velocity acquisition techniques. In-plane pixel resolution of the MRI slices was 0.25 mm\(^2\).

\(^c\)Technique uses phase contrast imaging with 0.0625 mm\(^2\) resolution and three-dimensional paraboloid fitting.

\(^d\)In-plane pixel resolution of the MRI slices was 0.47 mm\(^2\).

\(^e\)Measurements performed 2-3 mm from the flow divider.
Figure 2.1: Stationary WSS values in the large arterial vessels of conscious non-atherosclerotic humans. Note that the location determines the actual WSS values.
2.3. VARIATIONS IN MEAN WSS IN THE ARTERIAL SYSTEM

between different arteries. Wu et al. showed significant differences between the superficial femoral artery \((3.9 \pm 1.8 \text{ dyn/cm}^2)\), the brachial artery \((5.8 \pm 3.0 \text{ dyn/cm}^2)\), and the common carotid artery \((10.0 \pm 1.8 \text{ dyn/cm}^2)\) in one study within the same human subject and experimental protocol [275]. Dammers et al. also showed significant differences between the common carotid artery \((11.5 \pm 2.1 \text{ dyn/cm}^2)\) and the brachial artery \((4.8 \pm 1.5 \text{ dyn/cm}^2)\) within one study [30]. These observations confirm the concept of non-uniformity of mean WSS in the human arterial tree. The remainder of the data is summarized in Table 2.1. Furthermore, although the comparison between several arteries is conducted with data from separate studies, the mean WSS clearly differs between certain vascular locations, as the range values show no overlap (e.g. common carotid artery, range 9.5–15.0 dyn/cm^2 versus common femoral artery, range 2.9–6.5 dyn/cm^2).

2.3.2 Cross-species differences

In Table 2.3, the mean WSS values in the common carotid artery and the common femoral artery of different laboratory animals and of several investigators are compared. All the WSS values are calculated using in vivo data from measurements of both lumen and outer diameter of the blood vessel. If no lumen diameter is presented, it can be calculated from the outer diameter as: lumen diameter = 80% of outer diameter [11], thereby assuming that wall thickness is proportional to lumen diameter. In rodents, and in rabbits in particular, the outer diameter is often used for WSS calculations, whereas the lumen diameter provides a more correct value for the radius of a vessel. The radius of a vessel is inversely related to the WSS. Consequently, recalculations of the WSS values using lumen diameter instead of outer diameter yields higher WSS values for rabbits, rats and mice. Therefore, further discussion in this review will be concerning WSS calculated from the lumen diameter values only. All values are stationary WSS values omitting the pulsatile nature of the blood flow. The blood flow and velocity were measured in both conscious and anesthetized animals, by applying ultrasound techniques (Table 2.4).
Table 2.3: Mean wall shear stress levels in laboratory animals. The table shows data for different animals and blood vessels. Both outer diameter (OD) and lumen diameter (LD) are listed. New Zealand white rabbits is abbreviated to Rabbits (NZW). Other abbreviations as in Table 2.1.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Animals</th>
<th>Structure</th>
<th>Mean wall shear stress (dyn/cm²)</th>
<th>Vessel diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pohl et al. [195]</td>
<td>Dogs</td>
<td>CFA</td>
<td>4.9 (OD), 9.5 (LD)</td>
<td>4.7 (OD), 3.8 (LD)</td>
</tr>
<tr>
<td>Serhatlioglu et al. [222]</td>
<td>Dogs</td>
<td>CFA</td>
<td>5.6 (OD), 19.3 (LD)</td>
<td>4.6 (OD), 3.7 (LD)</td>
</tr>
<tr>
<td>Lie et al. [140]</td>
<td>Dogs</td>
<td>CFA</td>
<td>4.8 (OD), 10.0 (LD)</td>
<td>4.3 (OD), 3.4 (LD)</td>
</tr>
<tr>
<td>Kamiya and Togawa [105]</td>
<td>Dogs</td>
<td>CCA</td>
<td>15.8 (LD)</td>
<td>3.0 (LD)</td>
</tr>
<tr>
<td>Lee et al. [126]</td>
<td>Dogs</td>
<td>CCA</td>
<td>46.0 (LD)</td>
<td>3.0 (LD)</td>
</tr>
<tr>
<td>Lee et al. [126]</td>
<td>Dogs</td>
<td>CFA</td>
<td>44.0 (LD)</td>
<td>3.0 (LD)</td>
</tr>
<tr>
<td>Zderic et al. [282]</td>
<td>Rabbits (NZW)</td>
<td>CFA</td>
<td>15.8 (LD)</td>
<td>0.94 (LD)</td>
</tr>
<tr>
<td>Tronc et al. [252]</td>
<td>Rabbits (NZW)</td>
<td>CCA</td>
<td>19.4 (LD)</td>
<td>1.8 (LD)</td>
</tr>
<tr>
<td>Walpola et al. [268]</td>
<td>Rabbits (NZW)</td>
<td>CCA</td>
<td>12.2 (LD)</td>
<td>2.0 (LD)</td>
</tr>
<tr>
<td>Marano et al. [150]</td>
<td>Rabbits (NZW)</td>
<td>CCA</td>
<td>60.9 (LD)</td>
<td>1.6 (LD)</td>
</tr>
<tr>
<td>Langille et al. [122]</td>
<td>Rabbits (NZW)</td>
<td>CCA</td>
<td>13.8 (OD), 17.3 (LD)</td>
<td>2.6 (OD), 2.1 (LD)</td>
</tr>
<tr>
<td>Sho et al. [227]</td>
<td>Rabbits (Japanese White)</td>
<td>CCA</td>
<td>10.2 (LD)</td>
<td>? (LD)</td>
</tr>
<tr>
<td>Masuda et al. [152]</td>
<td>Rabbits (Japanese White)</td>
<td>CCA</td>
<td>12.0 (OD), 20.9 (LD)</td>
<td>2.1 (OD), 1.7 (LD)</td>
</tr>
<tr>
<td>Lu et al. [147]</td>
<td>Rats (Wistar)</td>
<td>CFA</td>
<td>53.7 (OD), 102.9 (LD)</td>
<td>0.78 (OD), 0.62 (LD)</td>
</tr>
<tr>
<td>Rose et al. [205]</td>
<td>Rats (Sprague-Dawley)</td>
<td>CFA</td>
<td>15.0 (OD), 28.8 (LD)</td>
<td>0.78 (assumed OD), 0.62 (assumed LD)</td>
</tr>
<tr>
<td>Ibrahim et al. [95]</td>
<td>Rats (Sprague-Dawley)</td>
<td>CCA</td>
<td>23.6 (OD), 40.8 (LD)</td>
<td>0.88 (OD), 0.70 (LD)</td>
</tr>
<tr>
<td>Ibrahim et al. [95]</td>
<td>Rats (Fisher)</td>
<td>CCA</td>
<td>25.7 (OD), 43.3 (LD)</td>
<td>0.82 (OD), 0.66 (LD)</td>
</tr>
<tr>
<td>Ibrahim et al. [95]</td>
<td>Rats (BN, hypertensive)</td>
<td>CCA</td>
<td>18.8 (OD), 48.4 (LD)</td>
<td>0.79 (OD), 0.63 (LD)</td>
</tr>
<tr>
<td>Ibrahim et al. [95]</td>
<td>Rats (SHR-SR, hypertensive)</td>
<td>CCA</td>
<td>22.4 (OD), 38.6 (LD)</td>
<td>0.90 (OD), 0.72 (LD)</td>
</tr>
<tr>
<td>Ross et al. [205]</td>
<td>Rats (Sprague-Dawley)</td>
<td>CCA</td>
<td>42.6 (OD), 84.0 (LD)</td>
<td>0.88 (assumed OD), 0.70 (assumed LD)</td>
</tr>
<tr>
<td>Miyashiro et al. [162]</td>
<td>Rats (Fisher)</td>
<td>CCA</td>
<td>22.1 (OD), 43.0 (LD)</td>
<td>0.99 (OD), 0.79 (LD)</td>
</tr>
<tr>
<td>Miyashiro et al. [162]</td>
<td>Rats (Fisher, juvenile)</td>
<td>CCA</td>
<td>28.9 (OD), 56.3 (LD)</td>
<td>0.80 (OD), 0.64 (LD)</td>
</tr>
<tr>
<td>Tohdai et al. [250]</td>
<td>Rats (Sprague-Dawley)</td>
<td>CCA</td>
<td>20.0 (LD)</td>
<td>? (LD)</td>
</tr>
<tr>
<td>Hartley et al. [64]</td>
<td>Mice (C57BI/6J)</td>
<td>CCA</td>
<td>36.0 (OD), 45.0 (LD)</td>
<td>0.50 (assumed OD), 0.4 (assumed LD)</td>
</tr>
<tr>
<td>Li et al. [137]</td>
<td>Mice (C57BI/6J)</td>
<td>CCA</td>
<td>81.0 (OD), 102.0 (LD)</td>
<td>0.50 (assumed OD), 0.4 (assumed LD)</td>
</tr>
<tr>
<td>Koshunov and Berk [114]</td>
<td>Mice (C57BI/6J)</td>
<td>CCA</td>
<td>40.0 (LD)</td>
<td>? (LD)</td>
</tr>
<tr>
<td>Castier et al. [17]</td>
<td>Mice (C57BI/6J)</td>
<td>CCA</td>
<td>37.0 (LD)</td>
<td>? (LD)</td>
</tr>
<tr>
<td>Rudic et al. [207]</td>
<td>Mice (C57BI/6J)</td>
<td>CCA</td>
<td>31.0 (OD), 60.0 (LD)</td>
<td>0.50 (assumed OD), 0.4 (assumed LD)</td>
</tr>
<tr>
<td>Sullivan and Hoying [234]</td>
<td>Mice</td>
<td>CCA</td>
<td>142.0 (LD)</td>
<td>0.30 (LD)</td>
</tr>
<tr>
<td>Schiffers et al. [214]</td>
<td>Mice (C57BI/6J and 129 SV)</td>
<td>CCA</td>
<td>14.0 (OD), 28.0 (LD)</td>
<td>0.50 (OD), 0.4 (LD)</td>
</tr>
</tbody>
</table>

*a*50% Black Swiss and 50% 129 SV
### Table 2.4: Details on laboratory animals and methods. The Flow is not given if the concerning data was not available (N.A.).

<table>
<thead>
<tr>
<th>Reference</th>
<th>Flow (ml/min)</th>
<th>Technique</th>
<th>Anesthesia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pohl et al. [195]</td>
<td>99.0</td>
<td>Electromagnetic flow probe (perivascular)</td>
<td>Nembutal</td>
</tr>
<tr>
<td>Serhatlioglu et al. [222]</td>
<td>N.A.</td>
<td>Doppler sonography</td>
<td>Awake animals</td>
</tr>
<tr>
<td>Lie et al. [140]</td>
<td>79.6</td>
<td>Electromagnetic flow probe (perivascular)</td>
<td>Nembutal</td>
</tr>
<tr>
<td>Kamiya and Togawa [105]</td>
<td>N.A.</td>
<td>Electromagnetic flow probe (perivascular)</td>
<td>Nembutal</td>
</tr>
<tr>
<td>Lee et al. [126]</td>
<td>N.A.</td>
<td>Doppler sonography</td>
<td>Awake animals</td>
</tr>
<tr>
<td>Lee et al. [126]</td>
<td>N.A.</td>
<td>Doppler sonography</td>
<td>Awake animals</td>
</tr>
<tr>
<td>Zderic et al. [282]</td>
<td>N.A.</td>
<td>Doppler sonography</td>
<td>Acepromazine</td>
</tr>
<tr>
<td>Tronc et al. [252]</td>
<td>22.2</td>
<td>Electromagnetic flow probe (perivascular)</td>
<td>Nembutal and ketamine hydrochloride</td>
</tr>
<tr>
<td>Walpola et al. [268]</td>
<td>13.9</td>
<td>Electromagnetic flow probe (perivascular)</td>
<td>Xylazine and Ketamine</td>
</tr>
<tr>
<td>Marano et al. [150]</td>
<td>42.0</td>
<td>Doppler sonography</td>
<td>Isoflurane</td>
</tr>
<tr>
<td>Langille et al. [122]</td>
<td>N.A.</td>
<td>Electromagnetic flow probe (perivascular)</td>
<td>Xylazine and Ketamine</td>
</tr>
<tr>
<td>Sho et al. [227]</td>
<td>?</td>
<td>Electromagnetic flow probe (perivascular)</td>
<td>Xylazine and ketamine, sevoflurane</td>
</tr>
<tr>
<td>Masuda et al. [152]</td>
<td>19.4</td>
<td>Electromagnetic flow probe (perivascular)</td>
<td>Xylazine and ketamine, sevoflurane</td>
</tr>
<tr>
<td>Lu et al. [147]</td>
<td>5</td>
<td>Electromagnetic flow probe (perivascular)</td>
<td>Hypononar and Dormicium</td>
</tr>
<tr>
<td>Ross et al. [205]</td>
<td>1.4</td>
<td>Electromagnetic flow probe (perivascular)</td>
<td>Pentobarbione sodium</td>
</tr>
<tr>
<td>Ibrahim et al. [95]</td>
<td>2.8</td>
<td>Electromagnetic flow probe (perivascular)</td>
<td>Xylazine, Ketamine, and Acepromazine</td>
</tr>
<tr>
<td>Ibrahim et al. [95]</td>
<td>2.4</td>
<td>Electromagnetic flow probe (perivascular)</td>
<td>Xylazine, Ketamine, and Acepromazine</td>
</tr>
<tr>
<td>Ibrahim et al. [95]</td>
<td>2.4</td>
<td>Electromagnetic flow probe (perivascular)</td>
<td>Xylazine, Ketamine, and Acepromazine</td>
</tr>
<tr>
<td>Ibrahim et al. [95]</td>
<td>2.7</td>
<td>Electromagnetic flow probe (perivascular)</td>
<td>Xylazine, Ketamine, and Acepromazine</td>
</tr>
<tr>
<td>Ross et al. [205]</td>
<td>5.7</td>
<td>Electromagnetic flow probe (perivascular)</td>
<td>Pentobarbione sodium</td>
</tr>
<tr>
<td>Miyashiro et al. [162]</td>
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<td>Electromagnetic flow probe (perivascular)</td>
<td>Xylazine, Ketamine, and Acepromazine</td>
</tr>
<tr>
<td>Miyashiro et al. [162]</td>
<td>2.9</td>
<td>Electromagnetic flow probe (perivascular)</td>
<td>Xylazine, Ketamine, and Acepromazine</td>
</tr>
<tr>
<td>Tohda et al. [250]</td>
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<td>Sodium pentobarbital</td>
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<td>Hartley et al. [84]</td>
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<td>Isolurane</td>
</tr>
<tr>
<td>Li et al. [137]</td>
<td>N.A.</td>
<td>Electromagnetic flow probe</td>
<td>Pentobarbital</td>
</tr>
<tr>
<td>Korshunov and Berk [114]</td>
<td>0.5</td>
<td>Electromagnetic flow probe (perivascular)</td>
<td>Xylazine, Ketamine</td>
</tr>
<tr>
<td>Castier et al. [17]</td>
<td>0.64</td>
<td>Electromagnetic flow probe</td>
<td>Xylazine, Ketamine</td>
</tr>
<tr>
<td>Rudic et al. [207]</td>
<td>0.75</td>
<td>Electromagnetic flow probe</td>
<td>Xylazine, Ketamine</td>
</tr>
<tr>
<td>Sullivan and Hoying [234]</td>
<td>0.75</td>
<td>Electromagnetic flow probe</td>
<td>Xylazine, Ketamine</td>
</tr>
<tr>
<td>Schiffers et al. [214]</td>
<td>0.35</td>
<td>Ultrasonic probe</td>
<td>Xylazine, Ketamine</td>
</tr>
</tbody>
</table>
In anesthetized animals, WSS levels in the dog common carotid artery are 15.8 dyn/cm$^2$, and ranged from 10.2 to 156.8 dyn/cm$^2$ in rabbits, from 20.0 to 84 dyn/cm$^2$ in rats, and from 28.0 to 142.0 dyn/cm$^2$ in mice. When calculating the average of these WSS values per species in the same type of artery, cross-species differences in mean WSS values are found (Fig. 2.2), which are 15.8 dyn/cm$^2$ in dogs, 23.3 dyn/cm$^2$ in rabbits, 46.6 dyn/cm$^2$ in rats, and 64.8 dyn/cm$^2$ in mice. Comparing these data to the average mean WSS in the common carotid artery in conscious humans (11.6 dyn/cm$^2$) reveals a higher WSS in smaller species (Fig. 2.2). In anesthetized animals, the average WSS in the common femoral artery is 9.8 dyn/cm$^2$ in dogs, 156.8 dyn/cm$^2$ in rabbits, and 65.9 dyn/cm$^2$ in rats, compared to 4.3 dyn/cm$^2$ in conscious humans. Together these data indicate a variation in WSS between species when the data are ordered per type of artery, with a tendency for WSS to increase in smaller animals. However, when interpreting the collected data we should take into account that in contrast to the data provided for humans, the majority of the experiments are conducted in an invasive manner using different anesthetics. Consequently, the data for one species for one type of vessel may vary between different studies (e.g. WSS ranges for the common carotid artery of the rabbit from 10.2 to 156.8 dyn/cm$^2$). For example, comparing the data acquired from the common femoral artery and the common carotid artery in dogs anesthetized with Nembutal (common femoral artery: 9.5 dyn/cm$^2$, common carotid artery: 15.8 dyn/cm$^2$) with the data from conscious animals (common femoral artery = 19.3 and 44.0 dyn/cm$^2$, common carotid artery = 46.0 dyn/cm$^2$), we found clear indications that the WSS levels are presumably higher in non-anesthetized dogs [105, 195, 222, 140, 126]. However, it remains unclear if this also holds true for different types of anesthetics in different laboratory animals, as the effect of anesthetics on the cardiovascular system can vary between species. Most of the commonly used anesthetics do have a heart rate lowering effect, which could lead to a reduction in cardiac output, thereby lowering the blood flow in the arterial tree. In rats, for example, the often used combination of ketamine-xylazine reduces the heart rate by $\sim 20\%$ up to one hour after administration [235]. A recent study conducted in mice compared the effect of different anesthetics (isoflurane, ketamine-xylazine and
2.3. VARIATIONS IN MEAN WSS IN THE ARTERIAL SYSTEM

Figure 2.2: Average mean WSS values in the common carotid artery of five different species.

pentobarbital sodium) on the cardiac output with the hemodynamic condition measured in conscious animals. The results indicated that the mean arterial pressure and the cardiac output were decreased during all anesthetic interventions, with the smallest effects observed for isoflurane and the largest for ketamine-xylazine [99]. As all mouse studies summarized in this review were carried out using one of these types of anesthetics, the mean WSS values in the conscious mice are likely to be higher than the values presented in Table 2.3. One should take into account that in Figure 2.2, the WSS values in conscious humans are compared to anesthetized animals, and that the mean WSS in at least conscious dogs, rats and mice is presumably higher than indicated.

Despite these restrictions, a trend was noticed between species, indicating an effect of body size, in addition to the effect of location on WSS values. As both observations have been ignored to a great extent, it is of importance to test these findings in one study. In our laboratory, mice experiments are routinely carried out under isoflurane anesthetics. We conducted a small experiment in which we non-invasively measured the blood velocity in wildtype C57BL/6J mice using a 30 MHz Doppler probe. The femoral artery and common carotid artery were compared. The measured velocity was subsequently used for WSS calculations, using an assumed luminal diameter for the common carotid artery and the femoral artery. We observed that under the conditions
of current experimental protocol: (i) the average WSS level in mice are indeed much higher than the WSS values observed in larger animals and humans, (ii) the average WSS of the femoral artery is $\sim 4$ times smaller than the average WSS in the common carotid artery ($39.6 \pm 2.7 \text{ dyn/cm}^2$ versus $170.6 \pm 14.8 \text{ dyn/cm}^2$, respectively) (Table 2.5). The last observation is comparable to the findings in healthy human subjects, where the average WSS level in the femoral artery is $\sim 3$ times smaller than in the common carotid artery.

Taken together, these observations are not in accordance with the widely believed concept of an optimal energy transfer in the arterial system. These points will be discussed in more details below.

### 2.4 Modification of Murray’s law may explain a non-uniform WSS

As indicated above, the concept regarding a constant WSS value is rooted in the principles of Murray’s law [172], stating that:

$$r_{\text{mother vessel}}^3 = r_{\text{daughter vessel}1}^3 + r_{\text{daughter vessel}2}^3$$  \hspace{1cm} (2.1)

In this equation $r$ represents the radius of the vessel lumen, and the 3rd power represents a value of physiological implication in the context of steady flow. The blood flow equals $v_{\text{mean}} \cdot \pi r^2$, where $v_{\text{mean}}$ is the averaged cross-sectional velocity. According to Murray’s law, the blood flow is related to
2.4. EXPLANATION OF A NON-UNIFORM WSS

Figure 2.3: Relation between mean WSS and vessel lumen diameter (A) in different types of arteries of non-atherosclerotic humans and (B) in the carotid artery of different non-atherosclerotic species.

$r^3$, hence $v_{mean}$ is linearly related to the radius. As a result, the flow profile is the same in mother and daughter vessels and so is the derivative $(dv/dr)$. Because the WSS equals $\mu \cdot dv/dr$, in which $\mu$ is the viscosity of the fluid, the WSS in these vessels is predicted to be constant. However, a statistically significant linear correlation between lumen diameter and WSS was found in the collected data from the human studies (Fig. 2.3A). In addition, the cross-species comparison of lumen diameter and the WSS of the carotid artery could be described by a hyperbolic function, for which the shear stress relates more closely to $r^2$ than $r^3$ (Fig. 2.3B).
These data are therefore not in accordance with the cube law. Several studies pointed out that a squared law describing radius versus blood flow might be more appropriate for the larger conduit arteries [280, 256, 59]. However, until now variations in measurement of wall shear stress did not allow discrimination between cube and squared laws [106]. In this overview, we combined measurements of shear stress at different locations within a single species and measurements at a single location across species. According to the \( r^2 \) law, blood flow is related to \( r^2 \), and WSS is predicted to vary inversely with the vessel radius, becoming larger when the diameter of the vessel decreases. Indeed, in Figure 2.3B, WSS is inversely related with vessel diameter. Apparently, a linear relationship was noted when different locations within a single species were compared (Fig. 2.3A). However, when placing the human values in the relationship for different species, the human data fit within the hyperbolic relation of lumen diameter and shear stress.

These observations have important implications for future studies in the shear stress field, as the normal shear stress values now depend on the diameter of the vessel under study. The mechanism for this finding is currently unknown, but can only be explained if the endothelial cells located in different vessels are “primed” to a different mean WSS value. This hypothesis is supported by findings in in vitro experiments on endothelial cells of cardiac and aortic origin. Culture of these endothelial cells under the same steady flow conditions resulted in different expression patterns of shear stress responsive genes between these cell types (Hierck et al., unpublished data). Comparison of endothelial cells derived from human umbilical vein (HUVEC) with endothelial cells derived from the human aorta (HAEC) that are exposed to the same level of shear stress, also show differences in the cytokine expression profile [170]. In addition, microarray analysis of HUVEC and HAEC show differences in the shear stress response [22, 63]. Thus, the endothelial layer in the regions with lower mean WSS is accustomed phenotypically to the local hemodynamic conditions. Recent publications have identified endothelial shear stress sensing mechanisms that can alter their capacity by adjusting their shear stress sensors in response to WSS (e.g. hyaluronic acid glycosaminoglycans in the glycocalyx [163, 74], and PECAM–1 [255, 26]). The endothelium may be attuned to local
WSS conditions by specific modification of these shear stress sensors. The priming of the endothelial cells to the local WSS conditions may take place during endothelial cell development. In adult endothelial cells, WSS regulates gene expression by inducing epigenetic modification of histones and activation of transcription complexes bearing acetyltransferase activity [96]. In a recent study, it was shown that WSS could also epigenetically modify histones and influence cell differentiation in mouse embryonic stem cells, committing them to become cardiovascular precursors [97]. It is interesting to hypothesize that perhaps during embryonic development, the geometry in the vasculature determines the fate of these vascular precursors by priming them to respond athero-protectively to the local WSS value, resulting in the adaptation of the subsequently formed endothelium.

2.5 Limitations of the assessed studies

Estimation of the magnitude of shear stress based on Doppler measurements indeed relies on the validity of Poiseuille’s law. This law is valid under a number of assumptions, including rigid vessel walls, sufficient inlet length, stationary Newtonian flow, and excluding bends/bifurcations of the vessel. These parameters have an effect on the velocity, and therefore the shear stress, distribution in arteries. All but one of these parameters were investigated in an excellent series of studies by the group of Hoeks. They measured velocity profiles in the common carotid artery, and they showed that the velocity profiles follow the Womersley profiles reasonably well. This implies that, although momentary velocity and shear stress patterns may deviate from the steady flow values based on Poiseuille’s law, the time averaged values are approximated fairly well by the steady flow assumptions. The one parameter that was not studied in the straight common carotid arteries, is the effect of geometrical variation. Indeed, the presence of bends and branching points may lead to local differences in shear stress that can be large. As these sites are not the territory we are interested in, we still believe that the observations in this review are valid.

Anesthetics and exercise may have an effect on the WSS values in Tables
2.1 and 2.3. Therefore, we only included WSS values of the human studies that were measured in a conscious, resting state in a lying down position in order to keep the WSS values comparable between the different human studies. The type of anesthetics used in the animal experiments was also reported. However, the WSS values presented in this paper depend on the mobile state and anatomical position of the subjects and could deviate from the WSS values in different conditions (e.g. during exercise).

2.6 Conclusion

In summary, by comparing the data provided from literature we have shown that: (i) the assumption of a physiological WSS level of \( \sim 15 \text{ dyn/cm}^2 \) for all the straight vessels in the arterial tree is incorrect; (ii) WSS is not constant throughout the vascular tree; (iii) WSS varies between species; (iv) WSS is approximately inversely related to the vessel diameter. These data support a “r\(^2\) law” rather than Murray’s r\(^3\) law for larger vessels in the arterial tree.
Chapter 3

Effect of shear stress on vascular inflammation and plaque development


3.1 Abstract

This review describes evidence that shear stress acts through modulation of inflammation and by that process affects atherogenesis and plaque composition. In low shear stress regions antiatherogenic transcription factors are downregulated and pro-atherogenic transcription factors are upregulated. Consequently, inflammatory cells may home low shear stress regions more easily to the plaques because of increased expression of adhesion factors, a decreased rolling speed and an increased expression of chemokines, thereby changing the composition of the plaques into a more vulnerable phenotype. In contrast, in advanced plaque development vascular lumen decreases and shear stress
increases, especially upstream of the plaques. The predominant upstream location of lipids induces a prevalent upstream location of inflammatory cells leading to localized plaque rupture. Shear stress has been shown to play a role in plaque induction, plaque progression and plaque rupture. The mechanism for plaque induction seems to differ from the role of shear stress for plaque rupture, whereby the former mechanism is induced by low shear stress and the latter by high shear stress.

3.2 Introduction

Atherosclerosis is the disease with the highest mortality in the western world. Despite its large socio-economical impact, the underlying mechanisms are only partially known. It has been accepted for decades that atherosclerosis is a lipid-driven disease, despite the fact that risk factors related to lipid metabolism only partially explain atherogenesis. Furthermore, new therapies specially focused upon lipid metabolism only partially reduce plaque size. Recently two concepts - inflammation and blood flow/shear stress - have undergone a renaissance and gained a lot of interest as complementary explanations for plaque formation and these concepts will be the topic of the present manuscript [249, 83, 75, 223].

The role of inflammation became apparent from a series of mouse studies in which systematically parts of the immune system were knocked down, before the induction of atherosclerosis [249, 83, 75]. These studies identified inflammation as an independent mechanism contributing to plaque formation, and based upon these results and further studies atherosclerosis is considered a lipid driven inflammatory disease. The effect of blood flow in atherosclerosis is based upon the observation that plaques are not evenly distributed over the arterial system [223, 228, 34]. These predilection sites are at or near side branches, where blood flow is nonuniform, or at the lesser curvature of bends, where blood velocity is relatively low. The effect of blood flow on the vessel-wall is through shear stress, which alters the physiology of endothelial cells. Shear stress (τ in N/m² or Pa) arises from the friction between two virtual layers in a fluid, and is induced by the difference in movement of the two layers
3.3 SENSING MECHANISM

\( \frac{dv}{dr} \) in s\(^{-1}\); in case of a cylindrical tube) and the “roughness” or viscosity \( \eta \) in Pa\( \cdot \)s between these layers \( \tau = \frac{dv}{dr} \cdot \eta \). Shear stress also arises at the interplay between blood and the endothelial layer, where it induces a shearing deformation of the endothelial cells. This shearing deformation affects the phenotype of the endothelial cells and thereby the inflammatory component and plaque progression/composition.

This paper describes the interaction between shear stress and inflammation. We will first describe recent findings on the sensing mechanism of shear stress by the endothelium. Subsequently, we will examine pro-inflammatory pathways modulated by shear stress in endothelial cells, followed by the effect of shear stress on plaque progression and plaque composition. At the end we will discuss new findings related to longitudinal plaque heterogeneity.

### 3.3 The sensing mechanism of endothelial cells

Shear stress is directed in the plane of the endothelial cells, in contrast to the pressure, which acts perpendicular to the endothelial cells. Its magnitude is up to 5 Pa which is a fraction of the 10000 Pa of the pressure. Hence, endothelial cells are specifically equipped with a dedicated sensing mechanism to detect shear stress. A way to amplify shear stress is by shear deformation of specialized cellular mechanotransduction elements of the cytoskeleton. Amplification occurs through a parallel activation of parts of the cytoskeleton. Furthermore, the cytoskeleton is coupled to the cellular membrane in a distributed manner by elements that include integrins, cell-cell adhesion molecules and receptors. Conformational changes of the specific proteins of these connecting complexes lead to activation of intracellular signalling molecules, of which the large family of small GTPases (including Ras, Rho, Arf, Rab and Ran GTPases) are of importance, which then leads to transcriptional activation of target genes (see below).

In epithelial cells cytoskeletal deformation is amplified by protrusion of a primary cilium into the lumen. This has been described for epithelial cells in the renal, bile, and pancreatic ducts, as well as for epithelial cells of the embryonic organizing centre, which determine left-right handedness of the body plan.
CHAPTER 3. EFFECT OF SHEAR STRESS

Figure 3.1: Theoretical description of new information regarding shear stress sensing by the endothelial cells. Indicated are new developments of shear stress sensing by cilia, new information regarding the pathways involved. All details of the different components are described in the text.

[7, 151, 212]. The primary cilium is a microtubule-filled, membrane covered, extension from the basal body that protrudes from the cell surface and is connected to the microtubular part of the cytoskeleton through the microtubule organizing centre. By bending the cilium, fluid shear stress induces a torque that can amplify fluid forces up to 600 times [79]. Primary cilia have been demonstrated on endothelial cells [98, 258] especially in regions of low and disturbed blood flow [258], and with high incidence at atherogenic predilection sites (Fig. 3.1) [258]. Here, they most likely function to elevate the shear responsiveness of endothelial cells to prevent atherogenic activation.
3.4 The role of shear stress on pro-inflammatory pathways in endothelial cells

Endothelial cells respond to different shear stress fields with a different gene expression profile as identified with a variety of techniques including microarrays. These studies [34, 156, 202, 128] have aroused the interest of the identification of transcription factors that regulate shear stress related gene expression including NF-κB, AP-1, KLF2 and Nrf2.

A large body of work has revealed the importance of NF-κB and AP-1 in regulating vascular inflammation, cell viability and other fundamental cellular activities. Indeed, several pro-inflammatory genes including VCAM-1, E-selectin and IL-8 require binding of both NF-κB and AP-1 for transcriptional activation. Several groups have examined the effects of shear stress on NF-κB and MAP kinase signalling pathways. Acute induction of laminar shear stress (5 min to 1 h) is known to activate JNK [277, 159] and NF-κB [6, 121, 35] and trigger the expression of ICAM-1 [169, 175], VCAM-1 [73] and E-selectin in endothelial cells, thus facilitating leukocyte adhesion. In contrast, more prolonged laminar shear stress (6–24 h) induces pronounced anti-inflammatory effects in cultured endothelial cells, associated with inhibition of JNK and p38 MAP kinase signalling and alteration of NF-κB function [189]. Thus endothelial cells exposed to laminar shear stress become refractory to the effects of pro-inflammatory cytokines or endotoxin [277, 225, 224, 237].

Other groups have examined the relationship between complex flow patterns and NF-κB activity. High levels of NF-κB molecules have been detected in endothelial cells at sites of low shear in the aortic arch in mice and pigs [190] suggesting that these regions may be primed for enhanced NF-κB activation. Furthermore, Parhar et al. [187] observed that the RelA NF-κB sub-unit localizes constitutively to the nucleus of murine aortic endothelial cells at sites exposed to disturbed flow. This finding was supported by our group who observed that constitutive NF-κB transcriptional activity is elevated in low shear stress regions in vivo [189]. In addition, Gimbrone’s group [67] found that application of a pulse wave pattern identified at atherosusceptible sites of the human carotid artery bifurcation triggered nuclear localization...
of NF-κB in cultured endothelial cells whereas an “atheroresistant” pattern did not. Overall, these studies indicate that regions of the vasculature exposed to oscillating/or low shear stress may be susceptible to inflammation due to constitutive activation of NF-κB.

Recent studies have identified KLF2 and Nrf2 transcription factors as central regulators of physiological responses to shear stress. KLF2 is induced in endothelial cells exposed to prolonged laminar shear [125, 40, 41, 39, 188] where it induces more than 1000 transcripts that regulate diverse processes such as vascular tone, cell migration, angiogenesis and inflammation [125, 40]. Shear stress also activates Nrf2, a transcription factor that regulates redox levels by activation of numerous antioxidant genes including HO–1 and ferritin. Overexpression of these transcription factors in endothelial cells have revealed that both KLF2 and Nrf2 possess the capacity to modulate pro-inflammatory signalling pathways [221, 270]. KLF–2 can suppress NF-κB activation by sequestering coactivators of transcription and has also been reported to suppress constitutive activation of ATF–2 [221]. In contrast, it has been suggested that Nrf2 suppresses the induction of pro-inflammatory transcripts in endothelial cells by inhibiting activation of p38, but it does not target NF-κB. Thus KLF2 and Nrf2 have been proposed as potential mediators of the anti-inflammatory effects of shear stress.

3.5 Pro-atherogenic shear stress profiles produce different plaque phenotypes

Despite the evidence that shear stress modifies the inflammatory gene expression pattern of endothelial cells, no direct confirmation is present that shear stress directly modifies plaque progression [29, 116, 115]. In order to study this question, we developed a method for chronic induction of low shear and oscillatory shear stress regions in atheroprotective arterial segments in vivo (for a detailed description see legend of Fig. 3.2). We subsequently induced a high cholesterol environment and studied the interaction of high cholesterol levels with the pro-atherogenic shear stress profiles over time. At each time
3.5. PLAQUE PHENOTYPES

point, low shear stress was a much stronger atherogenic stimulus than oscillatory shear stress (Fig. 3.3). In addition, low shear stress induced a different plaque composition than oscillatory shear stress (Fig. 3.4). Plaques in the low shear region consisted of low vascular smooth muscle cells, large lipid pools, less collagen in the cap and accumulation of many macrophages (a condition termed vulnerable plaque phenotype in the literature), while oscillatory shear stress induced small plaques with a more stable phenotype.

One of the striking differences between the two plaque phenotypes was the abundance of inflammatory cells in the vulnerable plaques. As low shear stress increases the expression of adhesion factors through a complex interplay between shear stress responsive transcription factors (see above) and shear stress reduces the rolling speed of inflammatory cells over the endothelium, we focused on the question that low shear stress increases the uptake of inflammatory cells. To that end, we evaluated the homing mechanism of inflammatory cells. First of all, we determined that VCAM–1 and ICAM–1 were selectively upregulated in low shear stress regions. Next we determined the amount of rolling and firm adhesion of leukocytes in the low shear regions by intravital microscopy. Indeed, in the first pilot studies many cells adhered to the endothelium in low shear stress regions and not in high or oscillatory shear regions. Third, as arrest of inflammatory cells on the endothelium in the arterial system is caused by activated integrins, we determined the expression of chemokines in low shear stress induced plaques. The family of chemokines are known to activate integrins and induce arrest of inflammatory cells, even at high shear stress values. Out of a screen of 13 chemokines we found that Fractalkin was specifically upregulated in low shear stress induced vulnerable plaques. Furthermore, inhibition of Fractalkin reduced the uptake of macrophages to vulnerable plaques significantly, and changed the vulnerable plaque into a stable plaque. These experiments indicate that low shear stress increases the inflammatory component during atherogenesis and thereby changes plaques composition into a vulnerable phenotype.
Figure 3.2: Description of the method to induce atherogenic velocity profiles in vivo.  
(a) Based upon computational fluid dynamics, a geometry was selected to induce low, high and oscillatory shear stress regions in a straight arterial segment in vivo. A cast was manufactured of thermoplastic polyetherketon, consisting of two longitudinal halves of a cylinder with a cone shaped lumen (not shown). The upstream outer diameter is 500 μm (nonconstrictive) and gradually declines to 250 μm at the downstream side of the cast (approximately 70% inner diameter constriction) resulting in a shear stress range of approximately 300%. (b) The downstream area will be exposed to temporal oscillations in shear stress (oscillatory shear stress region as measured by velocity reversal. Panel (a) is modified with permission from Cheng et al. [25]. Atherosclerotic lesion size and vulnerability are determined by patterns of fluid shear stress. Circulation. Published by the American Heart Association, 2006.
3.5. **PLAQUE PHENOTYPES**

Figure 3.3: Plaque development studied in apoE mice over time after induction of low shear stress, and oscillatory shear stress regions. The mice were placed on a Western diet two weeks prior to cast placement to ensure steady state hypercholesteremia. In those conditions, both low shear stress and oscillatory shear stress induce atherosclerosis, but low shear stress appeared to be much stronger than oscillatory shear stress. Modified with permission from Cheng et al. [24]. Shear stress-induced changes in atherosclerotic plaque composition are modulated by chemokines. Journal of Clinical Investigation. Published by the American Society for Clinical Investigation; 2007. Grey indicates Control; white indicates low shear stress; black indicates oscillatory shear stress.
Figure 3.4: Plaque composition studied after 9 weeks of cast placement. This is at a time point when differences in plaque size between low and oscillatory shear stress regions were maximal (Fig. 3.3). (a) The different plaque components in the low shear stress region for a single animal. The plaques consisted clearly of many macrophages, large lipid core, few smooth muscle cells and a thin fibrous cap. This is defined in human pathology as a thin cap fibroadenoma (TCFA). (b) The induction of vulnerable plaques is reproducible in a large series of animals. Furthermore the differences in plaque composition between low shear and oscillatory shear stress regions are clearly indicated. Modified with permission from Cheng et al. [25]. Atherosclerotic lesion size and vulnerability are determined by patterns of fluid shear stress. Circulation. Published by the American Heart Association, 2006.
3.6 The role of shear stress in plaque heterogeneity

While cross sectional plaque composition has been characterized extensively, longitudinal plaque composition has received little attention. Some human pathology studies of carotid specimen found a preferential location of plaque rupture upstream of the narrowest location of the plaque. This was associated with an upstream location of macrophages in the vessel wall and thrombus at the sub endothelium [42, 146, 274]. Because such a spatial organization might be explained by local variations in shear stress, we determined the role of shear stress in longitudinal plaque formation, by combining a newly developed three-dimensional histology technique with computational fluid dynamics. The experiments indicate that a proximal accumulation of oxidized LDL was associated with the accumulation of foamy macrophages, MMPs and high shear stress regions. Selection on basis of low/high shear stress indicated a predominant accumulation of macrophages in the high shear stress regions (Fig. 3.5) [220]. As this was the first finding of an unbeneficial effect of high shear stress during atherosclerosis, we were reluctant to publish these findings. A recent theoretical study, however, pointed to a possible deleterious effect of high shear stress over plaque endothelium [26]. Hence we performed a second analysis in which we focused upon different reasons for the upstream plaque composition, by comparing near wall resident time with local shear stress values (Fig. 3.6; unpublished data) [220, 144]. From this first analysis it seems that the delivery of lipid particles or an increased permeability might be more important to explain the upstream location of these plaques than the inflammation.

3.7 Conclusion

This manuscript describes novel experimental findings in the relationship between blood flow, inflammation and atherogenesis. It is clear now that low or oscillatory shear stress is pro-atherogenic and induces plaques through an inflammatory mechanism. Furthermore, high shear stress may induce vulnerable plaques by the production of reactive oxygen species and oxidized LDL
Figure 3.5: Plaque composition studied in atherosclerotic rabbits after eight weeks of atherogenic diet. In this animal model a new three dimensional histology method was developed and validated [220]. This method indicated a large plaque heterogeneity in these animals similar to those found in patients. Three-dimensional calculations of shear stress and coupling of shear stress to the 3-D histology enabled to identify regions with high shear stress (1.05 times average shear stress) and low shear stress (0.95 times average shear stress). It is indicated that high shear stress identifies regions with inflammation, while low shear stress regions identify stable regions. Oscillatory shear stress regions were not defined in these experiments.
Figure 3.6: The role of near wall residence time and shear stress in lipid accumulation. Example of the role of near wall resident time (NWRT) (a), according to Longest et al. [144] in the accumulation of lipid particles (b). For comparison a shear stress profile is depicted (c). Lipid accumulation was quantified with a recently developed 3-D histology technique [220]. NWRT was calculated according to Longest et al. [144]. It can be seen from inspection that a closer association is apparent between NWRT and lipid accumulation than with shear stress.
upstream of plaques. The mechanism is unknown but may relate to the activation of transcription factors in endothelial cells.
Chapter 4

Gelatinolytic activity in atherosclerotic plaques is highly localized and is associated with both macrophages and smooth muscle cells in vivo

CHAPTER 4. GELATINOLYTIC ACTIVITY

4.1 Abstract

Atherosclerosis is considered an inflammatory disease. Recent studies provided evidence for a predominant upstream location of plaque inflammation. The present study introduces a novel technique that evaluates the underlying mechanism of this spatial organization. In hypercholesterolemic rabbits, atherosclerosis of the infrarenal aorta was induced by a combination of endothelial denudation and a high-cholesterol diet (2% cholesterol for 2 months). At the time of death, aortic vessel segments were dissected and reconstructed with a new technique that preserved the original intravascular ultrasound-derived lumen geometry. This enabled us to study the spatial relation of histological markers like macrophages, smooth muscle cells, lipids, gelatinolytic activity, and oxidized low-density lipoprotein. Results showed a predominant upstream localization of macrophages and gelatinase activity. Colocalization studies indicated that gelatinase activity was associated with macrophages and smooth muscle cells. Further analysis revealed that this was caused by subsets of smooth muscle cells and macrophages, which were associated with oxidized low-density lipoprotein accumulation. Upstream localization of a vulnerable plaque phenotype is probably due to an accumulation of oxidized low-density lipoprotein, which activates/induces subsets of smooth muscle cells and macrophages to gelatinase production.

4.2 Introduction

Atherosclerosis is nowadays considered a lipid-driven inflammatory disease. Inflammation has been associated with plaque progression, plaque rupture, thrombosis, and subsequent myocardial infarction [32, 213, 259, 176]. Several studies indicated that plaque inflammation is unevenly distributed over its length, with a predominant upstream presence of inflammatory cells and/or a location in the plaque shoulders [259, 48, 146, 42, 251, 216]. These observations indicate a spatially oriented mechanism, which to date has not received much attention. To study the underlying mechanism of such a highly spatially localizing mechanism, there is a need for a precise, quantitative technique, en-
4.2. INTRODUCTION

abling the study of plaque heterogeneity in experimental atherosclerosis. The first aim of the present study was to present a three-dimensional histological technique permitting the study of plaque heterogeneity in more detail than before.

Both plaque progression and plaque rupture have been associated with a larger infiltration of macrophages, irrespective of the underlying plaque morphology [260, 191]. Activated macrophages produce numerous factors, including matrix metalloproteinases (MMP) [61]. MMP belong to a family of zinc-activated proteases modulating the extracellular matrix in the vascular wall [203, 102, 178]. The activity of some family members induces weak spots in the extracellular matrix, thereby introducing a condition sensitive to plaque rupture [61, 62, 138, 139]. Only a relatively small fraction of macrophages can be measured in the entire plaque [192] and therefore, the process of plaque weakening may either be very localized, or other cell types may be involved in the process. Indeed, several studies have indicated that, besides macrophages, endothelial cells and smooth muscle cells (SMCs) may produce MMP when brought into an inflammatory, atherogenic environment [28, 217, 276].

Recently, a new technique has been introduced to measure gelatinolytic activity in histological cross sections with a high spatial resolution [165, 278]. We have adopted this method for vascular tissue and incorporated it into three-dimensional histological reconstructions. Our second aim was to evaluate, by combining above techniques, whether MMP are active very locally or that multiple cell types are involved in experimental atherosclerosis in vivo.

Low-density lipoprotein (LDL) accumulates in the vessel wall, where it may become oxidized (oxLDL). OxLDL is known to be involved in many processes related to atherosclerosis, including stimulation of macrophage infiltration and foam cell formation, stimulation of vascular smooth muscle cell migration and proliferation, and endothelial cell apoptosis [158, 4, 37, 94, 180]. Recent studies indicated that oxLDL is associated with plaque instability [180]. This observation might be explained by the modulation of activation of some MMP family members by oxLDL [178]. Most of the oxLDL-related studies, however, have been conducted on isolated cells in vitro, which are devoid of the complex environment of the atherosclerotic vessel wall, thereby identifying the
need to study the role of oxLDL in vivo. It is presently unknown whether oxLDL is distributed heterogeneously within the plaque or if it is associated locally with gelatinolytic activity in plaques in vivo. Therefore, the third aim of the present study was to evaluate whether oxLDL might be the cause of the spatially restricted accumulation of activated macrophages in plaques in vivo.

4.3 Methods

4.3.1 Instrumentation

Three days before baseline measurements, male New Zealand white rabbits (n = 8; weight, 3.6 ± 0.2 kg; Harlan Netherlands BV, Horst, The Netherlands) were fed a high (2%) cholesterol diet for a 2-month period. At experimentation, rabbits were anesthetized with an intramuscular injection of ketamine (Sanaket 10%, 25 mg/kg, Anisane BV, Raamsdonkveer, The Netherlands) and a subcutaneous injection of medetomidine (Domitor, 0.5 mg/kg, Orion, Espoo, Finland). The marginal ear artery was cannulated for arterial pressure measurement with a fluid-filled catheter (Amatek, US Gauge, Feasterville, USA) and for arterial blood withdrawal. A 4 French guiding catheter was advanced from the left femoral artery up to the level of the renal artery ostium. After angiography, a 40 MHz intravascular ultrasound catheter (CVUS, Boston Scientific, Maastricht, The Netherlands) was advanced through the guiding catheter and located 1 cm upstream of the lower of the two renal arteries. Subsequently, a motorized pullback was performed at a speed of 0.5 mm/s spanning an arterial segment of 7 cm, which was recorded on high-resolution videotapes. Endothelial denudation was performed within this predefined segment by twisting and pulling back an inflated 3 French Fogarty balloon (Applied Cardiac Systems, Inc. Laguna Hills, USA) over a length of 5 cm.

4.3.2 Follow-up

After 8 weeks of follow-up, rabbits were anesthetized as described above. Next, the right femoral artery was dissected for the introduction of a 4 French sheath. An angiographic overview of the infrarenal aorta was performed, and
radiopaque markers were located subcutaneously to indicate the previously
denuded region. Subsequently, the intravascular ultrasound pullback was re-
peated at the location of the previously denuded segment. Then, the abdomen
was opened and a longitudinal marker and two transverse markers were placed
externally on the aortic vessel wall, the lumen was filled with OCT compound
(Tissue-Tek, Sakura Finetek Inc., Torrance, USA); and the arterial segment
of interest was dissected, collected, and snap-frozen in liquid nitrogen. The
distance between both transverse markers was measured before and after exci-
sion and was used to calculate a correction factor for shrinkage resulting from
arterial elasticity.

All experiments were performed in accordance with institutional regula-
tions and the “Guide for the Care and Use of Laboratory Animals” published
by the US as approved by the Council of the American Physiological Society.

4.3.3 Plasma lipids

Lipid profiles were measured according to well-established enzymatic calori-
metric methods (Roche Diagnostics, Pleasanton, USA). Cholesterol levels were
determined at the 8-week follow-up in the present hypercholesterolemic group
and in a normocholesterolemic control group, which consisted of sex- and age-
matched rabbits.

4.3.4 Intravascular ultrasound

The high-resolution videotaped intravascular ultrasound data were digitized
at intervals of 0.5 mm with a resolution of 800 × 600 pixels and stored on
a standard personal computer. Next, the lumen and the acoustic interface
between media and the adventitial layer were traced semiautomatically by a
well-validated software package [115]; then the lumen area and media bounded
area were calculated from these contours. The difference between these two
was defined as the wall area.
4.3.5 Tissue harvesting and histological analysis

From the excised 5 cm aortic segment, tissue blocks were prepared every 2 mm with a cutting device developed in-house. This resulted in approximately 20 to 25 blocks per blood vessel, depending on the extent of shrinkage. Immunohistochemistry was performed on 5 μm cryosections obtained from the middle of the 2 mm tissue blocks. Sections were stained for macrophages (RAM–11, Dako Diagnostics BV, Glostrup, Denmark), smooth muscle cells (α-actin, Dako Diagnostics BV, Glostrup, Denmark) and for oxidized LDL (epitope against apolipoprotein B100 [91]) and lipids (Oil Red O, Sigma, Rotterdam, The Netherlands). After staining, sections were digitized with a high-resolution charge-coupled camera (Zeiss Axiocam HR, Jena, Germany) and quantitatively analyzed with commercial imageanalysis software (Clemex Technologies Inc., Longueuil, Canada).

4.3.6 In situ zymography

Gelatinolytic activity was demonstrated in unfixed cryosections with DQ-gelatin as a substrate (EnzChek, Molecular Probes, Eugene, USA). Sections were air-dried for 60 minutes, while during that period DQ-gelatin was dissolved in a concentration of 1 mg/ml in Milli-Q and then diluted 1:10 in 1 % (wt/vol) low-gelling-temperature agarose (Sigma, Rotterdam, The Netherlands) in phosphate-buffered saline. Subsequently, 25 μl of this mixture was placed on each cryosection and incubated for two hours at room temperature following placement of a cover slip. Fluorescence was detected with a confocal microscope (Zeiss, LSM 510 Meta, Jena, Germany) using an argon laser for excitation at 488 nm and emission collection at 512 nm to 542 nm, applying appropriate filters, background and autofluorescence correction. Detailed testing for specificity of the in situ zymography was described in literature [165].
4.3. METHODS

4.3.7 Three-dimensional histology

The three-dimensional reconstruction of histological cross sections consisted of the following steps: (1) acquisition of quantitative image analysis data from histological sections; (2) rotation of sections on the basis of an externally placed longitudinal marker, which was still present after histological processing; (3) stacking of image data and correction for shrinkage in the longitudinal and radial directions; and (4) rotation of the entire stack of data with respect to the renal artery and mapping of histology on three-dimensional intravascular ultrasound reconstructions.

4.3.8 Data and statistical analyses

As a first approach, we averaged each histological variable (plaque area, macrophages, SMCs, lipids, gelatinolytic activity, and oxLDL) per cross section and displayed the longitudinal heterogeneity per blood vessel. Subsequently, plaque areas of each blood vessel were shifted so that maximal plaque areas of all vessels were aligned. The shift for each individual blood vessel was applied to all its histological variables, which eventually were spatially averaged. To study spatial differences in relation to the histological variables described above and in relation to plaque area, spatial averages were calculated upstream and downstream of the maximum plaque area for each individual animal. Differences between these averages were then evaluated by an exact Wilcoxon signed rank test.

To further explore underlying mechanisms, we performed colocalization studies of the reordered data and linear regression analyses. Colocalization was defined as the existence of two variables in the same radial segment. The colocalized pixels per histological variable were counted and divided by the number of total elements in the segment of interest. Differences between colocalization of macrophage- gelatinolytic activity and SMC-gelatinolytic activity and of macrophage-oxLDL and SMC-oxLDL were tested. In addition, percentage macrophages (area/area) and percentage SMC (area/area) were calculated, and differences in colocalization were tested as described above.

The authors of this article had full access to all of the data in the study
and take responsibility for the integrity of the data and the accuracy of the data analysis.

4.4 Results

4.4.1 Animal characteristics

Systolic, diastolic, and mean arterial blood pressures were 88 ± 1 mmHg, 60 ± 3 mmHg, and 69 ± 1 mmHg, respectively. These values remained unchanged during the experimental procedures. The 2% cholesterol-rich diet significantly increased total plasma cholesterol (from 1.4 ± 0.2 mmol/l to 33.4 ± 15.7 mmol/l), high density lipoprotein (from 0.7 ± 0.1 mmol/l to 14.5 ± 2.7 mmol/l), and LDL (from 0.2 ± 0.1 mmol/l to 30.9 ± 10.8 mmol/l). Triglyceride levels remained unchanged.

4.4.2 Longitudinal plaque heterogeneity displays an upstream location of inflammatory cells, gelatinolytic activity, and a vulnerable phenotype

In plaques generated in the aorta of a representative hypercholesterolemic rabbit, longitudinal plaque heterogeneity of lipid particles, macrophages, and SMCs was clearly present (Fig. 4.1). Surprisingly, a similar upstream accumulation of gelatinolytic activity and oxLDL was measured (Fig. 4.2). When the data were shifted and spatially averaged (see above), the predominant accumulation of macrophages was demonstrated for all animals (Fig. 4.3). Distribution of each variable with plaque area revealed a higher accumulation of macrophages upstream (11.6%) compared with downstream (7.9%; \( p = 0.016 \)) of the plaque. In contrast, a more diffuse distribution of vascular SMCs (26.6% versus 27.1%) and lipids (2.9% versus 2.5%) was measured. As a consequence, a local vulnerability index (modified from Shiomi et al. [226]) displayed a maximum upstream of maximal plaque area compared with downstream (3.2 versus 2.8; \( p = 0.039 \)) (Fig. 4.4). This higher vulnerability upstream is similar to observations in the carotid arteries of patients [42].
Figure 4.1: Vascular lumen reconstructions (three-dimensional) of a rabbit aorta obtained by combining intravascular ultrasound with histological data. Top, Histology projected onto the 3-D intravascular ultrasound lumen reconstruction; bottom, the same data on a flat plane. From left to right are displayed lipid distribution, macrophage distribution, SMC distribution, and intravascular ultrasound-derived wall thickness distribution. Flow direction is from bottom to top. Note the predominant upstream location of inflammatory markers.
Figure 4.2: Reconstructions (3-D) of the same aorta as presented in Figure 4.1. Presented are histological markers projected on a flat plane only. From left to right are displayed oxLDL distribution, macrophage distribution, distribution of metalloproteinase gelatinolytic activity, and SMC distribution. Flow direction is from bottom to top. Note the predominant upstream location of all components, similar to that in Figure 4.1.
Figure 4.3: Longitudinal heterogeneity of plaque area (A) and macrophage (B), SMC (C), and lipid (D) distribution. Displayed in each figure is the longitudinal plaque area distribution as averaged for all rabbits. Note that all variables are located predominantly upstream of the plaque.
Figure 4.4: Local distribution of the vulnerability index (VI-index) adapted from Shiomi et al. [226] displayed as a function of longitudinal location in a series of atherosclerotic rabbit aortas (n = 10). A high value signifies a more vulnerable phenotype; a low value, a more stable phenotype. The highest values are displayed upstream of the plaque.
4.4.3 In vivo gelatinolytic activity is colocalized with both SMCs and macrophages

Inspection of histological cross sections revealed that both SMCs and macrophages were associated with gelatinolytic activity (Fig. 4.5). Quantitative analysis confirmed these findings because the total amount of gelatinolytic activity associated with SMCs and macrophages was $85 \pm 10\%$, distributed into an equal SMC-gelatinase fraction of $42 \pm 7\%$ and macrophage-gelatinase fraction of $43 \pm 9\%$ (Fig. 4.6). Although this pointed to a similar contribution of SMC and macrophages to overall gelatinolytic activity, these fractions represent only a minor fraction of overall macrophage ($23 \pm 7\%$) and SMC ($22 \pm 7\%$) content, which were similar for both cell types. When we reexamined the cross section containing gelatinase-producing vascular SMCs and macrophages, we identified foamy SMCs and foamy macrophages (Fig. 4.5, right).

4.4.4 OxLDL identifies subsets of gelatinase-producing SMCs and macrophages

Inspection of histological cross sections revealed that both SMCs and macrophages were associated with oxLDL accumulation (Fig. 4.5). These single observations were confirmed by quantitative analysis; OxLDL almost entirely ($98 \pm 19\%$) colocalizes with both macrophages and SMCs, with an even distribution between both cell types (SMC: $54 \pm 9\%$ and macrophages: $44 \pm 7\%$) (Fig. 4.6). These colocalization studies identified similar subsets of SMCs ($28 \pm 4\%$) and macrophages ($28 \pm 8\%$) that were spatially associated with oxLDL.

4.5 Discussion

Atherosclerosis is considered a lipid-driven inflammatory disease. Several studies indicated a strong heterogeneity of the inflammatory process [191, 82, 231], consisting of an accumulation of inflammatory cells in the shoulders and/or
Figure 4.5: Nearly consecutive cross sections of a single atherosclerotic rabbit aorta at low magnification showing macrophage accumulation (A), gelatinolytic activity (B; dashed white line indicates the tunica media), and SMC distribution (C). Note that near the cap the gelatinolytic activity is high. At this location, both SMCs and macrophages are identified. A $100 \times$ magnification of nearly consecutive cross sections indicates that foam cells (Oil Red O staining; E) belong to the macrophage fraction (RAM–11; D) and to the SMC fraction ($\alpha$-actin; F). Dashed black lines indicate an area where foamy SMCs are present.
4.5. DISCUSSION

Figure 4.6: Pie diagrams of gelatinolytic activity (left) and oxLDL accumulation distribution with respect to SMCs and macrophages. The distribution is calculated as a percentage of SMC or macrophage colocalized with gelatinase activity or oxLDL divided by total gelatinase or total oxLDL, respectively. Unknown refers to the amount of either gelatinolytic activity or oxLDL accumulation not associated with either SMCs or macrophages.

upstream of the plaque \[42\]. Not much attention has been paid to this heterogeneity in experimental atherosclerosis, probably because of the lack of a suitable technique to study such phenomena in animals.

With a new 3-D reconstruction technique for histology, we were able to show that the inflammatory component in the atherosclerotic plaque was spatially located upstream of the maximal cross-sectional plaque area, similar to that reported for human conditions \[42\]. In addition, we demonstrated that gelatinolytic activities also were spatially confined to the same region and were associated with macrophages and SMCs. This tight spatial localization of gelatinases enabled us to study colocalization with other cell types. We found that not only macrophages but also SMCs contributed significantly to gelatinolytic activity in vivo. Previous studies indicated that SMCs in culture produce pro-MMP–2 after stimulation and, at the same time, reduce their production of tissue inhibitor of metalloproteinase, leading to a higher MMP–2 activity \[253\]. Furthermore, macrophages stimulated with oxLDL decrease their tissue inhibitor of metalloproteinase–1 release and increase pro-MMP–9 release, leading to MMP–9 activation \[276\]. The imbalance between tissue inhibitor of metalloproteinase and pro-MMP release may explain the high gelatinase activity found in the present study. Thus, from the present findings,
one may postulate that local weak spots in the extracellular matrix occur up-
stream of the plaque because of a highly localized gelatinolytic concentration
produced by both (foamy) macrophages and (foamy) SMCs.

The reason that such a localized accumulation of cells in atherosclerotic plaques occurs is currently unknown, but several lines of evidence indicate that oxLDL is involved. OxLDL has been measured in (vulnerable) plaques, where it modulates macrophage accumulation and foam cell formation through the expression of adhesion factors, secretion of monocyte chemotactic protein–1, migration of SMCs, and apoptosis of SMCs and endothelial cells [37, 94, 180, 262]. Furthermore, several studies identified a modulating effect of oxLDL on MMP activation by macrophages and SMCs in vitro [103, 167, 86, 5, 93]. Therefore, we tested whether oxLDL colocalized with macrophages and SMCs. Only 25% of either cell type was associated with oxLDL, but these two cell types accounted for all oxLDL colocalization and all gelatinolytic activity in our plaques. This indicates that the total oxLDL is taken up by SMCs and macrophages in approximately similar amounts but that this uptake is performed by subsets of both cell types.

Highly evolutionary preserved subsets of monocytes have been identified in the blood of humans and mice [127, 236], which results in different macrophage phenotypes as maturation occurs [236, 45]. These circulating subsets express different chemokine receptors [236, 142] and different scavenger receptors [236, 45]. These studies may offer an explanation for the finding of a subset of macrophages with a preferential location upstream of the plaque, combined with a particular predominance of foam cell differentiation.

Subsets of SMCs may change into foam cells and therefore their role in atherogenesis may have been underestimated [142, 3]. It has been demonstrated that discrete clones of SMCs exist in human vessels, which differentially accumulate cholesteryl esters when exposed to oxidized lipoproteins [3, 134]. Once turned into foamy SMC, they start to produce cytokines and express chemokine receptors [148], providing an explanation for their tight colocalization within the plaque. The fact that foamy SMCs and foamy macrophages are located in similar vessel segments might be explained by the evidence that macrophages secrete factors enhancing uptake of cholesteryl ester by vascular
SMCs [266]. To the best of our knowledge, the association of foamy SMC subsets, foamy macrophage subsets, and gelatinolytic activity in atherosclerotic plaques in vivo has not been described before.

4.6 Conclusion

A specific spatial colocalization of macrophages, lipids, and SMCs was demonstrated upstream of the plaque, similar to that found in patients with proven upstream plaque ruptures. This upstream plaque composition is characterized by an accumulation of (subsets of) macrophages and SMCs, oxLDL, and gelatinolytic activity. We hypothesize that activation of these subsets by oxLDL induces gelatinolytic activity, followed by breakdown of the extracellular matrix and subsequent weakening of the plaque. The unexpected, important role of a subset of SMCs in this process warrants further studies.
Chapter 5

Diameter changes of atherosclerotic rabbit aorta after vascular shunting measured by magnetic resonance imaging

Frank Helderman, Timo Baks, Piotr A. Wielopolski, Luc C. A. van Damme, Thijs van Aken, Antonius F. W. van der Steen, and Rob Krams. This chapter is based on a publication in preparation.

5.1 Abstract

Healthy blood vessels adjust their luminal diameter by keeping mean wall shear stress constant. It has been proposed that this mechanism is also of importance in remodeling of atherosclerotic vessels, but data on this topic are sparse and contra dictionary. We increased blood flow in five atherosclerotic
rabbits and recorded time series of the aortic luminal diameter by magnetic resonance imaging. The diameter increased on average $15 \pm 5.4\%$ over the period of twenty days. The experiments demonstrated that thin walls remodel faster than thick walls. Wall shear stress in the atherosclerotic aortas did not return to its reference level.

5.2 Introduction

Blood vessels possess the capacity to adjust their diameter to meet flow demands of the organs they perfuse [140]. This is of importance during development from fetus to adult, during exercise and after surgical intervention. Large arteries cannot directly sense the flow, instead, the endothelial layer is detecting the shear stress by an as yet unknown mechanism [33]. As a consequence, luminal diameter is adapted in such ways that mean wall shear stress is kept within limits [69, 104, 71].

In hypercholesteremic conditions, lipids accumulate in the inner wall and affect the endothelial layer leading to endothelial dysfunction. The endothelial layer becomes less sensitive to vasoactive stimuli, expresses more adhesion factors and produces pro-atherogenic signals to the vessel wall [47, 9]. In further advanced stages of disease, the presence of neointima within atherosclerotic vessels will increase diffusion distances of signaling molecules and/or changes the local wall stress in the smooth muscle layer disturbing another mechanism involved in outward remodeling. As a consequence, when atherosclerotic lesion occupies forty percent of the internal elastic lamina area, outward remodeling seems hampered and lumen narrowing is initiated [70]. This conversion from outward to inward remodeling is an essential switch in vessel adaptation. Hence, it is interesting to understand the mechanism underlying remodeling in atherosclerotic vessels.

During the development of atherosclerosis, many processes are coupled and depend on each other. A simulation model can offer great help for understanding the individual processes and the role of atherosclerosis therein. Several simulation models describing vascular remodeling have been presented in the literature [239, 204, 264, 2]. One of the first models used finite elas-
ticity theory to compute aortic growth, which depended linearly on smooth
muscle fiber stress and on the shear stress due to blood flow on the endothe-
lium [239]. Rodriquez et al. presented more advanced finite element models of
arteries with realistic three-dimensional geometries [204]. Also an anisotropic
model for tissue growth and remodeling during early development of cerebral
aneurysms has been developed [264]. Further refinement included addition of
particular features of arteries such as their multilayered structure and residual
stresses [2]. Despite the fact that these models have reached a high level of
sophistication, they describe remodeling by assuming shear or wall stress nor-
malization, a feature that is affected during development of atherosclerosis, as
indicated above.

Currently, quantitative measurements that enable simulation models to be
tested is lacking. Hence, we will describe quantitative experiments performed
in an atherosclerotic animal model that enables to study vascular remodeling
in disease.

5.3 Methods

5.3.1 Animals

For the atherosclerotic animal model we used five New Zealand white rabbits
(Harlan Netherlands BV, Horst, The Netherlands) of twenty months of age,
weighting three to four kilograms.

Atherosclerosis was induced by increasing lipid accumulation. To that end,
the animals were fed a two percent cholesterol diet for two months. During
the first week the amount of cholesterol was gradually increased to acclima-
tize the animals to the diet. After this period of habituation the endothelial
layer was removed to increase the permeability of the wall to lipids. At the
day of experimentation, animals were anesthetized with an intramuscular in-
jection of Ketamine (Sanaket 10\%, 25 mg/kg, Anisane BV, Raamsdonkveer,
The Netherlands) and a subcutaneous injection of Medetomidine (Domitor,
0.5 mg/kg, Orion, Esoo, Finland). The denudation was done by twisting and
pulling back an inflated three French Fogarty balloon (Applied Cardiac Sys-
tems, Inc. Laguna Hills, USA) over a distance of fifty millimeter between the renal arteries and the aortic bifurcation for at least three times.

We have shown before that after two months the endothelial layer had grown back over the entire surface area [271, 43, 44]. As the two percent cholesterol diet led to discomfort of animals after two months, the diet was further continued on a one percent cholesterol level for a period of three weeks.

Flow was increased by establishment of a side-to-side anastomosis. The animals were anesthetized applying the protocol described in section 5.3.1. They were attached to the operating table and laid down on a heating pad, to compensate for a fall in temperature due to the anesthetics. A digital thermometer was placed rectally to measure the temperature constantly. The animals received extra oxygen through an O$_2$-mask (300 ml/min), which was monitored every thirty minutes. Meanwhile the groin was exposed by a longitudinal incision and a side-to-side anastomosis between the femoral artery and vein was created, applying standard surgical procedures. During the postoperative period, the animals received Aspegic to prevent blood clotting, Temgesic 0.6 mg/kg as a pain reliever and they were given subcutaneously a mixture of electrolytes and ten percent glucose to help recovery.

All experiments were performed in accordance with institutional regulations and the Guide for the Care and Use of Laboratory Animals as approved by the Council of the American Physiological Society.

5.3.2 Measurements

The animals were anesthetized as described in section 5.3.1. Next, the bladder was emptied by gently pushing the abdomen for improvement of the MRI quality. The rabbit aorta was monitored with a 3 T MR scanner of General Electric Medical Systems using an eight-channel brain coil. Consequently, we were able to obtain images of the cross sectional area of the aorta with an in-plane pixel spacing of 156 $\mu$m. The geometry of the aorta was captured by taking three-millimeter slices over the whole length of the infra-renal aorta applying a protocol with fat saturation. Typical MRI parameters were: 90$^\circ$ flip angle, 80 mm $\times$ 80 mm field of view, 512 $\times$ 320 matrix size, 11.7 ms echo time,
5.3. METHODS

5500 ms repetition time. The acquisition plane was always perpendicular to the axial direction of the aorta. The velocity profile of blood flow through the aorta was measured just downstream of the renal arteries with a phase contrast velocity mapping protocol. Acquisition parameters were: 60 cm/s velocity encoding at baseline, 150 cm/s at follow up, 20° flip angle, 80 mm × 80 mm field of view, 256 × 256 matrix size, 4.2 ms echo time, 17 ms repetition time. This ECG-gated flow quantification in the aorta produced velocity profiles in twenty phases over a cardiac cycle. During the measurements the ears of the rabbit were protected against noise and the rabbit was given extra oxygen (900 ml/min). After the procedure, a mixture of electrolyte and ten percent glucose was injected subcutaneously to increase recovery.

5.3.3 Calculations

Wall shear stress was calculated with the finite element package SEPRAN (Sepra, Delft, The Netherlands). For the finite element method we had to create a mesh in the shape of the vessel geometry. This geometry was obtained by manually drawing the inner and outer contours of the vessel wall in MRI-images with Brutus 1.21 (Brutus, The Netherlands). The mesh had sixty-one cross sections in axial direction and the boundary between lumen and wall was presented by sixteen circumferential points. The time average of the MRI-derived velocity profiles was used as a boundary condition. Further boundary conditions for the Navier-Stokes solver included no slip at the vessel wall and a constant pressure gradient at the exit of the mesh. Furthermore, blood was treated as a Newtonian fluid with a viscosity of 0.003 Pa·s. Shear stress was taken as the product of shear rate and the viscosity.

5.3.4 Study design

For each animal a baseline measurement was performed, consisting of MRI based aortic geometry and velocity profile. The next day a side-to-side anastomosis between the femoral artery and vein was established. This enabled blood to flow directly from the high-pressure artery to the low-pressure vein with very low resistance. Consequently, the cardiac output was increased,
Figure 5.1: The lumen boundary of an aorta is displayed for a single rabbit, captured during baseline (A) and follow up (B-D) measurements. The colours indicate the wall shear stress (N/m²). At baseline, shear stress was at its reference level. After vascular shunting wall shear stress was elevated severally (B). The diameter increased but wall shear stress remained high (C and D).

raising flow and shear stress in the rabbit aorta (Fig. 5.1). Follow up measurements were performed at two, fourteen and twenty days after the side-to-side anastomosis was established.

5.3.5 Analysis and statistical methods

There were four variables studied in detail in this study: wall thickness, luminal diameter, mean wall shear stress and aortic flow. Local wall thickness was calculated as the distance in radial direction between lumen boundary and
outer boundary. Diameter was the distance between two facing points on the lumen boundary. Wall shear stress represented the local shear stress between aortic lumen and vessel wall. Flow was always the time averaged flow during a measurement.

**Baseline data**

Baseline data of the wall thickness, diameter and shear stress are presented as average with the standard deviation (SD).

**Changes in variables**

Due to vascular shunting the variables of interest were expected to change. We compared for each animal the flow $Q$ at follow up with the flow at baseline $Q_{\text{baseline}}$. We calculated the relative change in flow $\Delta Q$ for all time points with the following equation.

$$\Delta Q = 100 \cdot \left( \frac{Q}{Q_{\text{baseline}}} - 1 \right)$$

Likewise, we calculated for each animal the relative change in wall thickness, diameter and shear stress. Though, for these variables, the values were averaged over the whole aorta in advance. Changes in the variables were averaged over the animals and are presented with the standard error of the mean (SEM) and if necessarily with a 95% confidence interval (CI).

It was expected that a thin wall will adapt the diameter faster. To confirm this prediction experimentally, variables were averaged per cross section. Then, correlation between wall thickness and diameter change could be calculated for each animal. Some care had to be taken for combining the results of all animals. First, for each animal, the data per cross section were corrected by their subject-specific mean value. Then, the corrected data was combined into a single file, and the relation between diameter change and wall thickness was evaluated by linear regression.
Table 5.1: Baseline data (mean ± SD).

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Wall thickness (mm)</th>
<th>Luminal diameter (mm)</th>
<th>Wall shear stress (N/m²)</th>
<th>Flow (l/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.29 ± 0.05</td>
<td>3.00 ± 0.24</td>
<td>0.70 ± 0.19</td>
<td>0.040</td>
</tr>
<tr>
<td>2</td>
<td>0.32 ± 0.04</td>
<td>3.10 ± 0.21</td>
<td>0.94 ± 0.22</td>
<td>0.058</td>
</tr>
<tr>
<td>3</td>
<td>0.34 ± 0.05</td>
<td>3.00 ± 0.18</td>
<td>0.82 ± 0.18</td>
<td>0.046</td>
</tr>
<tr>
<td>4</td>
<td>0.43 ± 0.09</td>
<td>3.20 ± 0.14</td>
<td>1.06 ± 0.20</td>
<td>0.073</td>
</tr>
<tr>
<td>5</td>
<td>0.44 ± 0.07</td>
<td>3.10 ± 0.17</td>
<td>0.77 ± 0.14</td>
<td>0.045</td>
</tr>
</tbody>
</table>

5.4 Results

Care was taken to perform measurements as scheduled. One follow up scheduled at day fourteen was performed at day six. This measurement was not included for calculation of changes in variables. As a consequence, there were five measurements during baseline and the first follow up, four measurements at 14 days and five at 21 days.

5.4.1 Baseline data

At baseline the hemodynamic conditions in the aorta were reasonably comparable among the rabbits (Table 5.1). The mean aortic wall thickness varied between rabbits. Moreover some aortic walls displayed more variation in wall thickness (i.e. a larger standard deviation) than other aortas. There was hardly any difference in diameter. The same was expected for shear stress. However, in our study wall shear stress varied between rabbits (Table 5.1).

5.4.2 Changes in variables

At day two, the averaged flow had increased 221% compared with baseline (95% CI of 68–373%). There was no difference in flow between follow up measurements (Fig. 5.2A). Wall thickness did not change in our study (Fig. 5.2B). The 95% CI of change in wall thickness was -23% to 14% at day two, -10% to 2% at day fourteen and -23% to 3% at day twenty. The diameter increased af-
ter vascular shunting (Fig. 5.2C). At day fourteen the diameter was larger than at baseline and day two (16% versus 5%, \( p < 0.05 \)). However, no difference in diameter could be established between day fourteen and day twenty. There was no difference in shear stress between follow up measurements suggesting minimal adaptation of the vessel diameter. At the last follow up, twenty days after vascular shunting, wall shear stress was still 159\% (95\% CI of 18–300\%) above reference level (Fig. 5.2D).

On average, the wall thickness was 0.35 mm and the diameter changed 0.75\% per day. Rabbits with sufficient variation in wall thickness displayed a relationship between diameter change and wall thickness, indicating that vessel diameter changed faster for thinner walls. The regression analysis showed that for every millimetre increase in wall thickness diameter change was reduced by 1.8\% per day. The correlation between wall thickness and diameter change was moderate but significant (-0.33, \( p < 0.001 \)).

5.5 Discussion

We measured vascular remodeling in hypercholesteremic conditions and showed that remodeling was strongly reduced and shear stress remained above resting conditions, which is contradictory to conditions in healthy blood vessels [149, 119, 69, 104]. We further found that thin vessel walls remodel faster than thick walls. A phenomenon assumed to be of importance before hand and which was confirmed in the current study.

It is widely accepted that wall shear stress is kept constant in healthy blood vessel [69, 104, 71]. We measured whether this mechanism was still active in atherosclerotic vessels [281]. Our experiments with atherosclerotic rabbits revealed that the remodeling of atherosclerotic aortas stagnated after a severe flow increase. Therefore, shear stress did not return to baseline level. We can think of three explanations for this phenomenon.

Firstly, the time series were too short to record the slow remodeling of diseased vessels. Indeed, literature indicate that the speed of adaptation is decreased in atherosclerotic vessels [56]. The latter might be due to the diffusion distance for a generic signalling molecule. If plaque burden increases only
Figure 5.2: Measured changes of the variables after vascular shunting. The bars show the average of all animals at each follow up. The values are relative to the baseline data and expressed as percentages. At the top of each bar the standard error of the mean is shown. (A) Flow increased after vascular shunting. (B) There was no change in wall thickness visible. (C) The increase in luminal diameter stagnated after day fourteen. (D) This was not expected while wall shear stress was still above reference level.
5.5. DISCUSSION

A part of the medial layer is activated producing a reduced response. During the subsequent reduced response the vessel wall becomes thinner. Because of this, outer layers of the vessel wall can still be exposed to the generic signalling molecule. This will lead to remodeling at lower pace, but at similar magnitudes.

Secondly, the endothelial layer had not grown back over the entire surface area. Although we showed in previous studies that the protocol produced re-growth of endothelial cells, this was not measure routinely in the current experiments. An absent endothelial layer would increases wall stiffness and potentially counteract outward remodeling [51].

Thirdly, the reference value for wall shear stress might not be similar for dysfunctional endothelium as for healthy endothelium and it may change during the progress of disease. If the diameter of the aorta increases, more endothelial cells are needed to cover the inner surface of the aorta. New endothelial cells will have to settle down and adapt to the shear stress. While shear stress was still elevated, we now assume that these new endothelial cells use the elevated wall shear stress as their reference level. The result would be a shift in reference level and a stagnated growth. To explain our measurements 6% of the endothelial cells had to be replaced daily. Normal daily endothelial cell turnover rate is roughly 0.1% in humans but an activated endothelial cell phenotype can have 1% to 10% replications per day [68, 130]. In normal rat the daily cell replication of aortic endothelium is 13% at birth [219]. Thus in situations of rapid growth a turnover rate of 6% may be realistic. Moreover in atherosclerotic vessels endothelial cells can have larger turnover rates than endothelial cells in healthy vessels [19][183]. Therefore, this phenomenon might be more visible in atherosclerotic vessels.

In conclusion, numerical models for predicting remodeling of atherosclerotic arteries assume wall shear stress normalization while that might not happen in reality. Measurements showed stagnated enlargement of the luminal diameter and persistent elevated mean wall shear stress. This might indicate that the reference level for wall shear stress can change.
Chapter 6

Pulmonary artery size and function after Fontan operation at a young age


6.1 Abstract

Purpose: To assess pulmonary artery (PA) size, flow variables, and wall shear stress (WSS) in patients after Fontan operation at a young age. Flow in the branch PA was obtained with phase contrast velocity-encoded cardiovascular magnetic resonance imaging in 14 patients before and after low-dose dobutamine stress (7.5 μg/kg/min) and in 17 healthy controls at rest. At rest, stroke index, total flow, average, and peak flow rate were all statistically significantly lower in patients than in controls ($p < 0.001$). With stress-testing, all variables increased in patients ($p < 0.001$), apart from stroke index, which
did not change. At rest, branch PA area did not differ between patients and
controls. Distensibility was lower in patients than in controls ($p < 0.001$).
With stress testing, area and distensibility did not change. At rest, WSS was
lower in patients than in controls ($p < 0.001$). WSS increased with stress-
testing ($p < 0.001$), but not to the same levels as during resting conditions
of the control group. PA size is normal long-term after Fontan operation at
a young age. Flow variables, distensibility, and WSS are significantly lower
compared to healthy controls, and do not show adequate reactions with stress-
testing, which is suggestive of pulmonary artery endothelial and/or vascular
dysfunction.

6.2 Introduction

After Fontan completion for a functionally univentricular heart, the systemic
venous return is directly connected to the pulmonary arteries (PAs), resulting
in substantial or total loss of pulsatile flow into the lung [196, 118, 198, 110].
The long-term effects of this abnormal flow pattern on PA growth and function
are a matter of concern. Several groups have studied the effects of a bidirec-
tional Glenn or Fontan pathway on PA growth and diameters [197, 15, 245],
but have shown equivocal results.

Wall shear stress (WSS), the force per unit area induced by the relative
movement of blood and endothelium, is an important determinant of vascular
function [201], and altered levels of WSS are associated with a variety of dis-
ease processes [23, 272, 20]. WSS is inversely related to vessel diameter and
alterations in WSS induce vascular remodeling [23]. After Fontan operation,
increased WSS was found in one study [168] and endothelial dysfunction has
been reported that may interfere with the normal remodeling process of the
PA [118, 107, 129].

Recent studies on the effects of exercise on caval vein and pulmonary artery
flow after Fontan operation have emphasized the need for evaluation of the
Fontan circulation under exercise conditions [273, 194]. In Fontan patients,
PA flow has been studied directly after supine bicycle exercise with magnetic
resonance imaging (MRI) [194]. However, this study did not look into the re-
action of the PAs on an increase in flow. Therefore, the objectives of this study were: 1) to assess the size of a branch of the PA, its local flow pattern, and the local WSS after Fontan operation performed at a young age using phase contrast velocity-encoded cardiovascular magnetic resonance imaging (CMR); and 2) to simulate the effects of exercise on PAs with low-dose dobutamine stress.

6.3 Materials and methods

6.3.1 Subjects

Fourteen patients were included in this study (Table 6.1). All patients had been subject to an atriopulmonary connection (APC) or to a total cavopulmonary connection (TCPC), completed before 7 years of age. Follow-up time after Fontan completion was at least 5 years. In 2 patients the initial TCPC was fenestrated, but the fenestration had been interventionally closed during follow-up. Patients did not have contraindications for MRI or dobutamine administration. Medical records were reviewed for anatomical and operative details. Mean PA pressure before Fontan completion was 10.0 mmHg (7–14 mmHg, n = 12). Mean PA pressure after Fontan completion was 10.5 mmHg (6–16 mmHg, n = 8). These invasive measurements were obtained 2.3 (1.2–5.6) years after Fontan completion and 4.9 (2.0–13.4) years before participation in this study. The postoperative measurements were part of standard invasive investigations at least 1 year after Fontan completion.

Seventeen healthy children (nine boys) were included as controls for this study. The mean age was 13.3 ± 2.3 years, mean body surface area (BSA) was 1.54 ± 0.20 m². There was no statistically significant difference between the controls and patients in age, gender, and BSA. The study was approved by the Institutional Review Boards and by the Dutch Central Committee on Research Involving Human Subjects. All subjects and/or their parents (if required) gave informed consent.
### Table 6.1: Characteristics of the patients. Data are given as frequencies, or median with the range within parentheses.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients / males</td>
<td>14 / 9</td>
</tr>
<tr>
<td>Age (years)</td>
<td>11.9 (7.5–20.1)</td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>1.35 (0.93–2.09)</td>
</tr>
<tr>
<td>Follow-up after Fontan completion (years)</td>
<td>8.2 (5.4–16.8)</td>
</tr>
<tr>
<td>Age at Fontan completion (years)</td>
<td>3.5 (1.0–6.8)</td>
</tr>
<tr>
<td>Fontan type:</td>
<td></td>
</tr>
<tr>
<td>APC(^a)</td>
<td>3</td>
</tr>
<tr>
<td>TCPC(^b), lateral tunnel</td>
<td>8</td>
</tr>
<tr>
<td>TCPC, extracardiac conduit</td>
<td>3</td>
</tr>
<tr>
<td>Dominant ventricle:</td>
<td></td>
</tr>
<tr>
<td>right</td>
<td>4</td>
</tr>
<tr>
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<td>10</td>
</tr>
<tr>
<td>Pre-Fontan procedures:</td>
<td></td>
</tr>
<tr>
<td>Blalock-Taussig shunt</td>
<td>6</td>
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<tr>
<td>Pulmonary artery banding</td>
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<td>Norwood</td>
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<td>Glenn anastomosis</td>
<td>9</td>
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<tr>
<td>Branch pulmonary artery augmentation</td>
<td>2</td>
</tr>
<tr>
<td>Post-Fontan procedures:</td>
<td></td>
</tr>
<tr>
<td>Bentall procedure</td>
<td>1</td>
</tr>
<tr>
<td>Atrial level shunt closure</td>
<td>2</td>
</tr>
<tr>
<td>Extracardiac conduit replacement</td>
<td>1</td>
</tr>
</tbody>
</table>

\(^a\)Atriopulmonary connection.  
\(^b\)Total cavopulmonary connection.
6.3.2 MRI

A Signa 1.5 T whole-body MR imaging system was used (General Electric, Milwaukee, WI) for all patients, in combination with a dedicated phased-array cardiac surface coil that was placed over the thorax. All patients were monitored by vector cardiogram gating and blood pressure monitoring.

The PAs were localized on an axial set through the thorax, using a steady-state free precession (SSFP) sequence. On this axial localizer the PAs were cut longitudinally (Fig. 6.1A). The subsequent image together with the axial localizer was used to plan a flow measurement perpendicular to the flow, using phase-contrast velocity-encoded imaging. The imaging plane was set halfway between the origin (ie, point of the Fontan anastomoses in patients) and the first branching point of the PA (Fig. 6.1B). The branching points could be well visualized and there was no evidence of proximal PA stenosis on this double-oblique localizer. Flow measurements were obtained over one cardiac cycle and were divided into 24 phases. Imaging parameters were TR 5–6 ms, TE 3 ms, flip angle 20°, 7 mm slice thickness, 6 views/segment, scanning matrix of 256 × 128.

It is known that the better the velocity encoding matches the real velocity in the region of interest, the more precise the measurement becomes [145]. In patients, unidirectional velocity encoding was set at 30 cm/s and adjusted in case of aliasing. The maximal velocity encoding used was 80 cm/s. To minimize the effect of breathing, flow measurements were made without breath-hold using three signal averages. When the study protocol had been completed, dobutamine-hydrochloride (Centrafarm Services, Etten-Leur, The Netherlands) was administered by continuous infusion into an antecubital vein at 7.5 μg/kg/min. After 15 minutes, when a new steady-state in heart rate and blood pressure had been obtained, a second flow measurement was acquired using the imaging parameters specified above. Dobutamine infusion was lowered to 5 μg/kg/min if any of the following events occurred: 1) heart rate, systolic, or diastolic blood pressure of more than 150% baseline; 2) heart rate, systolic, or diastolic blood pressure of less than 80% baseline [38].

In healthy controls, flow measurements in the PAs were performed as part of another study protocol. In this study, unidirectional velocity encoding was
Figure 6.1: SSFP images of PAs on an axial localizer (A, top row) of a healthy control (left), patient with an APC (middle), and patient with a TCPC (right). The red line indicates the imaging plane in the RPA (left panel), and LPA (middle and right panel) for the subsequent localizer (B, lowest row). On this double oblique image the flow measurements were planned halfway to the origin of the PA and the first branching point (red line).
150 cm/s and acquisitions were taken with breath-hold in end-expiration. Flow measurements were done at rest only. Difference in acquisition parameters between the patient and control group were accepted, since others have demonstrated clinically nonsignificant differences in absolute blood flow and peak velocity in the branch PAs between expiratory breath-hold versus free-breathing acquisitions [131, 132].

6.3.3 Image analysis
CMR studies were analyzed on a commercially available Advanced Windows workstation (General Electric Medical Systems) using the Flow analysis software package V3.1 (Medis Medical Imaging Systems, Leiden, The Netherlands). The following variables were determined: time averaged intraluminal area; maximal and minimal intraluminal area; peak flow rate, minimal and time averaged flow rate; stroke index (stroke volume divided by BSA), and total flow per minute indexed for BSA.

6.3.4 Calculations
To compare our results with the results of a previous study [168], distensibility of the PA was approximated by the formula: (maximal area − minimal area) / maximal area.

6.3.5 WSS determination
WSS was determined according to the method of Wentzel et al. [272]. All 24 phases of the velocity measurement were processed to obtain WSS data. We applied a moving average filter (3 × 3) to reduce noise. Shear rate, being the change in velocity per unit distance, was calculated according to the following method: for each pixel the velocity value of the left and right neighbor pixel and the upper and lower neighbor pixel were taken and half of the velocity difference was divided by the pixel size. This resulted in a velocity gradient in two orthogonal directions for each pixel. Those two velocity gradients were squared. The final shear rate for each pixel was taken as the square root from
the squared sum of these values. To determine the shear rate at the vessel wall, we separated the wall into 12 parts. Each part was determined by the outer 10% of the vessel radius and 30° in the circumferential direction. The highest shear rate found in each part was considered representative for the wall shear rate in the corresponding part. The wall shear rate \( s^{-1} \) was multiplied with the viscosity \( 0.003 \text{ N·s/m}^2 \) to get the WSS \( \text{N/m}^2 \). Average WSS per cardiac phase was the arithmetic mean from the WSS of the 12 parts. Mean WSS of the whole cardiac cycle was the arithmetic mean from the average WSS of all 24 phases.

### 6.3.6 Statistical analysis

Data are expressed as frequencies, mean (standard deviation), or median (range) as appropriate. Comparisons between groups were made using the appropriate \( t \)-test. A p-value < 0.05 was considered to indicate statistical significance.

### 6.4 Results

The study protocol was well tolerated and completed by all subjects. In three patients the dobutamine infusion was lowered to 5 \( \mu \text{g/kg/min} \), since the heart rate was more than 50% above baseline with a dosage of 7.5 \( \mu \text{g/kg/min} \), although this was well tolerated.

In patients, flow measurements in the right pulmonary artery (RPA) were not always possible due to the short distance between the connection with the superior vena cava and the first branching point. Therefore, all flow measurements were additionally performed in the left pulmonary artery (LPA). In eight patients there was an adequate flow measurement in the RPA. Statistical analysis of flow variables did not show any difference between the RPA and LPA (Table 6.2).

In the control group the first branching point of the LPA was closer to the bifurcation than the branching point of the RPA. This led to difficulties with WSS determination in half of the controls since, because of vessel movement
Table 6.2: Comparison of flow results in the RPA and LPA. Data are given as mean with the standard deviation within parentheses.

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=9)</th>
<th>Patients (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RPA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>LPA&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Average area (mm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>197 (45)</td>
<td>216 (29)</td>
</tr>
<tr>
<td>Maximal area (mm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>253 (58)</td>
<td>267 (40)</td>
</tr>
<tr>
<td>Average flow (ml/s)</td>
<td>52 (12)</td>
<td>46 (13)</td>
</tr>
<tr>
<td>Peak flow (ml/s)</td>
<td>177 (31)</td>
<td>171 (32)</td>
</tr>
<tr>
<td>SVI (ml/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>29 (5)</td>
<td>25 (4)</td>
</tr>
<tr>
<td>Total flow (ml/min/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>2007 (383)</td>
<td>1761 (395)</td>
</tr>
<tr>
<td>Average area (mm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>203 (61)</td>
<td>210 (57)</td>
</tr>
<tr>
<td>Maximal area (mm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>229 (70)</td>
<td>232 (63)</td>
</tr>
<tr>
<td>Average flow (ml/s)</td>
<td>28 (8)</td>
<td>29 (8)</td>
</tr>
<tr>
<td>Peak flow (ml/s)</td>
<td>59 (40)</td>
<td>59 (36)</td>
</tr>
<tr>
<td>SVI (ml/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>19 (6)</td>
<td>20 (9)</td>
</tr>
<tr>
<td>Total flow (ml/min/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>1243 (182)</td>
<td>1284 (285)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Right pulmonary artery
<sup>b</sup>Left pulmonary artery
Table 6.3: Comparison of branch pulmonary flow variables in patients (LPA results) and controls (RPA results). Data are given as mean with the standard deviation within parentheses. If the p-value was not significant (NS) the value is not listed.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Controls</th>
<th>Patients (rest)</th>
<th>Patients (stress)</th>
<th>P-value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>P-value&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>72 (12)</td>
<td>69 (12)</td>
<td>93 (17)</td>
<td>NS</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Stroke index (ml/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>31 (7)</td>
<td>19 (7)</td>
<td>19 (7)</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
<tr>
<td>Total flow (ml/min/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>2189 (463)</td>
<td>1244 (274)</td>
<td>1705 (308)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Average flow (ml/s)</td>
<td>56 (15)</td>
<td>28 (6)</td>
<td>39 (13)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Peak flow (ml/s)</td>
<td>187 (48)</td>
<td>55 (31)</td>
<td>71 (44)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Average area (mm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>207 (42)</td>
<td>183 (63)</td>
<td>186 (66)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Maximal area (mm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>263 (59)</td>
<td>206 (70)</td>
<td>209 (78)</td>
<td>0.02</td>
<td>NS</td>
</tr>
<tr>
<td>Distensibility</td>
<td>0.41 (0.09)</td>
<td>0.22 (0.06)</td>
<td>0.20 (0.07)</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
<tr>
<td>Average WSS&lt;sup&gt;c&lt;/sup&gt; (N/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>0.84 (0.14)</td>
<td>0.38 (0.15)</td>
<td>0.50 (0.18)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<sup>a</sup>Between controls and patients at rest.

<sup>b</sup>Between patients at rest and patients with stress-testing.

<sup>c</sup>Wall shear stress.

during the cardiac cycle, the flow signal of the LPA was disturbed by flow signals of branch vessels. Therefore, all flow measurements were done in the RPA. Statistical analysis of flow variables in the RPA and LPA of the controls also did not show any difference (Table 6.2). In Table 6.3 the results of measurements in the branch PA are shown (i.e., the RPA for healthy controls and the LPA for Fontan patients).

### 6.4.1 Flow measurements

At rest, stroke index, total flow, average flow rate, and peak flow rate were statistically significantly higher in controls than in Fontan patients (Table 6.3). With dobutamine stress-testing in Fontan patients the stroke index did not change. Total flow per minute increased significantly with increased heart rate (Table 6.3). However, all variables remained below the levels of controls during rest.
6.4.2 Pulmonary artery area and distensibility

At rest, average area of the branch PA halfway to the origin and the first branching point did not differ between patients and controls. In contrast, there was a statistically significantly higher maximal area in the controls compared to patients, which was partly related to a higher distensibility in the control group (Table 6.3). With stress-testing in patients, area and distensibility did not change, despite an increase in blood flow (Table 6.3).

6.4.3 WSS determination

At rest, WSS was significantly lower in the patient group compared to controls. When the spatial average WSS per cardiac phase was plotted against cardiac phase, three distinct patterns were visible in controls, TCPC patients, and APC patients, in agreement with the flow patterns in these groups (Fig. 6.2). In controls, peak WSS occurred at peak systole, while in TCPC patients there was only little variation in WSS throughout the cardiac cycle. In contrast, in APC patients there was an increase in WSS in late diastole, coinciding with atrial contraction. When WSS was plotted against area of the branch PA, Fontan patients showed a WSS that was too low for the area (Fig. 6.3). With stress-testing in patients, WSS increased but not to the level of the controls at rest (Table 6.3).

6.5 Discussion

In this study we demonstrated that normal PA area’s after Fontan operation coincide with a decreased distensibility and WSS. Furthermore, pulsatility of different parameters (flow, WSS) was reduced considerably. We propose that these observations are suggestive of endothelial and/or vascular dysfunction in Fontan patients.

In the present study, loss of pulsatility was demonstrated by the flow curves and WSS curves, as presented in Figure 6.2. Loss of pulsatility has been linked to pulmonary endothelial dysfunction in a few recent studies [118, 198, 110]. After a bidirectional Glenn procedure, Kurotobi et al. [118] found a significant
Figure 6.2: WSS (a) and flow rate (b) throughout the cardiac cycle of representative study subjects. APC, atriopulmonary connection; TCPC, total cavopulmonary connection; WSS, wall shear stress.
6.5. DISCUSSION

Figure 6.3: WSS versus branch PA diameter. Triangles represent the control group, squares the Fontan group. PA, pulmonary artery; WSS, wall shear stress.

correlation between loss of pulsatility and significantly impaired endothelium-dependent relaxation (by acetylcholine) in the lower lobe PA. Others have observed an abnormal pulmonary vascular response to exogenous nitric oxide in Fontan patients, in some part related to a lack of pulsatility in the pulmonary circulation [107].

The long-term effects of total or substantial loss of pulsatility of flow in the PAs after Fontan operation remain unclear. Results from a few studies focusing on the pulmonary vasculature in Fontan patients are suggestive of pulmonary endothelial dysfunction, particularly in the nitric oxide pathway. Pulsatile stretch and WSS are important for the release of nitric oxide by the endothelium [16, 81]. Nitric oxide, a locally acting vasodilator, contributes to the maintenance of low pulmonary vascular resistance in healthy children [18]. Low pulmonary vascular resistance might be essential for optimal functioning of the Fontan circulation.

Previous studies have measured decreased PA sizes after Fontan operation [197, 15, 245, 168]. However, in these studies, PA size was determined at different locations than in the present study. Furthermore, Reddy et al. [197], Buheitel et al. [15], and Tatum et al. [245] determined maximal PA diameter on angiograms, while Morgan et al. [168] determined maximal PA area on
CMR flow measurements (perpendicular to the axial axis of the vessel). It is known that the cross-sectional area of a PA is not a perfect circular shape, but rather oval shaped. Determination of the diameter on angiograms in posteroanterior projections can therefore give an inaccurate estimate of the real diameter or area of the vessel. In our study we used the average and maximal crosssectional area of the branch PA (halfway between the origin and the first branching point) on CMR flow measurements. Although there was a statistically significant difference in maximal area, time averaged area did not differ between patients and controls. In our opinion, average PA area is a more representative measure of PA size. Pulmonary artery flow, in Fontan patients is not primarily in systole, but continuously throughout the cardiac cycle. Although pressure was not measured, it is to be expected that this signal will follow the flow signal. Hence, a lower maximal area of a PA in a Fontan patient reflects the lack of pulsatility of pressure and an impaired distensibility of the vessel, but does not necessarily mean the vessel is too small. Therefore, we did not calculate the McGoon ratio or the Nakata index in this study group, since both these parameters use maximal PA diameter.

In our study, lower distensibility in Fontan patients at rest might be explained by the decreased pressure range after the Fontan operation. Distensibility, however, is not only affected by pulse pressure but also by properties of the vessel wall. Hence, the current findings might also be explained by altered muscle mechanics due to different tone of the smooth muscle cells. Tone of the smooth muscle cells is partly determined by the blood flow. However, with an increase in blood flow during low-dose dobutamine stress, distensibility did not change, arguing against an important effect of blood flow on distensibility. In healthy adults, pulmonary arteries distend ∼2% of their initial diameter with every millimeter mercury increase in transmural pressure with increased flow during exercise [199]. Possibly the PA is already maximally dilated at rest to ensure adequate pulmonary blood flow and cannot expand with increased flow. A second explanation might be endothelial dysfunction, preventing the vessel from dilating. This might explain the inability to increase the stroke index with stress-testing and, therefore, the cardiac index can only be increased by increasing the heart rate.
In our study, time and spatially averaged WSS was significantly lower in the Fontan patients than in the control group, which is consistent with a reduced blood flow at rest, while vessel area was normal. Throughout the cardiac cycle, distinct WSS-patterns were seen in healthy controls, TCPC patients, and APC patients, comparable with the flow curves in these groups (Fig. 6.2). Physiologic and pulsatile WSS constitutes the most potent stimulus for continuous production of nitric oxide by the endothelium [20]. Low WSS reduces the bioavailability of nitric oxide by decreasing the expression of nitric oxide synthase. This will lead to an increase in pulmonary vascular resistance, as has been demonstrated in patients late after a Fontan-type operation by Khambadkone et al. [107]. Levy et al. [129] demonstrated weak expression of nitric oxide synthase in Fontan patients with a good surgical outcome, but overexpression of nitric oxide synthase in patients after Fontan failure. They hypothesized that this overexpression could be due to an attempt to improve the pulmonary vascular resistance and facilitate Fontan circulation. The mechanism, however, cannot be explained by the low WSS in this circulation and warrants further investigation.

Recently, Cheng et al. [23] postulated that average WSS is not constant throughout the vascular tree—as has been assumed—but is inversely related to the vessel diameter. This new theory explains the variation in average WSS observed in vessels of varying sizes. In our study group, average WSS in Fontan patients was too low for the corresponding area when compared to controls (Fig. 6.3). According to the theory of Cheng et al, WSS in these Fontan patients should either be higher, or vessel area should be larger. The mismatch between blood flow and area may indicate a lack of appropriate vascular remodeling by WSS.

When comparing our results with the results of the study of Morgan et al. [168], also investigating PA size, blood flow, and WSS in Fontan patients, we found: 1) a higher total flow and branch PA flow in controls and patients, 2) no difference in PA area between patients and controls, 3) significantly lower distensibility in patients, and 4) lower WSS in patients at rest and even during stress with different WSS patterns. The findings of Morgan et al are somewhat unexpected and counterintuitive in light of the current knowledge.
of the pulmonary circulation after Fontan operation. Important differences in study group and methods might explain these discrepant findings. In our study, patients were younger at Fontan completion, follow-up age was lower, and follow-up time after Fontan completion was longer. Flow measurements were planned on a double-oblique cross-section of the PA—halfway between the origin and the first branching point—to plan the measurements perpendicular to the flow, and to limit the influence of bifurcations on the flow pattern. In the study by Morgan et al. [168], WSS was determined immediately after the bifurcation of the main PA, which is an area with swirling flow, possibly responsible for the increase in WSS they found.

In our group of patients, all after Fontan completion at a young age and in which the majority had a TCPC, the average PA area is normal—an important finding for patients operated on according to this treatment strategy nowadays. The implications of the results that are suggestive of PA endothelial dysfunction and inappropriate vascular remodeling should be made with caution. The inability of the pulmonary vascular bed to expand with increasing blood flow might be an important limitation for maintenance of adequate ventricular preload in the long term. However, more information is needed on the long-term effects of this pulmonary vascular dysfunction, e.g., the effects on the peripheral pulmonary vasculature and resistance, and will require serial follow-ups. CMR imaging is a safe, noninvasive, and easily applicable modality for this assessment of PA size and function in selected patients.

This study is limited by the small size and inhomogeneity of the study group, preventing us from analyzing the effects of diagnosis, pre-Fontan procedures, and Fontan type on the measured variables. In theory, endothelial function can be different in patients with an APC because of the different flow profile and higher energy losses. Our results are not applicable to patients with different operative courses (e.g., Fontan completion at older age). Although there was no clinical evidence of increased PA pressure or resistance, this was not assessed invasively.

Acquisition parameters were different between patients and controls. This could introduce an error in the comparison of WSS in patients and controls. However, studies have shown differences of only 1% to 10% in flow velocity
between free breathing and breath-hold acquisitions [131, 132]. Since the error in WSS is proportionate to the error in flow velocity, and differences in flow velocity between patients and controls were much higher in this study, we consider this error negligible. Adenosine, a vasodilator commonly used for myocardial perfusion imaging in patients with coronary artery disease, may be a better pharmacological agent to test a vessel’s distensibility. However, since our objective was to simulate physical exercise, we chose to use dobutamine for its positive inotropic and chronotropic effects.

Velocity-encoding in three directions might be more accurate and informative on the flow pattern. All flow measurements were planned in the branch PA before the first branching point. The smaller peripheral pulmonary vasculature was not visualized, and therefore an absence of stenosis in this area cannot be excluded, even as possible effects on blood flow and endothelial function in the proximal branch PA. Temporal resolution of the flow measurements can be improved and results in more accurate measurements [1]. However, this also increases acquisition time. Depending on the study subject, an acceptable balance between temporal and spatial resolution and acquisition time should be sought.

In conclusion, long-term after Fontan completion at a young age, patients have a normal average PA size when compared to controls. However, pulmonary blood flow, distensibility, and WSS are all decreased in these patients and do not show adequate reactions with lowdose dobutamine stress testing, suggesting endothelial and/or vascular dysfunction.
CHAPTER 6. FONTAN OPERATION
Chapter 7

Early onset of retrograde flow in the main pulmonary artery is a characteristic of pulmonary arterial hypertension


7.1 Abstract

Purpose: To evaluate if early onset of retrograde flow in the main pulmonary artery is a characteristic of pulmonary arterial hypertension (PAH). Fifty-five patients with suspected pulmonary hypertension (PH) underwent rightsided heart catheterization and retrospectively ECG-gated MR phase-contrast ve-
velocity quantification in the main pulmonary artery. Pulmonary hypertension was defined by a mean pulmonary artery pressure being larger than 25 mmHg. The onset time of the retrograde flow relative to the cardiac cycle duration (Relative Onset Time = ROT) was compared with mean pulmonary artery pressure. By the catheterization, 38 patients were identified as having PAH. The ROT for these PAH patients was significantly smaller than the ROT found in the 17 non-PH subjects (0.14 ± 0.06 versus 0.37 ± 0.06, \( p < 0.001 \)). The mean pulmonary artery pressure was related to the ROT \( (R^2 = 0.62, \ p < 0.001) \) and could be estimated from the ROT with a standard deviation of 11.7 mmHg. With a cutoff value of 0.25, the ROT distinguished PAH patients from non-PH subjects. Early onset of retrograde flow in the main pulmonary artery is a characteristic of pulmonary arterial hypertension and is visible by standard MR phase-contrast velocity quantification.

### 7.2 Introduction

Pulmonary hypertension (PH) is a progressive disease defined by chronically elevated mean pulmonary artery pressure that exceeds 25 mmHg at rest \[157\]. Various studies have shown that in PH patients the flow pattern in the main pulmonary artery differs from that seen in healthy people \[108, 182, 112, 171, 200\]. One of the main findings is the appearance of retrograde flow at the right dorsal side of the main pulmonary artery. Okamoto et al. suggested that this indicates the appearance of a vortex \[182\]. Recently, Reiter et al. found that the vortex duration is related to the mean pulmonary arterial pressure \( (R^2 = 0.88) \) \[200\]. Long vortex duration may be associated with early onset of retrograde flow. If so, then early onset of retrograde flow could indicate the presence of PH.

Many patients which are suspected of PH, undergo MR phase-contrast velocity quantification in the main pulmonary artery for the assessment of stroke volume \[155, 181\]. The measured velocity images show retrograde flow if present. The question is whether early onset of this retrograde flow indicates the presence of PH and what cutoff value should be used.

Therefore, we conducted a study in pulmonary arterial hypertension (PAH)
patients and subjects suspected of having PH, in whom both right heart catheterization and MRI are performed.

7.3  Materials and methods

7.3.1  Study population

Our sample comprised of patients diagnosed with pulmonary arterial hypertension (PAH) and non-PH subjects. PH was diagnosed using right heart catheterization, whereby an mPAP larger than 25 mmHg indicated PH. Non-PH subjects were suspected of PH but turned out to have a mean pulmonary artery pressure (mPAP) smaller than 25 mmHg. All patients in this study were referred to the VU University Medical Center, from January 2003 until April 2009. The inclusion criterion was the existence of right heart catheterization and MRI velocity quantification measurements within a time interval of three weeks. Exclusion criteria were reception of pneumonectomy and poor MRI image quality. Some patients with PH can have characteristics other than pressure that can affect pulmonary flow. Therefore, we decided for clarity to confine to only patients with pulmonary arterial hypertension.

7.3.2  Right heart catheterisation

Right-sided heart catheterization (RHC) was performed with a balloon tipped, flow directed 7 French Swan-Ganz catheter (131HF7, Baxter Healthcare Corp, Irvine, CA). The patients were in stable condition, lying supine and breathing room air, while their heart rate was continuously monitored. Measurements were made of mean right atrial pressure, right ventricular pressure, pulmonary artery pressure and pulmonary capillary wedge pressure (PCWP). Cardiac output (CO) was obtained by thermodilution or by direct Fick method, from arterial and mixed venous oxygen saturation and $O_2$ consumption. Pulmonary vascular resistance was calculated as (mPAP − PCWP) / CO.
CHAPTER 7. PULMONARY FLOW

7.3.3 MR velocity quantification

MRI was performed with either a Siemens 1.5 T “Sonata” or 1.5 T “Avanto” whole body scanner (Siemens Healthcare, Erlangen, Germany) equipped with a phased-array body coil. One investigator performed all MR acquisitions. Phase-contrast velocity imaging was performed without averaging during continuous breathing. We performed a prospectively electrocardiograph (ECG)-gated, spoiled gradient-echo MRI sequence, with through-plane velocity encoding and a velocity sensitivity of 120 cm/s. The orientation of the image plane was orthogonal to the main pulmonary artery (Fig. 7.1). The flow sequence was run with the following parameters: slice thickness 6 mm, field of view 240 mm $\times$ 320 mm, matrix size 140 $\times$ 256, echo time 4.8 ms, repetition time 11 ms, temporal resolution 22 ms, flip angle 25°. If breathing artifacts were obvious, the acquisition was repeated. Phase offset errors were corrected by subtracting the velocity images measured at a stationary water-phantom using the same acquisition settings and an artificial ECG mimicking the patients ECG.

7.3.4 Image analysis

A semiautomatic program, based on studies published by Li et al. [135, 136], using Matlab R2008a (The Mathworks, Inc., Natick, MA) was used to find the lumen boundary of the main pulmonary artery (MPA). The software reduced operator bias by using a minimum cost algorithm. This algorithm is very robust to image noise because it can sense the whole lumen boundary at once. Therefore, even if the vessel wall was only partly visible, this algorithm could find the contour that marked the lumen boundary. This tested and well-validated technique was developed for ultrasound images. A summary of the wall detection is shown in Figure 7.2.

Our software created thirty starting points randomly around the marks selected by the operator. This way, 30 different contours rescinded the influence of the operator. From these contours, an average contour was calculated. The middle of the average contour was an estimator for the middle of the vessel in the next image. Again, 30 starting point were generated and the minimal
Figure 7.1: a-c: Steady-state free precession MR images to localize the pulmonary trunk (repetition time, 3.2 ms; echo time, 1.6 ms; flip angle, 65°; section thickness, 6 mm; matrix, 256 × 96). First, an image was prescribed perpendicular to a transversal view (a) that included the pulmonary artery. This resulted in an oblique-sagittal image as shown in b. A third localizer image shown in c was acquired orthogonal to b, and through the pulmonary artery and then used together with b to obtain the image plane for the through-plane velocity measurement in the main pulmonary artery (MPA). d: The cross-section of the MPA, acquired as the magnitude image in the flow measurement. This acquisition was with a gradient-echo sequence with through-plane phase-contrast velocity quantification (see text for details).
Figure 7.2: Detection of the arterial wall. a: The user only once marks the centre of the vessel and a reference point outside the vessel. b: Sixty radial lines scan the intensity of the magnitude image. c: The sixty radial lines are transformed to an image showing the bright lumen in the bottom half. d: The gradient of signal intensity (SI) in the top-down direction of the image in c. A negative gradient corresponds to low SI (“dark”), and thus to low cost. e: Cumulative cost image showing the path (yellow line) of minimal cost in the left-right direction of the gradient image in d. f: Transformation of the path of minimal cost to the original magnitude image. The path indicates the inside of the arterial wall.
cost algorithm was applied to the second image. After a contour was found for every image, a filter smoothed all contours whereby preserving the vessel shape. The operator, unaware of RHC results, visually checked all contours and performed all analyses.

### 7.3.5 Variables in the study

The contours that marked the lumen boundary during the cardiac cycle were essential to calculate the variables in this study. The cross-sectional area (CSA) was the smallest cross-sectional area of the MPA during the cardiac cycle. This was easily found as the smallest area enclosed by any contour. The average distance between the contour and its centre was multiplied by two to get the diameter. The difference in length between the longest and shortest contour was divided by the length of the shortest contour to get MPA strain. The MR velocity images showed velocity perpendicular to the image plane on a pixel-by-pixel basis. Therefore, it was possible to locate and quantify retrograde flow. Flow (ml/s) was calculated by summing the product of velocity and pixel size. By keeping track of the flow direction for each pixel, antegrade, retrograde, and net flow were calculated (Fig. 7.3). Net flow was identical to the antegrade flow minus the retrograde flow. For some variables in this study, we had to know when the pulmonary valve opened and closed or when flow reached a maximum. These data were automatically detected based on the net flow. Valve opening was identified by net flow reaching 5% of its maximum. Valve closure was identified by zero net flow or a minimum in net flow. Time between valve opening and maximal net flow was the acceleration time. Time between valve opening and valve closure was the ejection time. Onset of retrograde flow was identified by retrograde flow reaching 5% of its maximum. Relative retrograde flow (RRF) was the sum of systolic retrograde flow relative to the sum of systolic antegrade flow. Furthermore, we calculated the averaged and peak velocities over the CSA during systole. The most important variable in our study was the relative onset time (ROT). The ROT indicated the onset time of retrograde flow relative to the duration of the cardiac cycle (Fig. 7.3). The duration of the cardiac cycle was retrieved from
CHAPTER 7. PULMONARY FLOW

Figure 7.3: Antegrade and retrograde flow in the main pulmonary artery during one cardiac cycle. The antegrade flow (red line) rapidly increases from the beginning of the cardiac cycle and reaches a maximum during systole. The retrograde flow (blue line) starts after an onset time and often shows two peaks. The first peak is during systole and was only seen in PAH patients. The second peak is at valve closure and was visible in all subjects. The inset shows the velocity profile perpendicular to the image plane for maximal flow. This illustrates the occurrence of antegrade and retrograde flow at the same time.

the data that accompanied the MR images.

7.3.6 Location of retrograde flow

Retrograde flow in the MPA is a complex phenomenon because the amount of flow depends on time and location (Fig. 7.4). Furthermore, during the cardiac cycle the MPA stretches and shows some motion. We decided to express location of retrograde flow as one simple variable independent of time. Hence, in the coordination system of the velocity images the location was expressed as an angle between $0^\circ$ and $360^\circ$ as commonly done in a circle. To determine this angle data processing was required.

The phase-contrast data in the MPA was averaged over the whole cardiac cycle. Hereby, the motion of the MPA was corrected by shifting the centre
7.3. MATERIALS AND METHODS

Figure 7.4: Early onset of retrograde flow in PAH. Examples of phase-contrast velocity quantification in the main pulmonary artery for a PAH patient (mPAP = 66 mmHg) on the top row and a non-PH subject (mPAP = 7 mmHg) below. Time after opening of the pulmonary valves was 22, 73, 123, 174, and 224 ms (left to right). All images show an area of 5 cm × 5 cm. The grey scale indicates the through-plane velocity. White corresponds with antegrade velocities, black corresponds with retrograde velocities. The images show early retrograde flow in the PAH patient and absence of retrograde flow in the non-PH subject.

of the lumen of each phase-image to the same location. Then we calculated the weighted mean of retrograde time averaged flow. Finally, the angle of retrograde flow in relation to the centre of the lumen was calculated.

7.3.7 Statistical analysis

Differences between patient groups were evaluated with an independent sample t-test. We compared mPAP with MRI-derived variables. Statistical test included descriptive statistics, independent t-tests, linear regression and ROC-curves. All tests were twotailed and a p-value smaller than 0.05 was considered significant. Some data were presented with a 95% confidence interval (CI). In addition to presenting the mean and standard deviation of the location of retrograde flow, a figure visualized the distribution. For this purpose, subjects were classified in intervals of 10 degrees and the distribution was shown in a bar plot and a circular plot. Analyses were performed with SPSS for Windows (version 15.0.0, SPSS Inc., Chicago, USA).
7.4 Results

7.4.1 Study population

This study included 38 PAH patients and 17 non-PH subjects. The average time interval between MRI and RHC was 5 days. As expected, the mPAP, systolic pulmonary artery pressure, mean right atrial pressure, pulmonary vascular resistance, mixed venous oxygen saturation, cardiac output, and heart rate at RHC were significantly different between PAH patients and non-PH subjects. There were no significant differences in terms of age or sex distribution, body surface area, time interval between procedures, or pulmonary capillary wedge pressure (Table 7.1).
Table 7.1: Demographic and hemodynamic data (mean ± SD). Data within parentheses are percentages.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total</th>
<th>Patients without PH</th>
<th>Patients with PAH</th>
<th>P-value$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>55</td>
<td>17</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>50 ± 15</td>
<td>54 ± 14</td>
<td>49 ± 16</td>
<td>0.25</td>
</tr>
<tr>
<td>No. of female patients</td>
<td>44 (80)</td>
<td>14 (82)</td>
<td>30 (79)</td>
<td>0.9</td>
</tr>
<tr>
<td>Time interval between MRI and RHC (days)</td>
<td>0 ± 5</td>
<td>0 ± 6</td>
<td>0 ± 5</td>
<td>0.42</td>
</tr>
<tr>
<td>Body surface area (m$^2$)</td>
<td>1.8 ± 0.3</td>
<td>1.9 ± 0.3</td>
<td>1.8 ± 0.3</td>
<td>0.85</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>81 ± 14</td>
<td>74 ± 12</td>
<td>84 ± 13</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Mean right atrial pressure (mmHg)</td>
<td>6 ± 4</td>
<td>3 ± 2</td>
<td>7 ± 4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic pulmonary artery pressure (mmHg)</td>
<td>61 ± 30</td>
<td>26 ± 8</td>
<td>77 ± 21</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic pulmonary artery pressure (mmHg)</td>
<td>23 ± 13</td>
<td>8 ± 3</td>
<td>30 ± 11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean pulmonary artery pressure (mmHg)</td>
<td>39 ± 19</td>
<td>16 ± 5</td>
<td>49 ± 13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pulmonary capillary wedge pressure (mmHg)</td>
<td>8 ± 4</td>
<td>7 ± 3</td>
<td>8 ± 4</td>
<td>0.19</td>
</tr>
<tr>
<td>Pulmonary vascular resistance (dyn·s/cm$^5$)</td>
<td>543 ± 412</td>
<td>134 ± 99</td>
<td>724 ± 365</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cardiac index (l/min/m$^2$)</td>
<td>3.2 ± 1.1</td>
<td>4.1 ± 1.3</td>
<td>2.8 ± 0.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mixed venous oxygen saturation (%)</td>
<td>69 ± 7</td>
<td>75 ± 6</td>
<td>66 ± 6</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

$^a$Comparison between patients without pulmonary hypertension (PH) and patients with pulmonary arterial hypertension (PAH).
Table 7.2: Data derived from MRI velocity quantification in the pulmonary artery (mean ± SD).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total</th>
<th>Patients without PH</th>
<th>Patients with PAH</th>
<th>P-value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average velocity (m/s)</td>
<td>0.23 ± 0.12</td>
<td>0.38 ± 0.10</td>
<td>0.16 ± 0.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Peak velocity (m/s)</td>
<td>0.69 ± 0.25</td>
<td>0.88 ± 0.22</td>
<td>0.60 ± 0.21</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MPA strain (%)</td>
<td>9 ± 8</td>
<td>16 ± 10</td>
<td>6 ± 3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diameter (mm)</td>
<td>32 ± 6</td>
<td>25 ± 3</td>
<td>36 ± 4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cross sectional area (mm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>854 ± 316</td>
<td>487 ± 123</td>
<td>1017 ± 223</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Acceleration time (ms)</td>
<td>95 ± 28</td>
<td>107 ± 20</td>
<td>89 ± 29</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Acceleration time/ejection time</td>
<td>0.31 ± 0.07</td>
<td>0.34 ± 0.07</td>
<td>0.29 ± 0.07</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Relative onset time</td>
<td>0.21 ± 0.12</td>
<td>0.37 ± 0.06</td>
<td>0.14 ± 0.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Relative retrograde flow</td>
<td>0.06 ± 0.06</td>
<td>0.01 ± 0.01</td>
<td>0.09 ± 0.05</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<sup>a</sup>Comparison between patients without pulmonary hypertension (PH) and patients with pulmonary arterial hypertension (PAH).

7.4.2 Variables in the study

The ROT, average velocity, smallest CSA and RRF of PAH patients were significant different from those found in non-PH subjects (Table 7.2).

7.4.3 The location of retrograde flow

Retrograde flow was found at an angle of 261° (95% CI = 254–268°) with a standard deviation of 27°. The angle found in PAH patients (264 ± 27°) did not differ from those found in non-PH subjects (254 ± 26°). The location of retrograde flow corresponded to the dorsal side of the pulmonary artery and was slightly to the right side (Fig. 7.5).

7.4.4 Relation of variables with pressure

At pulmonary valve closure, the retrograde flow showed a peak in all subjects. In PAH patients, the retrograde flow often showed a second peak (Fig. 7.3). The ROT of retrograde flow was related to mPAP ($R^2 = 0.62$, mPAP = $-125 \times$ ROT + 66). Thus, large pressures corresponded with small ROT (Fig. 7.6). The standard deviation of pressure around the linear fit was 11.7 mmHg. There
Figure 7.5: Location of retrograde flow. a: The histogram shows the location of retrograde flow for every subject. The number of subjects was classified in intervals of ten degrees. b: A circular plot showing the histogram with the location of retrograde flow at the right angle. Related to anatomical directions 0° is the left side, 90° the ventral side, 180° the right side, and 270° the dorsal side.
was also a relation between mPAP and average velocity ($R^2 = 0.60$, mPAP = $-119 \times $ velocity + 66), CSA ($R^2 = 0.57$, mPAP = $0.046 \times$ CSA) and RRF ($R^2 = 0.54$, mPAP = $238 \times $ RRF + 24).

### 7.4.5 Distinguishing PAH patients from non-PH subjects by ROT and CSA

Cutoff values of 0.25 for ROT and 626 mm$^2$ for the CSA, gave the best sensitivity and specificity, in our preselected population, to predict pulmonary hypertension to be present. By combining ROT with CSA data, the PAH pa-
7.5. DISCUSSION

Figure 7.7: Distinguishing PAH patients from non-PH subjects by ROT and CSA. 
\textbf{a}: Mean pulmonary arterial pressure as function of ROT. Black dots indicate PAH patients and grey squares indicate non-PH subjects. The vertical line indicates the cutoff value of 0.25. \textbf{b}: By combining ROT with CSA data, the PAH patients can be distinguished more clearly from the non-PH subjects.

Patients could be distinguished more clearly from the non-PH subjects (Fig. 7.7).

7.5 Discussion

We studied the retrograde flow in the pulmonary artery and found a relation between onset time and pulmonary artery pressure. Furthermore, relative onset time of retrograde flow in PAH patients was significant smaller than those found in non-PH subjects. In our study, the cut off value for ROT of 0.25 distinguished PAH patients from all non-PH subjects (Fig. 7.7a).

Sanz et al. found that of many variables, average velocity ($R^2 = 0.53$) and CSA ($R^2 = 0.42$) related best with pressure [211]. The relation between pressure and ROT is consistent with, but inferior to the relation between pressure and the vortex duration of Reiter et al. ($R^2 = 0.62$ versus $R^2 = 0.88$). The large variation of pressure around the linear fit between ROT and mPAP is a limiting factor for using ROT as an mPAP estimate. The ROT is
visible by standard MR phasecontrast velocity quantification (Fig. 7.4).

Mild retrograde flow is normal in late systole (2% of the total flow), but in PH patients, the retrograde flow is on average 26% of the total flow [8]. Our study confirms that relative retrograde flow was significantly larger in PAH patients than in non-PH subjects (9% versus 1%, \( p < 0.001 \)). Retrograde flow in our study was less than found by Bogren et al. However, our study calculated this figure based on systole only while Bogren et al. considered the whole cardiac cycle. Various investigators have documented this reversal of flow during systole and diastole in patients with PH [182, 8, 112, 171, 200]. Retrograde flow can show one or two peaks during the cardiac cycle. One peak always appears at end-systole and represents valvular regurgitation. In some subjects another peak of retrograde flow appeared at mid systole [112]. This mid systolic peak was not reported later, only the peak representing valvular closing has been detected [171, 200]. Our study, however, clearly shows the mid systolic peak in patients with PAH. The described retrograde flow is presumably a manifestation of a vortex, in patients with PH [200]. The vortex rotates at the same location as where retrograde flow was detected: at the dorsal side of the MPA [182, 112, 171]. Blood flows forward along the vortex at the ventral side of the MPA.

Flow or more precisely the velocity field is always the result of pressure gradients. Just after opening of the pulmonary valves, the pressure in the right ventricle is larger than pulmonary pressure. This pressure gradient causes blood to accelerate. Obviously, blood reaches maximal velocity during systole just before the pressure gradient switches. The highest forward velocity is close to the middle of the vessel, while close to the wall velocity is small. After the moment of maximal forward velocity, the adverse pressure gradient causes blood to decelerate. Because of the large diameter and velocities, flow in the MPA is unsteady (Womersley number > 10). This means that velocity and the pressure gradient are out of phase. While blood in the middle of the vessel still decelerates, blood closer to the wall already accelerates in the other direction toward the ventricle.

The question is why in PAH patients retrograde flow starts earlier. Patients with PH usually have an enlarged MPA. This means flow in PH is more
unsteady (larger Womersley number). Furthermore, if the CSA is much larger than the valve opening then flow will separate into a jet and boundary layer [243]. The boundary layer in an enlarged MPA is expected to be much larger than in a normal MPA. This favors the start of retrograde flow. Furthermore, the pressure gradient possibly switches earlier in PH. Two mechanisms for this early switch of the pressure gradient are proposed. First, because of the conservation of mass, velocity has to slow down as blood proceeds through the widened MPA. The velocity can only change due to pressure gradients. Therefore, the widened MPA adds an adverse pressure gradient. As a result, the total pressure gradient will switch earlier. Second, due to the elastic properties of the vessel wall, a pulse wave travels down the MPA and returns to the ventricle after reflecting in the periphery. The reflected pulse wave adds an adverse pressure gradient to the pressure in the MPA [254]. Because the wave speed is proportional to pressure [160] and inverse proportional to compliance [123], the pulse wave returns faster in PH. Therefore, the pressure gradient will switch earlier.

The shape of the MPA influences the location of retrograde flow. In a curved vessel, the jet tends to flow straight to the outer wall. Therefore, the boundary layer at the inner curve becomes larger while at the outer wall the boundary layer flattens. The large boundary layer at the inner curve is susceptible to retrograde flow and the formation of a vortex (Fig. 7.8) [200].

In conclusion, early onset of retrograde flow in the main pulmonary artery is a characteristic of pulmonary arterial hypertension.
Figure 7.8: Flow in the main pulmonary artery. The illustration shows a cross-section of the MPA in axial direction. Drawn are streamlines, the velocity vectors at the image plane, velocity profiles, and the large boundary layer at the inner curve. Retrograde flow in the boundary layer is part of the vortex.
Chapter 8

Progressive dilatation of the main pulmonary artery is a characteristic of pulmonary arterial hypertension and is not related to changes in pressure

8.1 Abstract

Pulmonary artery (PA) dilatation is one of the consequences of pulmonary arterial hypertension (PAH) and is used for noninvasive detection. However, it is unclear how the size of the PA behaves over time and whether it is related to pressure changes. The aim of this study was to evaluate PA size during follow-up in treated patients with PAH and whether it reflects pulmonary vascular hemodynamics. Fifty-one patients with PAH who underwent at least two right-sided heart catheterizations (RHCs) together with cardiac MRI (CMR) were included in this study. Eighteen patients who had normal pressure at RHC were included for comparison at baseline. From RHC, we derived PA pressures and cardiac output. From the CMR images we derived PA diameter (PAD) and the ratio of the PAD and ascending aorta diameter. The PAD was significantly larger in patients with PAH than in patients without PAH \( p < 0.001 \). A ratio of the PAD and ascending aorta diameter > 1 had a positive predictive value of 92% for PAH. Mean follow-up time was 942 days, and there was a significant dilatation during this period \( p < 0.001 \). The change of the PAD did not correlate with the changes in pressure or cardiac output. A moderate correlation with follow-up time was found \( r = 0.56; p < 0.001 \). A dilatated PA is useful for identifying patients with PAH. However, during patient follow-up, progressive dilatation of the PA is independent of the change in PA pressure and cardiac output and might become independent from hemodynamics.

8.2 Introduction

Pulmonary arterial hypertension (PAH) is a clinical syndrome characterized by an increase in pulmonary vascular resistance (PVR) leading to right-side heart failure and, ultimately, death [157]. Diagnosis early in the course of the disease is difficult because of its nonspecific nature and symptoms, such as dyspnea, exercise intolerance, and fatigue. Several secondary effects of abnormally elevated pulmonary artery pressure (PAP) on right-sided structures, such as pulmonary artery (PA) dilatation and right ventricular hypertrophy, may be
helpful in the diagnosis of PAH. PA diameter (PAD) can be obtained noninvasively and is, therefore, one of the parameters in PAH that has been studied throughout the years. Early investigators found through chest radiography reasonable correlations between right descending PAD and PAP [246]. After the introduction of helical CT imaging, several studies have been performed to measure the PAD and showed that an increased main PAD is a reliable indicator of PAH, especially when the ratio of PAD and ascending aorta diameter (rPAD/AAD) is used [80, 117, 179, 215, 87, 166, 78]. More recently, using cardiac MRI (CMR), various authors showed that PAD or rPAD/AAD is useful in the noninvasive detection of PAH. On the basis of these studies, it can be concluded that PAD or rPAD/AAD is useful in the noninvasive detection of PAH, but correlations of diameter and pressure vary considerably among the studies possibly because of differences in study populations [54, 174, 211, 210, 12].

Although PAP is supposed to be the driving force to dilate the PA, the influence of other parameters, such as time and flow, is unknown. In addition, it is unknown whether the changes in PAP are followed by a similar change in PAD. Therefore, the aim of this study is to investigate whether change in PAD over time reflects changes in pressure, cardiac output (CO), or both in patients with PAH.

8.3 Materials and methods

8.3.1 Study group

This study is part of a large prospective study aimed to evaluate the role of MRI in PAH. This study has institutional research board approval, and all patients gave informed consent to the procedures. Fifty-six patients with PAH were initially included in our study. All had a baseline evaluation before the start of therapy and a follow-up evaluation of therapy at least 8 months after. Each evaluation consisted of a right-sided heart catherization (RHC) and CMR measurement separated on average two days from each other. In five patients, the quality of one of the MRI images did not permit measurement because of respiratory artifacts.
CHAPTER 8. PROGRESSIVE DILATATION

Table 8.1: Treatment of the 51 patients with pulmonary arterial hypertension

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of Patients$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambrisentan</td>
<td>2</td>
</tr>
<tr>
<td>Bosentan</td>
<td>36</td>
</tr>
<tr>
<td>Sitaxentan</td>
<td>11</td>
</tr>
<tr>
<td>Calcium channel blocker</td>
<td>3</td>
</tr>
<tr>
<td>Sildenafil</td>
<td>41</td>
</tr>
<tr>
<td>Epoprostenol (IV)</td>
<td>20</td>
</tr>
<tr>
<td>Treprostenil (subcutaneous)</td>
<td>13</td>
</tr>
<tr>
<td>Iloprost (inhaled)</td>
<td>1</td>
</tr>
</tbody>
</table>

$^a$The total number of treatments exceeds the number of patients, indicating combined treatment or changes in treatment.

Consequently, 51 patients with PAH were included this study. All patients were classified in group 1 of the clinical classification of PAH,[60] with 41 having idiopathic PAH; nine, collagen vascular disease-associated PAH; and one, HIV-associated PAH. During follow-up, patients were treated according to the standard in PAH (Table 8.1). Additionally, for baseline analysis, we included 18 patients suspected of having PAH who underwent RHC and MRI once but had normal PAP at RHC (i.e., normotensive patients).

8.3.2 CMR protocol

In this study, we used CMR images that were part of the routine clinical evaluation of the patients or part of former studies in this center. CMR was performed with either a Siemens 1.5 T Sonata or Avanto (Siemens Medical Solutions; Erlangen, Germany) whole-body scanner equipped with a phased-array body coil. PAD was assessed from magnitude images used for phasecontrast imaging because these images were assessed in all patients in this retrospective study (Fig 8.1). The image plane for measuring the ascending aorta (AA) was determined as follows: A set of coronal localizer images was acquired, and then the image that showed the AA was selected. On this coronal image, a
8.3. MATERIALS AND METHODS

set of axial images was acquired that intersected the AA. From these axial images, the image was selected that showed the AA in a circular cross section. This axial plane was at the level of the right PA. During the follow-up acquisition, position of the orthogonal slice of the main PA at baseline measurements was used for positioning the orthogonal slice at follow-up of the main PA to minimize differences in measurement localization from baseline.

8.3.3 RHC protocol

RHC was performed with a balloon, flow-directed 7 French Swan-Ganz catheter. The patients were in stable condition, lying supine and breathing room air, while heart rate was continuously monitored. Measurements were made of mean right atrial pressure, right ventricular pressure, systolic PAP, diastolic PAP (dPAP), mean PAP (mPAP), and pulmonary capillary wedge pressure (PCWP). CO obtained by either thermodilution or direct Fick method from arterial and mixed venous oxygen saturation and oxygen consumption. Afterward, PVR was calculated as (mPAP − PCWP)/CO. The median interval from CMR to RHC was 2 days (range, 2-21 days).

8.3.4 Image analysis

In the magnitude images, the wall of the PA is automatically detected. A semi-automatic wall detection program, based on a study published by Li et al. [136] using Matlab R2008a (The Mathworks, Inc., Natick, MA) was used to acquire more-accurate measurements of the PA, reduce operator bias, and minimize detection difficulties due to low signal intensity during diastole. Cross-sectional areas (CSAs) were obtained through the entire cardiac cycle (25–60 images) and visually checked by the operator. The minimal CSA was considered the diastolic CSA. From the CSA of this contour, the average diameter was calculated and used in this study. For scaling to aorta size, we measured the AAD in the same way as the PAD at end diastole at the level of the PA bifurcation.

All analyses were performed by one investigator who was unaware of RHC results. Ten patients were randomly selected for repeated measurements by both the first investigator and a second blinded investigator in order to test
Figure 8.1: A-C, Steady-state free precession cardiac MRI (CMR) images to localize the pulmonary trunk (repetition time/echo time, 3.2/1.6 ms; flip angle, 65°; section thickness, 6 mm; matrix, 256 × 96). A, An image was prescribed perpendicular to a transversal view that included the PA. B, An oblique-sagittal image results. C, A third localizer image was acquired according to the straight line in B and used together to obtain the image plane for the throughplane velocity measurement in the main PA. D, The cross section of the main PA acquired as the magnitude image in the flow measurement was obtained with a gradient-echo sequence with through-plane phase-contrast velocity quantification (see text for details). In addition, A shows the plane orthogonal to the AA in which the aortic diameter is measured is used for normalization of the PA size with respect to the aorta. AA = ascending aorta; PA = pulmonary artery.
intra- and interobserver variability.

8.3.5 Statistical analysis

Hemodynamic values are presented as mean ± SD or median (interquartile ranges). Differences between patient groups were evaluated with an independent-sample *t*-test or a Mann-Whitney *U* test when not normally distributed. The ability of the rPAD/AAD to predict PAH was tested using a receiver operating characteristic curve. Differences between baseline and follow-up were evaluated with a paired-sample *t*-test. For analysis of tertile differences, a one-way analysis of variance with post hoc Bonferroni correction was used. Intra- and interobserver agreement was assessed by Bland-Altman analysis. All tests were two-tailed, and a *p* < 0.05 was considered significant. Analyses were performed with statistical software SPSS for Windows, version 15.0.0 (SPSS Inc; Chicago, IL).

8.4 Results

8.4.1 RHC results

RHC measurements confirmed the diagnosis of PAH in the 51 study patients and normal pressures in the 18 normotensive patients. The demographic and hemodynamic characteristics obtained at RHC of both patients groups are shown in Table 8.2. As expected, mPAP, systolic PAP, dPAP, mean right atrial pressure, PVR and heart rate at RHC were significantly higher in patients with PAH than in the normotensive patients. In addition, CO, cardiac index, stroke volume, and mixed venous oxygen saturation were significantly lower in patients with PAH. Between the study groups, there were no significant differences in terms of sex distribution, body surface area, arterial oxygen saturation, or PCWP. The normotensive patients were significantly older than the patients with PAH.
Table 8.2: Baseline hemodynamic and demographic characteristics. Data are presented as mean ± SD or median (interquartile range). Data within parentheses are percentages. The p-value for data between the PAH group and normotensive group is not given if the p-value was not significant (NS).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Total Patients (No.)</th>
<th>Patients with PAH&lt;sup&gt;a&lt;/sup&gt; (No.)</th>
<th>Normotensive patients (No.)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (No.)</td>
<td>69</td>
<td>51</td>
<td>18</td>
<td>...</td>
</tr>
<tr>
<td>Age (y)</td>
<td>44.8 ± 15</td>
<td>41.6 ± 13</td>
<td>53.7 ± 16.9</td>
<td>0.004</td>
</tr>
<tr>
<td>Female patients (No.)</td>
<td>53 (77)</td>
<td>38 (75)</td>
<td>15 (83)</td>
<td>NS</td>
</tr>
<tr>
<td>Body surface area (m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>1.86 ± 0.21</td>
<td>1.90 ± 0.2</td>
<td>1.85 ± 0.16</td>
<td>NS</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>83 ± 15</td>
<td>87 ± 14</td>
<td>73 ± 10</td>
<td>0.003</td>
</tr>
<tr>
<td>Systolic PAP&lt;sup&gt;b&lt;/sup&gt; (mmHg)</td>
<td>66.6 ± 30.3</td>
<td>80.5 ± 21.9</td>
<td>27.1 ± 6.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Diastolic PAP (mmHg)</td>
<td>25.9 ± 13.2</td>
<td>32.2 ± 8.9</td>
<td>8.1 ± 3.7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Mean PAP (mmHg)</td>
<td>42.2 ± 19.1</td>
<td>51.5 ± 12.5</td>
<td>15.9 ± 4.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>PCWP&lt;sup&gt;c&lt;/sup&gt; (mmHg)</td>
<td>8.2 ± 4.7</td>
<td>8.5 ± 5.1</td>
<td>7.3 ± 3.1</td>
<td>NS</td>
</tr>
<tr>
<td>PVR&lt;sup&gt;d&lt;/sup&gt; (dyn·s/cm&lt;sup&gt;5&lt;/sup&gt;)</td>
<td>660 ± 465</td>
<td>837 ± 401</td>
<td>130 ± 77</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Mean right atrial pressure (mmHg)</td>
<td>7.7 ± 5.2</td>
<td>8.9 ± 5.1</td>
<td>3.4 ± 2.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Right ventricular diastolic pressure (mmHg)</td>
<td>9.4 ± 6.9</td>
<td>11.4 ± 6.3</td>
<td>2.0 ± 2.4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Cardiac output (l/min)</td>
<td>5.3 ± 1.8</td>
<td>4.8 ± 1.65</td>
<td>6.7 ± 1.7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Cardiac index (l/min/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>2.9 ± 1.1</td>
<td>2.6 ± 1.0</td>
<td>3.7 ± 1.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Stroke volume (ml)</td>
<td>66.7 ± 27.4</td>
<td>55.8 ± 24.1</td>
<td>92.0 ± 22.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Arterial oxygen saturation (%)</td>
<td>95.6 ± 3.0</td>
<td>95.6 ± 2.6</td>
<td>95.7 ± 4.3</td>
<td>NS</td>
</tr>
<tr>
<td>Mixed venous oxygen saturation (%)</td>
<td>67.1 ± 9.1</td>
<td>65.2 ± 9.0</td>
<td>72.9 ± 6.5</td>
<td>0.002</td>
</tr>
<tr>
<td>PAD&lt;sup&gt;e&lt;/sup&gt; (mm)</td>
<td>31.9 ± 5.8</td>
<td>33.7 ± 5.3</td>
<td>25.0 ± 6.8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>AAD&lt;sup&gt;f&lt;/sup&gt; (mm)</td>
<td>28.3 ± 5.4</td>
<td>27.0 ± 4.4</td>
<td>31.8 ± 6.1</td>
<td>0.02</td>
</tr>
<tr>
<td>rPAD/AAD&lt;sup&gt;g&lt;/sup&gt;</td>
<td>1.16 ± 0.27</td>
<td>1.26 ± 0.22</td>
<td>0.87 ± 0.17</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

<sup>a</sup>Pulmonary arterial hypertension.
<sup>b</sup>PAP = pulmonary artery pressure.
<sup>c</sup>Pulmonary capillary wedge pressure.
<sup>d</sup>Pulmonary vascular resistance.
<sup>e</sup>Pulmonary artery diameter.
<sup>f</sup>Ascending aorta diameter.
<sup>g</sup>Ratio of the PAD and AAD.
8.4. RESULTS

Figure 8.2: Graphs showing the results of regression analysis in the study group for pulmonary artery diameter versus mPAP (A) and the PAD in proportion to the ascending aorta diameter (ratio PAD/AAD) versus mPAP. ○ = normotensive patients; ● = patients with PA hypertension; AAD = ascending aorta diameter; mPAP = mean pulmonary artery pressure; PAD = pulmonary artery diameter. See Figure 8.1 for expansion of the other abbreviations.

8.4.2 PAD and pressure

The diastolic PAD was significantly larger in patients with PAH than in normotensive patients (Table 8.2). In the entire group at baseline, the correlation coefficient between PAD and mPAP was 0.58 \( (p < 0.001) \) (Fig 8.2A). In all patients with PAH, there was only a weak, but significant relation \( (r = 0.29; p = 0.04) \) between PAD and mPAP. A significant difference was found between the rPAD/AAD in the PAH group and normotensive group. In the entire group, the correlation coefficient of rPAD/AAD with mPAP was 0.71 \( (p < 0.001) \) (Fig 8.2B). Again, for patients with PAH, the relation between rPAD/AAD and dPAP was weak \( (r = 0.49; p < 0.001) \). The correlation coefficient of rPAD/AAD with dPAP was 0.69 \( (p < 0.001) \). The area under the receiver operating characteristic curve for the rPAD/AAD in the detection of PAH was 0.93 \( (CI, 0.86–0.99) \) (Fig 8.3). The optimal rPAD/AAD was 1.1, with a sensitivity of 80% and a specificity of 94%. For an rPAD/AAD of 1, we found a sensitivity of 92%, a specificity of 72%, and a positive predictive value of 92%.
Figure 8.3: The receiver operating characteristic curve of the ability of the ratio of PAD and AAD to detect an mPAP of > 25 mmHg. AUC = area under the curve. See Figure 8.1 and 8.2 legends for expansion of the other abbreviations.
8.4.3 Changes of PAD during follow-up

Mean follow-up time was 942 days (range, 242–2359 days). During follow-up, the PAD increased significantly (Table 8.3). In 37 (73%) patients with PAH, the diameter increased (mean, 3 ± 3 mm; range, 0.2 mm to 17 mm). In 11 (22%) patients, the diameter decreased (mean, -2 ± 2 mm; range, -0.2 mm to 5.4 mm). In three patients, the diameter remained unchanged. PVR decreased, and CO increased, both significantly, under treatment during follow-up (Table 8.3). Figure 8.4 shows a plot of the change of PAD against the change in mPAP. Of the 11 patients with a decreased diameter, only three had a decrease > 2 mm. The mPAP in these three patients was normalized or almost normalized under treatment; the mPAP at follow-up ranged from 15 mmHg to 28 mmHg. In patients with an increased PAD at follow-up, the pressure either increased or decreased, and no relation was found between PAD change and pressure change. Figure 8.5 shows that there were no significant differences between tertiles regarding CO, mPAP at baseline, and PAD at baseline. In contrast, diameter change across tertiles of follow-up time differed significantly for the outermost tertiles, whereas the changes in CO and mPAP were not different over the tertiles. Overall, a moderate correlation between follow-up time and diameter change was found ($r = 0.56; p < 0.001$). Neither a relation between changes in PAD and pulse pressure at baseline ($r = -0.21; p = 0.14$) nor a relation between changes in PAD and pulse pressure changes during follow-up ($r = 0.16; p = 0.27$) was found.

8.4.4 Reproducibility of PAD measurements

Although the detection of the PAD is semiautomatic and the program used reduces operator bias, intra- and interobserver variability was checked. Bland-Altman analysis revealed an intraobserver bias of 0.1 ± 0.1 mm and an interobserver variability bias of 0.1 ± 0.2 mm, showing good reproducibility of PAD measurements.
Figure 8.4: Change in pulmonary artery diameter versus the change in mean pulmonary artery pressure (Δ mPAP), indicating the change in pressure and diameter between right-sided heart catheterization and CMR evaluation in 59 patients. Four patients (highlighted) showed a decrease in diameter of more than 2 mm. In all four patients, the pressure was (almost) normalized during follow-up.
8.4. RESULTS

Figure 8.5: Diameter change against the different tertiles of PAD at baseline (A). Mean pulmonary arterial pressure at baseline (B). Changes in cardiac output (C). Duration of follow-up (D). See Figure 8.1 and 8.2 legends for expansion of the other abbreviations.
Table 8.3: Hemodynamic characteristics and PAD at baseline and follow-up of 51 patients. Mean follow-up time was 942 days (range: 224–2359 days). Abbreviations as in Table 8.2.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Follow-up</th>
<th>P-value$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAD (mm)</td>
<td>33.7 ± 5.3</td>
<td>35.7 ± 6.5</td>
<td>0.001</td>
</tr>
<tr>
<td>Mean PAP (mmHg)</td>
<td>51.5 ± 12.5</td>
<td>49.2 ± 13.9</td>
<td>0.15</td>
</tr>
<tr>
<td>Cardiac output (l/min)</td>
<td>4.8 ± 1.65</td>
<td>5.2 ± 1.2</td>
<td>0.005</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>87 ± 14</td>
<td>83.5 ± 12.5</td>
<td>0.13</td>
</tr>
<tr>
<td>Stroke volume (ml)</td>
<td>55.8 ± 24.1</td>
<td>60.0 ± 23.3</td>
<td>0.6</td>
</tr>
<tr>
<td>PVR (dyn·s/cm$^5$)</td>
<td>837 ± 401</td>
<td>730 ± 365</td>
<td>0.026</td>
</tr>
</tbody>
</table>

$^a$Paired-sample t-test.

8.5 Discussion

In this study, we show that PAD and rPAD/AAD are useful in discriminating patients with PAH from patients without PAH. This finding is in line with previous reports. However, during follow-up, most PAs showed progressive dilatation, which is not related to the changes in pressure. Furthermore, we found that the change in PAD was not related to changes in flow.

8.5.1 PAD and pressure

Our results showed that the diastolic PAD has diagnostic value in PAH. We chose the diastolic diameter because it is the customary choice in CT images. The diameters used were averaged diameters derived from the CSA. There was no difference in the relation of rPAD/AAD with dPAP and mPAP, which can be explained by the strong proportional relation between dPAP and mPAP [21, 238]. The mPAP was used in this study because diagnosis customarily is based on it. As seen in prior studies, we found that an enlarged PAD is related to the presence of PAH and, thus, can be used in the detection of PAH. We found that an rPAD/AAD > 1.1 yields the highest diagnostic accuracy. The use of the rPAD/AAD also is recommended because its relationship with PAP is independent of body surface area and sex [179]. This cut-off point of 1.1 is
different from earlier studies that found that a ratio of 1 is the best diagnostic cut-off point. The explanation of this discrepancy might be that these studies used 20 mmHg of mPAP as the diagnostic criterion for PAH instead of the currently used criterion of 25 mmHg [157]. However, if we apply the cut-off point of 1.0, a reasonable sensitivity of 92% and specificity of 72% were found. The diagnostic accuracy of the rPAD/AAD might be overestimated because there is a relatively large hiatus in the levels of mPAP between the normotensive patients and this well-defined group of patients with PAH. In this study group, there were no patients with aortic abnormalities or systemic hypertension, which could affect the size of the AA and clearly could affect the reliability of the rPAD/AAD.

8.5.2 Changes of PAD during follow-up

During follow-up, the PA dilated in most of the patients. The changes of the PAD between the two CMR measurements did not reflect changes in mPAP between the corresponding RHCs. In all the patients in whom the pressure was higher than initially, the PAD had increased. However, if the pressure was lower than initially, the majority of this group’s PADs still increased. Only in three patients did the PAD decrease > 2 mm; in all these patients, the pressure was normalized or almost normalized.

Thus, a progressive dilatation was found in the patients with PAH, which was not explained by pressure changes. Even in the majority of the patients in which the pressure decreased, there was an ongoing dilatation of the PA. Figure 8.5A suggests that a PA with a large diameter at baseline tends toward greater dilatation during follow-up. It seems logical that if dilatation during follow-up becomes independent of hemodynamics, a larger diameter at baseline will lead to larger dilatation during the follow-up. However, this relation was not found to be statistically significant. The finding that neither pressure nor flow are related to the progressive dilatation in most of the patients shows that other explanations than changes in pulmonary hemodynamics underlie this progressive dilatation, a phenomenon well-known from aneurysms of the aorta. Although systemic hypertension is an important underlying cause of
this disease, a further dilatation of the aorta in aneurysms is independent of systemic BP [13]. The main question was to investigate whether during follow-up changes in PA dilatation exist and whether they are related to changes in pressure. For this reason, we did not evaluate specific other hemodynamic parameters as possible explanations for changes in PAD. Nevertheless, we found that the diameter increase did not correlate with age, use of epoprostanol, and pulsatility in PAD.

Absence of a direct relation between changes in pressure or flow and changes in diameter does not exclude that increased PAP or reduced flow is the cause of PA dilatation. Although there is an absence of radiologic studies investigating the structure of the PA wall, recent histologic studies of the proximal parts of the PAs provide evidence that significant remodeling of the proximal PA wall occurs in PAH [111, 120]. Structural changes in elastin and collagen under the influence of an increased PAP might eventually become a cause of PA dilatation, irrespective of changes in pressure and flow. In addition, altered flow in PAH affects wall shear stress and, subsequently, matrix properties of the vessel wall [10] that might lead to PA dilatation.

8.5.3 Clinical implications
Our findings have several clinical implications. First, the PAD, although useful in the diagnosis of PAH, is not useful for follow-up of the disease or evaluation of treatment effects. Second, we show that the dilatation of the PA in PAH is more related to follow-up time than to pressure change, indicating that the PA needs time to dilate. A severe dilated PA at the time of diagnosis thus indicates that the PAH was already present for a long period. Third, our data indicate that although increased PAP leads to dilation of the PA, further dilation is a process most likely due to a change of the intrinsic vessel properties, which is independent of the pulmonary hemodynamics.

8.5.4 Limitations
There are some study limitations that might have influenced our results. First, pressure and CMR measurements could not be performed simultaneously for
8.5. DISCUSSION

logistic and patient reasons and might have affected the strength of the associations we found. However, given the average time of follow-up (942 days), it is unlikely that a median interval of two days between RHC and CMR measurements affected the conclusions. Second, given the conus-shaped main PA, differences in level of image acquisition on the different time points might have created a bias. We tried to overcome this limitation by using an experienced investigator to acquire all CMR images and by using reference points acquired at the baseline measurement during the follow-up measurement.

In conclusion, in patients with PAH, the rPAD/AAD can be used for the detection of PAH. During follow-up, dilatation of the PA does not reflect changes in pressure or flow; therefore, changes in PAD cannot be used in clinical practice for the evaluation of the course of the disease, therapeutic response, or estimation of pressure.
Chapter 9

A numerical model to predict abdominal aortic aneurysm expansion based on local wall stress and stiffness


9.1 Abstract

Aneurysms of the abdominal aorta enlarge until rupture occurs. We assume that this is the result of remodeling to restore wall stress. We developed a numerical model to predict aneurysm expansion based on this assumption.
In addition, we obtained aneurysm geometry of 11 patients from computed tomography angiographic images to obtain patient specific calculations. The assumption of a wall stress related expansion indeed resulted in a series of local expansions, adjusting global geometry in an exponential fashion similar as in patients. Furthermore, it revealed that location of peak wall stress changed over time. The assumptions of this model are discussed in detail in this manuscript, and the implications are related to literature findings.

9.2 Introduction

Abdominal aortic aneurysm is a disease occurring in 5–7% of people over 60 years [52]. The largest problem of this disease is the chance of rupture, which increases with aneurysm diameter. Recent studies provided a mechanical explanation for rupture as it has been shown that the stress at which the aneurysm ruptures is close to the stresses present in advanced aneurysm formation [50, 77, 265].

While those studies highlight the role of local wall stress during the process of rupture, wall stress has also been implicated in vascular remodeling [55, 232]. remodeling, i.e. a structural change of the vessel wall occurs when local wall stress exceeds a reference value [76]. This stress related remodeling has been studied extensively in hypertensive conditions [55, 76].

However, little is known about the remodeling process in aneurysm formation. The vessel wall in a long standing aneurysm consists mainly of acellular material interspersed in abundant extracellular matrix [247]. Consequently, some studies have indicated that the local strength of the aneurysm tissue is determined by the amount of extracellular matrix, which again is affected by the balance between synthesis and breakdown of this matrix [133]. On a cellular level this balance is regulated by the activity of smooth muscle cells, which synthesize collagen, and by macrophages, which synthesize proteases, including matrix metalloproteinases (MMPs) which breakdown extracellular matrix. LOX–1 positive foamy macrophages, which are characteristic for aneurysm formation, produce large amount of MMPs [177]. Numerous studies indicate that the activity of both cell types is affected by wall stress/wall stretch. For
example vascular smooth muscle cells when stretched in vitro, proliferate, synthe-
thesize collagen and MMPs with a small net effect of collagen synthesis [133].
While, macrophages when placed under strain produce MMPs and cytokines
[153, 242]. Hence, both cell types increase the break down of collagen. All
these changes may be summarised as that areas of large stretch/stress leads to
a changed balance of extracellular matrix turnover ("remodeling") resulting
in expansion of the aneurysm (Fig. 9.1). In order to provide evidence for the
feasibility of the concept that a wall stress induced change in the stiffness of
the aneurysm may induce expansion we developed a finite element model that
incorporates this concept. This manuscript describes its design and some first
results.

9.3 Methods

9.3.1 Images

Computed tomography angiographic (CTA) images of eleven patients diag-
nosed with an abdominal aortic aneurysm (AAA) were collected from an ex-
isting database on basis of the presence of AAA and the absence of rupture
[218]. All patients were scanned in the recumbent position without cardiac
triggering on a Siemens multi-slice CT scanner. The image resolution varied
for this group but was in the order of 1 mm × 1 mm × 1 mm.

9.3.2 Segmentation of aneurysms

The segmentation software used a semi-automatic approach. Operator depen-
dent orientation points were placed just below the renal arteries, in both the
left and right common iliac arteries and at the bifurcation point. Based upon
these points a wave front propagation was calculated from the gradient of the
in slice image intensity. When the endpoint of the wave front was reached, the
lumen centreline was reconstructed by back tracking the minimal cost path
of the wave front cost function. Subsequently, a tube was placed around the
centreline consisting of a collection of non-planar simplex faces called 3-D ac-
tive objects. The 3-D active objects deformed iteratively using forces based on
Figure 9.1: Conceptual model underlying the numerical model. The accumulation of inflammatory cells and apoptosis of vascular smooth muscle cells in atherosclerotic tissue leads to multiple weak spots especially at the shoulder of the plaques. Local wall stress peaks may occur, that further increase the activity of inflammatory cells, and endothelial cells to secrete active proteases. Similarly, the vascular smooth muscle cells start to produce collagen and MMPs. Due to the resulting breakdown of the extracellular matrix, the Young’s modulus decreases and this leads to a local bulging of the vessel wall. As a consequence, local wall stress decreases thereby allowing the vessel wall to restore the extracellular matrix at the location of local weakening. However, at remote regions wall stress increases and inflammatory cells are activated, producing proteases and inducing remote local bulging. This cycle repeats itself resulting in global diameter increase.
image features and shape regularization until it reached the vessel wall. This method has been evaluated [36] and maximal diameter could be reproduced with an error below 5\% and maximal wall stress was reproducible within 10\%.

### 9.3.3 Numerical methods

Calculations were done with well-validated commercially available (Sepra, Delft, The Netherlands) finite element methods (FEM) [65, 66, 257]. We deformed a tube shaped mesh with quadrilateral elements to the surface of the 3-D active objects obtained above. Wall thickness was set on two millimeter in the radial direction [50, 49]. The number of nodes in the mesh of the vessel wall was 2280, 19 axial, 24 circumferential and 5 radial. The elements had typical dimensions of $5.5 \text{ mm} \times 5 \text{ mm} \times 0.5 \text{ mm}$ in axial, circumferential and radial directions, respectively. Based upon this finite element mesh, wall stress distribution was calculated treating the tissue initially as homogeneously and linear elastic. Consequently, the mechanical behavior of the tissue could be characterized by a single Young’s modulus of $2 \times 10^6 \text{ N/m}^2$ and a Poisson ratio of 0.45. The lumen boundary was pressurized with the measured difference between systolic and diastolic pressure while both ends of the aneurysm were fixed in all directions.

### 9.3.4 Expansion mechanism

The biological mechanism of aneurysm expansion can be translated to a mechanical description (Fig. 9.2). We proposed that the material properties of the vessel wall were initially in a reference state. In this reference state, wall stress and strain were in a normal range and elasticity was homogeneous. An additional increment in blood pressure stretched the material and due to geometry or wall thickness, some parts were stretched minimally (panel A) while other parts have stretched more extensively (panel B1). With a higher stretch, a larger wall stress is induced, which is associated with a higher protease activity and a larger breakdown of collagen. As a result the Young’s modulus became lower and the material will be stretched even more (panel B2). Due to the local nature of the process, a reduction in Young’s modulus associates with
Figure 9.2: Mechanical description of expansion. The material properties are initially in a reference state. Blood pressure stretches the material (A). Some parts are stretched more due to a thinner wall or less wall curvature (B1). If material is stretched more, stress will be higher. In high stress regions material properties change due to biological activity. The Young’s modulus becomes lower and the material is stretched even more (B2). Because of the lower Young’s modulus, the stress level might easily drop to the reference value and collagen can be built up again. This newly built up collagen is not stretched and mixes with the remaining stretched collagen. Therefore the material properties are the same as the reference (B3). Interestingly before and after the remodeling the material is in a reference state. However the pressurized geometry is adjusted from U1 to U2.

The following protocol was implemented to simulate expansion of the aneurysm. First, the local wall stress and local deformation field were calculated in a patient-specific 3-D AAA geometry. Second, if the wall stress locally exceeded $3 \times 10^4$ N/m$^2$, the Young’s modulus was locally lowered by 25%,
9.3. METHODS

Figure 9.3: Flow chart of the expansion mechanism. After obtaining a patient-specific geometry with CTA, a mesh was generated. Local wall stress and displacements (U1) were calculated. If the wall stress exceeded a predefined threshold level, the young’s modulus was lowered locally by 25%. With these heterogeneous conditions, new displacements (U2) were calculated. The difference between the displacements was taken as the expansion and used to reshape the mesh of the aneurysm.

mimicking collagen breakdown by proteases and the calculations were repeated with variable stiffness. The difference in the deformation from the homogeneous and heterogeneous calculations was used to reshape the mesh of the aneurysm. In this way we updated the mesh iteratively simulating the process in time (Fig. 9.3). The simulations were stopped if the calculations exceeded one hundred iterations or if maximal diameter exceeded 10 cm. These stopping criteria were applied to all simulations in this article.

9.3.5 Quality of 3-D geometry: Operator dependence

To create a mesh with a realistic 3-D geometry of an aneurysm, the operator had to select four initial orientation points. This may induce operator dependency, which was evaluated by two operators who created meshes for five
patient-specific aneurysms. Differences between operators were quantified, by calculating for each aneurysm the maximal absolute and relative difference in diameter measured in anterior posterior direction during the entire dilation process.

9.3.6 Quality of wall stress/deformation calculations

With FEM the accuracy of the calculations depends on several factors, including the mesh density. A higher density results in a better accuracy at the cost of more computation time. Therefore, the mesh density should be just high enough to ensure small errors compared with other uncertainties, while keeping calculation time within predefined acceptable limits. To test the influence of the mesh density we simulated expansion at mesh resolutions with $19 \times 24 \times 5$, $31 \times 32 \times 5$ and $43 \times 40 \times 5$ nodes. These meshes were constructed in such a way that there were $7 \times 8 \times 5$ nodes in both meshes with initially the same coordinates. We compared for each of these nodes the difference in position during the expansion process.

9.3.7 Change of reference parameters: Sensitivity analysis

The expansion mechanism was based on a step like change in elasticity, if the stress locally exceeded a threshold level of $3 \times 10^4 \text{ N/m}^2$ the Young’s modulus was locally lowered by 25 percent. As we could not find any literature regarding the choice of the actual values, we performed a detailed sensitivity analysis to evaluate the effect of changes in these parameters. For three patient-specific aneurysms we performed simulations with threshold stress levels of $2.0 \times 10^4$, $2.5 \times 10^4$, $3.0 \times 10^4$, $3.5 \times 10^4$ and $4.0 \times 10^4 \text{ N/m}^2$ keeping the Young’s modulus at $1.5 \times 10^6 \text{ N/m}^2$. Likewise we performed simulations with local Young’s moduli of $1.0 \times 10^6$, $1.2 \times 10^6$, $1.4 \times 10^6$, $1.6 \times 10^6$ and $1.8 \times 10^6 \text{ N/m}^2$ keeping the threshold at $3.0 \times 10^4 \text{ N/m}^2$. To quantify the results, we fitted an exponential function of the following form to the data:

$$d = c_1 + c_2 \cdot e^{(c_3 \cdot t)}$$  \hspace{1cm} (9.1)
In this equation $d$ is the diameter, $t$ is time and $c_1$, $c_2$ and $c_3$ are constants. The inverse of $c_3$ is the time constant. The time constant is the amount of time it takes for the aneurysm to increase a factor $e$ in diameter. We took the time constant as a measure for expansion rate.

### 9.3.8 Influence of initial 3-D geometry on expansion rate

One of our main goals was to evaluate whether the AAA expansion rate depends on the initial 3-D geometry. We therefore simulated expansion curves for eleven patientspecific aneurysms. To evaluate effects of 3-D geometry on expansion rate, we compared time constant with aneurysm length.

### 9.4 Results

#### 9.4.1 Quality of 3-D geometry: Operator dependence

The largest absolute difference in diameter during simulated exponential expansions between two operators was $3.2 \pm 2.1$ mm ($4.2 \pm 2.8\%$). One operator systematically created shorter meshes for the aneurysms of $13.1 \pm 1.0\%$, partially explaining these differences.

#### 9.4.2 Quality of wall stress/deformation calculations

Comparing the $19 \times 24 \times 5$ mesh with the $31 \times 32 \times 5$ mesh resulted in a maximal $0.37$ mm difference in position during the expansion simulations. Between the $31 \times 32 \times 5$ mesh and $43 \times 40 \times 5$ mesh the differences were even smaller. These errors were much less than those induced by operator dependence (see above) and were small given the error in diagnostic uncertainty. To save computation time we chose the lowest mesh density for further calculations.
CHAPTER 9. A NUMERICAL MODEL

Figure 9.4: Sensitivity of the exponential expansion rate to changes of the Young’s modulus. The expansion model assumed a step like change in Young’s modulus after stress exceeded a threshold level. The reference Young’s modulus was $2 \times 10^6$ N/m$^2$. The threshold stress level was set at $3 \times 10^4$ N/m$^2$. The effect of the lowered Young’s modulus on expansion rate was tested. In the figure the time constant (in loops) was plotted against the lowered Young’s modulus. A small time constant indicates a large expansion rate. The relation between lowered Young’s modulus and time constant is different for each aneurysm.

9.4.3 Change of reference parameters: Sensitivity analysis

For all three patient-specific aneurysms, variation in threshold stress affected the time constant less than variation of the Young’s modulus. The largest change in time constant over the threshold range of $2.0 \times 10^4$, $2.5 \times 10^4$, $3.0 \times 10^4$, $3.5 \times 10^4$ and $4.0 \times 10^4$ N/m$^2$ was 8.2%, while the time constant varied from 15.5 to 28.3 months for a Young’s modulus of $1.0 \times 10^6$ N/m$^2$ and from 156.6 to 256.9, for a Young’s modulus of $1.8 \times 10^6$ N/m$^2$ (Fig. 9.4).
Figure 9.5: Progression of aneurysm expansion and peak stress drift. In this figure the state of two aneurysms was displayed at different time points. The panels show the aneurysm for $t = 0, 20, 40, 60$ and $80$ loops. Over time, the diameter of the aneurysm increased. The aneurysm became more spherical during expansion indicating a large amount of dilating areas. The location with the largest stress changed during expansion. The gray scale indicates the relative stress level.

### 9.4.4 Influence of initial 3-D geometry on expansion rate

The progression of aneurysm formation of two representative patients is displayed in Figure 9.5. Note that the locations of peak stress changed over time and were patient-specific. All simulations exhibited an exponential relation of maximal anterior-posterior diameter versus time ($\text{RMSE} = 0.40 \pm 0.28 \text{ mm}$). The time constant, which characterizes exponential curves, varied from patient to patient by a factor of three. This was related ($R^2 = 0.53$) to length of the aneurysmal sac (Fig. 9.6).
Figure 9.6: Aneurysm expansion rate as a function of initial aneurysm length. In this figure the time constant was plotted against the aneurysm length. The time constant was lower for longer aneurysms ($R^2 = 0.53$). Two points in the plot were regarded as outliers.
9.5 Discussion

In this study, we developed a framework to provide evidence that wall stress induced changes in aneurysm stiffness might be associated with aneurysm expansion. The first simulation indicated that this mechanism indeed occurs. Furthermore, it produced exponential expansion similar as found in patients [89].

In order to evaluate the mechanism we developed a patient-specific numerical approach. Patient-specificity depended upon the quality of the 3-D reconstructions and their meshes. Accuracy of 3-D reconstructions was evaluated by comparing the results of two independent operators. The simulated diameters by these different operators were in reasonable agreement considering diagnostic uncertainty of 2 mm as reported in literature [13]. Furthermore, mesh density is important for the accuracy of numerical calculations. The variation of mesh density showed differences in aneurysm geometry that were much less than those induced by operator dependence and were small given the error in diagnostic uncertainty. The calculations of expansion were more sensitive for the reduction in wall stiffness than for the used threshold stress level. The reason for this observation is that wall stress in aneurysms is so high that for each threshold stress level, local wall stress was above reference level in most locations.

Very interestingly the simulations revealed that locations of peak wall stress changed over time. While this is a consequence of a first approach with many assumptions (see below), it implies that methods aiming at prediction of location and time of aneurysm rupture should probably take this mechanism into account. Although the remodeling mechanism acted locally and no assumption of exponential expansion was introduced into the model, all simulations exhibited an exponential relation between diameter and time. This behavior is in agreement with the experimental study of Brady [13] and Schouten [218]. This exponential relation was also found for echo derived maximal diameters measurements [263]. The expansion rate was related to the length of the aneurysm. Longer aneurysms tended to expand faster than shorter ones. This effect has already been reported by Hatakeyama [85] who found a correlation
Several assumptions needed to be included in the model to perform these calculations. Simple linear relations were assumed in the numerical model. While this is a gross simplification, the aim of this study was to focus on a wall stress driven expansion mechanism. Non-linearity will probably change the expansion mechanism and this is necessary to include after comparing this approach to real measurements.

The patients were scanned without cardiac triggering. As a result the initial geometry was averaged over the cardiac cycle and represented the aneurysm at average pressure. Furthermore, Computer Tomography images are not suited for accurate wall thickness determination and tissue characterization. The main problem is not lack of resolution but insufficient contrast between wall and surrounding tissue. Therefore, we used a constant wall thickness and a constant Young’s modulus for the initial mesh. As local wall stress depends on wall thickness and material properties, the calculations will benefit if detailed wall thickness values and tissue characteristics could be retrieved.

We did not apply pressure on the outer wall of the aneurysm. Surrounding organs, muscles and bones should result in some pressure on the aneurismal sac. Boundary conditions could be applied to represent these effects to some extent in the near future.

The threshold stress level in our model was set at $3.0 \times 10^4$ N/m$^2$. This value was based on healthy blood vessels. We are unaware of any information regarding the threshold stress levels in atherosclerotic vessels or aneurysms and consider this assumption as a first approach for setting up these models.

We further assumed that if wall stress exceeded the threshold level, the Young’s modulus was locally lowered by 25%, mimicking breakdown of the extracellular matrix by high protease activity. We do not know, however, how much the Young’s modulus would decrease as a result of extracellular matrix breakdown. Hence, we determined the stiffness variation in the aneurysmal wall from literature [248], which was in the order of 25–30%. According to the present framework this variation is due to vascular remodeling and a maximal range of 25% was realized in our model.

We assumed that aneurysm tissue exhibited a linear stress-strain relation-
ship, while many studies have shown biological tissue to be non-linear. The assumption was considered not far from reality, as deformation in each iteration was relatively small. Hence, tissue could be described with an incremental linear elastic modulus during those conditions.

Furthermore, we assumed that remodeling aims at restoring its original tissue stress and actually restores tissue strain. Thus at the beginning of each iteration tissue stress was homogeneous, while strain and stiffness were at a reference level. The additional blood pressure during the cardiac cycle leaded to stress differences and additional deformation. Expansion occurred by repeating a sequence of steps over time. These iterations are not directly related to time and a constant factor between time and iteration may be missing.

9.6 Conclusion

In conclusion, we have developed a first simplified model that can describe aneurysm expansion based on a stress regulating mechanism. The simulations with this model showed exponential expansion of aneurysms and revealed that location of peak wall stress drifts over time. The next step is incorporation of risk factors to get a multi factorial prediction model. New studies are necessary to validate the outcome of this model.
Chapter 10

Predicting patient-specific expansion of abdominal aortic aneurysms


10.1 Abstract

Objective: Local anatomy and the patient’s risk profile independently affect the expansion rate of an abdominal aortic aneurysm. We describe a hybrid method that combines finite element modelling and statistical methods to predict patient-specific aneurysm expansion. The 3-D geometry of the aneurysm was imaged with computed tomography. We used finite element methods to calculate wall stress and aneurysm expansion. Expansion rate was adjusted by risk factors obtained from a database of 80 patients. Aneurysm diameters predicted with and without the risk profiles were compared with diameters
measured with ultrasound for 11 patients. For this specific group of patients, local anatomy contributed 62% and the risk profile 38% to the aneurysmal expansion rate. Predictions with risk profiles resulted in smaller root mean square errors than predictions without risk profiles (2.9 mm versus 4.0 mm, \( p < 0.01 \)). This hybrid approach predicted aneurysmal expansion for a period of 30 months with high accuracy.

### 10.2 Introduction

In current clinical practice, the chance of rupture of abdominal aortic aneurysm (AAA) is estimated based on maximum aortic diameter only [52], with 55 mm or more being the generally accepted cut-off point for consideration of elective repair. In a study by Lederle et al. however, a significant number of patients with a maximal AAA diameter larger than 55 mm never experienced a rupture [124], whereas, in another study, rupture did occur in aneurysms smaller than 55 mm [14].

Due to these observations, several new criteria for the decision of elective surgery have been proposed [49, 109]. These newer criteria are derived from the assumption that rupture occurs when tissue stress exceeds a critical level [77]. Hence, several laboratories have developed methods to estimate the chance of rupture on basis of wall stress distribution and failure stress [261, 267]. Recent studies indicate that even peak wall stress alone is a better predictor of rupture than maximal diameter [49, 109].

The rate of aneurysm expansion has been studied often [13], because this factor determines surveillance interval and time to intervention. If aneurysmal expansion in a 6–12 month period is much greater than expected, the risk of rupture may also be higher. The expansion rate depends on several modulating factors including smoking, diabetes and gender [13, 164]. Recently, we published that local remodeling of the vessel wall, which is induced when local wall stress deviates from a reference value, might be an important and yet underrated factor [88]. The aim of the present study is to extend the above-mentioned wall stress concept by including aneurysmal expansion. Towards this end, a hybrid model consisting of both a wall stress remodeling rule
and risk factors is proposed and tested to predict patientspecific aneurysm expansion.

10.3  Methods

10.3.1  Patient population and screening protocol

Between January 2001 and 2006, all patients who presented with an identified AAA in our hospital were screened for cardiac risk factors, renal failure, chronic obstructive pulmonary disease (COPD), smoking, diabetes mellitus and symptoms of other peripheral disease. For each patient, a radiologist or a trained sonographer measured the maximal diameter of the aneurysm by ultrasonography every 6–12 months. The selected patient group was partitioned into a test group (n = 80) for building a risk model and a validation group (n = 11) for comparison of patientspecific predictions with measurements. Subjects in the validation group were scanned with computed tomography angiography (CTA) and at least three times with ultrasonography during follow-up. To test for homogeneity between the test and validation groups, data were compared with a \( t \)-test for continuous variables and with a chi-square test for binary variables.

10.3.2  Segmentation of aneurysm

Angiographic images were acquired with a Siemens multislice computed tomography (CT) scanner. An operator processed these images and located the renal arteries, the left and right common iliac arteries and the aortic bifurcation. Based on these landmarks, a virtual tube was positioned in the aorta starting at the renal arteries and ending at both iliac common arteries. Subsequently, the tube deformed iteratively until it matched the borders of the lumen. The operator inspected the quality of segmentation. If necessary, a few parameters could be adjusted to optimise the segmentation. This technique has been described in detail elsewhere and has a reproducibility of 3–5\% [36].
10.3.3 Numerical methods

Initial conditions

We calculated local wall stress with commercially available finite element method (FEM; Sepran, Sepra, Delft, The Netherlands) [65, 66, 257]. Local wall stress depends on the geometry of the aneurysm, local tissue properties and blood pressure. The geometry of the aneurysm was represented by a finite element mesh. We set the wall thickness of the mesh at 2 mm in the radial direction as others have done [50, 49]. After creating a finite element mesh, wall stress distribution was calculated treating the tissue initially as homogeneous and linearly elastic. Due to homogeneity, the tissue could be characterised by a single stiffness value of $2 \times 10^6$ N/m$^2$ [88]. The lumen boundary was pressurised with $5 \times 10^3$ N/m$^2$, while both ends of the aneurysm were clamped in all directions. The initial geometry corresponded to an aneurysm that in reality was already pressurised. Therefore, the applied pressure was, in fact, only the change in pressure during the cardiac cycle. The calculated wall stress was therefore the change in stress over the cardiac cycle. The intra-luminal thrombus was neglected in the model. The stiffness of thrombus is an order of magnitude lower than the stiffness of the aneurysm wall [64]. Thus, thrombus does not play an important role in bearing the load. Furthermore, thrombus is porous for fluid. Therefore, blood pressure acts directly on the aneurysm wall as implemented in the model. No pressure was applied to the outer boundary. Further details of this method have been described before [88].

Mechanism of expansion

We propose the following mechanism for aneurysm expansion. Initially, the aneurysm wall is in a reference state, where wall stress, wall strain and elasticity are homogeneous. An increment in blood pressure will now stretch the wall and elevate the wall stress. However, due to the complex geometry of the aneurysm, the wall strain and wall stress vary locally. We assumed that in areas with high wall stress the tissue stiffness decreases and, as a consequence, the wall will stretch further. As a consequence, the wall will curve locally to a greater extent, and this leads to a relief of wall stress, enabling collagen fibres
10.3. METHODS

This remodeling enables the vessel wall to return to its reference state, while the pressurised geometry is adjusted.

The following protocol was implemented in our model to simulate expansion of the aneurysm as described above. First, the local wall stress and deformation were calculated for the aneurysm. Second, if the local wall stress exceeded \( 3 \times 10^4 \text{ N/m}^2 \), the stiffness of the wall decreased locally; the calculations were repeated with variable stiffness. The difference in the deformation from the homogeneous and heterogeneous calculations was used to reshape the mesh of the aneurysm.

**Rate of expansion**

The rate of expansion is commonly expressed in mm per year. A given increase in diameter is relatively larger for a small aneurysm than for a large aneurysm. Thus, it is more convenient to look at the amount of time needed for the aneurysm to enlarge by a certain factor. In this study, we therefore expressed expansion rate in months. It is the amount of time needed to become 2.71 (Euler’s number) times larger.

**10.3.4 Risk profiles from the test group**

Recently, risk factors have been identified [124, 13, 218] as important modifiers of the rate of expansion of AAA, independent of local wall stress. We determined the contribution of all factors displayed in Table 10.1. Thus, we compared aneurysm expansion in subjects who had a clinical risk factor with aneurysm expansion in subjects who were free of this risk factor. Only significant factors were included in the final model (Table 10.2). All statistics were performed with SPSS.

**10.3.5 Conversion from model iterations to time**

For the validation group, we simulated aneurysm expansion without the risk factors. The average expansion rate found in the simulations was assumed to be equal to the average expansion rate obtained from the risk model. From this comparison, we retrieved the time period of every iteration.
10.3.6 Patient-specific expansion predicted from the model

We varied the percentage with which the wall stiffness was lowered in elevated stress areas. In this way, we obtained, for each patient, a relation between wall stiffness and expansion rate. The change in expansion rate due to the risk profile could therefore be transferred to an adjustment in wall stiffness.

10.3.7 Validation of the patient-specific model predictions

To validate the model, we compared the measured diameters with simulated maximum aortic diameters in the validation group. We calculated the absolute and relative difference in diameter. We calculated root mean square errors (RMSEs) to see if predictions with risk profile are more valid than predictions without risk profile.

10.3.8 Model predictions for single patients

We varied the risk factors for single patients and studied the effect on the aneurysmal expansion rate for that patient. In addition, time to intervention, which signifies the time necessary for maximal diameter to reach 55 mm, was calculated for patients of the validation group in the absence and presence of their specific risk profiles.

10.4 Results

10.4.1 Patient population

The characteristics of the patients are displayed in Table 10.1. Partitioning of the data resulted into a test group and a validation group that were similar to each other, with the exception of statin usage (Table 10.1).
Table 10.1: Demographic data and risk factors of the patient population divided in a test group and a validation group.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Test group</th>
<th>Validation group</th>
<th>P-value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=80</td>
<td>n=11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender (m/f)</td>
<td>70/10</td>
<td>11/0</td>
<td>0.13</td>
</tr>
<tr>
<td>Age (years)</td>
<td>67.8 ± 8.4</td>
<td>67.6 ± 7.1</td>
<td>0.93</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>13.8</td>
<td>5.9</td>
<td>0.38</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>46.3</td>
<td>47.1</td>
<td>0.95</td>
</tr>
<tr>
<td>Peripheral arterial disease (%)</td>
<td>17.5</td>
<td>5.9</td>
<td>0.23</td>
</tr>
<tr>
<td>Cerebrovascular accident (%)</td>
<td>10.0</td>
<td>11.8</td>
<td>0.83</td>
</tr>
<tr>
<td>Ischemic heart disease (%)</td>
<td>47.5</td>
<td>52.9</td>
<td>0.69</td>
</tr>
<tr>
<td>Pulmonary disease (%)</td>
<td>81.3</td>
<td>76.5</td>
<td>0.66</td>
</tr>
<tr>
<td>Nitrates (%)</td>
<td>20.0</td>
<td>17.6</td>
<td>0.83</td>
</tr>
<tr>
<td>Statins (%)</td>
<td>30.0</td>
<td>58.8</td>
<td>0.02</td>
</tr>
<tr>
<td>Prednisone (%)</td>
<td>6.3</td>
<td>5.9</td>
<td>0.96</td>
</tr>
<tr>
<td>Atrovent (%)</td>
<td>12.5</td>
<td>17.6</td>
<td>0.58</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>81.3</td>
<td>64.7</td>
<td>0.48</td>
</tr>
<tr>
<td>Chronic obstructive pulmonary disease (%)</td>
<td>31.3</td>
<td>35.3</td>
<td>0.75</td>
</tr>
<tr>
<td>Myocardial infarction (%)</td>
<td>35.0</td>
<td>35.3</td>
<td>0.98</td>
</tr>
<tr>
<td>Deccordi (%)</td>
<td>7.6</td>
<td>17.7</td>
<td>0.06</td>
</tr>
<tr>
<td>Angina pectoris (%)</td>
<td>22.5</td>
<td>23.5</td>
<td>0.93</td>
</tr>
</tbody>
</table>

<sup>a</sup>If $p > 0.05$ the difference between the prediction and the test group can be Ignored.
Table 10.2: Risk factors that influence expansion rate.

<table>
<thead>
<tr>
<th>Factor</th>
<th>R-square $^b$</th>
<th>P-value $^c$</th>
<th>Expansion rate $^d$ (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.66</td>
<td>0.00</td>
<td>196</td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>0.01</td>
<td>0.00</td>
<td>-40</td>
</tr>
<tr>
<td>Nitrates</td>
<td>0.01</td>
<td>0.00</td>
<td>+80</td>
</tr>
<tr>
<td>Peripheral arterial disease</td>
<td>0.01</td>
<td>0.01</td>
<td>+40</td>
</tr>
<tr>
<td>Gender</td>
<td>0.004</td>
<td>0.04</td>
<td>+72</td>
</tr>
</tbody>
</table>

$^a$These factors were found with a multivariate linear regression using a stepwise method performed on the test group ($n = 80$).

$^b$R-square indicates the proportion of variation in the corresponding factor explained by the regression model.

$^c$If $p < 0.05$, the null hypothesis is rejected.

$^d$This indicates the progression of aneurysm formation without the influence of any known factors.

10.4.2 Risk profiles from the test group

In our study, only few risk factors influenced the expansion rate (Table 10.2). It is clear from the last column in Table 10.2 that ischemic heart disease accelerated AAA expansion, while being male, peripheral arterial disease (PAD) and nitrates decelerated AAA expansion in our population.

10.4.3 Patient-specific expansion predicted from the model

Expansion rates ranged from 142 to 420 months (Fig. 10.1). With our approach, we were able to study the separate contributions of 3-D geometry and risk profile to the variability of the expansion rate. Simulations in the absence of a risk profile resulted in an expansion rate of $196 \pm 64$ months. Here, the variety in expansion rate was caused by differences in the geometry of the aneurysm. The statistical risk model showed an expansion rate of $254 \pm 39$ months for patients in the validation group. We calculated from these data that geometry contributed $62\%$ and the risk profile contributed $38\%$ to the variation in expansion rate. The regions of wall stress exceeding the thresh-
10.4. RESULTS

Figure 10.1: Simulations of maximal aneurysm diameter for individual patients versus duration of disease ($\Delta$ time). Note the difference in expansion rate for different patients.

old value are displayed for one aneurysm in Figure 10.2. It can be seen that locations of high wall stress shifted during aneurysm formation.

10.4.4 Validation of the patient-specific model predictions

The average difference between simulated and measured diameter was $3.7 \pm 3.1$ mm or $8.3 \pm 6.3\%$ of maximal diameter. Absolute and relative differences in diameter were independent of time (Fig. 10.3). Further analysis showed that incorporation of risk profiles resulted in a significantly smaller RMSE between prediction and measurement than simulations without risk profiles ($2.9$ mm versus $4.0$ mm, $p < 0.01$).
Chapter 10. Predicting Expansion

Figure 10.2: Wall stress calculations for a single aneurysm over time. Wall stress exceeding the threshold is displayed in red. Note that the locations of high wall stress shift with time.

Figure 10.3: The differences between simulated and measured maximal diameters were plotted versus time (Δt) in absolute terms (panel A; mm) and relative to echo diameter (panel B; %). Note that the errors are independent of time.
10.5 DISCUSSION

Figure 10.4: Effect of changes in risk profile on aneurysmal expansion rates for two patients. Displayed is the maximal diameter versus time ($\Delta t$). Circles are the expansion rate without risk factors; triangles the expansion rate in the presence of heart disease; squares the expansion rate in the presence of peripheral arterial disease and diamond the expansion rate in the presence of nitrates. Note that each risk factor has a different effect on expansion rate and that two patients differ in their responses.

10.4.5 Model predictions for single patients

To demonstrate applications of this model, we evaluated the effect of adding single risk factors to the expansion rate of two patients. One can see the differences in expansion rate between omitting medical intervention (i.e., nitrates) and the presence of ischemic heart disease for individual patients (Fig. 10.4). In addition, the risk profiles changed time to intervention significantly and varied strongly from patient to patient (Fig. 10.5).

10.5 Discussion

Patient-specific diagnostics and therapies is a field of large interest. Patient-specific predictions of aneurysm expansion might lead to improvement in surgical management of aneurysms. If predictions of aneurysm expansion can be combined with risk of rupture, then models can be developed to predict
Figure 10.5: Time to intervention was plotted versus initial diameter for individual patients. The line represents the averaged time to intervention in absence of risk factors. In general, a larger initial diameter decreases the time to intervention. The risk profiles changed the time to intervention and varied from patient to patient.
10.5. DISCUSSION

moment of rupture. In a recent article, we introduced the concept that vessel wall remodeling affected aneurysm expansion rate [88]. In the present study, we explored this method to predict patient-specific expansion rates enabling to validate the method against patient-specific measurements. For this purpose, the existing model was extended with clinical risk factors. This hybrid approach resulted in accurate prediction of maximal diameter growth over a period of 36 months.

The current approach enabled to evaluate the effect of several factors on expansion rate. First, we evaluated the role of remodeling and risk profiles on the variability of inter-individual expansion rates. It could be shown that in our small data set, remodeling accounted for two-thirds, while risk profiles accounted for one-third of this variation. This might imply that remodeling is a more important factor in determining 3-D aneurysm geometry. Second, we evaluated the individual effect of risk factors on expansion rate for single patients. The simulations indicate that changes in drugs usage may affect the expansion rate of the aneurysm. The present approach may calculate the benefit of interventions for a single patient. Third, the simulations also predicted that, during expansion, high-stress regions shifted. This was due to the proposed remodeling law that adjusted the stress distribution. This prediction is essential for further validation of the current model.

We only had 80 subjects to find the relevant risk factors. Thus, our study might have identified other factors than those found by others. In our study, expansion rate depended on the usage of nitrates, gender, symptomatic PAD and ischemic heart diseases. In several other studies gender was also found to be a risk factor [164, 218]. A study by Brady revealed that aneurysm expansion was lower in those with low ankle/brachial pressure index [13]. This might support our finding that PAD reduced expansion rate. Brady further found that growth rate was lower in those with diabetes and higher for current smokers. It seems reasonable that medical intervention such as usage of nitrates can influence the expansion rate. A study by Schouten already showed that statins are associated with a reduced aneurysm growth [218].
10.5.1 Limitations of methods

The validation group was selected on the basis of at least three diameter measurements with ultrasonography. As more follow-up visits are expected when an aneurysm expands slowly, we might have identified a validation group with relatively slow expansion rates.

The model, based on CTA, was validated with diameters measured with ultrasonography. These might differ from CTA-derived diameters. Validation using series of CTA measurements may be more appropriate and are necessary for model improvements. Furthermore, CT images are not suited for accurate wall thickness determination and tissue characterisation. The main problem is insufficient contrast between the wall and the surrounding tissue. Therefore, we used a constant wall thickness and a constant stiffness for the initial mesh. Both parameters can have much variation and the assumed values may be far from reality. As local wall stress depends on wall thickness and material properties, the calculations will benefit if detailed wall thickness values and tissue characteristics could be retrieved. We did not apply pressure on the outer wall of the aneurysm. Surrounding organs, muscles and bones should result in some pressure on the aneurismal sac. To implement the boundary conditions well, the surrounding tissues should be characterised. Although this might be difficult and laborious, this is really a recommendation for future work. This is because our study shows the importance of the geometry in aneurysm expansion and the surrounding tissues most likely have an influence on the geometry. We neglected the intra-luminal thrombus because, mechanically, its contribution to load-bearing is small. However, thrombus can reduce peak wall stress [269, 230]. It should be noted that thrombus might also influence aneurysm expansion by a biological mechanism. The influence of thrombus on aneurysm expansion is more likely by this biological mechanism than by mechanics [230]. We assumed that in areas with high wall stress, the tissue stiffness decreases and, as a consequence, the wall will stretch further. This assumption was based on in vitro studies. Those studies have shown that high wall stress is associated with a higher protease activity and a larger breakdown of collagen [133, 153, 242]. There is still no proof that in vivo proteolytic enzymes usually associated with aneurysm wall degradation are increased by wall stress, neither
in animal models nor in human aneurysms. The risk profiles were based on 80 patients in the database of the Erasmus MC, which is comparatively low. A larger follow-up study is warranted for strengthening the present study.

10.6 Conclusion

In conclusion, we have introduced an approach to incorporate risk factors in a numerical model to predict aneurysm expansion. This hybrid approach predicted expansion rate accurately, and may help to predict modifications of drug treatment and changes in lifestyle on patient-specific expansion rates.
Chapter 11

Conclusion

Although the remodeling of large arteries has been extensively studied in the past few decades, the remodeling adaptation to pathology has remained relatively unexplored. In this work, the remodeling and disease progression of the abdominal aorta and the main pulmonary artery have been presented and discussed in detail. In the next paragraphs I will summarize our findings (section 11.1). Then practical applications and implications will be discussed in section 11.2 followed by recommendations for future research (section 11.3).

11.1 Summary

In chapter 2 we evaluated the reference level of wall shear stress. Wall shear stress is an important determinant of vascular function. It is generally assumed that wall shear stress remains constant at a reference value of $\sim 15 \text{ dyn/cm}^2$. In a study of small rodents, we realized that this assumption could not be valid. We presented an overview of recent studies in large and small animals where shear stress was measured, derived from velocity measurements or otherwise, in large vessels.

The data show that large variations exist within a single species (human: variation of 2–16 N/m²). Moreover, when we compared different species at the same location within the arterial tree, an inverse relationship between
animal size and wall shear stress was noted. When we related wall shear stress to diameter, a unique relationship was derived for all species studied. This relationship could not be described by the well-known $r^3$ law of Murray [172], but by the $r^2$ law introduced by Zamir et al. [280]. In summary, by comparing data from the literature, we have shown that: (i) the assumption of a physiological wall shear stress level of $\sim 15$ dyn/cm$^2$ for all straight vessels in the arterial tree is incorrect; (ii) wall shear stress is not constant throughout the vascular tree; (iii) wall shear stress varies between species; (iv) wall shear stress is approximately inversely related to the vessel diameter. These data support an “$r^2$ law” rather than Murray’s $r^3$ law for the larger vessels in the arterial tree.

In chapter 3 we reviewed literature and described the complicated role of shear stress in atherosclerosis. Shear stress has been shown to play a role in plaque induction, plaque progression and plaque rupture. The mechanism for plaque induction seems to differ from the role of shear stress for plaque rupture, whereby the former mechanism is induced by low shear stress and the latter by high shear stress.

It is clear now that low or oscillatory shear stress is pro-atherogenic and induces plaques through an inflammatory mechanism. Furthermore, high shear stress may induce vulnerable plaques by the production of reactive oxygen species and oxidized LDL upstream of plaques. The mechanism is unknown but may relate to the activation of transcription factors in endothelial cells.

In chapter 4 we studied the spatial relation between histological markers in atherosclerotic plaques. Recent studies provided evidence for a predominant upstream location of plaque inflammation. We introduced a novel technique that evaluates the underlying mechanism of this spatial organization. In hypercholesterolemic rabbits, atherosclerosis of the infrarenal aorta was induced by a combination of endothelial denudation and a high-cholesterol diet. At the time of death, aortic vessel segments were dissected and reconstructed with a new technique that preserved the original intravascular ultrasound-derived lumen geometry. This enabled us to study the spatial relation of histological markers like macrophages, smooth muscle cells, lipids, gelatinolytic activity, and oxidized LDL.
11.1. SUMMARY

There was a predominant upstream localization of macrophages and gelatinase activity. Colocalization studies indicated that gelatinase activity was associated with macrophages and smooth muscle cells. Further analysis revealed that this was caused by subsets of smooth muscle cells and macrophages, which were associated with oxidized LDL accumulation. Upstream localization of a vulnerable plaque phenotype is probably due to an accumulation of oxidized LDL, which activates or induces subsets of smooth muscle cells and macrophages to gelatinase production.

In chapter 5 we described the diameter changes of atherosclerotic rabbit aorta after vascular shunting measured by magnetic resonance imaging. Healthy blood vessels adjust their luminal diameter by keeping mean wall shear stress constant. It has been proposed that this mechanism is also of importance in remodeling of atherosclerotic vessels, but data on this topic are sparse and contra dictionary. Therefore, we increased blood flow in five atherosclerotic rabbits by vascular shunting and recorded time series of the aortic luminal diameter by magnetic resonance imaging.

After vascular shunting flow through the aorta was 200–300% higher than at baseline. The diameter increased on average 15% over the period of twenty days. This was less than expected and the wall shear stress did not return to its reference level.

In chapter 6 we studied the remodeling of pulmonary arteries after an intervention. The purpose was to assess pulmonary artery size, flow variables, and wall shear stress in patients after Fontan operation at a young age. Flow in the branch pulmonary artery was obtained with phase contrast velocity-encoded cardiovascular magnetic resonance imaging in 14 patients (on the left side) before and after low-dose dobutamine stress and in 17 healthy controls (on the right side) at rest.

At rest, stroke volume (divided by the body surface area), total flow, average flow, peak flow and wall shear stress were all statistically significantly lower in patients than in controls. Branch pulmonary artery area did not differ between patients and controls. Distensibility was lower in patients than in controls. With stress-testing, total flow, average, and peak flow increased in patients. Wall shear stress increased with stress-testing, but was still lower
than levels found in the control group at rest. Stroke volume, area and dis-
tensibility did not change. We concluded that although pulmonary artery
size remained normal after a Fontan operation at a young age, the operation
probable causes pulmonary artery endothelial and/or vascular dysfunction.

In chapter 7 we studied pulmonary flow. The purpose was to evaluate if
early onset of retrograde flow in the main pulmonary artery is a characteristic
of pulmonary arterial hypertension. Fifty-five patients with suspected pul-
monary hypertension underwent right-sided heart catheterization and ECG-
gated MR phase-contrast velocity quantification in the main pulmonary artery.
Pulmonary hypertension was defined by a mean pulmonary artery pressure be-
ing larger than 25 mmHg. The onset time of the retrograde flow relative to the
cardiac cycle duration was compared with mean pulmonary artery pressure.

By the catheterization, 38 patients were identified as having pulmonary ar-
terial hypertension. The relative onset time of retrograde flow in these patients
was significantly smaller than found in the 17 subjects without pulmonary hy-
pertension, i.e. a large relative onset time of retrograde flow corresponded with
low mean pulmonary artery pressure. The relation found, is consistent with
the relation between pressure and the vortex duration of Reiter et al. [200].
The location of retrograde flow corresponded to the dorsal side of the pul-
monary artery and was slightly to the right side. This location coincide with
the inner curve of the main pulmonary artery. With a cutoff value of 0.25, the
relative onset time of retrograde flow distinguished patients with pulmonary
arterial hypertension from all subjects without pulmonary hypertension. We
concluded that early onset of retrograde flow in the main pulmonary artery is
a characteristic of pulmonary arterial hypertension.

In chapter 8 we studied the effect of pressure on the dilatation of the
main pulmonary artery. Dilatation of the pulmonary artery is one of the
consequences of pulmonary arterial hypertension and is used for noninvasive
detection. However, it is unclear how the size of the pulmonary artery behaves
over time and whether it is related to pressure changes. The aim of this study
was to evaluate pulmonary artery size during follow-up in treated patients with
pulmonary arterial hypertension and whether it reflects pulmonary vascular
hemodynamics. Fifty-one patients with pulmonary arterial hypertension who
underwent at least two right-sided heart catheterizations together with cardiac MRI were included in this study. Eighteen patients who had normal pressure at catheterization were included for comparison at baseline. By catheterization, we measured pulmonary artery pressures and obtained cardiac output by either thermodilution or direct Fick method. From the cardiac MR images we derived the diameter of the main pulmonary artery and the diameter in proportion to the ascending aortic diameter (the relative diameter).

The diameter of the pulmonary artery was significantly larger in patients with pulmonary arterial hypertension than in patients with normal blood pressure (33.7 mm versus 25 mm). In the entire group at baseline, the correlation coefficient between the diameter and mean pulmonary artery pressure (mPAP) was 0.58. In all patients with pulmonary arterial hypertension, there was only a weak, but significant relation between the diameter of the pulmonary artery and mPAP ($r = 0.29$). A significant difference was found between the diameter in proportion to the ascending aortic diameter in the group with pulmonary arterial hypertension and the normotensive group. In the entire group, the correlation coefficient of this relative diameter with mPAP was 0.71. Again, for patients with pulmonary arterial hypertension, the relation between the relative diameter and diastolic pulmonary artery pressure was weak ($r = 0.49$).

The area under the receiver operating characteristic curve for the relative diameter in the detection of pulmonary arterial hypertension was 0.93. We found that a relative diameter > 1.1 yields the highest diagnostic accuracy. This cut-off point of 1.1 is different from earlier studies that found that a ratio of 1 is the best diagnostic cut-off point. The explanation of this discrepancy might be that these studies used 20 mmHg of mPAP as the diagnostic criterion for pulmonary arterial hypertension instead of the currently used criterion of 25 mmHg [157]. However, if we apply the cut-off point of 1.0, a reasonable sensitivity of 92% and specificity of 72% were found.

Mean follow-up time was 942 days, and there was a significant dilatation during this period. A dilatated pulmonary artery is useful for identifying patients with pulmonary arterial hypertension. However, during patient follow-up, progressive dilatation of the pulmonary artery was independent of the change in pulmonary artery pressure and cardiac output and might be inde-
pendent from hemodynamics. Absence of a direct relation between changes in pressure or flow and changes in diameter does not exclude that increased PAP or reduced flow is the cause of pulmonary artery dilatation. Although there is an absence of radiologic studies investigating the structure of the pulmonary arterial wall, recent histologic studies of the proximal parts of the pulmonary arteries provide evidence that significant remodeling of the proximal pulmonary arterial wall occurs in pulmonary arterial hypertension [111, 120]. Structural changes in elastin and collagen under the influence of an increased PAP might eventually become a cause of pulmonary artery dilatation, irrespective of changes in pressure and flow. In addition, altered flow in pulmonary arterial hypertension affects wall shear stress and, subsequently, matrix properties of the vessel wall [10] that might lead to dilatation of the pulmonary artery.

In chapter 9 we introduced a numerical model for describing aortic aneurism expansion. The goal was to provide evidence that wall stress induced changes in aneurysm stiffness might be associated with aneurysm expansion. We assumed wall stress peaks induce break down of the extracellular matrix. Consequently the wall stiffness decreases locally and this leads to local bulging of the vessel wall. We developed a numerical model to predict aneurysm expansion based on this assumption. In addition, we obtained aneurysm geometry of 11 patients from computed tomography angiographic images to obtain patient specific calculations.

The assumption of a wall stress related expansion indeed resulted in a series of local expansions, adjusting global geometry. Although the remodeling mechanism acted locally and no assumption of exponential expansion was introduced into the model, all simulations exhibited an exponential relation between diameter and time. This behavior is in agreement with the experimental studies of Brady [13], Schouten [218] and Vardulaki [263]. Longer aneurysms were predicted to expand to expand faster than shorter ones. This prediction was in agreement with a study of Hatakeyama [85]. Furthermore, the calculations showed that location of peak wall stress changed over time. Thus, the concept of wall stress induced changes in stiffness seems to demonstrate realistic aneurysm expansion.
In chapter 10 the numerical model was extended with risk profiles. Local anatomy and the patient’s risk profile independently affect the expansion rate of an abdominal aortic aneurysm. Therefore, we combined finite element modelling with patient specific risk factors such as ischemic heart disease to predict patient-specific aneurysm expansion. The three-dimensional geometry of the aneurysm was imaged with computed tomography. Risk factors were obtained from a database of 80 patients. Aneurysm diameters predicted with and without the risk profiles were compared with diameters measured with ultrasound for 11 patients.

In our study, only few risk factors influenced the expansion rate. Ischemic heart disease accelerated the expansion rate, while being male, peripheral arterial disease and nitrates decelerated the expansion rate. Local anatomy contributed 62% and the risk profile 38% to the aneurysmal expansion rate. The average difference between simulated and measured diameter was 3.7 mm or 8.3% of maximal diameter. Predictions with risk profiles resulted in smaller root mean square errors than predictions without risk profiles (2.9 mm versus 4.0 mm). This hybrid approach predicted aneurysmal expansion for a period of 30 months with high accuracy.

11.2 Practical applications, implications or recommendations

The observations in chapter 2 have important implications for future studies in the shear stress field, as the normal shear stress values now depend on the diameter of the vessel under study. The mechanism for this finding is currently unknown, but can only be explained if the endothelial cells located in different vessels are “primed” to a different mean wall shear stress value.

Vulnerable plaques have raised great clinical interest because they are prone to rupture, leading to massive clotting and causing 70% of sudden cardiac deaths in humans. Heterogeneity in single plaques is of great importance because plaques usually do not rupture at random over their entire length. Instead, they rupture more locally and usually upstream of maximal plaque
location. The latter result might be of importance for interventional procedures. The results of chapter 4 show that such a localized vulnerability is due in part to the localized gelatinolytic activity in this area. In addition, we demonstrated that this plaque-weakening activity is not restricted to the macrophage but also occurs in a subset of vascular smooth muscle cells. As a consequence, smooth muscle cells should be placed in a wider perspective in which they are not solely the producing cells of stabilizing collagen fibers. The two faces of the smooth muscle cell are of significance in the concept of plaque weakening and thus in the development of new pharmaceutical and interventional procedures.

The study on diameter changes of atherosclerotic rabbit aorta in chapter 5 showed stagnated enlargement of the luminal diameter and persistent elevated mean wall shear stress. This might indicate that in the aorta the reference level for wall shear stress can change.

The study on early onset time of retrograde flow has clinical implications. Many patients which are suspected of pulmonary hypertension, undergo standard MR phase-contrast velocity quantification in the main pulmonary artery for the assessment of stroke volume. The measured velocity images show retrograde flow if present. With retrograde flow we could distinguished all patients with pulmonary arterial hypertension from subjects without pulmonary hypertension.

In chapter 8, we showed that the diameter of the pulmonary artery and the diameter in proportion to the ascending aortic diameter are also useful in discriminating patients with pulmonary arterial hypertension from patients with normal blood pressure. The use of the relative diameter is recommended because its relationship with pulmonary artery pressure is independent of body surface area and sex [179]. Progressive dilatation of the pulmonary artery is independent of the change in pulmonary artery pressure and cardiac output and might become independent from hemodynamics.

Our findings have several clinical implications. First, the diameter of the pulmonary artery, although useful in the diagnosis of pulmonary arterial hypertension, is not useful for follow-up of the disease or evaluation of treatment effects. Second, we show that the dilatation of the pulmonary artery in pul-
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Pulmonary arterial hypertension is more related to follow-up time than to changes in blood pressure, indicating that the pulmonary artery needs time to dilate. A severe dilated pulmonary artery at the time of diagnosis thus indicates that pulmonary arterial hypertension was already present for a long period. Third, our data indicate that although increased pulmonary artery pressure leads to dilation of the pulmonary artery, further dilation is a process most likely due to a change of the intrinsic vessel properties, which is independent of the pulmonary hemodynamics.

Very interestingly the numerical model to predict the expansion of the abdominal aorta revealed that locations of peak wall stress changed over time. It implies that methods aiming at prediction of location and time of aneurysm rupture should probably take this mechanism into account.

To demonstrate applications of a model that incorporates risk factors, we evaluated the effect of adding single risk factors to the expansion rate of two patients. There was a difference in expansion rate between omitting medical intervention (nitrates) and the presence of ischemic heart disease for individual patients. In addition, the risk profiles changed time to intervention significantly and varied strongly between the patients. Our approach enabled evaluating the effect of several factors on expansion rate. First, we evaluated the role of remodeling and risk profiles on the variability of inter-individual expansion rates. It could be shown that in our small data set, remodeling accounted for two-thirds, while risk profiles accounted for one-third of this variation. This might imply that remodeling is a more important factor in determining 3-D aneurysm geometry. Second, we evaluated the individual effect of risk factors on expansion rate for single patients. The simulations indicate that differences in drugs use may affect the expansion rate of the aneurysm. The present approach may calculate the benefit of interventions for a single patient.

### 11.3 Recommendations for future research

Chapters 2 and 5 show that the reference level for wall shear stress is not just a constant value. How can the reference level for wall shear stress be different
throughout the vascular tree? How exactly does an endothelial cell adjust the reference level? One of the first steps to be taken is ascertain whether the reference level in a specific blood vessel can change due to growth.

In chapter 4 a specific spatial colocalization of macrophages, lipids, and smooth muscle cells was demonstrated upstream of the plaque, similar to that found in patients with proven upstream plaque ruptures. This upstream plaque composition is characterized by an accumulation of (subsets of) macrophages and smooth muscle cells, oxidized LDL, and gelatinolytic activity. We hypothesize that activation of these subsets by oxLDL induces gelatinolytic activity, followed by breakdown of the extracellular matrix and subsequent weakening of the plaque. The unexpected, important role of a subset of smooth muscle cells in this process warrants further study.

The long-term effects of total or substantial loss of pulsatility of flow in the pulmonary arteries after Fontan operation remain unclear. Results from a few studies focusing on the pulmonary vasculature in Fontan patients are suggestive of pulmonary endothelial dysfunction, particularly in the nitric oxide pathway. Pulsatile stretch and wall shear stress are important for the release of nitric oxide by the endothelium [16, 81]. Nitric oxide, a locally acting vasodilator, contributes to the maintenance of low pulmonary vascular resistance in healthy children [18]. Low pulmonary vascular resistance might be essential for optimal functioning of the Fontan circulation.

Time and spatially averaged wall shear stress was significantly lower in the Fontan patients than in the control group, which is consistent with a reduced blood flow at rest, while vessel area was normal. Throughout the cardiac cycle, distinct wall shear stress-patterns were seen in healthy controls, patients with a total cavopulmonary connection, and patients with an atropulmonary connection, comparable with the flow curves in these groups. Physiologic and pulsatile wall shear stress constitutes the most potent stimulus for continuous production of nitric oxide by the endothelium [20]. Low wall shear stress reduces the bioavailability of nitric oxide by decreasing the expression of nitric oxide synthase. This will lead to an increase in pulmonary vascular resistance, as has been demonstrated in patients late after a Fontan-type operation by Khambadkone et al. [107]. Levy et al. [129] demonstrated weak expression
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of nitric oxide synthase in Fontan patients with a good surgical outcome, but overexpression of nitric oxide synthase in patients after Fontan failure. They hypothesized that this overexpression could be due to an attempt to improve the pulmonary vascular resistance and facilitate Fontan circulation. The mechanism, however, cannot be explained by the low wall shear stress in this circulation and warrants further investigation.

In chapter 8, a progressive dilatation was found in the patients with pulmonary arterial hypertension, which was not explained by pressure changes. Even in the majority of the patients in which the pressure decreased, there was an ongoing dilatation of the pulmonary artery. The finding that neither pressure nor flow are related to the progressive dilatation in most of the patients shows that other explanations than changes in pulmonary hemodynamics underlie this progressive dilatation, a phenomenon well-known from aneurysms of the aorta. Although systemic hypertension is an important underlying cause of this disease, a further dilatation of the aorta in aneurysms is independent of systemic blood pressure [13]. In chapter 9 and 10 wall stress induced changes in stiffness predicted aneurysm expansion accurately. This concept was not tested for the main pulmonary artery. Numerical quantitative models should be developed to study progressive dilatation of the main pulmonary artery.

Models to predict aneurysm expansion can be improved. CT images are not suited for accurate wall thickness determination and tissue characterization. As local wall stress depends on wall thickness and material properties, the calculations will benefit if detailed wall thickness values and tissue characteristics could be retrieved.

We did not apply pressure on the outer wall of the aneurysm. Surrounding organs, muscles and bones should result in some pressure on the aneurysmal sac. Boundary conditions could be applied to represent these effects to some extent. To implement the boundary conditions well, the surrounding tissues should be characterised. Although this might be difficult and laborious, this is really a recommendation for future work. This is because our study shows the importance of the geometry in aneurysm expansion and the surrounding tissues most likely have an influence on the geometry.

In vitro studies have shown that high wall stress is associated with a higher
protease activity and a larger breakdown of collagen [133, 153, 242]. I recommend an in vivo study to proof that proteolytic enzymes usually associated with aneurysm wall degradation are increased by wall stress.
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