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**NON-INVASIVE ASSESSMENT OF ARTERIAL
REMODELLING IN ARTERIOVENOUS FISTULAS
FOR HEMODIALYSIS**

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NON-INVASIVE ASSESSMENT OF ARTERIAL REMODELLING IN ARTERIOVENOUS FISTULAS FOR HEMODIALYSIS

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Universiteit Maastricht,
op gezag van de Rector Magnificus,
prof. dr. A.C. Nieuwenhuijzen Kruseman,
volgens het besluit van het College van Decanen,
in het openbaar te verdedigen
op donderdag 27 november 2003 om 14.00 uur

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The task is not so much to see what no one has yet seen,
but to think what nobody has yet thought about that,
which everybody sees

Erwin Schrödinger (1887 – 1961)

Voor mijn ouders

Omdat alles relatief is en gepaste nederigheid niet misstaat

Aan Diana

*This is my secret. It is very easy: It is only with the heart that one can see rightly.
What is essential is invisible to the eye.*

Antoine-Marie-Roger de Saint-Exupéry (1900 – 1944)

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List of abbreviations

ACh	Acetylcholine
ADP	Adenosine diphosphate
ANOVA	Analysis of variance
ANCOVA	Analysis of covariance
AP-1	Activator protein 1
ATP	Adenosine triphosphate
AVF	Arteriovenous fistula
BAEC	Bovine aorta endothelial cell
Ca ²⁺	Calcium ion
[Ca ²⁺]	Calcium concentration
cAMP	Cyclic adenosine monophosphate
CC	Compliance coefficient
cGMP	Cyclic guanosine monophosphate
Cl ⁻	Chloride ion
CNP	C-type natriuretic peptide
CO	Cardiac output
CWS	Circumferential wall stress = wall tension
DAG	Diacylglycerol
DC	Distensibility coefficient
EC	Endothelial cell
ECE	Endothelin-converting enzyme
ECG	Electrocardiogram
EDHF	Endothelium-derived hyperpolarizing factor
EDRF	Endothelium-derived relaxing factor
<i>E_m</i>	Young's modulus
eNOS	Endothelial nitric oxide synthase
ELISA	Enzyme-linked immunosorbent assay
ERK	Extracellular signal-regulated kinase
ESRD	End-stage renal disease
ET-1	Endothelin 1
ET _A	Endothelin receptor A
ET _B	Endothelin receptor B
FMD	Flow-mediated vasodilation
GDP	Guanosine diphosphate
G-protein	GTP-binding regulatory protein
GTN	Glycerin trinitrate
GTP	Guanosine triphosphate
HUVEC	Human umbilical vein endothelial cell
ICAM-1	Intracellular adhesion molecule-1
<i>IMT</i>	Intima-media thickness = wall thickness
iNOS	Inducible nitric oxide synthase
IP ₃	Inositol-1,4,5-triphosphate
JNK	c-jun amino-terminal kinase
K ⁺	Potassium ion
[K ⁺]	Potassium concentration

MAPK	Mitogen-activated protein kinase
MCP-1	Monocyte chemoattractant protein-1
MMP	Matrix metalloproteinase
mRNA	Messenger ribonucleic acid
NF- κ B	Nuclear factor- κ B
NO	Nitric oxide
NPR-B	Natriuretic peptide receptor-B
NPR-C	Natriuretic peptide receptor-C
PAI-1	Platelet activator inhibitor-1
PC	Personal computer
PDGF	Platelet-derived growth factor
PGI ₂	Prostacyclin
PKA	Protein kinase A
PKG	Protein kinase G
PLA ₂	Phospholipase A ₂
PLC	Phospholipase C
PTFE	Polytetrafluoroethylene
rf	Radio-frequency
SD	Standard deviation
TF	Tissue factor
TGF- β	Transforming growth factor- β
TNF- α	Tumor necrosis factor- α
TIMP	Tissue-inhibitor of metalloproteinase
VSMC	Vascular smooth muscle cell
vWF	Von Willebrand factor
WBV	Whole blood viscosity

CHAPTER 1

General introduction and aims of the study

The human kidney extracts nitrogenous waste products and excess fluid, amongst others, from the circulation through dialysis. The principles of dialysis, separating colloids from solutions containing smaller molecules by the difference in rate at which they pass through a semipermeable membrane, were already described by the Scottish chemist Thomas Graham in 1854.¹ The accumulation of nitrogen metabolism waste products, particularly creatinine and urea, and volume overload are the principal features of renal failure that, when left untreated, would eventually lead to death.^{2,3}

Present treatment modalities for end-stage renal disease (ESRD) consist of kidney transplantation or renal replacement therapy, such as peritoneal dialysis and hemodialysis. Although blood purification through hemodialysis was first performed by the German surgeon Georg Haas,⁴ it was not until the invention of the first truly artificial kidney by Willem Kolff, a Dutch internist, that modern hemodialysis therapy was established.⁵⁻⁷ In a challenging time during World War II, his work is illustrative of how medical science and practitioners can work in a dynamic manner to meet clinical needs. The first clinical successful hemodialysis in a human patient was performed and published in 1943⁸ and was later republished in English in early 1944 in *Acta Medica Scandinavica*.⁹ Lacking a reliable vascular access at that time, the long-term utility for adequate hemodialysis was limited, however.

VASCULAR ACCESS

Initially, an intermittent method of hemodialysis access was used; blood was removed from the patient via arterial puncture, dialysed, and then manually returned to a vein. Soon, in 1949, Alwall and his colleagues described the first use of an arteriovenous fistula for hemodialysis by placing a silicone tube between an artery and a vein.^{10,11} Permanent vascular access was, however, still not feasible, due to infectious and thrombotic complications.

In 1960, the combined forces of internal medicine, surgery, and engineering, lead to the construction of the earliest permanent arteriovenous fistula, the so-called Scribner shunt. Classically, this was an external loop connection between the radial artery and the cephalic vein and consisted of two rigid Teflon cannulas that were inserted into the vessels and a piece of flexible Silastic tubing interconnecting the two cannulas.¹² During dialysis the interconnecting tube was detached and the artificial kidney was attached to both cannulas. Although the external shunt provided a rapid effective access for chronic dialysis, prolonged fistula exploitation still was largely hampered by thrombosis and infection.

In 1966, Brescia and Cimino reported their experience with the classic autogenous radial-cephalic direct wrist access¹³ (exemplified in figure 1) in 13 patients with chronic ESRD.¹⁴ In fact, it was the surgeon Kenneth Appel who performed the first surgically created subcutaneous connection between the radial artery and cephalic vein. Since then, this fistula has remained the permanent angio-access of choice, primarily owing to its superior long-term patency of up to 78% at 4 years.¹⁵ Although initially cannulated immediately after surgery,¹⁴ these fistulas are now allowed to mature for a period of at least 4 to 6 weeks until there is sufficient venous dilation.¹⁶ Since comorbid factors such as age and the incidence of

diabetes have increased in the renal failure population, prosthetic graft interposition was increasingly required due to the lack of suitable arteries or veins due to arteriosclerosis.^{17,18}

Despite the vast variety of biologic (cow mesenteric vein, sheep ovine graft, human umbilical vein) and synthetic (polyester, polyurethane and PTFE) materials available for graft construction, the use of expanded PTFE has superseded all others. ePTFE is non-thrombogenic and allows tissue and capillary ingrowth through the graft's pores.^{19,20} Long-term patency rates approximate 50% at 3 years.²¹ To date, ePTFE graft insertion has become the procedure of choice for prosthetic straight or looped bridge accesses.

Figure 1. Example of an autogenous radial-cephalic direct wrist access. In this picture, the cephalic vein is anastomosed to the radial artery in an side-to-side fashion (AV-fistula).

Complications

An estimated 520,240 ESRD patients will be on hemodialysis in the United States in 2010, with a current incidence of about 100,000 patients per year.²² Approximately 0.79 – 1.6 interventions per patient per year are needed to maintain adequate vascular access for hemodialysis,^{23,24} accounting for 25% of the causes of hospitalisation and a cost burden exceeding \$1.0 billion on U.S. health services per year.²⁵

Both autogenous and prosthetic fistula function is endangered by various complications, of which thrombotic occlusion is the most frequent (up to 80%).²⁶ Furthermore, fistula function is jeopardized by infection, aneurysm formation, venous hypertension, distal (hand) ischemia, and cardiac failure.^{26,27} In addition, autogenous fistula function may be impaired by non-maturation.²⁸⁻³¹ In the context of the present thesis, we will only discuss the major complications of both autogenous and prosthetic fistulas.

Prosthetic bridge access

The most commonly occurring complication observed in prosthetic arteriovenous fistulas is thrombosis,²⁶ with an overall incidence of 0.8 – 1.25 per patient per year.³²⁻³⁴ Fistula thrombosis occurs more frequent (relative risk: 7.2) when the volumeflow drops below 600 mL.min⁻¹.³⁵ Also, it has been put forward that fistulas with a more than 35% decrease in vascular access bloodflow had a relative risk increase of 13.6 compared to fistulas with no change in bloodflow.³⁶ This decrease

in fistula flow can be caused by hypotension and stenosis. Hypotension during dialysis is a common feature, due to the extraction of excess fluid.³⁷ In case of severe hypotension ultimately fistula thrombosis might ensue.

Intimal hyperplasia is the basic source for stenosis development in arteriovenous fistulas,³⁸⁻⁴⁰ as it is also an important feature in peripheral and cardiac bypass grafting and restenosis.⁴¹⁻⁴³ Intimal hyperplasia is classically defined as the abnormal proliferation and intimal migration of medial VSMCs, accompanied by extracellular matrix deposition, such as proteoglycans, elastin, and collagens.⁴⁴ Very different mechanisms can induce intimal hyperplasia, for instance mechanical stimuli such as stretch and shear forces.⁴⁴⁻⁴⁸ The role of wall shear stress in intimal hyperplasia will be discussed in more detail in **CHAPTER 2**, along with vascular remodelling. Accumulating evidence suggests that vascular remodelling, i.e., the ability of an artery to accommodate neointimal growth, plays a key role in the pathobiology of (re)stenosis.⁴²

Autogenous radial-cephalic direct wrist fistula

Besides the aforementioned intimal hyperplasia, which also puts autogenous fistulas at risk, another major complication is non-maturation of the fistula that is observed in 10 – 30% of newly created autogenous fistulas.²⁸⁻³¹

Classically, autogenous fistula non-maturation depends on the quality and size of the vessels used for the arteriovenous anastomosis and the ability of vessel adaptation induced by the augmented bloodflow. In recent duplex ultrasound studies it has been forwarded that using a well-sized radial artery (> 1.6 mm) and cephalic vein (> 1.6 mm) would be beneficial for future autogenous radial-cephalic direct wrist access function.^{31,49-51} In fact, it has been shown that especially small cephalic vein diameters seem to be related to non-maturation.²⁹ Furthermore, radial artery flow augmentation at day 1 and 7 post surgery might predict good maturation.^{29,51} In a recent study also the prevalence of intimal hyperplasia in the radial artery before arteriovenous fistula creation has been related to failure to mature.⁵² In a high number of patients non-maturation might occur as a result of stenoses, mainly in the venous outflow tract. In the recent years it has been advocated to aggressively attack these lesions through surgical and/or radiological intervention.⁵³ Still, unexplained failure and non-maturation occurs despite anastomosing adequate vessels. It cannot be attributed to the vasculature baseline diameters since Bourquelot et al. have shown that even in very small children (!) it is possible to create autogenous fistulas for hemodialysis.⁵⁴ This prompted many research on the maturation of the vasculature itself by remodelling.

Remodelling, elaborated upon in **CHAPTER 2**, is the adaptive process by which an artery or a vein changes its lumen diameter and/or vessel wall thickness in response to a variety of stimuli, i.e., shear stress and circumferential wall stress (= wall tension), which will be discussed in the next section.^{42,44,45} Shortly, shear stress is in part dependent on the flow velocity, which obviously increases after arteriovenous fistula creation in both artery and vein.⁵⁵⁻⁵⁷ Wall tension, on the other hand, is in part dependent on mean arterial pressure which increases in the efferent vein after arteriovenous fistula creation, due to the evasion of peripheral vascular resistance.^{56,57}

PHYSIOLOGY OF ARTERIOVENOUS FISTULA BLOOD FLOW

In this section the basic biophysical principles of vascular wall properties, along with the characteristics of flowing blood in a pulsatile system, will be discussed, with emphasis on shear stress, the tangential force exerted on the vascular wall by the flowing blood. Subsequently, the hemodynamics of arteriovenous fistulas for hemodialysis will be detailed upon.

Basic biophysical principles; emphasis on shear stress

Vascular wall

The arterial wall is divided into three concentric zones: the tunica intima, media, and adventitia. The tunica intima consists of a single layer of vascular ECs and a thin layer of elastin and collagen fibres. Demarcation between the intima and media is provided by the internal elastic lamina; the outer elastic lamina separates the tunica media from the tunica adventitia, a region of collagen and elastin tissues merging with the surrounding connective tissue. The tunica media is the principle determinant of the mechanical properties of the vessel and primarily consists of VSMCs and elastin fibres. Because the vessel wall actively reacts on various stresses, shear stress and circumferential wall stress,^{42,44,45} and because it plays an important role in the development of different vascular disorders, research on the mechanical properties of the vascular wall has gained large interest. For example, atherosclerotic risk factors such as hypercholesterolemia, smoking, diabetes, amongst others are related to abnormal large artery properties,⁵⁸⁻⁶⁰ a greater wall thickness,^{61,62} and impaired vascular reactivity.⁶³⁻⁶⁵ Similar deviations are also observed in patients with ESRD.⁶⁶⁻⁷⁰

To quantify vascular wall mechanics different parameters have been introduced, of which only a few are used in the present thesis. To calculate vascular compliance, i.e., the storage capacity of a vessel per unit of length, the change in volume produced by a change in transmural pressure is measured, assuming a constant length of the vessel segment, which is a reasonable assumption because of tethering of the vessel to the surrounding tissue. The compliance coefficient CC [$\text{m}^2 \cdot \text{Pa}^{-1}$] is calculated through:

$$CC = \frac{\Delta A}{\Delta P} \cong \frac{\pi \Delta d \cdot d}{2 \Delta P} \quad (1-1)$$

where ΔA [m^2] is the increase in cross-sectional area brought upon by the pressure change ΔP [Pa], d is diameter [m], and Δd is distension [m]. If the cross-sectional area changes linearly with pressure, the incremental pressure change might be replaced by pulse pressure, defined as systolic minus diastolic blood pressure. Tightly related to vascular compliance, vascular distensibility DC [Pa^{-1}] reflects the elasticity, or “stiffness”, of the vessel wall and is expressed by:

$$DC = \frac{\Delta A / A}{\Delta P} \cong \frac{2 \Delta d / d}{\Delta P} \quad (1-2)$$

A parameter to quantify elasticity independent of vessel shape and size is the Young's modulus E_m [Pa]. This inverse measure of elasticity is calculated with:⁷¹

$$E_m = \frac{0.75d}{IMT \cdot DC} \quad (1-3)$$

where IMT [m] (intima media thickness) is a measure for the wall thickness. The morphology of the vessel wall can be approximated by the dimensionless ratio of vessel wall thickness and diameter (IMT/d).

The increase in circumference of a tube, caused by a distending pressure P , is proportional to the circumferential tension applied to it. For a very thin-walled cylindrical tube this tension is given by the law of Laplace. In an artery with a certain wall thickness IMT [m], the circumferential wall stress or wall tension CWS [Pa] at a given transmural pressure P [Pa] is calculated with Lamé's equation:⁷¹

$$CWS = \frac{P \cdot d}{2(IMT)} \quad (1-4)$$

Arterial bloodflow and shear stress

Blood contains blood cells (erythrocytes, platelets, white blood cells), lying in a protein-rich liquid substance, the blood plasma. The main function of the blood is to deliver nutrients, oxygen, and hormones to and extract waste products from the various body tissues. Typical characteristics of blood are hematocrit and viscosity, subdivided into plasma viscosity and whole blood viscosity. The hematocrit or relative blood volume is defined as the volume of erythrocytes per volume of whole blood; normal range 35 – 45%. The protein load of the plasma, i.e., fibrinogen, albumin, and globulins, mainly determines plasma viscosity; normal range 1.13 – 1.44 mPa·s.^{72,73} In turn, whole blood viscosity is estimated by both hematocrit and plasma viscosity. For normal hematocrit values, whole blood viscosity will increase linearly with hematocrit.⁷⁴⁻⁷⁶

Shear stress, the viscous drag exerted on the vessel wall by the flowing blood, has been of interest for many research groups, as the interaction between the blood flow and the vascular wall, more specific the ECs, is becoming more and more apparent.⁷⁷⁻⁸⁰ Vascular wall shear stress τ [Pa] depends on the radial velocity v_r [m·s⁻¹] profile of the flowing blood, the vessel radius r [m], and the whole blood viscosity WBV [Pa·s] as expressed in the following equation:⁷⁴

$$\tau = WBV \cdot \left. \frac{\Delta v_r}{\Delta r} \right|_{r=R} = WBV \cdot \gamma \quad (1-5)$$

γ or wall shear rate [s⁻¹] is defined as the derivative of the velocity profile at the radial position $r = R$, i.e., the lumen-wall transition (Figure 2).

In most *in vitro* as well as *in vivo* studies the wall shear stress is estimated with the use of Poiseuille's law that supposes a steady non-pulsatile parabolic laminar flow of a Newtonian fluid, i.e., independent of shear rate, in a rigid straight vessel.⁷⁴ This is at the least an inaccurate supposition as human large artery blood flow is pulsatile and the vascular system is a distensible instead of a rigid tube.^{81,82} Moreover, due to the viscoelastic properties of human blood, *WBV* might vary with the shear rate, thus influencing wall shear stress or *vice versa*.^{72,75}

Figure 2. Diagram of shear rate definition. A parabolic flow profile is shown. The maximum velocity ($v_{\max}$) lies at the parabola top. To calculate shear rate at the lumen-wall interface ($\gamma_{r=R}$) one considers the derivative of the velocity profile at that position, i.e., $r = R$ (equation 1-5).

Interestingly, shear stress in a Newtonian liquid can also be described as function of the pressure gradient $(P_1 - P_2)/L$ [Pa] in a vessel with length L [m] and radius $r = R$ [m]:⁷⁴

$$\tau = \frac{r(P_1 - P_2)}{2L} \Big|_{r=R} \quad (1-6)$$

Please note that this is only an approximation as blood is a viscoelastic fluid (i.e., non-Newtonian) as mentioned before. Thus, knowing the pressure gradient over a certain vessel with known length and diameter would enable one to approximate shear stress, or *vice versa*, the pressure loss over a certain length, as a measure of energy loss of the flowing blood, can be approximated when the shear stress is known.

The present thesis explicitly considers the measured *in vivo* time-dependent velocity distribution, and thus wall shear rate distribution, and calculated *WBV* to assess wall shear stress. More detail is provided in **CHAPTER 3** concerning the shear stress assessments.

Hemodynamics of arteriovenous fistulas for hemodialysis

The fundamental components of an arteriovenous fistula include a donor artery, an outflow conduit, a low-flow, high-resistance connection (the peripheral vascular

bed), and a high-flow, low-resistance connection (the fistula itself).⁸³ The “hemodynamic” changes after arteriovenous fistula creation can be subdivided into systemic and local changes, which will be discussed separately.

Systemic cardiovascular changes

As a result of the creation of a low-resistance arteriovenous fistula, the blood flow experienced by the donor artery will increase significantly (up to 6 times baseline flow).^{56,84-87} A similar process is seen when in physical activity arterial blood flow increases as a result of the increased skeletal musculature oxygen needs. The local carbon dioxide pressure and lactate concentration increase, with the subsequent decrease in pH value, cause the arterioles to dilate and hence decrease peripheral vascular resistance. This decrease in peripheral vascular resistance will induce an increased heart rate and cardiac stroke volume. In turn, this increase in *CO* results in higher arterial blood flows (up to 6 times baseline flow). Hence, in case of a low-resistance arteriovenous fistula, *CO* will increase. In this setting, Ori et al.⁸⁸ have shown that a vascular resistance decrease of 23.5% resulted in a *CO* augmentation of 19.0%. Eventually, one can imagine cardiac failure to develop.⁸⁹ This “high-output” cardiac failure as a result of arteriovenous fistula placement is a very uncommon complication in hemodialysis patients, and can only be cured by fistula banding or closure.⁹⁰⁻⁹⁵ Besides cardiac failure, it has even been proposed that patients with an arteriovenous fistula are at higher risk for myocardial ischemia.⁹⁶

Systemic blood pressure does not change markedly after arteriovenous fistula placement or closure,^{88,92,96} although directly after arteriovenous fistula placement systemic blood pressure drops, which is rapidly compensated for by an increased *CO* as mentioned above.⁹⁷

Distal to an arteriovenous fistula digital blood pressure decreases.⁹⁸ This can be illustrated by calculating the digital/brachial index (DBI), being the quotient of the digital artery systolic blood pressure via plethysmography and brachial artery systolic blood pressure via oscillometry.⁹⁹ This DBI is used as measure to predict the occurrence of peripheral ischemia, the so-called radial steal syndrome, which has an incidence of 2 – 8% of hemodialysis patients.^{97,99} The DBI cut-off value is set at 0.6 mmHg/mmHg⁻¹, with a sensitivity and specificity of 100% and 59%, respectively.⁹⁹ Radial steal will develop when the peripheral resistance is too high compared to the resistance of the arteriovenous fistula located proximal to that periphery.⁹⁷ The direction of the radial artery blood flow might become retrograde, i.e., away from the digital arteries, eventually leading to digital ischemia and even necrosis and gangrene.^{97,100} Different treatment modalities have been proposed for this complication, such as closure of the fistula, sympathectomy, banding, and even ligation of the radial artery distal to the fistula, with moderate to good results.^{100,101} Schanzer et al. have developed an innovative technique in which the donor artery is ligated just distal to the fistula and a bypass is created from the donor artery to the radial or ulnar artery.¹⁰² This technique has been baptized DRIL (distal revascularisation interval ligation) by other investigators for whom it has become the intervention of choice in case of critical hand ischemia.^{97,100,103-105}

Local hemodynamic changes

As described above, as a result of arteriovenous fistula creation the minimal pressure gradient that normally exists between neighbouring segments of an artery is changed. Instead, the large difference in pressure between arteries and veins causes an immense pressure drop over the arteriovenous fistula, resulting in a flow augmentation. The greater the anastomotic length, i.e., the fistula communication opening, the larger the increase in blood flow until the anastomotic length approaches 75% of the donor artery.⁸³

This sudden increase in blood flow will induce an acute diameter increase in both the proximal arterial and the distal venous system. Girerd et al. have shown that, after a 6-fold increase in blood flow, the radial artery diameter proximal to an arteriovenous fistula is increased 1.4-fold as compared to the contralateral radial artery.⁵⁶ Others have observed a similar diameter rise in arteries proximal to an arteriovenous fistula (1.1- to 2-fold),^{85,86,98,106,107} and an even more pronounced diameter increase in the cephalic outflow tract (1.5- to 2.5-fold).^{98,108} Some authors claim that the increased *CWS* (equation (1-4)) resulting from this FMD might induce eccentric hypertrophy, i.e., thickening of the vascular wall at increasing diameter.¹⁰⁸⁻¹¹⁰

At regions of disturbed flow the eccentric hypertrophy might be exaggerated in a way that intimal hyperplasia will develop.¹¹¹ With use of flow visualization and computational models, various authors have shown that in arteriovenous fistulas flow separation and turbulence occur frequently and at distinct places. Flow separation, for example, can be observed at the artery floor and anastomosis toe (Figure 3) where shear stress is increased or even oscillatory, with large spatial and temporal gradients. Just distal to the anastomosis mainly turbulence is observed, which can be explained by the great pressure drop caused by anastomosing an artery to a vein. This turbulence as a consequence of the pressure drop has a moderating effect on shear stresses in the course of the venous tract. Low shear stresses are also observed in the heel of an arteriovenous fistula.¹¹¹⁻¹²⁰

Figure 3. Diagram of an arteriovenous fistula. The blood flows from the proximal to the distal artery. Disturbed flow can be observed at the fistula heel, toe, and floor. [Adapted from flow visualization studies by Sivanesan et al.¹¹⁶]

In concordance with the previous observation various studies have reported that intimal hyperplasia at the venous anastomosis and the venous outflow tract of the graft is the major cause of prosthetic arteriovenous fistula stenosis.¹²¹⁻¹²³ Although many theories have been suggested and the exact mechanism of intimal hyperplasia remains tentative,^{44,45,111,124-128} the fluid dynamics within a vascular graft as described above are thought to have great influence on the initiation and development of intimal hyperplasia.

For example, high shear stress is known to inhibit VSMC proliferation,¹²⁹ yet high shear stress may also cause damage to the endothelium and thus inhibit the antiproliferative effect on smooth muscle cells.^{38,121} Low shear stress, on the other hand, triggers ECs to release mitogenic factors and hence might initiate intimal hyperplasia.^{48,111,124,130} Other factors, such as graft-vein anastomotic compliance and anastomotic angle geometry, have also been shown to be of importance in determining graft patency and local hemodynamics.^{111,131}

Over the years, different geometric configurations have been introduced to influence fluid hemodynamics and ultimately reduce shear stress induced intimal hyperplasia.^{48,132-140} One such configuration is graft tapering; both 4 – 7 mm and 6 – 8 mm prosthetic grafts have been used in experimental research and clinical trials.^{135,137-140} Although Fillinger et al. demonstrated less intimal hyperplasia at the venous anastomosis of 4 – 7 mm tapered prostheses in arteriovenous fistulas in mongrel dogs,¹³⁷ the overall results of these studies are still very contradictory.

AIMS OF THE STUDY

The main goal of the present thesis is to increase the knowledge regarding arteriovenous fistula maturation and long-term function. The basic question is how pre-operative EC function, vascular wall mechanics, hemodynamics, and extracellular matrix metabolism in both the brachial and radial artery relate to both maturation and function. We anticipated that these parameters could determine the prognosis regarding functional maturation of the arteriovenous fistula well before surgery.

OUTLINE OF THE THESIS

In **CHAPTER 2** vascular remodelling is reviewed from recent literature to set the problem and the development of intimal hyperplasia is presented. The materials and methods, regarding assessment of vascular wall properties, hemodynamics, and the determination of serum parameters are discussed in **CHAPTER 3**, together with the estimation of *WBV*. Brachial artery hemodynamic and vascular wall properties are presented in **CHAPTER 4**, also showing the reproducibility for these measurements. In **CHAPTER 5** brachial artery characteristics in patients with ESRD are compared to a healthy population. Furthermore, the role of ET-1, an EC marker, is discussed. In an observational study, **CHAPTER 6** provides insight in the vascular wall and hemodynamic changes that occur in both the brachial and radial artery after arteriovenous fistula placement. Serum levels of MMPs (**CHAPTER 7**) are also addressed regarding their effectiveness in predicting fistula non-maturation or malfunction. Brief insight into intracellular processes in intimal hyperplasia progression is provided in **CHAPTER 8**. The summary and general

discussion (**CHAPTER 9**) provides a comprehensible synopsis of the thesis and discusses the flaws assessing EC function. Furthermore, future biophysical, molecular, and clinical studies are proposed to offer possible answers to the still not completely understood physiology of remodelling and the development of intimal hyperplasia.

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CHAPTER 2

The endothelium as a signalling interface for
shear stress-induced arterial remodelling

Submitted:

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ABSTRACT

Hemodynamic shear stress is the principle force regulating acute mechanical signalling in endothelium that leads to altered vascular tone. In contrast, if altered mechanical loading is sustained, arteries undergo a slow reorganization of the vascular wall that involves changes in both cellular and extracellular elements, i.e., vascular remodelling. Thus, shear stress alterations invariably produce transformations in the vessel wall that aims to accommodate the new conditions and ultimately restore basal (physiological) levels of shear stress. The present review pursues to describe the acute and chronic changes that can occur due to changes in shear stress. Furthermore, mechanisms by which ECs are able to sense these changes will be described.

INTRODUCTION

The hemodynamic shear stress, defined as the tangential force exerted by the flowing blood, is the principle factor regulating acute mechanical signalling in the endothelium, leading to adapted vascular tone. However, in reaction to a sustained altered mechanical loading, arteries undergo a slow reorganization of the composition of the vascular wall, which involves changes in both the cellular and extracellular elements.¹⁻⁷ Vascular wall shear stress (τ ; Pa) depends on the radial gradient of the axial velocity profile (v_r ; $\text{m}\cdot\text{s}^{-1}$) of the flowing blood, the vessel radius (r ; m), and whole blood viscosity (WBV ; $\text{Pa}\cdot\text{s}$) as expressed by equation (1-5) and shown in figure 2.⁸

As a result of their unique location, endothelial cells (ECs) are directly exposed to shear stress and are believed to sense and respond to changes herein.^{1,9-13} The response comprises the acute release of vasoactive substances and more chronically changes in gene expression, cell metabolism, and cell morphology. The mechanisms by which the EC is able to sense changes in shear stress are as yet not completely understood. It is generally accepted, nevertheless, that the endothelial response should eventually lead to restoration of the shear stress to its baseline value, which is estimated to be approximately 1.0 – 1.5 Pa, corresponding to 10 – 15 $\text{dyne}\cdot\text{cm}^{-2}$.¹⁴⁻¹⁸

It is assumed that the shear stress has an important role in the development of pathologic vessel wall conditions such as atherosclerosis, restenosis, and intimal hyperplasia.¹⁹⁻²⁴ In the present review acute and long-term alterations in blood flow, and hence shear stress, and the subsequent (patho)physiologic adaptive processes will be discussed. Furthermore, a short overview of shear stress mechanoreceptors together with their downstream effectors is provided.

FLOW-MEDIATED ENDOTHELIAL SIGNAL TRANSDUCTION THROUGH MECHANORECEPTORS

Vascular tone, acute response to flow changes, arterial remodelling, and the creation of an anti-proliferative, anti-thrombotic, and anti-inflammatory milieu are accepted responsibilities of the EC and the blood vessel as a whole. Although evidence about the endothelial importance is accumulating rather swiftly, the basic question how the EC senses shear stress remains tentative.

Molecules at the luminal cell surface are candidate flow sensors since they are in direct contact with the flowing blood. These molecules can be activated directly by physical displacement or indirectly by mass transfer gradients.¹² Candidate membrane structures or mechanoreceptors include ion channels, G-protein linked receptors, tyrosine kinase receptors, integrins, and caveolae.^{1,3,25-32} These mechanoreceptors can generate a biochemical cascade by second messengers such as the adenylate cyclases cGMP and cAMP, and by the activation of protein kinases. Another way shear stress may be transduced, is through interactions of activated mechanoreceptors with cytoskeletal elements, such as focal adhesion sites and cell-cell junctions.^{25,31,33,34} Changes in ion permeability, IP_3 generation, intracellular free Ca^{2+} , and adenylate cyclase activity are rapid responses, on the order of seconds to minutes, whereas altered gene expression, cytoskeletal redistribution, and cell shape changes require many hours to develop.^{5,11,12,35} Berk et

al. have postulated that Ca^{2+} -dependent mechanisms may play an important role in the rapid responses, while Ca^{2+} -independent mechanisms are critical in the slower sustained responses.³⁵ The following sections address possible mechanoreceptor mechanisms.

Ion channels

The activation of flow-sensitive ion channels in ECs was first reported by Olesen et al., who used whole cell patch-clamp recordings to demonstrate that steady shear stress stimulates inward rectifying K^+ -channels,³⁶ leading to cell membrane hyperpolarization. Many authors have proposed various other shear-sensitive K^+ -channels.³⁷⁻³⁹ It has been shown that blockade of mechanosensitive K^+ -channels with barium chloride or tetraethylammonium blocked the shear-mediated NO production and the release of transforming growth factor β ,^{38,40} suggesting that transmembrane ion flux and intracellular ion homeostasis are important mediators of the endothelial response to shear stress changes. Shear stress also induces an outward rectifying Cl^- current in ECs, opposing the K^+ -channel-mediated hyperpolarization.^{26,41,42} Although both of these currents are activated rapidly on the initiation of flow, it is suggested that the flow-sensitive K^+ -channels attain full activation and/or desensitise more rapidly than the Cl^- -channels. Therefore, differences in activation or desensitisation between the two channel types may have important consequences for the vascular endothelial responsiveness to a flow stimulus.^{1,25,26} Recently, a mechanosensitive Ca^{2+} -permeable channel has been observed in freshly isolated ECs from rat aorta. This PKG-sensitive channel is activated by stretch but its activity correlates with a shear stress-induced Ca^{2+} signal.⁴³ There is some evidence that activation of K^+ -channels is associated with G-protein coupling, other than direct stimulation by shear stress.^{25,44}

G-protein linked receptors

G-protein linked receptors, which belong to a receptor family with seven transmembrane domains regulate activation of second messengers, which in turn influence the behaviour of other target proteins in the cell.⁴⁵ The interactions between the receptor and second messengers are mediated through enzymes or ion channels that are activated by GTP-binding regulatory proteins (or G-proteins). G-proteins are heterotrimers composed of an α chain loosely bound to a $\beta\gamma$ dimer that anchors the G-protein to the cytoplasmic face of the plasma membrane, whereas the α chain exchanges bound GDP or ADP for GTP and ATP, respectively, upon receptor activation (Figure 4), leading to the dissociation of the heterotrimer.^{25,45} A number of different G-protein families, each of them associated with different families of α , β , and γ subunits, have been identified. In ECs these include G_q -proteins, which activate PLC (Figure 5),^{25,45,46} G_s -proteins that activate Ca^{2+} -channels and adenylyl cyclase, both increasing cytosolic $[\text{Ca}^{2+}]$,^{25,28,29,45,47,48} and G_i -proteins that inhibit adenylyl cyclase and are thought to be involved in the stimulation of K^+ -channels in ECs, causing hyperpolarization (Figure 4).^{25,27,45}

Lipid membrane fluidity

Recent studies suggested that endothelial shear-responsive mechanotransduction is regulated through the cell membrane via shear stress-induced changes in fluidity.⁴⁹⁻⁵² By measuring fluorescence recovery after photobleaching (FRAP) Butler et al. demonstrated that in BAECs shear-induced changes in membrane fluidity are time

dependent and heterogeneous over the cell membrane.⁵³ Furthermore, these shear-dependent changes in membrane fluidity activate signalling molecules of the MAPK family, i.e., ERK and JNK.⁵⁴

Cytoskeleton, focal contact associated proteins, integrins and caveolae

Helmke and Davies proposed a more extended decentralized model of signal transduction, which is not exclusively limited to the luminal surface.^{31,33} In this model, forces acting on the cell surface are also transmitted by the cytoskeleton to other intracellular locations, such as focal adhesions, cell-cell junctions, the nuclear membrane, and cell surface caveolae, where signalling can occur. Supporting evidence of decentralized endothelial mechanotransduction includes the flow-induced remodelling of focal adhesion sites and activation of proteins localized at adhesion sites.^{55,56} The cytoskeleton reorganizes in response to shear stress and is able to directly transmit shearing forces by the rapid displacement and deformation of cytoskeletal filaments.^{31,57,58}

Focal adhesion sites are regions of the abluminal cell surface where membrane components (integrins) contact the extracellular matrix, e.g. fibronectin, collagen, or vitronectin.^{25,33,34,45,59} These sites are primarily responsible for cell adhesion and are involved in the regulation of EC signalling, morphology, proliferation, migration, and differentiation. They provide anchorage essential for the maintenance of tension within the cell by the association of actin stress fibres on the plasma membrane with an array of cytoplasmic proteins that link the cytoskeleton to transmembrane integrins. This provides a continuum between the intracellular cytoskeleton and extracellular adhesion proteins to which the integrins are bound.^{31,33,34,45,60} Biochemical evidence of adhesion site-specific responses to flow have been reported by several groups and includes phosphorylation of focal adhesion kinase, paxillin dephosphorylation, and movement of kinases from the cytosol to the adhesions, serving as a scaffold for recruitment of signalling molecules that play a role in the activation of MAPKs.^{4,34,35,56,61,62}

The contact surfaces between neighbouring cells experience shear stress as a torque on the cell body. These intercellular surfaces contain junctional complexes that regulate barrier function and vasoconducted responses.^{31,33,63,64} Both communication and structural junctions adapt their spatial distribution in response to altered shear stress. Proteins located in adherens junctions, including cadherin, catenins, and plakoglobin, localize to the ends of actin stress fibres following adaptation to shear stress.^{63,64} It seems likely that adherens junctions serve as a mechanical link between the cytoskeletal networks of adjacent cells.^{31,33,63}

Caveolae are specialized invaginated microdomains that abundantly appear on the endothelial luminal cell surface. They have been shown to act as dynamic carriers for the intracellular and transvascular transport of blood macromolecules.^{30,32,65} In addition, caveolae have been implicated in the organization of cell surface signalling molecules such as ion channels, G-proteins, and IP₃, and the shear stress-dependent activation of MAPKs.^{3,30-32,65,66} Spatial localization of signalling molecules that associate with actin stress fibres also implicate caveolae in directly transmitting force to the cytoskeleton.⁶⁷ It remains, however, questionable whether caveolae act as flow-sensing organelles through

their structure and overall organization or just contain flow-sensing proteins that initiate signalling events.

REACTION TO ACUTE WALL SHEAR STRESS CHANGES

To accommodate acute changes in blood flow and to restore shear stress values quickly, arteries, both large and small can either dilate or constrict. Dilation or constriction is usually due to the local release of vasoactive peptides that will be discussed separately. In large (conduit) arteries this will result in flow regulation and in small arteries this will lead to blood pressure regulation on the arteriolar level.

Figure 4. Vasodilatory signalling initiated by an acute increase in shear stress. The NO-, PGI₂-, CNP-, and EDHF-pathway are recapitulated.

Acute increase in wall shear stress

Furchgott and Zawadzki ascribed arterial dilation to the endothelium for the first time.⁶⁸ Their study showed that ACh, an established vasodilator, was ineffective if the endothelium was substantially damaged. They proposed that ACh acts on the muscarinic receptors of the EC, thereby stimulating the release of EDRFs. Subsequently, Furchgott as well as other investigators have proven that EDRF is

actually NO produced endogenously by the endothelium.⁶⁹⁻⁷¹ In response to a sudden flow increase various vasoactive substances, capable of inducing vasodilation, will be secreted.⁷²⁻⁷⁶ Figure 4, although incomplete, summarizes the different vasodilatory peptides as described below.

Nitric oxide (NO)

Since its discovery in 1847 by Ascanio Sobrero, NO has probably become the best-known vasodilator in clinical practice.^{77,78} Due to its rapid action and established efficacy, NO-donors such as nitroglycerin or GTN, is the treatment of choice for acutely relieving angina pectoris or chest pain due to cardiac ischemia.^{78,79} Furthermore, nitrates are used if heart failure patients do not respond adequately to diuretics, angiotensin converting enzyme inhibitors, and/or glycosid cardiotonics such as digoxin.^{80,81} Even chronic anal fissures are healed in about 60% of cases by nitrates through lowering anal pressure by relaxation of the anal sphincter muscle.^{82,83} Not only NO-donors, but also modulation of the NO axis through, for example, exogenous L-arginine and/or cGMP phosphodiesterase inhibitors has gained clinical use in for instance the treatment of erectile dysfunction, pulmonary hypertension, and peripheral vascular disease.⁸⁴⁻⁸⁷

Because of its instability ($t_{1/2} = 0.5 - 5$ s) NO is a local factor. It is formed (Figure 4), together with the side product L-citrulline from the amino acid L-arginine, catalyzed through an endothelial enzyme, the constitutively expressed eNOS.^{18,28,88-90} eNOS is Ca^{2+} - and calmodulin-dependent and an acute shear stress elevation can increase the intracellular $[\text{Ca}^{2+}]$, thereby activating eNOS.^{18,35,88,90} The relaxation induced by NO is largely due to the activation of soluble guanylyl cyclase in the VSMC with enhanced formation of cGMP. This second messenger causes a lowering of cytosolic $[\text{Ca}^{2+}]$ through PKG by blocking Ca^{2+} influx and by blocking phosphorylation of troponin, thereby inhibiting the activation of the Ca^{2+} -dependent myosin light chain kinase, effectuating VSMC relaxation and hence vasodilation (Figure 4).⁹¹⁻⁹⁴ Another mechanism by which NO might induce VSMC relaxation is by VSMC hyperpolarization through the activation of K^{+} -channels (Figure 4). Hyperpolarization causes a K^{+} -efflux to the extracellular space and decreases intracellular Ca^{2+} with vasodilation as a consequence.⁹⁵

The potency of the ECs to produce NO endogenously has become a hallmark in the discussion regarding endothelial function. Ever since the leading article by Celermajer et al. in *The Lancet*, where they show that in coronary artery diseased patients and hypercholesterolemic children endothelial dysfunction can be observed,⁹⁶ a great number of cardiovascular risk factors and diseases have been linked to endothelial dysfunction.⁹⁷⁻¹⁰⁴ Basically, these studies focus on the difference in vasodilatory capacity of the brachial artery after an induced flow increase and after GTN administration. In case of a diseased or dysfunctional endothelium the so-called brachial FMD is lower than the dilation brought upon by GTN, which is believed to be endothelium-independent. Although promising results have been obtained, this test is as yet not viable for the individual patient.¹⁰⁵⁻¹⁰⁹ It would therefore be better to assess endothelial (dys)function through, for example, plasma endothelial function markers, such as vWF, ET-1, ICAM-1, TF, and PAI-1 for example.¹¹⁰⁻¹¹³

Prostacyclin (PGI₂)

Vane and co-workers discovered that vascular tissue is capable of PGI₂ generation.^{18,47,114,115} PGI₂, a member of the vast family of prostaglandins, is synthesized from arachidonic acid via the cyclo-oxygenase pathway (Figure 4). Various researches have established PGI₂ as a potent arterial vasodilator by stimulating VSMC cAMP-synthesis through a G-protein-coupled mechanism *in vitro*.^{28,29,47,48} Subsequently, this cAMP up-regulation results in a [Ca²⁺] decrease through PKA in a close interplay with cGMP and PKG, which induces VSMC-relaxation and hence vasodilation.⁹²⁻⁹⁴

In vitro as well as *in vivo*, ECs can be triggered by shear stress changes to generate PGI₂ and henceforth to regulate vascular tone.^{28,73,74,116-118} Several mechanisms have been described, for example an increase in cyclo-oxygenase-1 and PGI₂-synthase and the subsequent augmented release of PGI₂ has been observed in HUVECs under elevated shear stress conditions.¹¹⁶ Furthermore, arachidonic acid may be liberated through the combined action of the Ca²⁺-dependent PLC and PLA₂ pathway (Figure 4).¹¹⁷ Supporting this mechanism, several *in vitro* studies have shown that endothelial intracellular [Ca²⁺] increases in response to elevated shear stress and especially shear stress oscillations.^{43,51,119} Hecker et al. even showed that an endothelial [Ca²⁺] increase stimulates PGI₂ secretion.⁵¹

At present, PGI₂ analogues are applied in clinical trials, addressing their effect in various cardiovascular diseases such as Buerger's disease, arteriosclerosis obliterans, pulmonary arterial hypertension, Raynaud's phenomenon, and peripheral vascular disease.¹²⁰⁻¹²³ Intravenous (for instance iloprost) and oral (for instance beraprost and cicaprost) variants can be administered and the results of large, phase III, placebo-controlled studies are expected shortly. However, its efficacy seems limited in the long-term treatments of the above diseases, hence well-designed trials are necessary to define the actual function of PGI₂ analogues.

Endothelium-derived hyperpolarizing factor (EDHF)

In many vessels, notably in the small arteries of the resistance vasculature, the pharmacological inhibition of NO or PGI₂ synthesis does not greatly affect the endothelium-dependent relaxation response. Because the cellular action of this non-nitric oxide and non-prostanoid mediator has been associated with endothelium-dependent hyperpolarization of the VSMC, the factor has been labelled EDHF.^{74,76,124} The contribution of EDHF-mediated responses is also of importance for conduit arteries.¹²⁵

In general, hyperpolarization of VSMCs through an efflux of K⁺ to the extracellular space produces relaxation by closure of voltage-dependent Ca²⁺-channels and the turnover of intracellular phosphatidylinositides, thus decreasing the intracellular [Ca²⁺] (Figure 4).¹²⁶ Subsequently, vascular tone is lost and VSMC relaxation occurs. Although the nature and identity of the elusive EDHF is as yet not established, a number of recent reviews summarize possible candidates.^{74,76,124,125,127,128} Interestingly, NO is considered due to its ability to mediate VSMC hyperpolarization (in)directly through activation of VSMC K⁺-channels. Other candidates for EDHF comprise metabolites of arachidonic acid, particularly 5,6-epoxyeicosatrienoic acid, K⁺, hydrogen peroxide, and gap junctions

(mainly connexins) through electrical coupling. Moreover, evidence is accumulating that endothelial hyperpolarization through a $[Ca^{2+}]$ -increase is an important factor in EDHF-mediated responses, possibly triggering one or more of the above mentioned candidates. Just recently, few reports suggest that shear stress can elicit endothelium-dependent hyperpolarization in HUVECs and in *ex vivo* rabbit and human small arteries.¹²⁸⁻¹³¹

C-type natriuretic peptide (CNP)

After CNP was identified in ECs and recognized as a vasorelaxing substance^{132,133} the mRNA expression of this newest member of the natriuretic peptide family was shown to be shear stress-dependent in HUVECs and BAECs.^{134,135} Interestingly, autocrine signalling through shear-induced NO production does not seem to be involved (Figure 4).¹³⁶

CNP activates the cGMP cascade in VSMCs through the CNP-selective guanylyl cyclase NPR-B, which is abundantly present on the VSMC cell membrane.¹³⁷ Furthermore, the local expression of CNP in rabbit balloon-injured carotid arteries enhances iNOS gene expression and NO production, which in turn activates the cGMP cascade of VSMCs through cytoplasmatic soluble guanylate cyclase.¹³⁸ The cGMP-increase induced by one of the above mechanisms results in VSMC relaxation and thus arterial dilation as described earlier.

An interesting finding, recently described by Chauhan et al., nicely illustrates that in rat mesenteric arteries the release of CNP accounts for the biological activity of EDHF. They hypothesized that activation of NPR-C by CNP results in a G-protein dependent activation of a K^+ -channel in VSMCs inducing hyperpolarization and, thereby, relaxation.⁷⁵ Although this pathway is ACh-induced at the endothelial level, it demonstrates another mechanism by which CNP could act after induction by increased shear stresses.

Acute decrease in wall shear stress

In a human *in vivo* study, Joannides and his colleagues have satisfactorily shown that by inducing an acute flow and thus shear stress decrease, the radial artery smooth muscle cell tone indeed increases significantly,¹³⁹ which resulted in a diameter decrease and in an increased wall thickness. It is assumed that the endothelium also modulates the contractile tone of the VSMCs thereby maintaining a rather constant shear stress, accentuating the eminent role of the ECs in the overall regulation of wall shear stress.¹⁴⁻¹⁸ In this setting, various studies have shown considerable cross-talk between constrictory and dilatory substances, suggesting a physiological equilibrium.¹⁴⁰⁻¹⁴⁴ Moreover, due to the lower shear stress, the flow-mediated release of vasodilatory substances is decreased, opposing vasodilation. Besides the above-mentioned vasodilators the endothelium is indeed capable of releasing ET1, a potent vasoconstrictory substance that is discussed below. Other peptides known for their vasoconstrictive nature, such as vasopressin, angiotensin II and urotensin II, have as yet not been linked to a decreased shear stress and will therefore not be conferred.¹⁴⁵⁻¹⁴⁸

Endothelin-1 (ET-1)

Yoshizumi et al. examined the effects of shear stress on ET-1 mRNA and ET-1 production in cultured porcine ECs,¹⁴⁹ shortly after the discovery of ET.¹⁵⁰ They

observed that, under low shear stress conditions, ECs increased their ET production and augmented their expression of ET mRNA. Later, others have shown that shear stress regulation of ET-1 gene expression and production is characterized by a biphasic response with an early transient stimulation of ET-1 activity followed by a sustained inhibition.^{144,151,152} Furthermore, this inhibitory effect of shear stress on ET-1 production appeared to be NO-dependent.^{144,153} Current opinion is that physiological shear stress upholds the secretion of dilatory peptides such as NO and PGI₂, thereby counteracting possible vasoconstrictors.^{140,154,155} When blood flow tends to become non-laminar or even turbulent the release of vasodilatory substances is frustrated, thus the dilatory-constrictory balance is disturbed and vasoconstriction occurs as a consequence. This is corroborated by the fact that oscillatory flow, which is believed to be atherogenic, only upregulates ET-1 gene expression, whereas unidirectional pulsatile flow upregulates both eNOS and ET-1 gene expression.^{151,156-158}

Endothelin is a 21-amino acid peptide occurring in three isoforms (ECs release primarily ET-1), which is derived from preproendothelin (or big endothelin) via proendothelin (Figure 5).^{114,159,160} The conversion to ET-1 is catalysed by ECE found on the EC membrane

Figure 5. The vasoconstrictory effect of endothelin (ET-1) mediated through shear stress, NO, and PGI₂.

The circulating plasma concentrations of ET-1 are very low, probably because it binds to its receptors locally. Furthermore, it is preferentially secreted abluminally by ECs and binds to three types of receptors among which ET_A and ET_B.^{114,159,160} The ET_A receptor can be found on the VSMC and mediates the vasoconstrictive response. It is coupled to a G-protein and activates PLC, leading to the formation of IP₃ and DAG from the polyphosphoinositide PIP through hydrolysis.⁴⁶ IP₃, in turn, binds to the ligand gated Ca²⁺-channel on the endoplasmatic reticulum causing the release of Ca²⁺ into the cytosol, thereby increasing [Ca²⁺].¹¹⁴ The ET-receptor might also be linked to a voltage-operated calcium channel via another G protein.^{159,160} Consequently, troponin can be phosphorylated and the actin-filaments are activated, resulting in contraction of the VSMC and hence vascular constriction. Binding of ET-1 to the ET_B receptor, mainly expressed by the endothelium, increases the release of NO and prostaglandins thereby antagonizing the effects exerted by the activated ET_A receptor (Figure 5).^{114,161-164}

Just recently, Masatsugu and his colleagues have shown that the expression of ET-1 and ECE through shear stress is regulated via the production of intracellular reactive oxygen species.¹⁶⁵

LONG-TERM WALL SHEAR STRESS CHANGES; EMPHASIS ON INTIMAL HYPERPLASIA

Apart from the control of vascular tone and lumen diameter, as described in the previous section, normal functioning of the endothelium is essential to maintain anticoagulant, antiproliferative, and anti-inflammatory properties, and the regulation of vascular permeability, in order to prevent pathological conditions such as atherosclerosis, intimal hyperplasia, and restenosis.¹⁶⁶⁻¹⁶⁸ Here we will discuss the molecular mechanisms in the development of intimal hyperplasia or inward remodelling.¹⁶⁹⁻¹⁷¹

Intimal hyperplasia is defined as the abnormal proliferation and intimal migration of medial VSMCs and adventitial fibroblasts, accompanied by extracellular matrix deposition, such as proteoglycans, elastin, and collagens.^{166,167} Intimal hyperplasia can be observed in closure of the ductus arteriosus, pulmonary hypertension, arteriovenous fistulas, or arterial bypass conduits.^{168,172-175} A locally altered shear stress may be one of the culprits in the development of intimal hyperplasia. Several investigators have demonstrated that ECs may actually be sensitive to the magnitude of the temporal and spatial shear gradient along the vessel, especially in regions with low flow and areas with flow reversals.^{4,176-179}

VSMC proliferation is increased in intimal hyperplasia and is likely stimulated by EC factors that are regulated by shear stress. The activation of MAPK by shear stress induces transcription factors (AP-1 and NF- κ B) and immediate early genes, ensuing VSMC proliferation or quiescence, and matrix-integrin interactions involved in the regulation of MMPs.^{3,12,35,61} In an arteriovenous fistula model Kraiss et al showed that endothelial PDGF-A mRNA levels and VSMC proliferation were increased in areas with low blood flow velocities.¹⁸⁰ ET-1, a VSMC mitogen acting synergistically with PDGF, was dramatically reduced in response to exposure to physiological shear stresses in a flow chamber study on HUVECs.¹⁵⁸ Moreover, NO

and TGF- β , both inhibitors of VSMC growth, are secreted by endothelial cells in response to shear stress.^{38,181,182} The expression of another potent mitogen, angiotensin II, is downregulated through exposure to physiological shear stress by reducing angiotensin-converting enzyme mRNA and activity.¹⁸³ In a flow chamber model, Akimoto and his colleagues have shown that steady laminar shear stress induces EC cell cycle arrest by upregulating the cell cycle progression inhibitor p21^{waf1}.¹⁸⁴ Through its ability to regulate disparate VSMC growth factors, shear stress likely plays a role in the development of intimal hyperplasia at regions with low shear stress and flow reversal.¹⁸⁵ The activity of MMPs is furthermore upregulated in areas with disturbed shear stress,^{185,186} facilitating migration of VSMCs and the subsequent development of intimal thickening or inward remodelling. This has been eloquently illustrated by different animal studies where an arteriovenous fistula was created between the internal jugular vein and the carotid artery of either rats or rabbits.^{171,187} Inhibition of MMPs resulted in non-maturation of the fistula, expressed by a hampered arterial enlargement.¹⁸⁷

CONCLUSION

The present review concisely addressed various mechanisms by which ECs are able to sense and to react to changes in shear stress. Acute responses such as vasodilation and vasoconstriction and the chronic development of intimal hyperplasia were discussed. The recent advances in cell and molecular biology contributed to the identification of molecular mechanisms by which ECs sense and respond to changes in shear stress. Understanding the complex interplay of shear stress-induced signal transduction and gene regulation in ECs and VSMCs will facilitate our knowledge of physiologic as well as pathologic vascular remodelling and will help to develop strategies to manage cardiovascular diseases.

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CHAPTER 3

Materials and methods

BASIC ULTRASOUND PRINCIPLES

Ultrasound for clinical application is sound in the rf domain (1 – 20 MHz). The principle of echography is that a burst of ultrasonic waves is generated and emitted into the human body. Parts of the emitted waves are reflected and/or scattered as the waves impose on internal structures. Ultrasound pressure waves are generated and received with a piezoelectric apparatus (transducer), able to convert electrical energy to mechanical energy and *vice versa*.

Pulsed ultrasound is based on the repetitive emission of short ultrasound pulses at a time interval of $1/\text{PRF}$ [s] (PRF = pulse repetition frequency) and the subsequent reception of the reflected echoes originating from acoustic impedance transitions of various tissues. The acoustic impedance Z [$\text{kg}\cdot\text{m}^{-2}\text{s}^{-1}$] can be calculated by multiplication of the tissue's density ρ [$\text{kg}\cdot\text{m}^{-3}$] and the propagation velocity of sound in the various tissues c [$\text{m}\cdot\text{s}^{-1}$], which is close to $1540\text{ m}\cdot\text{s}^{-1}$ in most soft tissue and is considered to be tissue- and subject-independent.¹ Reflection only occurs at acoustic impedance transitions larger than a few wavelengths λ [m], defined as:

$$\lambda = \frac{c}{f_c} \quad (3-1)$$

where f_c [s^{-1}] is the carrier frequency of the ultrasound waves. Sound waves impinging on structures smaller than λ are backscattered. The distance to the point of reflection s [m] can be obtained through:

$$s = \frac{c \cdot t_d}{2} \quad (3-2)$$

where t_d [s] is the time that sound waves require to travel the round trip to axial distance s [m], hence the divisor 2.

The carrier frequency f_c in part determines the spatial resolution, divided into a lateral and axial resolution, of the ultrasound system. An f_c of 6 MHz corresponds to a λ of about $250\text{ }\mu\text{m}$ (see equation (3-1)). The time-length of the emitted pulse, depending on f_c and the number of emitted wavelengths, determines the axial resolution, i.e., in the direction of the sound wave propagation. Thus, the longer the duration Δt [s] of the emitted pulse is, the smaller the bandwidth BW [s^{-1}] of the emitted waves will be, following the fundamental relation, thereby gaining axial resolution:^{2,3}

$$BW \cdot \Delta t \cong 1 \quad (3-3)$$

Lateral resolution, on the other hand, is dependent on the local beam width, which is determined by the size of the transducer and the position of the focal point. Maximal focus can be obtained by a transducer size of 40λ , resulting in a beam width of approximately 5λ at the focal point. If the size of the transducer in

wavelengths is constant, then a higher carrier frequency will result in a narrower beam, ensuing maximal lateral resolution.

If the emitted ultrasound pulse encounters a moving object, then the reflected or backscattered echo shows a shift in depth Δs [m] with respect to the previous pulse. The time between each pulse or rf-signal is $1/\text{PRF}$. Δs results in a shift in carrier frequency of the temporal signal or in other words the Doppler shift f_D [s^{-1}], depending on the velocity of the moving object v [$\text{m}\cdot\text{s}^{-1}$] and on the carrier frequency f_c [s^{-1}] of the originally emitted pulse:

$$f_D = \frac{v \cdot 2f_c \cdot \cos(\varphi)}{c} \quad (3-4)$$

where c [$\text{m}\cdot\text{s}^{-1}$] is the ultrasound velocity in the transmission medium and φ [rad] is the assumed angle between the ultrasound beam and the direction of the moving object. The factor 2 again compensates for the round trip of the sound waves.

The frequency spectrum of the Doppler signal is filtered and the mean velocity at a certain depth can be calculated using equation (3-4). With pulsed ultrasound is possible to measure velocities at a certain depth, which can be expanded to velocities at various depths simultaneously by multigated pulsed Doppler. It is then possible to assess the velocity distribution and to calculate the shear rate distribution as the radial derivative in an artery along a line of observation as function of time. For a more detailed overview of the various technical aspects of the shear rate measurements please be referred to Brands et al.⁴

WALL TRACK SYSTEM AND SHEAR RATE ESTIMATION SYSTEM (ART-LAB)

The Wall Track System

End-diastolic diameter d [m] and the change in diameter from diastole to systole (distension or Δd [m]) were measured by means of an off-line automated rf method based on M-line signal processing (wall track system) that has been described in detail before.⁴ Briefly, with an ultrasound system (Ultramark 9 plus, Advanced Technology Laboratories (ATL), Bellevue, WA, USA) a B-mode image was obtained in a longitudinal plane, after which an M-line was positioned perpendicular to the arterial walls. The raw digitised (20 MHz) rf-signals were transferred to a PC through an interface configuration consisting of an internal card in the ATL-system in combination with a custom built plug-in card for the PC. The latter card allows capturing segments of the rf-signals starting at a preselected depth and with a preselected width. Data acquisition was initiated synchronously with a trigger derived from the top of the R-wave of an ECG signal. Consecutively, the first rf line was displayed on the computer monitor, allowing identification of the lumen to detect automatically the positions of the anterior and posterior wall of the vessel.^{4,5} Dedicated signal processing results in the initial distance between both walls d [m] and the change in distance Δd [m] as function of time within a cardiac cycle. The intraobserver intrasession and interobserver intrasession reproducibility of the system for carotid investigations is $4.5 \pm 1.1\%$ and $8.6 \pm 1.9\%$ for diameter

and $7.9 \pm 1.6\%$ and $11.3 \pm 2.6\%$ for the change in diameter, respectively.⁶ The reproducibility for brachial artery investigations will be discussed in **CHAPTER 4**.

Using the same technique, also local *IMT* [m] could be measured off-line using the wall thickness program (Figure 6).⁷ The system settings were held constant, with a threshold of 0.03 for the spatial derivative of the amplitude, normalised with respect to the peak amplitude within the segment considered. The resolution for *IMT* detection proved to be approximately 40 μm in an earlier study out our laboratory.⁷

Figure 6. Characteristic image of the rf-signal envelope of a healthy subject's brachial artery.

The Shear Rate Estimation System

To assess the instantaneous blood velocity and shear rate distribution along a selected line of observation with high axial resolution, the ultrasound system with a broadband (5 – 9 MHz) curved array transducer was switched to a wide band M-mode with a high pulse repetition frequency (10 kHz).^{4,8} From a depth of approximately 2 cm, the returned acoustic signals had an effective bandwidth of about 2.5 MHz, which is equivalent to a spatial resolution of about 300 μm . Accordingly, each velocity estimate was based on half overlapping data segments of 300 μm in depth and 10 ms in time and was calculated by means of a modelled cross correlation function for the rf signals, resulting in an instantaneous velocity distribution as shown in Figure 7A.⁹ The velocity distribution provides systolic velocity v_{max} or SV [$\text{m}\cdot\text{s}^{-1}$] and mean velocity MV [$\text{m}\cdot\text{s}^{-1}$], averaged over a heart cycle, in the centre of the lumen. The latter is comparable with the mean velocity derived from a Doppler signal observed in the centre of the lumen with conventional pulsed Doppler systems. The instantaneous shear rate distribution

(Figure 7B) follows from the radial derivative of the velocity profile at each site and each time instant. The PC memory presently available allows a recording duration of 6 seconds for wall shear rate measurements and up to 20 minutes for diameter measurements, which is sufficient to capture data over five complete heartbeats. The intrasubject intrasession reproducibility of the system for common carotid artery peak and mean shear rate is about 15 and 12%, respectively.^{10,11} The reproducibility for brachial investigations will be discussed in **CHAPTER 4**.

Figure 7. (A) Velocity and (B) shear rate distribution in a healthy subject's brachial artery

ASSESSMENT OF BLOOD PARAMETERS, ENDOTHELIAL CELL MARKERS, AND MATRIX DEGRADING PROTEINS

For this thesis, venous blood samples were drawn 15 minutes before assessment of vascular wall properties and hemodynamic parameters in both the healthy and the patient population. The blood samples were always obtained without stasis from an antecubital vein of the dominant arm to prevent scarring of the venous outflow tract of the future arteriovenous fistula.

Routine blood parameters

Total cholesterol, high-density and low-density lipoprotein, triglycerides, glucose, urea and creatinine levels were determined for routine clinical use with in-hospital procedures. Furthermore the hemoglobin concentration and hematocrit were obtained through standard in-hospital protocols. More specifically, plasma viscosity η_0 [Pa·s] was determined in duplo with a glass capillary viscometer (Mikro-Ostwald Viskosimeter, Schott-Geräte GmbH, Hofheim, Germany; normal range 1.09–1.4 mPa·s).

Endothelial cell markers

In the present thesis we were interested in established EC markers such as PAI-1, vWF, and ET-1 levels. Blood samples for PAI-1 and vWF were collected in tubes containing 3.25% sodium citrate and spun immediately at 5,000g for 10 min at 4°C. PAI-1 activity was measured photometrically (Kabi Diagnostica, Sweden; intra-assay coefficient of variation (CV) of 6.0%; normal range 1 – 20 arbitrary units·mL⁻¹). Plasma levels of vWF antigen were determined by an ELISA using rabbit anti-human vWF (Dako A/S, Glostrup, Denmark; CV=7.4%; normal range 50 – 150%).

Blood samples for plasma ET-1 was collected in pre-chilled tubes containing EDTA and centrifuged immediately at 2,000g for 15 min at 4°C. Plasma ET-1 was determined by radioimmunoassay (Kit Catalog #RIK6901; Peninsula Laboratories Europe Ltd., Merseyside, England). This method gave an extraction recovery of 97% with a CV of 4.6% (normal range 1.6 – 4.9 pg·mL⁻¹). These assays were all conducted by technicians who were blinded to any characteristics of ESRD patients and controls.

Matrix degrading proteins

Blood samples for determination of the matrix metalloproteinases MMP-2 and -9 and their natural inhibitors TIMP-2 and -1, respectively, were collected in tubes containing 3.25% sodium citrate and spun immediately at 5,000g for 10 min at 4°C. Plasma levels of MMP-2 were determined by an ELISA (Human Biotrak ELISA System RPN2617, Amersham Biosciences, Buckinghamshire, United Kingdom; coefficient of variation (CV)=5.3 – 6.3%; normal range 365 – 649 ng·mL⁻¹). Plasma MMP-9 was also determined by an ELISA (RPN2614; CV=4.9 – 5.5%; normal range is below the sensitivity of the assay, i.e., 0.6 ng·mL⁻¹). Plasma levels of both TIMP-1 and -2 were also determined by ELISAs (RPN2611; CV=8.9 – 11.4%; normal range 569 – 1063 ng·mL⁻¹, and RPN2618; CV=2.8 – 5.4%; normal range 21 – 108 ng·mL⁻¹, respectively). The colour absorbance is measured at 450 nm; the results are referred to as optical densities. These optical densities can be converted into plasma concentrations by use of the calibration of a standard curve following the manufacturer's protocol.

CALCULATION OF WHOLE BLOOD VISCOSITY AND SHEAR STRESS

In order to calculate vascular wall shear stress τ [Pa] through equation (1-5) as described in **CHAPTER 1**, we had to determine WBV [Pa·s] from the measured wall shear rate γ [s⁻¹], plasma viscosity η_0 [Pa·s], and hematocrit Htc [%]. To estimate WBV , the approximation proposed by Weaver was used:¹²

$$\log WBV = \log \eta_0 + \{0.030 - 0.0076 \log \gamma\} \cdot Htc \quad (3-5)$$

In **CHAPTER 1** we already discussed the viscoelastic properties of human blood. Equation (3-5) indeed considers blood to be a shear-thinning fluid and is therefore more accurate in approximating shear stress *in vivo* than Poiseuille's law. In a previous study we have in fact shown that mainly shear rate contributes to large changes in WBV , while plasma viscosity and hematocrit inflict only slight changes (Table 1).¹³ To obtain the wall shear stress τ [Pa], the calculated WBV [Pa·s] was multiplied by the measured wall shear rate γ [s⁻¹]:

$$\tau = WBV \cdot \gamma \quad (3-6)$$

STUDY DESIGN

According to institutional guidelines, all healthy subjects and ESRD patients were aware of the investigational nature of the study and gave written informed consent.

The study was approved by the joint Medical Ethical Committee of the Maastricht University and the university hospital Maastricht. All volunteers were non-smokers and none of them reported medication intake or a history of cardiovascular, cerebrovascular, or peripheral vascular disease.

<i>MWSR</i> [s ⁻¹]	Change in <i>WBV</i> [%] for 1% change in <i>Htc</i>	Change in <i>WBV</i> [%] for 1% change in η_0
100	2.13	0.74
250	1.86	0.74
500	1.73	0.74
750	1.43	0.74
1000	1.31	0.74

Table 1. The effect of changes in hematocrit and/or plasma viscosity on whole blood viscosity at different shear rates calculated with Weaver’s approximation.¹² *MWSR* = mean wall shear rate; *WBV* = whole blood viscosity; *Htc* = hematocrit; and η_0 = plasma viscosity.

Healthy and patient population

The measurements were always performed in the morning between 08:00 and noon after an overnight fasting of at least 10 hours. The necessary medication intake of the patients was allowed. Blood samples were obtained without stasis from an antecubital vein. The subjects were examined after an acclimatisation period of 15 minutes in supine position in a room with a temperature of 22 – 24°C. On the non-dominant arm brachial artery blood pressure was measured every 5 minutes by a semi-automated oscillometric device (Dinamap, Critikon, Tampa, Florida, USA).

On the dominant arm in casu quo the arteriovenous fistula arm, the vessel of interest was visualised in B-mode with the arm in approximately 45° abduction and the palm of the hand facing upward. First, the ultrasound system was switched to M-mode and diameter and wall thickness recordings were taken at an angle of observation of 90°. Subsequently, a suitable line of sight was selected, crossing the artery at an angle of 70° with the longitudinal axis of the artery, herewith measuring wall shear rate. The measurement procedure for both diameter and wall shear rate, each with a length of 5 seconds covering about 6 cardiac cycles, was repeated 5 times with a time interval of 1 minute between the measurements.

Arterial remodelling in arteriovenous fistulas

All patients scheduled for arteriovenous fistula placement on the left arm for future hemodialysis were included. Vascular access was accomplished through creation of an autogenous radial-cephalic wrist access by anastomosing the cephalic vein to the radial artery approximately 2 cm proximal to the radial styloid. To avoid operating skills bias, the same vascular surgeon performed all surgical procedures.

The measurement procedure as described above was maintained for the complete follow-up period. Both the brachial and radial artery were considered for this thesis. For the brachial artery the measurements were performed preoperatively and repeated 1 day, 1, 2, 3, 6, 12, 24, and 52 weeks after surgery to map potential arterial remodelling. In order to allow recovery of the radial artery, the radial artery was not measured until 3 weeks post surgery.

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CHAPTER 4

Brachial artery shear stress is independent of gender or age and does not modify vessel wall mechanic properties

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Hoeks A.P.G.

ABSTRACT

The objective of the present study is to obtain brachial artery baseline shear stress values in healthy volunteers and relate this to gender and age. Peak and mean wall shear rate were non-invasively measured in 30 healthy subjects using a shear rate estimation system. Arterial diameter and wall characteristics were obtained with a wall tracking system. Shear stress was estimated from shear rate and calculated *WBV*. Intrasubject variability and the effects of age and gender were assessed.

Intrasubject variability of brachial artery peak and mean shear rate were 16.2% and 28.6%. Baseline peak ($\approx 3.0 \pm 0.7$ Pa) and mean shear stress ($\approx 0.5 \pm 0.2$ Pa) were not gender dependent, nor were they influenced by age. No vessel wall parameter related to brachial artery shear stress. No differences in brachial artery shear stress were observed between the genders and no influence of age was apparent. Our results indicate that the brachial artery adequately responds to chronic changes in blood flow.

INTRODUCTION

Changes in vessel wall characteristics, such as compliance and distensibility, have been related to increased risks of atherosclerosis.^{1,2} The compliance coefficient *CC* is defined as the storage capacity of the vessel per unit of length and is calculated through equation (1-1). The distensibility coefficient *DC* reflects the elasticity of the vessel wall and is calculated with equation (1-2). These characteristics have been shown to depend upon age, gender and vascular territory.³ Thus, vessel wall mechanics in the brachial artery, a muscular artery, differ from those in the elastic common carotid artery or aorta.^{3,4}

Diameter, and hence vessel wall characteristics, are believed to be influenced by the local hemodynamic parameters. Especially wall shear stress, the viscous drag exerted on the vessel wall by the flowing blood, appears to play an eminent role in acute adaptations to flow changes, vascular remodelling, and primary atherosclerosis.⁵

The endothelial lining of the vessel wall is thought to sense the wall shear stress and subsequently react to changes herein (**CHAPTER 2**). For example, the endothelium releases a number of vasoactive substances, such as NO and PGI₂, in reaction to a sudden increase in wall shear stress arising from increased blood flow.^{6,7} As a consequence, VSMC relaxation will occur, which has known effects on vascular wall mechanics.^{8,9} Furthermore, a sustained increase in blood flow induces outward vascular remodelling,¹⁰⁻¹² which is regulated by EC gene expression.^{13,14}

In elastic arteries, such as the common carotid artery, shear stress has been shown to be correlated with *IMT* and diameter.¹⁵⁻¹⁷ Although the influence of age and gender upon brachial artery wall mechanics has been described before,³ baseline hemodynamic parameters have not been reported. Only the association between shear stress and FMD in healthy male subjects has been shown by Gnasso et al.¹⁸ who assumed a parabolic velocity profile. The objective of this study was to obtain physiologic shear stress values in the brachial artery of healthy subjects and relate this to gender and age. Furthermore, we investigated if brachial artery wall mechanics (i.e., diameter, *IMT*, and *DC*) are dependent on shear stress.

MATERIALS AND METHODS

Subjects and subject characteristics

Thirty normotensive, normocholesterolemic, and normoglycemic volunteers (15 female) were included in the study. All volunteers were non-smokers and none of them reported medication intake or a history of cardiovascular, cerebrovascular, or peripheral vascular disease. According to institutional guidelines, all subjects were aware of the investigational nature of the study and gave written informed consent. The study was approved by the joint Medical Ethical Committee of the Maastricht University and the university hospital Maastricht. Table 2 summarises the volunteer characteristics; the subjects are divided in a younger (group 1: 30 – 47 years) and an older group (group 2: 48 – 65 years).

The measurements were always performed in the morning between 08:00 and noon after an overnight fasting of at least 10 hours. Blood samples were obtained without stasis from an antecubital vein for determination of total

cholesterol, high-density lipoprotein, low-density lipoprotein, triglycerides, glucose, hemoglobin, hematocrit and plasma viscosity (Table 2). The subjects were examined after an acclimatisation period of 15 minutes in supine position in a room with a temperature of 22 – 24°C. Brachial artery diameter and wall shear rate measurements were performed on the non-dominant arm. Furthermore, on the other arm brachial artery blood pressure was measured every 5 minutes by a semi-automated oscillometric device (Dinamap, Critikon, Tampa, Florida, USA).

	Male		Female	
	group 1	group 2	group 1	group 2
Age [years]	36.1 ± 4.7	59.3 ± 4.6	36.7 ± 5.6	58.0 ± 4.5
BMI [kg·m ⁻²]	25.2 ± 2.0	26.3 ± 4.8	26.2 ± 6.0	24.2 ± 2.0
SBP [mmHg]	119.7 ± 9.7	133.4 ± 17.6	120.7 ± 7.8	124.8 ± 6.0
DBP [mmHg]	69.0 ± 5.9	81.9 ± 7.2*	70.3 ± 8.9	73.3 ± 12.0
MAP [mmHg]	86.4 ± 7.3	102.8 ± 12.2*	88.3 ± 7.5	92.5 ± 9.9
HR [min ⁻¹]	60.0 ± 12.3	64.0 ± 7.5	62.4 ± 9.6	62.6 ± 10.4
Hemoglobin [mmol·L ⁻¹]	9.27 ± 0.29	9.35 ± 0.40	8.09 ± 0.26†	8.66 ± 0.45†*
Hematocrit [%]	44 ± 2.6	45 ± 2.8	39 ± 2.6†	41 ± 2.8†*
Total cholesterol [mmol·L ⁻¹]	5.0 ± 1.1	6.0 ± 0.8*	4.7 ± 0.8	5.6 ± 0.6*
HDL [mmol·L ⁻¹]	1.4 ± 0.3	1.3 ± 0.3	1.7 ± 0.3	1.8 ± 0.3
LDL [mmol·L ⁻¹]	3.2 ± 0.3	4.0 ± 0.6	2.7 ± 0.8	3.4 ± 0.6*
Triglycerides [mmol·L ⁻¹]	0.9 ± 0.3	1.6 ± 0.8	0.8 ± 0.3	0.9 ± 0.3‡
Glucose [mmol·L ⁻¹]	5.6 ± 0.5	5.7 ± 0.6	5.6 ± 0.5	5.5 ± 0.6
Plasma viscosity [mPa·s]	1.13 ± 0.02	1.12 ± 0.04	1.17 ± 0.07	1.16 ± 0.08

Table 2. Subject characteristics. Data presented as means ± SDs; group 1 = younger group, group 2 = older group; BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; MAP = mean arterial pressure; HR = heart rate; HDL = high density lipoprotein; LDL = low density lipoprotein; * = p < 0.05 group 1 vs. group 2; † = p < 0.001 men vs. women; ‡ = p < 0.05 men vs. women.

The Wall Track System and the Shear Rate Estimation System (ART-Lab)

The wall track system and the shear rate estimation system have been described extensively in CHAPTER 3 Materials and methods.

Measurement procedure

The brachial artery was visualised in B-mode with the arm in approximately 45° abduction and the palm of the hand facing upward. First, the ultrasound system was switched to M-mode and diameter and wall thickness recordings were taken at an angle of observation of 90°. Subsequently, a suitable line of sight was selected, crossing the artery at an angle of 70° with the longitudinal axis of the artery, herewith measuring wall shear rate. The measurement procedure for both diameter and wall shear rate, each with a length of 5 seconds covering about 6 cardiac cycles, was repeated 5 times with a time interval of 1 minute between the measurements.

Shear stress calculation

Shear stress was approximated according to equation (3-5) and (3-6) as described in CHAPTER 3.

Statistical analysis

Repeated measurements were averaged. Statistical management and analysis was performed using the SPSS 10.0 package (SPSS, Chicago, IL, USA). Group means are presented as mean $\pm$ SD. To test the influence of gender, an ANCOVA was used with factors for mean arterial pressure, pulse rate, and body mass index. Factors with a p-value > 0.10 were excluded from the model. Variables were also tested for equal variances within and between groups with Levene's test for homogeneity. If a variable was not homogeneously distributed the non-parametric Kruskal-Wallis test for independent samples was used instead of an ANCOVA. To get the relation between vessel wall properties, hemodynamic parameters and age, age was also added as a covariate. To investigate the difference of the age-effect among the genders, the interaction of age and gender was also tested for. Statistical significance was considered at a p-value < 0.05 .

The ratio of group standard deviation and group average was considered the intersubject variation coefficient for a variable x . Also, the intrasubject variability $\bar{\sigma}$ was determined by summing for each subject s over all registrations r the squared deviation of its mean value and averaging over all subjects:

$$\bar{\sigma} = \sqrt{\frac{\sum_s \sum_r (x_{sr} - \bar{x}_s)^2}{\sum_s (r_s - 1)}} \quad (4-1)$$

and similarly the corresponding mean value $\bar{\mu}$ was calculated:

$$\bar{\mu} = \frac{\sum_s \sum_r (\bar{x}_{sr})}{\sum_s r_s} \quad (4-2)$$

Subsequently, the variation coefficient VC [%] was computed through:

$$VC = \frac{100\bar{\sigma}}{\bar{\mu}} \quad (4-3)$$

RESULTS

Groups were equally distributed for gender, age, body mass index, blood pressure, and heart rate (Table 2). Group 1 (30 – 47 years) consisted of 7 female and 7 male volunteers. Group 2 (48 – 65 years) included 8 persons in both genders.

	mean $\pm$ SD	VC [%]
Diameter [mm]	3.9 $\pm$ 0.3	7.0
Δd [μ m]	66 $\pm$ 17	25.8
IMT [μ m]	355 $\pm$ 58	16.4
SV [mm·s ⁻¹]	504 $\pm$ 63	12.5
MV [mm·s ⁻¹]	77 $\pm$ 19	24.7
PSR [s ⁻¹]	625 $\pm$ 101	16.2
MSR [s ⁻¹]	107 $\pm$ 31	28.6

Table 3. Intrasubject intrasession estimates. Data presented as means $\pm$ SDs. VC = variation coefficient; Δd = distension; IMT = wall thickness; SV = systolic velocity; MV = mean velocity; PSR = peak shear rate; MSR = mean shear rate.

Intrasubject variability of measured brachial artery vessel wall parameters and hemodynamic parameters are depicted in Table 3. The VC of end-diastolic diameter and IMT were 7.0% and 16.4%, respectively. The calculated VC of the measured distension was 25.8%. Measurements of hemodynamic properties were done with a VC of 12.5% and 24.7% for the peak systolic velocity and mean velocity, respectively. Peak shear rate and mean shear rate were measured with a VC of 16.2% and 28.6%.

	Male		Female	
	group 1	group 2	group 1	group 2
Diameter [mm]	4.2 $\pm$ 0.5	4.4 $\pm$ 0.6	3.1 $\pm$ 0.4*	3.9 $\pm$ 0.6 [†]
IMT [μ m]	296 $\pm$ 38	365 $\pm$ 50 [†]	328 $\pm$ 60	409 $\pm$ 59 [†]
IMT/d [%]	7.1 $\pm$ 0.8	8.4 $\pm$ 1.6	10.7 $\pm$ 1.6*	10.1 $\pm$ 1.4*
E_m [MPa]	2.2 $\pm$ 1.5	2.3 $\pm$ 1.3	1.9 $\pm$ 1.5	2.4 $\pm$ 1.8
CC [mm ² ·kPa ⁻¹]	0.08 $\pm$ 0.03	0.08 $\pm$ 0.04	0.04 $\pm$ 0.02	0.05 $\pm$ 0.02 [‡]
DC [MPa ⁻¹]	6.0 $\pm$ 2.3	5.5 $\pm$ 2.4	4.8 $\pm$ 1.9	3.6 $\pm$ 1.3

Table 4. Vessel wall mechanical properties of the brachial artery. Data presented as means $\pm$ SDs; group 1 = younger group; group 2 = older group; IMT = wall thickness; IMT/d = IMT/diameter ratio; E_m = Young's modulus; CC = compliance; DC = distensibility. * = $p < 0.001$ men vs. women; [†] = $p < 0.05$ group 1 vs. group 2; [‡] = $p < 0.01$ group 1 vs. group 2.

In Table 4, vessel wall mechanic properties are summarised. The diameter of the brachial artery was significantly lower in the younger women compared to the group of young men (4.2 $\pm$ 0.5 mm and 3.1 $\pm$ 0.4 mm, respectively, $p < 0.001$). In women only, diameter was related to age (Figure 8), i.e., diameter increased with age (group1: 3.1 $\pm$ 0.4 mm, group 2: 3.9 $\pm$ 0.6 mm; $p < 0.05$). IMT was related to age rather than to gender (male: 296 $\pm$ 38 μ m and 365 $\pm$ 50 μ m, $p < 0.05$; female:

328 ± 60 μm and 409 ± 59 μm, $p < 0.05$; Figure 8). When comparing the ratio of wall thickness and diameter (IMT/d) as a measure of vessel wall morphology, the ratio was higher in younger women than in younger men ($p < 0.001$). This also held for the older group ($p < 0.05$). With increasing age this ratio remained constant in both genders. The E_m [Pa], an inverse measure of intrinsic elasticity and calculated with equation (1-3),¹⁹ remained at a constant level with increasing age in both men and women and did not differ between both sexes.

Figure 8. Diameter and wall thickness of the brachial artery. Box plot of diameter and wall thickness. Data are presented as median and interquartile ranges. The whiskers represent the intergroup spread.

Figure 9. Compliance and distensibility of the brachial artery. Box plot of compliance and distensibility of the brachial artery. Data are presented as median and interquartile ranges. The whiskers represent the intergroup spread.

CC and *DC*, as measures of storage capacity and vessel wall mechanic properties, were not dependent of age (Figure 9). However, *CC* was significantly lower in the younger women than in the men ($p < 0.01$).

Figure 7 in **CHAPTER 3** shows a typical velocity (A) and shear rate (B) profile as measured in the brachial artery of a healthy volunteer. Hemodynamic parameters (i.e., systolic velocity, mean velocity, peak shear stress and mean shear stress in the brachial artery) did not differ between the genders and were not influenced by age. This is depicted in Table 5, in which mean hemodynamic data and their SDs are given.

	Male		Female	
	group 1	group 2	group 1	group 2
<i>SV</i> [mm·s ⁻¹]	593 ± 152	494 ± 124	451 ± 149	459 ± 128
<i>MV</i> [mm·s ⁻¹]	75 ± 20	83 ± 64	64 ± 33	81 ± 43
<i>WBV</i> [mPa·s]	5.0 ± 0.2	5.0 ± 0.6	4.8 ± 1.3	4.9 ± 1.3
<i>PWSS</i> [Pa]	3.32 ± 0.74	3.26 ± 0.47	2.71 ± 0.57	2.89 ± 1.21
<i>MWSS</i> [Pa]	0.52 ± 0.09	0.53 ± 0.25	0.44 ± 0.20	0.49 ± 0.20

Table 5. Hemodynamic parameters of the brachial artery. Data presented as mean ± SDs; group = younger group; group 2 = older group; *SV* = systolic velocity; *MV* = mean velocity; *PWSS* = peak wall shear stress; *MWSS* = mean wall shear stress.

Mean wall shear stress was not related to *IMT* in neither men nor women ($r^2 = 0.003$, $p = 0.84$ and $r^2 = 0.21$, $p = 0.12$, respectively). Moreover, no other vessel wall parameter (*d*, *CC*, *DC*, *E_m*, or *d/IMT*) was related to mean wall shear stress.

DISCUSSION

The instantaneous wall shear stress distribution of the brachial artery could be assessed in a reproducible manner. Peak (≈ 3 Pa) and mean wall shear stresses (≈ 0.5 Pa) were found to be approximately the same in healthy men and women and were not influenced by age. Intrasubject variability for the various measures for brachial artery wall shear rate could be assessed reliably (*VC* = 16 – 28%) and was considerably lower than intersubject variability (27 - 41%), indicating that individual differences in brachial artery shear rate can be detected. Intrasubject variabilities of diameter, distension, and *IMT* of the brachial artery are moderate to good (7 – 26%), corroborating observations in the carotid artery.^{20,21} Intersubject variability of the vessel wall parameters were on the same order (≈ 15%).

Our observations regarding diameter, *IMT*, *CC*, and *DC* corroborate findings previously reported by van der Heijden-Spek and colleagues³ in a population study of 498 men and women 20 to 79 years of age.

Using the following expression, in which γ is the observed wall shear rate [s⁻¹], v is centre stream velocity [m·s⁻¹], and d is diameter [m], the bluntness factor n of the velocity profile can be approximated with:

$$\gamma = \frac{2nv}{d} \quad (4-4)$$

For $n = 2$ expression (4-4) describes a parabolic velocity profile as may develop under non-pulsatile conditions (Poiseuille's law). In 27 healthy male subject, Gnasso et al.¹⁸ reported a calculated peak shear stress of 3.7 Pa for the brachial artery, assuming a parabolic velocity profile ($n = 2$) and a fixed WBV ($\eta = 4.5$ mPa·s). However, it can be expected that velocity profiles in the human arterial tree are not parabolic because of pulsatile blood flow and changing arterial geometry.²² Moreover, the shape of the velocity profile, even over a single cardiac cycle, is not constant, due to the compliance of the arterial tree.^{23,24} Thus, as has been described before,²⁵ in pulsatile conditions the time dependent velocity profile is required in order to evaluate the time dependent behaviour of shear rate. When we calculate n from our data, we find a bluntness factor of 2.4 and 2.7 for peak and mean velocity, respectively, implying a blunted velocity profile for the brachial artery (Figure 7A). The higher bluntness factor has also been observed by Simon et al.,²⁶ who concluded to a bluntness factor of 2.3 for the brachial artery. The assumption of a parabolic velocity profile^{15,18} would therefore result in an underestimation of shear rate of at least 20%.

In the present study, wall shear rate is also underestimated because the observed shear rate distribution does not reach a discrete maximum at the vessel wall-lumen interface, the location of which is not exactly known.²⁷ This is because shear rates are measured within sample volumes with a finite length (300 μm) at an angle of 70°, causing ambiguity about the origin of the reflected signals. Since we are able to measure as close as 200 – 400 μm to the vessel wall, the observed shear rate will be a least estimate. Taking into account a diameter of about 4 mm this implies a relatively larger estimation error compared to measurements in the carotid artery.²⁰

The intrasubject variance in shear rate of about 20% can partly be explained by the spatial resolution, partly by vessel wall artefacts or by physiologic variation. The similar behaviour of the observed centre stream velocity (SV ; Table 3) and wall shear rate corroborates the conclusion that we are mainly dealing with physiological variations.

The absolute discrepancy between the peak shear stress of Gnasso et al.¹⁸ can be attributed to the assumed parabolic velocity profile, WBV and the assessment of centre stream velocity. The pulsatile nature of flow in the brachial artery, affects local WBV . Indeed, for the brachial artery we observed a WBV of 5.0 mPa·s at a mean shear rate of about 100 s^{-1} while for the carotid artery a viscosity of 3.2 mPa·s at a mean shear rate of about 400 s^{-1} is common.²⁰ Because of intrasubject differences in wall shear rate and blood characteristics (plasma viscosity and hematocrit) the assumption about a fixed WBV of 4.5 mPa·s at a shear rate of 225 s^{-1} is not justified. Moreover, the peak systolic centre stream velocity as measured by Gnasso et al.¹⁸ is about 30% higher than we observed. Because of system bandwidth and sample volume transit time considerations²⁸ the estimated maximum of the spectral envelope will be about 20% higher than the actual centre stream velocity.²⁵ This absolute difference, however, has no consequences for the relative evaluations.

The decrease in carotid artery mean shear stress with increasing age has been described extensively elsewhere,^{15,16,29} suggesting a degrading capability of the carotid artery to adapt to chronic changes. In contrast, we conclude that the brachial artery is able to adapt better to chronic changes in blood flow over time than the carotid artery,¹⁷ since both peak as well as mean shear stress do not change with increasing age.

Acute changes in wall shear stress, and the concomitant change in diameter, can be induced by an acute increase in flow, and for the brachial artery results in a FMD of about 8%^{30,31} of baseline diameter. Compared to the acute 9- to 10-fold increase in flow³¹ this is only a marginal adaptation. The extremities, both upper and lower, are exposed to many changes in activity during the day. A low baseline shear stress allows for a large acute rise in flow, and thus shear stress, without an exorbitant increase in diameter and without reaching the maximal tolerable shear stress of ≈ 35 Pa.³² This might support the finding of Gnasso et al.¹⁸ regarding the linear relation between baseline shear stress and the magnitude of diameter response to FMD.

In an earlier observation, Kornet et al.³³ have shown that *IMT* in the muscular femoral artery is inversely related to mean wall shear stress. We tested this hypothesis for the brachial artery also but we failed to find any significant correlation between *IMT* and hemodynamic parameters, probably because of the high variability in brachial artery wall thickness measurements (Table 3).

In conclusion, we measured vessel wall properties and hemodynamic parameters in the brachial artery in healthy volunteers. With respect to the hemodynamic parameters no differences were observed between women or men and no influence of age was apparent. Both diameter and *IMT* were not related to peak or mean wall shear stress. Our results therefore indicate that the brachial artery adequately responds to chronic changes in blood flow. Further research is needed to clarify the mechanism of action by which the brachial artery responds to acute and chronic changes in blood flow.

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CHAPTER 5

Endothelin-1 levels and conduit artery
mechanical properties in end-stage renal disease

Submitted:

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ABSTRACT

EC dysfunction markers are increased in ESRD. In the present study we focused on the relationship between EC markers and conduit artery wall mechanics and hemodynamics in ESRD. In 29 ESRD patients and 16 healthy controls brachial artery diameter, distension, and *IMT* was measured and *CWS* calculated. Using a shear rate estimating system shear stress was determined. Furthermore, vWF ET-1 levels were measured. vWF ($p = 0.002$) and ET-1 ($p < 0.001$) were higher in ESRD patients and vWF was related to ET-1 ($r = 0.70$, $p = 0.005$). In ESRD wall thickness was increased ($p = 0.015$), while other vessel wall characteristics were similar. Peak ($p = 0.001$) and mean shear stress ($p = 0.003$) were significantly lower in ESRD patients and ET-1 showed an inverse loglinear relation with both (peak: $r = -0.59$, $p = 0.016$; mean: $r = -0.64$, $p = 0.007$). Also, ET-1 was loglinearly related to *CWS* ($r = 0.58$, $p = 0.014$). These results show that, in ESRD, conduit artery *CWS* is increased and shear stress is lower, which might be secondary to an increased peripheral vascular resistance, caused by higher ET-1 levels.

INTRODUCTION

Chronic renal failure is often complicated by hypertension and changes in the vascular system.¹⁻³ The latter is characterized by abnormal large artery properties such as a reduced distensibility and a lower compliance.^{1,2,4,5} Furthermore, carotid artery *IMT*, related to cardiovascular morbidity, is found to be greater in patients with ESRD.³⁻⁷

Besides the large artery structural changes, arterial functionality seems to be deteriorated also.⁸⁻¹¹ This is demonstrated by endothelial dysfunction, allegedly present in ESRD, through impaired vascular reactivity^{8,10,11} and increased levels of plasma endothelial dysfunction markers (e.g. asymmetric dimethyl-L-arginine (ADMA),¹² ET-1,¹³ vWF,¹⁴ adhesion molecules and selectins,¹⁵ NO and its metabolites¹²). The cause of this disturbance in endothelial homeostasis is as yet not clarified, but recent reports suggest that factors specific to uremia itself may impair endothelial function. For example, accumulation of ADMA plasma levels inhibits endothelial NO synthesis.¹² High levels of homocysteine, frequently observed in chronic renal failure,¹⁶ also causes endothelial damage through oxidative stress.^{16,17} Interestingly, gene polymorphisms have recently also been related to endothelial dysfunction in ESRD.¹⁸

To illustrate a defect in EC function in uremic patients alone, several authors have shown that endothelium-dependent vasodilation in response to ACh-like substances is impaired whereas endothelium-independent vasodilation in response to sodium nitroprusside is not affected.^{8,10}

As a consequence of endothelial dysfunction, alterations in hemodynamic properties, i.e., shear stress, and pulse pressure are sensed to a lesser extent or aggravated. For example, the vascular response to an increase in flow is impaired in ESRD.^{11,19} Furthermore, in uremic rats the response of the intima to low flow and the response of the media to high flow, mediated by ET-1, are exaggerated.²⁰

Taken together, patients with ESRD or patients on hemodialysis display a markedly increased risk for cardiovascular death.⁶ Plasma markers of endothelial dysfunction, such as ET-1 and vWF have been linked to hypertension, cardiovascular remodelling, and cardiovascular mortality in patients with chronic renal disease.^{13,14} In the present study we hypothesised that a disturbance in EC markers such as vWF and ET-1 is related to altered vessel wall mechanic properties and wall shear stress in the brachial artery.

MATERIALS AND METHODS

Subjects and measurement procedure

After approval by the joint Medical Ethical Committee of the University of Maastricht and the university hospital Maastricht, 29 ESRD patients and 16 healthy control subjects were recruited. All healthy control subjects were non-smokers and none of them reported medication intake or a history of renal or vascular disease. In Table 6 patient and control group characteristics are depicted.

The causes of renal disease were divided into two groups, namely ESRD due to primary renal/urological disease (37.9%) and ESRD due to secondary disease (55.2%).²¹ In 2 cases the cause of renal disease remains unknown. The major causes of renal disease were nephrosclerosis (37.9%), diabetic nephropathy

(13.8%), and glomerulo-/pyelonephritis (10.3%). At the time of the study, none of the patient was or had been on continuous dialysis therapy. Six ESRD patients reported smoking (Fisher's exact test, $p=0.075$).

	ESRD patients	Control group
Age [years]	66.6 $\pm$ 9.7	58.6 $\pm$ 4.4 [†]
Male/female	18/11	8/8
SBP [mmHg]	153.8 $\pm$ 28.8	129.1 $\pm$ 13.5 [*]
DBP [mmHg]	82.7 $\pm$ 14.1	77.6 $\pm$ 10.5
MAP [mmHg]	108.7 $\pm$ 18.8	97.0 $\pm$ 12.4
Pulse pressure [mmHg]	71.0 $\pm$ 18.5	51.5 $\pm$ 9.2 [*]
Heart rate [min ⁻¹]	69 $\pm$ 17	63 $\pm$ 9
Body mass index [kg·m ⁻²]	25.1 $\pm$ 4.7	25.2 $\pm$ 3.8
Hemoglobin [mmol·L ⁻¹]	6.7 $\pm$ 0.9	9.0 $\pm$ 0.5 [‡]
Hematocrit [%]	32.6 $\pm$ 4.2	43.0 $\pm$ 2.6 [‡]
Plasma viscosity [mPa·s]	1.19 $\pm$ 0.12	1.14 $\pm$ 0.06
Glucose [mmol·L ⁻¹]	6.5 $\pm$ 2.7	5.6 $\pm$ 0.5
Total cholesterol [mmol·L ⁻¹]	4.9 $\pm$ 1.4	5.8 $\pm$ 0.7 [*]
Triglycerides [mmol·L ⁻¹]	1.82 $\pm$ 1.23	1.26 $\pm$ 0.72
Urea [mmol·L ⁻¹]	22.4 $\pm$ 8.8	4.8 $\pm$ 0.7 [‡]
Creatinine [μ mol·L ⁻¹]	552 $\pm$ 200	89 $\pm$ 10 [‡]

Table 6. Subject characteristics. Data presented as mean $\pm$ SDs. Statistical analysis was performed with an ANCOVA corrected for age and medication intake. Male/female distribution was analysed with the Fisher's exact test. *SBP* = systolic blood pressure; *DBP* = diastolic blood pressure; *MAP* = mean arterial pressure. * = $p < 0.05$; † = $p < 0.01$; ‡ = $p < 0.001$, ESRD patients ($n = 29$) vs. healthy subjects ($n = 16$).

The measurements were performed in the morning between 08:00 o'clock and noon after an overnight fasting of at least 10 hours. The necessary medication intake of the ESRD patients was allowed. The subjects were examined after an acclimatisation period of 15 minutes in supine position in a room with a temperature of 22 – 24°C. After blood sampling via venipuncture of an antecubital vein, brachial artery blood pressure of the dominant arm was measured every 5 minutes by a semi-automated oscillometric device (Dinamap 1846P, Critikon, Tampa, FL, USA). The non-dominant brachial artery was measured in approximately 45° abduction and the palm of the hand facing upward.

Blood sampling and assays for plasma endothelial markers

Details regarding venous blood samples and the different assays used have been described in **CHAPTER 3** Materials and methods. In brief, plasma viscosity was determined in duplo with a glass capillary viscometer. PAI-1 activity was measured photometrically and vWF plasma levels were determined by an ELISA. Additionally, plasma ET-1 was determined by radioimmunoassay. All assays were conducted by technicians who were blinded to any characteristics of ESRD patients and controls.

The Wall Track System and the Shear Rate Estimation System (ART-Lab)

The wall track system and the shear rate estimation system have been described in detail in **CHAPTER 3** Materials and methods.

Using the basic geometric parameters Δd [m], d [m], and IMT [m], as measured with the wall track system, and assuming a linear relation between pressure and wall characteristics, other vessel wall properties could be calculated. CC [$m^2 \cdot Pa^{-1}$], defined as the storage capacity of the vessel per unit of length, was calculated through equation (1-1). DC [Pa^{-1}], reflecting the elasticity of the vessel wall, is approximated with equation (1-2). Furthermore, the CWS [Pa] at a given transmural pressure P [Pa] is calculated with Lamé's equation (equation (1-4)).

Shear stress calculation

Shear stresses were approximated according to equation (3-5) and (3-6) as described in **CHAPTER 3**.

Statistical analysis

Statistical management and analysis was performed with SPSS 10.0 (SPSS, Chicago, IL, USA). Group means are presented as mean $\pm$ SD. To compare both groups, where appropriate, a χ^2 -test or an ANCOVA was used with factors for age, mean arterial pressure, cause of renal disease and medication intake. Simple linear and logistic regression was performed to relate vessel wall characteristics or hemodynamic factors to EC markers. A p-value of less than 0.05 was considered statistically significant.

RESULTS

ESRD patients were significantly older as is shown in Table 6. Systolic blood pressure was significantly higher in the patient group, and because of an equal diastolic blood pressure, pulse pressure was also significantly increased (Table 6). Although a significant difference was found in the use of β -blockers related to the different causes of ESRD (primary vs. secondary renal disease; Fisher's exact test, $p = 0.021$), there were no differences whatsoever regarding the measured and/or calculated parameters in this study after correction for β -blocker intake.

After correction for age, medication intake, and cause of renal disease, EC markers were all significantly different between patients and controls except PAI-1 ($\approx 13.6 \pm 7.5$ AU $\cdot$ mL $^{-1}$). vWF was $225 \pm 95\%$ in ESRD patients and $132 \pm 49\%$ in control subjects ($p = 0.002$). ET-1 was also significantly higher in the patient group (14.9 ± 6.6 pg $\cdot$ mL $^{-1}$) compared to healthy controls (1.9 ± 0.8 pg $\cdot$ mL $^{-1}$, $p < 0.001$).

Correcting for age, brachial artery vessel wall mechanic parameters were similar in both groups except for the IMT (Table 7). The brachial artery relative distension, defined as the ratio of the diameter change and the end-diastolic diameter ($\Delta d/d$), was higher in the ESRD patients when compared to the control group but did not reach significance ($2.2 \pm 1.1\%$ vs. $1.5 \pm 0.7\%$, $p = 0.30$). The wall thickness to diameter ratio (IMT/d) as a measure of vessel wall morphology was also comparable in the ESRD patients ($10.6 \pm 3.8\%$) and the healthy controls ($9.2 \pm 1.7\%$, $p = 0.37$).

	ESRD patients	Control group
Diameter [mm]	4.4 ± 0.7	4.2 ± 0.6
Distension [μm]	97 ± 50	64 ± 29
Wall thickness [μm]	433 ± 65	386 ± 57*
Compliance [mm ² ·kPa ⁻¹]	0.07 ± 0.04	0.06 ± 0.03
Distensibility [MPa ⁻¹]	4.8 ± 2.4	4.5 ± 2.1
CWS [kPa]	74.6 ± 20.9	73.7 ± 22.7

Table 7. Brachial artery vessel wall mechanical properties. Data presented as mean ± SDs. Statistical analysis was performed with an ANCOVA corrected for age and medication intake. CWS = circumferential wall stress; * = $p < 0.05$, ESRD patients ($n = 29$) vs. healthy subjects ($n = 16$).

Brachial artery hemodynamic parameters as measured with the shear rate estimation system are summarised in Table 8. No differences could be observed between the ESRD patients and the control group after correction for age, although mean shear rate tended to be lower in the ESRD patients group. Calculated brachial artery *WBV* (equation (3-5)) was significantly lower in renal patients compared to the control group (4.1 ± 0.9 vs. 5.2 ± 1.2 mPa·s, $p = 0.001$), mainly due to the lower hematocrit in the patient group (Table 5). Consequently, both peak and mean wall shear stress were significantly decreased in ESRD patients (peak: 2.2 ± 0.7 vs. 3.1 ± 0.9 Pa, $p = 0.001$; mean: 0.28 ± 0.16 vs. 0.51 ± 0.05 Pa, $p = 0.003$).

	ESRD patients	Control group
Systolic velocity [mm·s ⁻¹]	495 ± 178	478 ± 123
Mean velocity [mm·s ⁻¹]	63 ± 46	82 ± 54
Peak shear rate [s ⁻¹]	562 ± 193	616 ± 212
Mean shear rate [s ⁻¹]	76 ± 57	111 ± 69
Peak shear stress [Pa]	2.2 ± 0.7	3.1 ± 0.9†
Mean shear stress [Pa]	0.28 ± 0.16	0.51 ± 0.05*

Table 8. Brachial artery vessel wall parameters. Data presented as mean ± SDs. Statistical analysis was performed with an ANCOVA corrected for age and medication intake. * = $p < 0.01$; † = $p < 0.001$, ESRD patients ($n = 29$) vs. healthy controls ($n = 16$).

Creatinine and urea levels were both significantly higher in ESRD patients, obviously (Table 5). Creatinine levels were related to brachial artery *IMT* ($r = 0.43$, $p = 0.017$) and both urea and creatinine levels were inversely related to brachial artery peak wall shear stress ($r = -0.44$, $p = 0.016$ and $r = -0.53$, $p = 0.003$, respectively). Creatinine and urea levels both related to the EC function markers vWF ($r = 0.64$, $p = 0.005$ and $r = 0.79$, $p < 0.001$, respectively) and ET-1 ($r = 0.80$, $p < 0.001$ and $r = 0.58$, $p = 0.024$, respectively). vWF levels only correlated well with peak wall shear stress ($r = -0.52$, $p = 0.005$). All EC function markers correlated with each other ($p < 0.01$). ET-1 levels were related to pulse pressure ($r = 0.80$, $p < 0.001$) and mean arterial pressure ($r = 0.79$, $p < 0.001$). In line with this

observation, a loglinear relation ($r = 0.58$, $p = 0.014$) existed between ET-1 levels (x) and CWS (y); $y = 67 + 10\ln(x)$. Additionally, ET-1 levels were also loglinear inversely related to mean and peak wall shear stress (Figure 10) but not to shear rate or WBV . No relation was found between ET-1 and brachial artery relative distension or distensibility ($r = 0.18$, $p = 0.49$; and $r = 0.33$, $p = 0.19$, respectively).

Figure 10. Wall shear stress and endothelin-1 levels. The loglinear relation between endothelin-1 levels and peak (right) and mean (left) shear stress are illustrated. The accompanying graph function is shown in the upper right corner.

DISCUSSION

We have shown that increased levels of ET-1, directly related to uremia, in patients with renal disease are related to an augmented blood pressure. In line with this observation, brachial artery CWS was loglinearly related to ET-1 levels. Furthermore, brachial artery shear stresses were lower in patients with ESRD, and were inversely loglinear related to ET-1.

Brachial artery compliance and distensibility were not different between the two groups, although contralateral brachial artery pulse pressure was markedly higher in ESRD patients. Results in the common carotid artery, however, suggest primarily a decrease in distensibility due to stiffening of the vessel wall because of a decline in vessel wall distension despite an increase in pulse pressure.^{1,2,4} On the other hand, the compliance is maintained at physiological levels through a diameter enlargement, neutralizing the decrease in distension. In the brachial artery, similar results have been reported.²² Only for a subgroup of patients aged < 50 years van Guldener et al.¹¹ reported the brachial artery DC to be lower compared to healthy individuals. We observed a slightly higher brachial artery relative distension in our population with a mean age of 66.6 years, which is in accordance with the higher pulse pressure.

The wall thickness to diameter ratio (IMT/d) was similar between ESRD patients and healthy controls, although the IMT was significantly higher (Table 6). According to the Lamé equation (equation (1-4)) this phenomenon is explained by a vessel segment striving for a constant CWS . With increasing transmural pressure

(i.e., blood pressure) the vascular wall reacts through VSMC proliferation and matrix deposition, ensuing an increase in *IMT*, herewith counterbalancing the higher transmural pressure.²³⁻²⁵ This has been extensively corroborated by studies focusing on pulmonary hypertension^{23,26} or essential hypertension.²⁷ Indeed, the studied ESRD patients did not exhibit a different *CWS* compared to the control group. As far as we know, no other studies have addressed the brachial artery *IMT* of ESRD patients, although for the common carotid artery the *IMT* growth has been widely reported^{3,6,7} and has been related to cardiovascular morbidity.²⁸ Although not concluded for the common carotid artery *IMT* in a previous study,¹ the present data suggest that brachial artery *IMT* is related to creatinine levels. This might be in agreement with earlier studies, in which uremic status relates to *IMT*.^{5,7}

A number of mechanisms have been reported through which peripheral vascular resistance increases, thereby amplifying large artery blood pressure.^{29,30} Endothelial dysfunction, characterized by a misbalance between vasodilating and -constricting factors, appeared to play a pivotal role in the augmentation of vascular resistance.^{9,12-15,29,30} Several studies have shown a decrease in NO bioavailability,¹² whereas others focused on ET-1.¹³ ESRD patients indeed appear to have an impaired NO-signalling^{18,31} and our data show increased ET-1 in ESRD patients levels that relate to pulse pressure, mean arterial pressure, and brachial artery *CWS*. Considering, however, the mitogenic potential of ET-1,³² one might also hypothesise that the imbalance between NO and ET-1 in favour of ET-1 could directly induce wall hyperplasia.

Furthermore, the EC function markers vWF and ET-1 were both significantly higher in ESRD patients, which is in agreement with previous studies.^{13,14,33} As we have shown that creatinine and urea levels were both directly related to these EC function markers, we believe that uremia itself might provoke endothelial dysfunction, which was also reported before.^{10,19} We therefore infer that in renal disease increased ET-1 levels increase vascular resistance and thereby increase large artery mean arterial blood pressure.^{34,35} Interestingly, ET-1 levels were not related to brachial artery distensibility or relative distension, suggesting that in the present study the increase in ET-1 levels is not a local phenomenon, although it has been shown in HUVECs that cyclic strain induces ET-1 expression.³⁶ We therefore assume that the higher pulse pressure can also be attributed to an increased peripheral vascular resistance, in concordance with the increase in mean arterial pressure.

Besides the increase in ET-1, also vWF was significantly higher in ESRD patients, corroborating endothelial dysfunction. As early as 1979 it was already shown that vWF was significantly higher in ESRD patients; it was suggested that this might contribute to the premature arteriosclerosis in these patients.³³ To date, several studies relate higher vWF-antigen levels to large artery stiffness, decreased vascular responsiveness, and cardiovascular mortality.^{14,37} In the present study, we did not find a relation between vWF and brachial artery vessel wall mechanic properties. However, in a regression model vWF appeared to be inversely related to peak wall shear stress, but this approach does not allow any definite conclusions about possible relationships. Another EC marker, PAI-1, was not different at all between ESRD patients and healthy controls.

Regarding the hemodynamic parameters, both peak and mean shear stress were significantly lower in ESRD patients. This was mainly due to the lower brachial artery *WBV* (equation (3-5)), caused by a significant lower hematocrit. In addition, brachial artery mean wall shear rate tended to be lower in ESRD patients, which might be the result of an increased peripheral vascular resistance. In fact, the ET-1 level, which was related to blood pressure, was also inversely related to peak and mean wall shear stress, suggesting that through a higher vascular resistance the conduit artery blood flow is less, resulting in lower shear stresses.^{20,35} Normally, an artery would counteract this decrease in shear stress by vasoconstriction. However, the vessel wall has to balance between two hemodynamic parameters, namely shear stress and *CWS*.^{38,39} So, in order to maintain a physiological *CWS*,⁴⁰ we believe that shear stress is sacrificed.

Summarising, we have shown that ET-1 levels relate to *CWS* and inversely to shear stresses in the brachial artery. We hypothesise that this is a consequence of increased peripheral vascular resistance through higher ET-1 levels. Although endothelin receptor antagonists might have a beneficial effect on conduit artery mechanical properties by lowering vascular resistance,^{34,35} the role of endothelin receptor blockers in cardiovascular diseases in renal failure remains to be elucidated.

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CHAPTER 6

The effect of flow changes on the arterial system proximal to an arteriovenous fistula for hemodialysis

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ABSTRACT

Arterial remodelling in response to acute and chronic flow changes is believed to be controlled by the endothelium, sensing changes in wall shear stress. The present study focuses on the remodelling capacities of the brachial and radial artery of renal failure patients after placement of an autogenous radial-cephalic wrist access for hemodialysis (AVF). The acute and sustained effects on brachial and radial artery vessel wall mechanic and hemodynamic properties were evaluated in sixteen patients undergoing AVF creation. Pre- and postoperatively on predetermined time-points brachial and radial artery diameter, *IMT*, and mean shear stress were assessed. Statistical analysis was performed with Wilcoxon's signed-rank test and multivariate analysis. After AVF creation, acute increases in brachial artery mean shear stress ($p = 0.018$) and lumen diameter ($p = 0.028$) were seen. The brachial artery diameter further increased in the next year ($r = 0.41$, $p = 0.023$) whereas brachial artery mean shear stress remained unchanged ($r = 0.28$, $p = 0.44$). Brachial artery *IMT* exhibited no alterations. Mean shear stress in the radial artery increased acutely ($p = 0.005$) and remained unaltered after 1 year ($r = 0.16$, $p = 0.86$). Radial artery diameter also increased acutely ($p=0.005$), but unlike the brachial artery diameter, remained similar over the course of 1 year ($r = 0.13$, $p = 0.93$). Radial artery *IMT* tended to decrease acutely ($p = 0.059$) and increased steadily over 1 year ($r = 0.48$, $p = 0.008$). Brachial and radial artery diameters acutely increase after an acute rise in shear stress and remain increased after 1 year. Also, the vessel wall of the radial artery enlarges, indicating structural remodelling. Even after 1 year, however, these changes do not result in restoration of mean shear stress.

INTRODUCTION

Arterial remodelling can be subdivided into 1) acute remodelling or rather adaptation needed after an acute environmental change, i.e., a change in blood pressure and/or blood flow, and 2) structural remodelling after a sustained change in hemodynamic environment, where remodelling is defined as any change in artery size, measured as changes in internal or external elastic lamina area.¹ Both these processes are, at least partly, regulated by the endothelium, because of its strategic location at the lumen-wall interface.^{2,3} Increasing evidence indeed suggest the endothelium's ability to sense and respond to significant alterations in wall shear stress, i.e., the tangential force exerted by the flowing blood on the vessel wall.²⁻⁵

A widely accepted example for acute remodelling is arterial FMD, in which an acute increase in flow, and thus shear stress, after a short ischemic period causes a significant increase in vessel diameter.⁶⁻⁸ This diameter increase has been proven to be endothelium-dependent in both healthy subjects and patients with various cardiovascular diseases.^{8,9} It is believed that, due to the sudden increase in wall shear stress, NO is released from the ECs, inducing a rise in intracellular VSMC cGMP, causing relaxation of the vessel wall.

When, however, the hemodynamic milieu is persistently altered, structural vascular changes occur. For example, expansive remodelling occurs in arteries with atherosclerotic lesions to ensure physiological luminal capacity.¹⁰ This is probably due to an increased flow, and hence shear stress, at the lesion site, that prompts the vessel wall to at first dilate and eventually restructure.¹⁰⁻¹³ Another feature of remodelling is vessel wall thickening that can be observed in arteries of hypertensive patients¹⁴⁻¹⁶ and in veins used for peripheral bypass surgery.¹⁷⁻¹⁹ The most probable explanation of this feature is that through higher transmural pressure the VSMCs will proliferate, hypertrophy, and realign, thereby obeying the Laplace-Lamé law.^{11,20} Shear stress may also play a significant role in vessel wall thickening, for Buus et al. have shown that with increased flow, diameter expansion and hypertrophy of the vessel wall can be observed.¹²

Arterial remodelling as described above is believed to exist to remodel the artery in such a manner that physiological, or hemodynamic, parameters are restored to baseline physiological values. Interesting is the restoration of shear stress to physiological readings, which has been the subject of many studies.^{21,22} Presently, the ingrained belief is that the endothelium senses shear stress in order to be able to respond to any hemodynamic changes and to maintain an "ideal" shear stress.^{2,3,5,21,22}

The focus of the present study is to measure *in vivo* remodelling in both the brachial and radial artery. An autogenous radial-cephalic wrist access for hemodialysis served as a high-flow model. Both hemodynamic and vascular mechanic parameters were assessed.

PATIENTS AND METHODS

Patients and patient characteristics

The study was approved by the joint Medical Ethical Committee of the University of Maastricht and the university hospital Maastricht. Sixteen patients with ESRD, aware of the investigational nature of the study, gave written informed consent. The

pre-operative patient characteristics are summarised in Table 9. The major cause of renal disease in the study population was nephrosclerosis (31.3%) as shown in Table 10.

All patients were scheduled for AVF placement on the left arm for future hemodialysis. Vascular access was accomplished through creation of an autogenous radial-cephalic wrist access by anastomosing the cephalic vein to the radial artery approximately 2 cm proximal to the radial styloid. To overcome operating skills bias, the same vascular surgeon performed all surgical procedures. In the present study we employed the AVF as an *in vivo* high flow model with an acute and sustained increase in blood flow.

The measurements were always performed in the morning between 08:00 o'clock and noon after an overnight fasting of at least 10 hours. The necessary medication intake was allowed. The subjects were examined after an acclimatisation period of 15 minutes in supine position in a room with a temperature of 22 – 24°C. Artery diameter and wall shear rate measurements were performed on the left brachial and radial artery. The right arm brachial artery blood pressure was measured every 5 minutes by a semi-automated oscillometric device (Dinamap 1846P, Critikon, Tampa, FL, USA). The measurement procedure for both diameter and wall shear rate, each with a recording length of 5 seconds covering about 6 cardiac cycles, was repeated 5 times with a time interval of 1 minute between the recordings.

	n = 16
Age [years]	64.6 ± 10.2
Male/female	9/7
BMI [kg·m ⁻²]	25.3 ± 4.7
Heart rate [min ⁻¹]	64.5 ± 8.9
Mean arterial pressure [kPa]	15.1 ± 1.7
Pulse pressure [kPa]	9.2 ± 2.0
Hemoglobin [mmol·L ⁻¹]	6.9 ± 0.8
Hematocrit [%]	33.5 ± 3.5
Plasma viscosity η_0 [mPa·s]	1.16 ± 0.10
Urea [mmol·L ⁻¹]	25.2 ± 9.3
Creatinine [μmol·L ⁻¹]	520 ± 190

Table 9. Preoperative patient characteristics. Shown are mean ± SDs of preoperative patient characteristics. BMI = body mass index.

The Wall Track System and the Shear Rate Estimation System (ART-Lab)

The wall track system and the shear rate estimation system have been described extensively in **CHAPTER 3** Materials and methods.

Using the basic geometric parameters Δd [m], d [m], and IMT [m], as measured with the wall track system, and assuming a linear relation between pressure and wall characteristics, other vessel wall properties could be calculated. CC [m²·Pa⁻¹], defined as the storage capacity of the vessel per unit of length, was

calculated through equation (1-1). The DC [Pa^{-1}], reflecting the elasticity of the vessel wall, is approximated with equation (1-2). E_m [Pa], an inverse measure of elasticity, independent of vessel shape and size, was calculated with equation (1-3). Furthermore, the CWS [Pa] at a given transmural pressure P [Pa] is calculated with Lamé's equation (equation (1-4)).

	Number of patients (%)
Nephrosclerosis	5 (31.3%)
Polycystic kidney disease	2 (12.5%)
Glomerulo-/pyelonephritis	2 (12.5%)
Toxic nephropathy	2 (12.5%)
Unknown	2 (12.5%)
Others*	3 (18.7%)

Table 10. Causes of renal disease. Shown are the different causes of renal disease in the study population expressed as total number of patients (%). *Others include Wegener's disease, hydronephrosis, and nephrotic syndrome.

Measurement procedure in the brachial (BA) and radial artery (RA)

The brachial and radial artery of all 16 patients were visualized in B-mode with the arm in approximately 45° abduction and the palm of the hand facing upward. The BA measurements were performed 2 – 3 cm proximal to the elbow and the RA was measured just proximal to the fistula anastomosis. The ultrasound system was switched to M-mode and diameter and distension measurements were taken perpendicular to the wall. Subsequently, a suitable line of sight was selected, crossing the artery at an angle of 70° with the longitudinal axis of the artery, allowing the measurement of velocity and wall shear rate. For the brachial artery these measurements were performed preoperatively and were repeated 1 day, 1, 2, 3, 6, 12, 24, and 52 weeks after surgery. In order to allow recovery of the radial artery, it was not measured until 3 weeks post surgery.

Shear stress calculation

Shear stress was determined with equation (3-5) and (3-6) as described in CHAPTER 3.

Statistical analysis

Statistical management and analysis was performed using the SPSS 10.0 package (SPSS, Chicago, IL, USA). Group means are presented as mean $\pm$ SD. Baseline differences between the both arteries were tested with a Mann-Whitney-U signed-rank test. To analyse acute effects of increased flow, the paired Wilcoxon signed-rank test was used and to test sustained effects a multivariate analysis was used with time-point, mean arterial pressure, and pulse rate as covariates. The baseline values were excluded from the analysis as the acute effect might bias long-term remodelling. Factors with $p > 0.10$ were dropped from the model. Statistical significance was considered at a p -value < 0.05 .

RESULTS

Table 11 provides the (preoperative) baseline values of the assessed vessel wall mechanical and hemodynamic properties of both brachial and radial artery. Obviously, the brachial diameter is larger than that of the radial artery. Consequently, CC , E_m and CWS are correspondingly larger. Shear stress values are quite similar in both arteries.

One day postoperative brachial artery mean wall shear stress increased to 1.19 ± 0.21 Pa, an increase of 345% ($p = 0.018$). Peak wall shear stress was not affected although, peak-peak, i.e., maximal minus minimal, wall shear stress decreased significantly to 1.82 ± 0.64 Pa (37%; $p = 0.018$), illustrating that the acute flow increase in the setting of an AVF is mainly provided by the diastolic flow component. The brachial artery diameter dilated to 5.2 ± 0.8 mm, an increase of 16% ($p = 0.028$). No significant change in brachial artery IMT ($p = 0.11$), CC ($p = 0.28$), DC ($p = 0.50$), and E_m ($p = 0.091$) was observed in the acute phase, although the wall thickness-to-diameter ratio (IMT/d), as a measure of vessel wall morphology, decreased by 14% ($p = 0.018$). CWS , or wall tension, tended to increase (15%; $p = 0.043$), due to the equivalent rise in diameter.

	Radial artery	Brachial artery
Diameter [mm]	2.2 ± 0.5	$4.5 \pm 0.5^\dagger$
Wall thickness [μ m]	406 ± 88	411 ± 71
Compliance [$\text{mm}^2 \cdot \text{MPa}^{-1}$]	21.3 ± 12.6	$65.6 \pm 26.1^\dagger$
Distensibility [MPa^{-1}]	5.3 ± 2.8	4.2 ± 1.8
Young's modulus [MPa]	1.3 ± 1.2	$2.4 \pm 1.3^*$
CWS [kPa]	44 ± 18	$85 \pm 18^\dagger$
Peak wall shear stress [Pa]	1.74 ± 0.53	2.12 ± 0.65
Mean wall shear stress [Pa]	0.22 ± 0.11	0.27 ± 0.14
$PPWSS$ [Pa]	2.39 ± 0.70	2.89 ± 0.80

Table 11. Baseline vessel wall mechanic and hemodynamic properties. Data presented as means $\pm$ SDs. CWS = circumferential wall stress; $PPWSS$ = peak-peak wall shear stress. For statistical analysis a Mann-Whitney-U signed-rank test was used; $^\dagger = p < 0.01$ compared to the radial artery; $^* = p < 0.001$ compared to the radial artery.

Table 12 provides the follow-up data of several mechanic and hemodynamic parameters in both the radial and the brachial artery. Radial artery mean wall shear stress increased markedly at 3 weeks (1198%; $p = 0.005$) as observed for the brachial artery. Contrary to the brachial artery, the radial artery peak wall shear stress increased significantly (137%; $p = 0.007$) and peak-peak wall shear stress decreased non-significantly at 3 weeks post surgery (-21%; $p = 0.58$). Both arteries diameter increased significantly (21 and 80%, respectively). Although not significantly, radial artery IMT tended to decrease (-13%; $p = 0.059$). The IMT/d of the radial artery, however, decreased from 19% to 9% (-53%, $p = 0.007$). At 3 weeks the brachial artery IMT/d also decreased further (-23% compared to baseline) as a consequence of the ongoing diameter increase. Arterial compliance of both arteries at week 3 was significantly higher compared to preoperative values. This,

	Diameter [mm]		Wall thickness [μ m]		PWSS [Pa]		MWSS [Pa]	
	RA	BA	RA	BA	RA	BA	RA	BA
Baseline	2.2 $\pm$ 0.5	4.5 $\pm$ 0.5*	406 $\pm$ 88	411 $\pm$ 71	1.74 $\pm$ 0.53	2.12 $\pm$ 0.65	0.22 $\pm$ 0.11	0.27 $\pm$ 0.14
Day 1	---	5.2 $\pm$ 0.8*	---	399 $\pm$ 78	---	2.31 $\pm$ 0.52	---	1.19 $\pm$ 0.21*
Week 1	---	5.3 $\pm$ 0.7	---	383 $\pm$ 86	---	2.61 $\pm$ 0.60	---	1.43 $\pm$ 0.37
Week 3	4.0 $\pm$ 0.7*	5.5 $\pm$ 0.8	354 $\pm$ 36	378 $\pm$ 75	4.11 $\pm$ 1.85*	2.64 $\pm$ 0.61	2.82 $\pm$ 1.18*	1.40 $\pm$ 0.43
Week 6	4.2 $\pm$ 0.8	5.8 $\pm$ 0.7	391 $\pm$ 69	394 $\pm$ 67	4.70 $\pm$ 1.72	2.95 $\pm$ 0.78	2.87 $\pm$ 0.78	1.58 $\pm$ 0.63
Week 12	4.4 $\pm$ 0.9	6.2 $\pm$ 1.1	401 $\pm$ 98	386 $\pm$ 68	4.17 $\pm$ 1.70	2.95 $\pm$ 0.59	2.55 $\pm$ 0.59	1.44 $\pm$ 0.41
Week 52	4.1 $\pm$ 1.0	6.2 $\pm$ 1.1	487 $\pm$ 115	366 $\pm$ 111	4.43 $\pm$ 1.96	2.71 $\pm$ 1.20	2.51 $\pm$ 1.35	1.36 $\pm$ 0.59
r =	0.13	0.41	0.48	-0.22	-0.16	-0.28	-0.15	0.29
P =	0.93	0.023	0.008	0.74	0.86	0.44	0.88	0.39

Table 12. 1-Year follow-up of radial and brachial artery vessel wall mechanic and hemodynamic parameters. Data presented as means $\pm$ SDs. RA = radial artery; BA = brachial artery; PWSS = peak wall shear stress; and MWSS = mean wall shear stress. To analyse acute and sustained changes the Wilcoxon's signed-rank test and multivariate analysis was used, respectively; * = $p < 0.001$ compared to the radial artery; † = $p < 0.05$ compared to baseline values; and ‡ = $p < 0.01$ compared to baseline values.

of course, was mainly due to the increase in luminal diameter. The *CWS* of the radial artery increased considerably (73%; $p = 0.017$), and the *DC* and E_m exhibited no significant changes.

Over the course of one year the brachial diameter continued to increase, whereas the radial artery diameter did not change from week 3 postsurgery onwards (Table 12). Furthermore, radial artery *IMT* increased significantly to, whereas brachial *IMT* maintained at baseline value (Table 12). This was also expressed in both arteries' *IMT/d* that tended to increase in the radial artery ($r = 0.39$, $p = 0.079$) and remained decreased in the brachial artery after one year ($r = 0.30$, $p = 0.32$). Interestingly, mean wall shear stress did not change in either artery and remained at its increased value as is illustrated in Table 12. Peak wall shear stress of the brachial artery remained unchanged after one year, whereas the brachial artery peak-peak wall shear stress remained at a lower value. The radial artery showed no changes in peak-peak wall shear stress ($r = -0.23$, $p = 0.64$), while radial artery peak wall shear stress maintained its increased level (Table 12). Although the arterial *CC* and *CWS* of both arteries acutely increased, beyond week 3, no significant changes were observed. The *DC* and E_m of either artery did not change over the course of one year.

DISCUSSION

After creation of an autogenous radial-cephalic wrist access, i.e., an AVF, for hemodialysis the mean wall shear stress in the brachial artery acutely (one day postoperative) increased. At 3 weeks the high level persisted and the radial artery showed an even more pronounced increase in mean wall shear stress compared to preoperative values (Table 12). In reaction to this considerable shear stress increase both arteries responded through diameter enlargement. In the brachial artery this dilation was still ongoing after one year, whereas the radial artery diameter maintained the diameter, acquired at 3 weeks, at one year (Table 12). Regarding *IMT*, only the radial artery wall showed thickening at 1 year after an early decrease. On the other hand the brachial artery *IMT* decreased directly postoperative and remained at this level over the course of one year (Table 12). Despite the changes in both diameter and wall thickness of the two arteries the mean wall shear stress remained significantly increased.

Through creation of an AVF, which is a low-resistance connection between a donor artery and a venous outflow conduit, the blood flow experienced by the donor artery will increase significantly.²³ As wall shear stress under stationary flow conditions is in the first order proportional to the quotient of blood flow velocity and lumen diameter, the rise in wall shear stress as shown in the present study is expected. Theoretically, this increase would acutely lead to a FMD of the donor artery.^{6-8,12} We have indeed shown that the brachial artery in the acute setting (one day postoperative) shows a 16% diameter increase, which is on the same order as observed after 5 minutes of tourniquet occlusion of the forearm.⁸ The 16%-change in diameter is, however, quite extraordinary because different authors have observed a diminished FMD in patients with ESRD.²⁴⁻²⁷ Apparently, the brachial artery vascular wall responds adequately to a shear stress increase despite an alleged endothelial dysfunction in patients with ESRD.

Even though the brachial artery diameter increases instantaneously, mean wall shear stress is not restored, thus providing a sustained milieu of increased shear stress. Both the brachial and radial artery respond by increasing their lumen diameter (Table 12). Both arteries however, differ in the magnitude of their response, i.e., the radial artery diameter reaches its peak (80%) after 3 weeks, whereas the brachial artery diameter increases over the course of one year to a maximum of 38% above baseline level. Although the radial diameter stops dilating at 3 weeks it is interesting to observe that the *IMT* shows a significant continuing increase over the course of one year. Buus et al. have also shown the remodelling capacities of rat mesenteric arteries after a sustained increase in blood flow and thus shear stress.¹² *In vivo* VSMC proliferation and differentiation, resulting in an increased wall mass,¹² elsewhere referred to as "favoured expansive hypertrophic remodelling".¹ With the present study, within the limits of ultrasound resolution, we achieved the same results in the human setting, albeit for the radial artery only.

In our *in vivo* model, however, mean wall shear stress did not return to its baseline physiological value despite remodelling of both the brachial and radial artery. One might therefore question the ingrained belief that shear stress is always restored to baseline values.^{2,3,5,21,22} This belief is based on the assumption that shear stress must be similar throughout the vascular system,^{21,22,28-30} although previous studies at our laboratory illustrate just the opposite.³¹⁻³⁵

The discussion regarding restoration of shear stress is confounded by the fact that most of the studies were performed in elastic arteries,^{10,28,36,37} while we considered muscular conduit arteries. In fact, we have already demonstrated that in muscular conduit arteries mean shear stress is lower than in elastic arteries such as the common carotid artery.³³ We believe that conduit arteries such as the brachial, radial, and femoral artery maintain a low baseline shear stress, allowing a large acute shear stress rise, with only a minor flow-dependent diameter increase. Thus, minimal work is needed to keep shear stress within physiological ranges.

Furthermore, most studies refer to Poiseuille's law when calculating the shear stress, which in our opinion is inaccurate as large artery blood flow is pulsatile, and the vascular system is distensible. For example, Girerd et al. have shown that the radial artery wall shear stresses between the donor artery of an AVF and the contralateral arm were not significantly different, despite a lumen diameter difference of 57%.³⁸ This would suggest a shear stress correction mechanism in a system obeying Poiseuille's law, which supposes a constant blood viscosity. However, *in vivo* *WBV* is also influenced by the shear rate, thus by blood flow (equation (3-5)).³³ Since blood flow is markedly increased in the donor artery of an AVF, as a consequence shear rate will also be higher. Using the Weaver equation (equation (3-5)) this would thus imply that *WBV* should decrease at that part of the vascular tree, in an attempt to lower shear stress.

The observed lumen diameter increase of both the brachial and radial artery and the *IMT* increase of the radial artery corroborate an active corrective mechanism. As shown this does not result in the expected decrease in mean wall shear stress. It might therefore be inferred that the sustained increase in blood flow exceeds the adaptation capacity of the investigated conduit arteries. Furthermore, the regulation process of the mean wall shear stress may be in competition with the

adaptation of other parameters. The *CWS* is a good candidate, but in the present study *CWS* remained elevated after one year. Even the radial artery *IMT* increase was not enough to restore *CWS* to normal (equation (1-4)).

Summarising, we have shown that the autogenous radial-cephalic wrist access for hemodialysis is an acceptable model to observe human *in vivo* vascular remodelling by an increase in brachial and radial artery lumen diameter in response to elevated wall shear stress. Eventually, also radial artery wall thickness increases. However, the challenge imposed to the arterial system greatly exceeds its adaptation capacity.

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CHAPTER 7

Matrix metalloproteinases-2 is an important regulator of blood flow-induced radial artery enlargement in human arteriovenous fistulas for hemodialysis

Submitted:

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ABSTRACT

Maturation of autogenous radial-cephalic arteriovenous fistulas for hemodialysis is believed to depend on radial artery remodelling capacities, initiated by a sudden increase in shear stress resulting in an acute lumen increase and subsequent structural alterations. We hypothesised that MMPs and their inhibitors (TIMPs) play an important role in radial artery remodelling. In 16 patients planned for AVF creation, radial artery diameter and shear stress were assessed before and 3, 6, 24, and 52 weeks after surgery. Plasma MMP-9, MMP-2, TIMP-1, and TIMP-2 were determined before and 3 weeks after surgery. For statistical analysis Wilcoxon's signed-rank test and multiple regression were used. Radial artery shear stress and diameter were increased 3 weeks after AVF creation ($p = 0.005$) and remained increased after 52 weeks. The MMP-2/TIMP-2 ratio, as a measure of MMP-2 activation, could not be demonstrated to increase significantly after surgery (85 vs. 197%, $p = 0.12$). The baseline diameter and the diameter at week 3 and 6 related to the pre- and postoperative MMP-2/TIMP-2 ratio, respectively ($r = 0.92$, $p = 0.001$; and $r = 0.80$, $p = 0.016$). The change in MMP-2/TIMP-2 ratio in response to surgery was inversely proportional to the diameter at 52 weeks ($r = -0.75$, $p = 0.031$). Furthermore, the diameter change over the course of one year was inversely proportional to both the pre- and postoperative MMP-2/TIMP-2 ratio ($r = 0.74$, $p = 0.035$; $r = -0.90$, $p = 0.003$, respectively). These observations were not confounded by heart rate, or mean arterial pressure. These data suggest that long-term blood flow-induced radial artery enlargement may be influenced by the expression of MMP-2 and its inhibitor, although further research is needed to clarify the role of the differential expression of other MMPs.

INTRODUCTION

The phenomenon of arterial enlargement after an acute increase in blood flow, FMD, is well established.^{1,2} It is believed that ECs, laying at the luminal-wall interface, play a pivotal role in this process. Via several mechanisms they are able to sense and respond to changes in shear stress, i.e., the viscous drag exerted by the flowing blood.³ When the blood flow, and hence shear stress increases, the endothelium reacts acutely by releasing various potent vasodilators such as NO and PGI₂, causing VSMC relaxation and thus dilation.⁴⁻⁶

If, however, the shear stress remains at a higher than normal level, it is known that the vessel wall will present enduring changes in vessel size and composition, i.e., remodelling, in order to adapt to the new hemodynamic environment.⁷⁻¹⁰ Again, the endothelium, exposed to this excessive shear stress, plays a fundamental role by the increased release of NO, growth factors and MMPs.^{7,10,11} In an excellent recent review, Galis and Khatri summarised the different stimuli presently known that can cause vessel reshaping under influence of MMPs.¹²

An *in vivo* model of sustained high blood flow can be obtained by creating an AVF, i.e., a large artery directly draining off an anastomosed vein thereby bypassing the peripheral vascular bed. Recently, different animal models have been proposed in which the role of several MMPs has been investigated. Sho et al. have shown that shortly after creation of an AVF in rabbits MMP-2 and -9 become up regulated in both ECs and VSMCs.¹³ Interestingly, MMP-9 activity rapidly declined whereas MMP-2 remained upregulated through a disproportional upregulation of MT1-MMP and TIMP-2. Anastomosing the carotid artery and the jugular vein in rabbits, Tronc and co-workers verified these results and they even stated that blood flow-induced MMP activation is critical in flow-induced vascular enlargement.¹¹ Interestingly, they additionally found NO, secreted by the endothelium, to participate in MMP activation.¹¹

Two studies from the same research group show experiments with an MMP inhibitor, RS-113,456, in a rat AVF model. They concluded that flow-mediated arterial enlargement can be limited by competitive MMP inhibition, further lending support to the idea that MMPs play a critical role in arterial remodelling.^{14,15}

Prompted by these reports we addressed the role of MMPs in human remodelling studying AVFs in patients with ESRD, planned for hemodialysis. As far as we know this is the first report on human arterial structural remodelling in a model of chronic flow augmentation. We measured *in vivo* radial artery shear stress and diameter, besides measuring plasma MMP-2 and -9, and their natural inhibitors (TIMP-2 and -1).

PATIENTS AND METHODS

Subjects and measurement procedure

This study was approved by the joint Medical Ethical Committee of the University of Maastricht and the University hospital Maastricht. After giving their written informed consent, 16 patients with ESRD, needing hemodialysis in the near future, were recruited. For the preoperative patient characteristics we refer to Table 13.

The measurements were performed in the morning between 08:00 o'clock and noon after an overnight fasting of at least 10 hours. The patients' necessary medication intake was allowed. The measurements were performed after an acclimatisation period of 15 minutes in supine position in a room with a temperature of 22 – 24°C. After blood sampling via venipuncture of an antecubital vein, brachial artery blood pressure of the dominant arm was measured every 5 minutes by a semi-automated oscillometric device (Dinamap 1846P, Critikon, Tampa, FL, USA). The radial artery of the non-dominant arm, i.e., the arm to be operated upon, was measured as described below with the arm in approximately 45° abduction and the palm of the hand facing upward.

Age [years]	64.6 ± 10.2
Male/female	9/7
Heart rate [min ⁻¹]	64.5 ± 8.9
Mean arterial pressure [mmHg]	113 ± 13
Hemoglobin [mmol·L ⁻¹]	6.9 ± 0.8
Hematocrit [%]	33.5 ± 3.5
Plasma viscosity [mPa·s]	1.16 ± 0.10
Urea [mmol·L ⁻¹]	25.2 ± 9.3
Creatinine [μmol·L ⁻¹]	520 ± 190

Table 13. Preoperative patient characteristics. Shown are mean values ± SDs.

Assessment of arterial hemodynamic and wall properties was performed before surgery and 3, 6, 24, and 52 weeks after operation. Blood samples for the determination of MMPs and TIMPs were obtained pre-operatively and 3 weeks after surgery.

Surgical procedure

Vascular access for hemodialysis was established through the creation of an autogenous radial-cephalic direct wrist access in the non-dominant arm. The surgical procedure is performed under regional anaesthesia. Approximately 2 cm proximal to the radial styloid a longitudinal or transverse skin incision (3 – 4 cm) at the ventral side of the forearm is made. The radial artery and cephalic vein are exposed, and the cephalic vein is separated from its surrounding tissue. After clamping, the cephalic vein is ligated as distal as possible and is subsequently anastomosed to the radial artery in an end-to-side fashion with a running prolene 7x0. All surgical procedures were performed by a single vascular surgeon.

Blood sampling and assays for plasma MMP-2 and -9, and TIMP-2 and -1

Venous blood samples for the determination of plasma hemoglobin concentration, hematocrit, urea and creatinine levels, plasma viscosity, and MMP-2 and -9 concentration, and their inhibitors TIMP-2 and -1, respectively, were drawn 15 minutes before assessment of vascular wall properties and hemodynamic parameters. Plasma viscosity was determined in duplo with a glass capillary viscometer. Plasma levels of MMPs and TIMPs were determined by an ELISA. In

addition, as a measure of free active MMP we calculated the MMP/TIMP ratio.¹² For a more detailed description of the techniques we refer to **CHAPTER 3** Materials and methods.

The Wall Track System and the Shear Rate Estimation System (ART-Lab)

The wall track system and the shear rate estimation system have been described extensively in **CHAPTER 3** Materials and methods.

Shear stress calculation

Shear stress was determined with equation (3-5) and (3-6) as described in **CHAPTER 3**.

Statistical analysis

Statistical management and analysis was performed with the SPSS 10.0 statistical package (SPSS, Chicago, IL, USA). Group means are presented as mean $\pm$ SD. To compare diameter changes, hemodynamic changes, or changes in MMPs and TIMPs, Wilcoxon's signed-rank test was used. To analyse long-term diameter or hemodynamic changes with respect to (changes in) matrix degrading proteins, a multivariate analysis was used with time-point, heart rate, and mean arterial pressure as covariates. A two-sided p-value of less than 0.05 was considered statistically significant.

RESULTS

After creation of the autogenous radial-cephalic direct wrist fistula (AVF) radial artery mean wall shear stress acutely increased from 0.22 ± 0.11 Pa before to 2.82 ± 1.18 Pa 3 weeks post surgery, an increase of 1198% ($p = 0.005$). Mean wall shear stress continued to be at this high level until 52 weeks (2.51 ± 1.35 Pa; $r = -0.16$, $p = 0.86$). Peak wall shear stress also increased significantly (1.77 ± 0.60 vs. 4.37 ± 1.55 Pa, i.e., before vs. 3 weeks post surgery; $p = 0.003$) and did not change further over the course of one year (4.43 ± 1.96 Pa; $r = 0.15$, $p = 0.88$). The radial artery diameter had increased significantly from 2.2 ± 0.5 mm at baseline to 4.0 ± 0.7 mm 3 weeks post AVF creation ($p = 0.005$), as is illustrated in Figure 11, but did not change further over the course of one year (4.1 ± 1.0 mm; $r = 0.13$, $p = 0.93$).

In Table 14 a summary of the measured MMPs and TIMPs before and 3 weeks post surgery is given. Shown are optical densities and concentrations. For the TIMP-1 assay no concentrations could be given, because a standard curve could not be obtained. Also depicted is the MMP/TIMP ratio. Strikingly, in these patients with ESRD both MMP-2 and -9 are higher than normal. After 3 weeks, MMP-9 tended to be increased when compared to baseline values, but this difference did not reach significance ($p = 0.09$). The same holds for the MMP-2/TIMP-2 ratio that seems higher post surgery ($p = 0.12$).

Figure 11. Radial artery diameter alterations after AVF creation. Shown are mean values $\pm$ SD. * $p=0.005$ compared to preoperative value (Wilcoxon's signed-rank test).

		Optical density	Concentration [ng·mL ⁻¹]
MMP-2	Baseline	1.57 $\pm$ 0.42	1155 $\pm$ 267
	3 weeks	1.58 $\pm$ 0.42	1178 $\pm$ 318
TIMP-2	Baseline	0.12 $\pm$ 0.03	16.2 $\pm$ 17.4
	3 weeks	0.12 $\pm$ 0.04	16.2 $\pm$ 18.5
MMP-2/TIMP-2	Baseline	13.6 $\pm$ 3.4%	85 $\pm$ 58%
	3 weeks	13.7 $\pm$ 4.4%	197 $\pm$ 184%
MMP-9	Baseline	0.011 $\pm$ 0.011	39.7 $\pm$ 10.0
	3 weeks	0.019 $\pm$ 0.020	46.7 $\pm$ 19.2
TIMP-1	Baseline	0.58 $\pm$ 0.10	---
	3 weeks	0.62 $\pm$ 0.09	---
MMP-9/TIMP-1	Baseline	0.021 $\pm$ 0.025%	---
	3 weeks	0.030 $\pm$ 0.034%	---

Table 14. MMP and TIMP values before and 3 weeks after AVF creation. Shown are mean values $\pm$ SDs of optical densities and concentrations of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) prior to and 3 weeks after surgery. MMP-2/TIMP-2 and MMP-9/TIMP-1 ratios are measures of active MMP-2 and -9, respectively and are expressed in percentages.

In a multivariate regression model the preoperative radial artery diameter was directly related to the MMP-2/TIMP-2 ratio prior to surgery ($r = 0.92$, $p = 0.001$). Interestingly, the plasma MMP-2/TIMP-2 ratio 3 weeks after surgery was related to the radial artery diameter at 3 and 6 weeks after surgery ($r = 0.80$, $p =$

0.016). In turn, however, the baseline diameter was not related at any time-point to any changes in diameter after surgery. Shear stress values were also not related to any changes in diameter at the measured time-points, illustrating that other factors might determine the long-term radial artery diameter enlargement. Furthermore, the diameter change over the course of one year was inversely proportional to the MMP-2/TIMP-2 ratio before and after AVF placement ($r = -0.74$, $p = 0.035$; $r = -0.90$, $p = 0.003$, respectively). Strikingly, the difference in MMP-2 before and 3 weeks after surgery was only related to the change in peak wall shear stress ($r = 0.90$, $p = 0.002$). Furthermore, the change between pre-operative and mean wall shear stress 3 and 52 weeks post surgery was related to the change in TIMP-1 ($r = 0.95$, $p < 0.001$; and $r = 0.73$, $p = 0.026$, respectively).

DISCUSSION

In the present study the human radial artery was exposed to a sustained increase in blood flow and therefore a 12-fold increase in mean and a 1.5-fold increase in peak shear stress occurred through creation of an autogenous radial-cephalic wrist access for hemodialysis. Both MMP-2 and MMP-9 were non-significantly higher 3 weeks after surgery. However, the pre-operative plasma MMP-2/TIMP-2 ratio as measure for free active MMP-2 correlated well with the baseline diameter. Furthermore, three weeks after surgery this ratio was proportional to the change in diameter at that time-point, suggesting that mainly the MMP-2 increase, instigated through the change in peak wall shear stress, accounts for the radial artery diameter enlargement in patients with ESRD.

Previous *in vivo* high-flow studies with AVFs in both rats^{14,15} and rabbits^{11,13} obtained similar results. In their rat model, Tronc et al. created an AVF between the left common carotid artery and the left external jugular vein and observed a shear stress increase.¹¹ Due to the concomitant carotid artery diameter enlargement the shear stress returned to baseline values. Interestingly, both proforms of MMP-2 and MMP-9 and active MMP-2 were shown to be upregulated 3 days post surgery. Fifteen days after surgery these returned to baseline values. In the report by Sho et al. MMP-2 appeared to continuously increase even after 7 days post AVF creation.¹³ Another study also demonstrated an upward trend in MMP-2 after 21 days in a rat AVF model involving the left common femoral artery and vein.¹⁵ This turned out to be dose-dependent and directly proportional to an aortic and left iliac diameter increase. In an earlier study, the same authors had already illustrated that inhibition of MMP activity by RS-113,456 diminished flow-mediated arterial enlargement in the same rat model.¹⁴

As far as we know, the present *in vivo* study is the first to report MMP involvement in arteriovenous fistula maturation in human subjects. The obvious disadvantage of our study is that MMP-2 and -9 and TIMP-2 and -1, respectively, were measured in plasma by ELISA only. Although we cannot be sure whether the measured concentrations of plasma MMP-2/TIMP-2 ratio correspond to MMP-2 activity through zymography, histology, or PCR in the vessel wall itself, we have shown that this ratio relates well to the diameter enlargement that we observed using the wall track system. Interestingly, the diameter changes were inversely related to the MMP-2/TIMP-2 ratios both before and after surgery, which we feel

reflects that the higher the baseline diameter, the less enlargement can be achieved.^{2,16,17}

A sustained increase in shear stress induces several changes in the vessel wall. For example, ECs are stimulated to secrete larger amounts of NO,^{7,11} triggering the VSMCs to leave their quiescent phase and start proliferating,¹⁸ whereas a steady laminar shear stress inhibits smooth muscle cell proliferation.¹⁹ Also by the release of NO, the release of MMPs are somehow regulated.¹¹ Another candidate is TGF- β , which is also capable of upregulating the expression of or even activation of MMP-2 and -9.^{13,20} When MMP-2 and -9, and most likely other members of the matrix degrading family, are expressed by ECs and maybe VSMCs and become activated, the cell basement membrane and the internal elastic lamina will be degraded. Interestingly, shear stress has been reported to inhibit membrane type 1-MMP (MT1-MMP) expression,²¹ which is needed to activate MMP-2.^{22,23} Our results regarding the MMP-2 increase, due to a rise in peak wall shear stress, are in concordance with these preceding findings. This creates a milieu where the VSMCs can freely proliferate and migrate to ensure arterial enlargement, herewith trying to restore shear stress to normal values. The wall shear stress of our ESRD population, however, showed no correction to baseline values, which is in disagreement with an earlier study.²⁴

Summarising we have illustrated *in vivo* that the radial artery diameter enlargement in human arteriovenous fistulas is at least in part influenced by the activity of MMP-2 in plasma. Despite the drawbacks of the present study, the results are in concordance with previous reports in animal studies and therefore we feel that the arteriovenous fistula model can be used to unravel the underlying mechanisms of arteriovenous fistula maturation and arterial remodelling.

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CHAPTER 8

Detection of cell-cycle regulators in failed arteriovenous fistulas for hemodialysis

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ABSTRACT

Chronic renal failure patients rely on AVFs for hemodialysis vascular access. Intimal hyperplastic stenoses result in failure of AVFs and frequent interventions are required to maintain vascular access. The extent of intimal hyperplasia depends on the interplay between cyclins and cyclin-dependent kinases (e.g. cdk2), positively regulating cell-cycle progression. cdk activity is negatively modulated by the interaction with cdk inhibitory proteins, such as p21^{Waf1} and p27^{Kip1}. Little is known about the expression of these proteins in the development of intimal hyperplasia in AVFs. p21^{Waf1}, p27^{Kip1}, cdk2, and Proliferating Cell Nuclear Antigen immunoreactivity was determined in 18 failed AVFs from 16 ESRD patients and 10 non-diseased vessels (5 arteries and 5 veins). The percentage p21^{Waf1}-positive cells was significantly lower in AVFs ($3 \pm 1\%$), compared with normal veins and arteries ($62 \pm 4\%$ and $63 \pm 4\%$, respectively; $p < 0.001$). cdk2-positive cells were significantly higher in AVFs ($40.7 \pm 3.7\%$) than in normal veins and arteries ($2 \pm 1\%$ and $0 \pm 0\%$, respectively; $p < 0.001$). Although no difference in p27^{Kip1} immunoreactivity was found between AVFs ($37 \pm 17\%$) and veins ($23 \pm 8\%$; $p = 0.21$), it was lower in healthy arteries ($17 \pm 11\%$; $p = 0.037$). These data suggest that in failed AVFs p21^{Waf1}, but not p27^{Kip1}, is related to intimal hyperplasia. This is the first report to show involvement of cell-cycle regulators in AVF-related human intimal hyperplasia.

INTRODUCTION

Intimal hyperplastic stenoses in surgically created AVFs for hemodialysis remain a serious problem in maintaining vascular access. One-year primary patency rates vary from 53 – 88% for autogenous radio-cephalic wrist fistulas to 37 – 70% when prosthetic grafts are used.¹ Pathological proliferation and migration of VSMCs resulting in intimal thickening is the main cause of graft stenosis and occlusion within the first year after AVF construction.^{2,3} In normal vascular tissue, quiescent VSMCs are found in the G₀ phase of the cell cycle and VSMCs proliferate at a low rate. However, on stimulation VSMCs can exit their quiescent state and progress through the G₁ and G₁/S transition of the cell cycle.^{4,5}

Cell-cycle progression is positively regulated by the formation of complexes comprised of cyclin-dependent kinases (cdk) and their activating subunits, the cyclins. cdk activity is, in turn, negatively modulated by the interaction with cdk inhibitory proteins (CKIs), resulting in cell-cycle arrest.⁶ Progression through the first gap (G₁) phase requires both cyclin D-cdk4/6 (early G₁) and cyclin E-cdk2 (late G₁).⁵ Specific CKIs (p21^{Waf1} and p27^{Kip1}) are capable of blocking cell-cycle progression by binding to and inhibiting the activation of cyclin E-associated-cdk complexes.⁷ Both p21^{Waf1} and p27^{Kip1} inhibit VSMC proliferation by reducing cdk2 activity.⁸

Expression of these CDKs and CKIs in arterial balloon injury models and the role of these cell-cycle proteins in VSMC proliferation have been investigated in cultured bovine, porcine, and human ECs and VSMCs as well as in human atherosclerotic lesions and normal porcine arteries.^{9,10} However, it is not known how the expression of these cell cycle regulators relate to venous intimal hyperplasia as a result of hemodynamic forces that are relevant in AVFs. Therefore, we investigated the expression of p21^{Waf1}, p27^{Kip1}, cdk2, and Proliferating Cell Nuclear Antigen (PCNA) in AVFs and non-diseased vascular tissue.

MATERIAL AND METHODS

Tissue Specimens

Eighteen specimens of stenotic lesions of failing AVFs were obtained from 16 patients (eight men and eight women; age 59.8 ± 3.4 years) undergoing AVF revision at the surgical department of the University Hospital Maastricht. Two patients underwent a second revision during which primarily stenosed tissue was collected. Hypertension was diagnosed in 12 patients (75%) and only one patient presented with non-insulin-dependent diabetes. The diagnosis of stenosis was based on generally accepted Duplex ultrasound criteria and confirmed by angiography.¹¹ The study was approved by the joint Medical Ethical Committee of the Maastricht University and the University Hospital Maastricht and the patients were included after written informed consent.

In 14 of 18 AVFs vascular access was created through a prosthetic brachial-antecubital forearm loop access, while in the four other cases, an autogenous radial-cephalic direct wrist fistula was constructed. AVF revision was performed 27 months (range 1 – 80 months) after primary access creation. Surgical repair was performed by placement of an interposition graft, allowing collection of the stenotic process and adjacent vessel segments. In all patients, the stenotic lesion

was focal and narrowing or occluding the lumen. All 18 specimens of stenotic lesions were taken from the direct vicinity of the venous anastomotic site distal to the AVF. During revision, the stenotic lesions were obtained and fixed in 10% formalin for 4 hours, followed by 18 hours in 70% ethanol, and paraffin-embedded for immunocytochemistry. Sections (4- μ m thick) were stained with hematoxylin and eosin, and immunostained with p21^{Waf1}, p27^{Kip1}, cdk2, and PCNA antibodies.

Five normal arteries were obtained during obduction from patients who died of non-cardiovascular cause. Remnants of vein grafts used for arterial revascularisation were obtained (n = 5). Warm ischemia time was less than 5 minutes in all veins. Both non-diseased arteries and veins were formalin fixed, paraffin-embedded and stained with afore mentioned antibodies.

Immunocytochemistry

Serial sections of the tissues were incubated with the following primary antibodies: a mouse monoclonal anti-human p21^{Waf1} antibody, 1:50 dilution (NeoMarkers, Inc., Fremont, CA, USA); a mouse monoclonal anti-human p27^{Kip1} antibody, 1:50 dilution (NeoMarkers); a mouse monoclonal anti-human cdk2 antibody, 1:50 dilution (NeoMarkers); and a mouse monoclonal anti-human PCNA antibody, 1:30 dilution (NeoMarkers). Sections (4- μ m thick) were deparaffinised in three changes of xylene, and rehydrated in 90, 70, and 50% ethanol. Slides were boiled in citrate buffer for 20 min, and incubated in 2% BSA/PBS/tween for 20 minutes to block aspecific binding activity. Primary antibodies were diluted in TBS and applied to the slides for 60 minutes at room temperature. After three washes with TBS for 5 minutes, a biotinylated horse anti-mouse secondary antibody (1:400 dilution, Dako) was applied for 30 minutes at room temperature, followed by a streptavidin amplification reagent (Dako) for 30 minutes at room temperature. Finally, Fast Red was used for 10 minutes at room temperature, yielding a red reaction product. Visualisation of all nuclei in the tissue sections was realised with a hematoxylin counterstain.

Total nuclei, and positive nuclei were counted in the (neo-)intima of the different vascular segments by means of a microscope-based video image analysing system (Analysis®, Soft Imaging System, Münster, Germany). Four random high-power fields of each specimen were analysed.

Statistical analysis

Data management and statistical analysis were performed with the SPSS 10.0 package for Windows (SPSS, Chicago, IL., USA). Data are presented as mean values and standard errors of the mean (SEMs). Mean percentages in the patient group and control tissues were compared by means of ANOVA with Bonferroni correction. An ANCOVA was performed to correlate patient characteristics (such as sex, age, and present cardiovascular risk factors) and graft survival to cell cycle regulating proteins.

RESULTS

All vascular segments were stained with p21^{Waf1}, p27^{Kip1}, cdk2, and PCNA antibodies and total nucleic, and immunoreactive nucleic count was determined in four random high-power fields. The average number of nuclei counted was 4,416 in AVFs, 1,363 in non-diseased veins, and 1,262 in non-diseased arteries.

Whereas p21^{Waf1} was clearly expressed to a greater extent in non-diseased veins and arteries, cdk2- and PCNA-positive cells were more frequently detected in failed AVFs (Figure 12). p27^{Kip1} immunoreactivity was observed both in non-diseased and intimal hyperplastic tissue (Figure 12). Figure 13 shows the percentage of positively stained cells for p21^{Waf1}, p27^{Kip1}, cdk2, and PCNA, respectively. The percentage p21^{Waf1}-positive cells was significantly lower in AVFs ($3 \pm 1\%$) compared with non-diseased veins and arteries ($62 \pm 9\%$ and $63 \pm 8\%$, respectively; $p < 0.001$). In contrast, cdk2- and PCNA-positive cells were abundantly present in AVFs ($41 \pm 16\%$ and $37 \pm 15\%$, respectively) whereas immunopositive cells were almost absent in non-diseased veins ($0 \pm 0\%$, and $0 \pm 0\%$, respectively; $p < 0.001$) and arteries ($2 \pm 2\%$, and $1 \pm 0.4\%$, respectively; $p < 0.001$). p27^{Kip1} immunoreactivity was lower in arteries compared with failed AVFs ($17 \pm 11\%$ and $37 \pm 14\%$, respectively; $p = 0.037$). However, between veins and AVFs no difference was found in p27^{Kip1} immunoreactivity ($23 \pm 8\%$; $p = 0.21$). No variations were observed between autogenous and prosthetic accesses concerning any cell-cycle regulating protein. Therefore, we decided not to report on these two types of access separately. Correction for the variables age, sex, and cardiovascular risk factors had no influence on the level of significance. No relation was found between the different cell-cycle regulating proteins and patient history of former AVFs or the number of revisions. Furthermore, no relation was found between age, sex, cardiovascular risk factors, and cell-cycle regulating proteins.

Figure 12. Representative immunocytochemical findings of cell-cycle regulators in normal arterial and venous tissue, and neointima of failed AVFs. Fast Red-stained nuclei are shown in normal veins: (A) p21^{Waf1}, (B) p27^{Kip1}, (C) cdk2, and (D) PCNA; normal arteries: (E) p21^{Waf1}, (F) p27^{Kip1}, (G) cdk2, and (H) PCNA; AVFs: (I) p21^{Waf1}, (J) p27^{Kip1}, (K) cdk2, and (L) PCNA expression. p21^{Waf1} and p27^{Kip1} immunoreactivity is higher in normal arteries and veins, compared to AVFs (A and B, E and F, I and J) versus higher cdk2 and PCNA immunoreactivity in AVFs compared to arteries and veins (C and D, G and H, K and L). Bar, 100 μm .

Figure 13. Expression of p21^{waf1}, p27^{kip1}, cdk2, and PCNA in arteriovenous fistulas for hemodialysis, normal veins and arteries. Data are presented as mean percentages $\pm$ standard error of the means. * = $p < 0.001$ compared to expression in AVFs; † = $p < 0.05$ compared to expression in AVFs.

DISCUSSION

Although cell-cycle regulator expression in normal, balloon-injured, and atherosclerotic vascular tissue has been described,^{9,10} this has not yet been reported in human intimal hyperplastic lesions.

Normal human arteries and veins are mainly characterised by the presence of quiescent VSMCs in the media. This is supported by our finding that p21^{waf1} and p27^{kip1} are abundantly expressed in non-diseased human arteries and veins. In contrast, cdk2 expression was absent in veins and minimally expressed in arteries. Considering the aforementioned characteristics of the cell-cycle machinery, these results agree with earlier studies, showing a correlation between CKI (p21^{waf1}, p27^{kip1}) expression and VSMC quiescence.^{8,9,12} We did not obtain any non-stenosed venous material from the direct vicinity of the stenosed lesion. This tissue would probably be more suitable as a control specimen, since high flow conditions in this part of the fistula might also initiate changes in the expression of cell-cycle proteins. However, ethical constraints would arise from clinical unnecessary excision of tissue indispensable for the patient.

Intimal hyperplasia, ensuing stenosis is characterised by an increased proliferation and migration of VSMCs, which is the main cell type present in the neointima of AVFs.¹³ p27^{kip1} and p21^{waf1} expression might be expected to be reduced in intimal hyperplasia. However, in the present study p27^{kip1} does not seem to play a significant role. In contrast, p21^{waf1} expression was considerably lower in

stenotic regions of failing AVFs. These findings support the notion that p21^{Waf1} is a negative regulator of VSMC proliferation and thus intimal hyperplasia in AVFs.^{8,9,12} The prevailing theory of p21^{Waf1} negatively modulating cdk2 expression is reflected by our finding that cdk2 is profusely expressed in intimal hyperplastic lesions.

Wei et al.¹⁴ have shown a temporally and spatially coordinated expression of cdk2, cyclins E and A, and PCNA in a rat balloon-injury model. Unfortunately, we were not able to examine cell-cycle protein expression through time due to the retrospective nature of the study. Further investigations in time-dependent cell-cycle protein expression are needed to elucidate cell-cycle protein function in intimal hyperplasia and eventually providing a site for intervention.

Recently, there has been a growing interest in the role of therapeutical means in preventing restenosis after vascular reconstruction. For example, rapamycin has been shown to inhibit VSMC proliferation and migration *in vitro* and arterial intimal thickening after balloon injury *in vivo* by increasing p27^{Kip1} levels, and inhibiting pRb phosphorylation within the vessel wall.¹⁵ The exact mechanism of action of rapamycin on p27^{Kip1} levels is unclear. Our results show that p27^{Kip1} is not suppressed in AVFs. Since rapamycin induces p27^{Kip1} expression, this prospective therapeutical agent might not be the ideal choice for the prevention of stenoses in AVFs. Another possible agent, tranilast, has been found to inhibit proliferation and migration of VSMCs and collagen synthesis¹⁶ by induction of p21^{Waf1} and p53, preventing restenosis after percutaneous transluminal coronary angioplasty.¹⁷ As a result of the induction of p21^{Waf1} levels, and subsequent decrease of cdk2 activity, tranilast blocks cell-cycle progression in the G₁ phase. In the present paper, we demonstrated dramatic reduction of p21^{Waf1} levels in occluded AVFs. It might be anticipated that an imposed stabilisation or even up-regulation of p21^{Waf1} might inhibit VSMC proliferation, thereby preventing AVF stenosis.

The altered expression of several cell-cycle proteins in failed AVFs contributes to intimal hyperplasia. Since the cell-cycle machinery is accessible for therapeutic intervention, it is our opinion that it might be an attractive target to prevent intimal hyperplasia in AVFs. Large randomised placebo-controlled intervention studies are required to prove noteworthy reduction in intimal hyperplasia after AVF construction.

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CHAPTER 9

General discussion:

Hemodynamic and biochemical changes that influence arteriovenous fistula maturation; findings on arterial remodelling *in vivo*

INTRODUCTION

The main goal of the present thesis was increasing the knowledge regarding hemodialysis arteriovenous fistula maturation. We were primarily interested in the relation or concerted action of hemodynamic and vessel wall properties of the arterial component of the arteriovenous fistula, hypothesising those properties could evoke functional arteriovenous fistula maturation. Furthermore, endothelial function, determined through functional or biochemical parameters, and extracellular matrix metabolism were of interest, assuming those could predict in any way arteriovenous fistula maturation. We anticipated that these parameters could determine the prognosis regarding functional maturation of the arteriovenous fistula before and shortly after surgery.

BRACHIAL ARTERY HEMODYNAMIC AND VASCULAR WALL PROPERTIES IN HEALTHY CONTROLS AND END-STAGE RENAL DISEASE PATIENTS

The custom-built ART-Lab system connected to an ultrasound device as described by Brands et al.¹ (**CHAPTER 3**) has already been widely used in various studies. Most of these studies were performed in the common carotid artery due to its undemanding localisation and its elastic properties, providing the possibility of extrapolating these data to the aorta. For their applicability in the common carotid artery, both the wall track system and the shear rate estimation system have been tested in previous studies at our laboratory.²⁻⁵ The common carotid artery has been of particular interest because of the relationship between vessel wall mechanic properties (such as compliance, distensibility, and *IMT*), shear stress, and cardiovascular disease and its risk factors in general.⁶⁻¹⁸

Because of our interest in the non-invasive assessment of arterial remodelling in arteriovenous fistulas we first needed to test the applicability of the ART-Lab system for measurements in a small calibre artery with minor distension such as the brachial artery. In this way we would be able to establish the reproducibility of future brachial artery measurements. We combined these efforts with, as far as we know, the first study to measure brachial artery shear stress *in vivo* in different age groups (**CHAPTER 4**). We observed an intrasubject variability for the various measures for brachial artery wall shear rate of 16 – 28% (Table 3), which is on the order of common carotid artery shear rate measurements.³ Furthermore, regarding vessel wall mechanic characteristics we also ascertained similar intrasubject variations (Table 3; 7 – 26%) as observed in the common carotid artery previously.^{2,5} Since we were only able, however, to measure as close as 200 – 400 μm to the vessel wall, the observed shear rate will be a least estimate. Taking into account a diameter of about 4 mm this implies a somewhat larger estimation error compared to measurements in the carotid artery.³

Interestingly, a mean wall shear stress of approximately 0.5 Pa was found that was equal in both healthy men and women and was not influenced by age. As common carotid artery mean wall shear stress approximates 1.5 Pa, this was a remarkable low finding. Others believe that mean shear stress is equal throughout the vascular system,¹⁹⁻²⁵ although in an additional study, beyond the scope of the thesis, we have proven that there is indeed a difference in brachial and common

carotid artery mean shear stress.¹¹ This inconsistency with the literature can only partly be explained by the fact that other studies calculate wall shear stress with Poiseuille's law. This law, however, supposes a steady non-pulsatile parabolic laminar flow of a Newtonian fluid, i.e., independent of shear rate, in a rigid straight vessel.²⁶ We believe that this is at the least an inaccurate supposition as human large artery blood flow is pulsatile and the vascular system is a distensible tube.^{27,28} Moreover, due to the viscoelastic properties of human blood, *WBV* might vary with the shear rate, thus influencing wall shear stress.^{29,30} In the present thesis, therefore, we approximated whole blood viscosity by implementing the measured shear rate (ART-Lab) in equation (3-5),³¹ considering blood to be a shear-thinning fluid. With the approximated whole blood viscosity, dependent on local shear rate, we then calculated shear stress with equation (3-6). The second difference explaining the different shear stress in various vessels can be found in the fact that it is merely the peripheral vascular bed that determines conduit artery blood flow. As is further elaborated upon below and in **CHAPTER 5**.

After establishing acceptable reproducibility we were interested in possible hemodynamic and vessel wall differences between healthy subjects and patients with ESRD who had not been on dialysis yet. In **CHAPTER 5** we observed that, after correction for age, patients with ESRD merely appeared to have a significantly thicker brachial artery wall compared to healthy subjects and the mean shear stress was about 50% lower, viz. 0.28 Pa (Table 7 and 8).

Although a thicker carotid artery *IMT*, i.e., wall thickness, had already been described in ESRD patients,³²⁻³⁴ we were the first to confirm these findings for the brachial artery. Moreover, we have shown a direct relationship between the brachial artery *IMT* and creatinine levels. Although Konings et al. could not confirm this,³⁵ others have previously shown that carotid artery wall thickness related to uremic status,^{33,36} corroborating our findings in the brachial artery.

Studies in the common carotid artery have shown that not only the *IMT* increases but also that the artery's distensibility decreases due to stiffening of the vessel wall.^{35,37,38} The compliance, however, is maintained at a constant level through diameter enlargement, further decreasing distensibility. Results in the brachial artery are scarce but few studies have indeed shown a lower brachial artery distensibility.^{39,40} Although we found the same absolute outcomes regarding distensibility ($\pm 5 \text{ MPa}^{-1}$) and distension ($\pm 60 \mu\text{m}$), we were not able to confirm differences with the healthy controls. We used a comparable technique to measure distension, and thus distensibility, and the patient groups were equally large. The principal difference between these studies, however, was the patients' age. The patients in our studies were approximately 65 years of age, whereas others presented their results in patients and controls of about 40 years of age. In this light van Guldener et al. have shown that only in a subgroup of patients aged younger than 50 years brachial artery distensibility was significantly lower.⁴¹ Apparently patients with ESRD display stiffening of the brachial artery earlier in life.

FLOW-INDUCED *IN VIVO* ARTERIAL REMODELLING

CHAPTER 6 and **7** describe the actual arterial remodelling taking place after creation of an arteriovenous fistula for hemodialysis. Both the brachial and the radial artery increase their diameter in response to increased blood flow as is shown in Table 12. Both arteries however, differ in the magnitude of their response, i.e., the radial artery diameter reaches its peak (80%) after 3 weeks, whereas the brachial artery diameter increases over the course of one year to a maximum of 38% above baseline level. Interestingly, however, the *IMT* of the radial artery tends to be lower at 3 weeks (354 μm) compared to baseline (406 μm) and increases significantly to its eventual thickness at one year (487 μm ; $p < 0.005$), whereas the brachial artery's wall thickness does not change, or even tends to decrease.

Despite these morphological changes in this non-invasive *in vivo* human remodelling "model", the shear stress increase after arteriovenous fistula creation (Table 12) is not restored to baseline "physiological" values in neither the brachial nor the radial artery. This finding disagrees with the common understanding that the vascular system always tries to restore shear stress to baseline values.^{20-22,42-45} Most studies, however, are based on data acquired from elastic arteries such as the common carotid artery, the aorta, and coronary arteries in both animals and humans and are therefore difficult to compare with our results in muscular conduit arteries such as the brachial and radial artery. We hypothesise, nevertheless, that muscular conduit arteries, connected to peripheral vascular beds that are subject to great changes in metabolic demands, maintain a lower baseline shear stress,⁴¹ so that they can accommodate large acute shear stress increases. The maximal tolerable shear stress for the vessel wall would therefore never be exceeded despite the minor flow-dependent diameter increase. This implies that only minimal work is needed to keep shear stress within physiological ranges. In a recent article Schmidt-Trücksass et al. presented results corroborating our hypothesis.⁴⁶ They observed that the femoral artery mean shear rate of paraplegic patients was significantly higher compared to endurance-trained athletes. Furthermore, they noted that the femoral artery diameter was considerably higher in athletes. However, the common carotid artery of paraplegics and endurance-trained athletes had the same diameter and mean shear rate.

The structural adaptation, i.e., the diameter enlargement, observed in the radial artery was related to pre-operative plasma matrix degrading protein content (**CHAPTER 7**). It appeared that mainly an increase in MMP-2 activity, instigated through the change in peak wall shear stress, accounted for the radial artery diameter enlargement in patients with ESRD. A major limitation of this study was that we were not able to confirm these results through zymography or PCR in vascular tissue obtained from arteriovenous fistulas, merely due to the fact that this would be unethical in the human setting. Also, we measured plasma levels of MMP-2 and -9, and their inhibitors of which we can never be sure that the arteriovenous fistula is the only or main cause of the changes in plasma levels of these matrix-degrading proteins. Nevertheless, our data are the first to link MMP-activation to human arteriovenous fistula maturation, in other words *in vivo* remodelling. With several animal studies claiming similar results,⁴⁷⁻⁵⁰ we believe

that further research needs to be performed. Especially, the inhibition of MMPs in the setting of arteriovenous fistula maturation^{47,48} and the development of intimal hyperplasia might launch new therapeutic means. Unfortunately, we were not able to relate pre-operative MMP-measures to long-term maturation, concluding that these cannot be used for diagnostic purposes.

PRE-OPERATIVE ASSESSMENT OF BRACHIAL ARTERY ENDOTHELIAL FUNCTION

Although one major objective of the present thesis had been satisfied, viz. evaluating the role of hemodynamic and vascular wall characteristics in *in vivo* human arterial remodelling, we failed in demonstrating a functional role for pre-operative endothelial function assessment.

Firstly, we performed several studies to characterise the reproducibility of the well-known FMD test. This test, ever since the leading article by Celermajer et al.⁵¹ has found great appliance in the cardiovascular research field.⁵²⁻⁵⁹ Basically, the FMD test focuses on the difference in vasodilatory capacity of the brachial artery after an induced flow increase and after administration of NO. In case of a diseased or dysfunctional endothelium the so-called brachial FMD is lower than the dilation brought upon by exogenous NO.

Stift et al. at our laboratory have shown a standard deviation of 2.9% with a mean brachial artery FMD of 5.9%, resulting in a variation coefficient of approximately 50% for this outcome measure.⁶⁰ This study was, however, performed to study the stimulus-response relationship of brachial artery flow increase and dilation simultaneously. Therefore, we executed another study, employing the wall track system, in which we subjected 14 young and completely healthy individuals to FMD. All measurements were performed under the same conditions and were repeated six times for every individual. Summarising, we obtained intrasubject intersession variation coefficients of 56 and 29%, for FMD and NO-mediated dilation, respectively (unpublished results). This corroborated with very recently published findings by de Roos et al., demonstrating intrasubject variation coefficients of about 50% for brachial artery FMD established via video-image processing.⁶¹ Although many authors show promising results, we believe that this test is as yet not viable for the individual patient⁶⁰⁻⁶⁴ and therefore we decided not to exploit the FMD test as a measure of endothelial (dys)function in our population.

As an alternative, other endothelial dysfunction parameters were considered, viz. the determination of plasma EC function markers such as vWF, PAI-1, and ET-1. In short, as described in **CHAPTER 5**, we were not able to find any differences whatsoever in PAI-1 levels between healthy subjects and patients with ESRD. Moreover, pre-operative PAI-1 levels were not related to the maturation of the arteriovenous fistulas in our studies. This also held for ET-1 and vWF, although both were significantly higher in the patient group.

INTIMAL HYPERPLASIA IN ARTERIOVENOUS FISTULAS

In a previous study at our department, Hofstra et al. illustrated that intimal hyperplastic lesions in both arteriovenous fistulas and peripheral bypasses displayed

enhanced cellular proliferation that was positively correlated to endothelial coverage.⁶⁵ Prompted by these findings we were interested which cell-cycle regulators would play a significant role in this intimal hyperplastic proliferation. Since the cell-cycle machinery is accessible for therapeutic intervention, it was our opinion that it might provide an attractive target to prevent intimal hyperplasia in arteriovenous fistulas for hemodialysis. The results in **CHAPTER 8** demonstrated for the first time that p21^{waf1} expression was considerably lower (and cdk2 significantly higher) in stenotic regions of failing fistulas, supporting the notion that p21^{waf1} is a negative regulator of VSMC proliferation and thus intimal hyperplasia in arteriovenous fistulas.⁶⁶⁻⁶⁸

THE RESULTS IN A CLINICAL PERSPECTIVE AND FUTURE RESEARCH

The problems of non-maturation of hemodialysis arteriovenous fistulas and the development of intimal hyperplasia are still seriously influencing dialysis care and compose a great burden on both patients and health caretakers. It would therefore be of utmost importance to develop means to prevent these dreaded complications through, for example, better pre-operative diagnosis and care, new surgical techniques, or pharmaceutical interventions.

We propose new and innovative insights in the remodelling or in other words maturation process of arteriovenous fistulas, especially regarding the role of shear stress and matrix degrading proteins. For this purpose we have shown that the ART-Lab system is a feasible tool to measure *in vivo* brachial artery shear stress successfully.

Future studies should focus on improving pre-operative (vascular) screening and possible pharmaceuticals to prevent and/or attack non-maturation and intimal hyperplasia. Duplex ultrasound scanning has proven its use in the past.⁶⁹⁻⁷¹ This technique is, however, greatly technician-dependent and more-objective, non-operator-dependent methods like magnetic resonance imaging might improve the accuracy of both the determination of vessel wall and hemodynamic characteristics, that are important for vessel adaptation.⁷²

Furthermore, alternative tests to establish endothelial (dys)function or vascular (dys)function in general should be developed or sophisticated. At present, ultrasound techniques and software protocols are designed to establish less variation in artery diameter and distension measurements and therewith in the determination of FMD. Woodman et al., for example, have developed an edge-detection and wall-tracking software protocol, tested both *in vitro* and *in vivo*, and promises more reliable accuracy in the measurement of FMD.⁷³ At our laboratory, Meinders et al. used fast B-mode ultrasonography and by implementing spatial and temporal variations they were able to eliminate the contribution of measurement conditions, thereby reducing both the spatial and temporal variation coefficients to about 3.2 and 2.0%, respectively.⁵ Although this fast B-mode technique has not yet been tested for FMD studies it imposes a great challenge and potential in this setting. Phase-contrast magnetic resonance angiography has also been forwarded to enhance the assessment of brachial artery FMD and hence vascular endothelial function.⁷⁴

Forearm vascular resistance measurements by strain gauge plethysmography has already proven its merit and is in our opinion more reliable at the present time in the determination of vascular function. However, it is more invasive than the techniques described above.^{75,76} Forearm vascular responses to brachial artery infusions of N(G)-monomethyl-L- arginine (L-NMMA), sodium nitroprusside, ACh, clonidine, and phenylephrine can easily be determined and provide information regarding endothelial function.

Alternatively, one may make use of the characteristics of dysfunctional endothelium, i.e., an endothelial surface displaying a prothrombotic, proinflammatory, and vasoconstrictory state. Plasma markers of endothelial (dys)function might thus be determined, such as vWF, platelet activating factor, P-selectin, ICAM-1, interleukin-8, MCP-1, TNF- α , TF, PAI-1, and phosphatidyl serine for example, providing data on the endothelial "health status".^{77,78}

Pharmaceutics that interfere with the cell cycle or that inhibit matrix degradation might prove effective. In this light, sirolimus for example has shown its applicability in preventing restenosis after percutaneous transluminal coronary angioplasty.^{79,80} In compliance with our study, tacrolimus might pose a good pharmaceutical means to prevent or stop the development of intimal hyperplasia, because its promoting effect on p21^{waf1}.⁸¹

More recent research aims at influencing the development of intimal hyperplasia. Radiation therapy or brachytherapy have already shown its inhibitory effect on reducing intimal hyperplastic growth in animal models. However, further studies are necessary to determine a suitable isotope and dosage for clinical application.^{82,83} The same can be settled on endovascular photodynamic therapy and intraluminal gene transfer, which impose great expectations for the future therapy of vascular disease showing abundant VSMC growth.⁸⁴⁻⁸⁸

Future research should be performed in close cooperation with medical sciences, such as biophysics and physiology, and medical practitioners. Especially, the collaboration of vascular surgeons, nephrologists, radiologists, and cardiologists is needed, because the problem of VSMC proliferation, in the course of remodelling, restenosis, intimal hyperplasia, and even atherosclerosis is affecting patients in all of these fields.

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SUMMARY/SAMENVATTING

SUMMARY

The human kidney extracts waste products and excess fluid from the circulation through the principle of dialysis. Accumulation of waste products and volume overload are the principal features of renal failure that, when left untreated, would eventually lead to death. Present treatment modalities for end-stage renal disease are kidney transplantation or renal replacement therapy, amongst which hemodialysis. To perform hemodialysis, however, adequate vascular access is indispensable. This is accomplished through a surgical connection of an artery to a vein, thus creating a high flow velocity arteriovenous fistula or AVF.

This AVF is, however, not without faults. In some cases the fistula does not mature, characterised by increased blood flow velocity and vessel dimension, or if it does mature its function is hampered by the development of so-called intimal hyperplasia. Intimal hyperplasia can be described as an increase of the vascular wall mass through proliferation of vascular smooth muscle. At these intimal hyperplastic regions thrombosis might occur, obstructing the AVF and therewith corrupting hemodialysis therapy.

The main goal of the present thesis was to increase the knowledge regarding the process of AVF maturation. We hypothesised that the reaction and interaction of hemodynamic, i.e., the dynamics of flowing blood, and vessel wall properties of the arterial component of the AVF in response to the sudden and chronic increase in blood volume flow are important factors in functional maturation. In the present thesis, special interest was given to shear stress, which is the drag generated by the flowing blood on the inner wall endothelial cells. Furthermore, we anticipated that the functioning of the endothelial cells could affect the prognosis regarding functional maturation. **CHAPTER 1** provides the clinical and physical background, leading to the main objectives of this thesis.

CHAPTER 2 reviews the role of the endothelial cells regarding vascular adaptation (e.g. the lumen diameter by wall tone adjustment) or remodelling (changes in structure and composition of the wall) to hemodynamic changes. It concisely addresses different mechanisms by which these cells are able to sense and to react adequately to blood flow, or more precise to changes in shear stress. It touches upon the mechanisms of flow-dependent vasodilation and constriction, broadening the perspective of the thesis.

CHAPTER 3 discusses the basic ultrasound principles and techniques (ART-Lab system) to assess non-invasively shear stress. Furthermore, it describes the procedures by which various markers for the functioning of the endothelium and components of the vascular wall were obtained from human blood. Moreover, it clarifies the relationship between whole blood viscosity and shear stress. It seems that whole blood viscosity is mainly dictated by changes in hematocrit, being the relative amount of blood components such as red blood cells and proteins. Finally, the study design of this thesis is presented.

As the study focused on the adaptation of the upper arm artery (brachial artery), having a rather small diameter (about 4 mm), it was of interest whether the ART-Lab system would be suitable for this application. Thirty healthy subjects, ranging from 30 – 60 years in age, were included for this purpose. Indeed, both hemodynamic and vessel wall properties could be determined reliably. For the shear rate, an indirect measure of shear stress, an intrasubject variability, the variation that one observer might observe within one person, of approximately 22% was observed. This figure corroborates findings in the common carotid artery. Furthermore, **CHAPTER 4** shows that in both healthy men and women the mean shear stress is approximately 0.5 Pa, independent of age. This is a surprisingly low value because it is commonly assumed that in large arteries shear stress is roughly 1.5 Pa.

CHAPTER 5 concentrates on possible hemodynamic and vessel wall differences between healthy individuals and patients with end-stage renal disease without dialysis therapy. In 29 patients with end-stage renal disease and 16 healthy controls, it was observed that the patient group had a thicker brachial artery wall, viz. 433 vs. 386 μm . This was directly related to the creatinine level, an indirect measure of end-stage renal disease gravity. Thus the higher the creatinine level, the thicker the vessel wall. Furthermore, in patients mean shear stress was 50% lower ($= 0.28 \text{ Pa}$), which was attributed to a higher peripheral vascular resistance.

In **CHAPTER 6** and **7** the actual arterial remodelling (maturation) after creation of an AVF for hemodialysis is considered. In a total of 16 patients, two arteries were investigated, viz. the brachial artery and the radial artery, which is one of the lower arm arteries. After AVF creation the diameter of both arteries increases dramatically in response to the chronic increase in blood flow velocity and, hence, in shear stress. The rate, however, differs significantly. The radial artery reaches its maximum diameter (approximately 1.8x its baseline) after only 3 weeks, whereas the brachial artery is still expanding after 1 year to 1.4x its baseline. The shear stress increased to a maximum of 11 and 5x the baseline value of the radial and brachial artery, respectively. However, the diameter increase of both arteries was not sufficient to decrease shear stress. Due to the swift radial artery diameter change, the wall thickness initially decreases drastically (from 406 to 354 μm). This is compensated for by “growth” of the vascular wall over the course of one year (487 μm).

A protein, called MMP-2, which is able to degrade vascular wall components, could be linked to the process of radial artery diameter enlargement, supporting the notion that AVF maturation is stimulated by MMP.

CHAPTER 8 illustrates for the first time that the expression of p21^{WAF1}, a cell-cycle regulator, is associated with the extent of intimal hyperplasia in obstructed and dysfunctional AVFs. This cell-cycle inhibitor was considerably lower in 18 intimal hyperplastic vessels (3%) compared with 10 normal vessels (63%). Another protein, cdk2, stimulating cell-cycle progression, was shown to be

higher in the intimal hyperplastic vessels (43%) than in the healthy blood vessels (2%).

The results of the different chapters are discussed in **CHAPTER 9**. Since all previous chapters are related to endothelial cell function, also current methods of its assessment are reviewed. It is concluded that these tests are inadequate because they are applied to an artery with a rather inert nature and they do not evaluate the stimulus-response relationship. In a study that included 14 healthy subjects at six repeated measurements, an intrasubject variation coefficient of 56% was found.

Due to the fact that only one of the created AVFs during the study period failed to mature, it proved statistically impossible to relate preoperative hemodynamic and vessel wall properties to adequate functional maturation of individual AVFs. However, the current study results in new and innovative insights in the maturation of AVFs. Especially the role of shear stress, matrix-degrading proteins, for example MMP-2, and the cell-cycle machinery, might yield new therapeutic targets to ensure functional maturation and to prevent intimal hyperplasia.

SAMENVATTING

Door middel van dialyse kan de nier zowel afvalproducten als overtollig water uit de bloedsomloop halen. Stapeling van afvalproducten en overvulling zijn kenmerken van nierfalen die, indien onbehandeld, uiteindelijk leiden tot overlijden. Enkele huidige niervervangende therapieën zijn niertransplantatie en dialyse via een kunstnier, de hemodialyse. Voorwaarde voor een goede hemodialyse is echter een adequate toegang tot de bloedbaan, die gerealiseerd wordt door een chirurgische verbinding tussen een slagader en een ader. Zo wordt er een zogenaamde hoge flow, hoge snelheid arterioveneuze fistel of AVF gecreeërd.

Zo'n AVF gaat helaas niet zonder problemen of complicaties. In sommige gevallen "rijpt" de fistel niet. Met rijpen wordt een toename van bloedsnelheid en vaatdimensie bedoeld. Als de AVF wel rijpt, kan de functie toch worden verstoord door de ontwikkeling van intima hyperplasie. Intima hyperplasie kan worden omschreven als een toename van de vaatwandmassa door de ontspoorde groei van spiercellen die oorspronkelijk in de vaatwand huizen. Plaatsen met intima hyperplasie zijn gevoelig voor het ontstaan van thrombose, waardoor de AVF verstopt raakt en hemodialyse dus niet meer mogelijk is.

Het belangrijkste doel van deze disseratie lag erin om de kennis omtrent de rijping van AV fistels te vergroten. Onze hypothese was dat de reactie en interactie tussen de hemodynamica, d.w.z. de dynamica van het stromende bloed, en de vaatwandeigenschappen van het slagaderlijke deel van de AVF, na de acute en chronische toename in bloedstroomsnelheid en -volume, belangrijke factoren zijn in het proces van AVF-rijping. Met name de shear stress, de afschuivende kracht die het stromende bloed teweeg brengt op de endotheelcellen van de vaatwand, werd uitvoerig bestudeerd. Wij anticipeerden dan ook dat de endotheelcelfunctie een prognostische factor zouden kunnen zijn aangaande een normale maturatie. In **Hoofdstuk 1** wordt de klinische en natuurkundige achtergrond hierover beschreven.

Hoofdstuk 2 geeft een samenvatting aangaande de adaptieve eigenschappen van de endotheelcel op veranderingen in hemodynamica. Zowel op korte termijn, door bijvoorbeeld de spierspanning te variëren, als op lange termijn, het zogenaamde remodeleren, waarbij de structuur van de vaatwand ingrijpend kan veranderen. Er wordt kort in gegaan op de verschillende mechanismen, waarmee deze cellen veranderingen in bloedstroom, en dus shear stress, kunnen waarnemen. Tevens wordt het mechanisme van vaatverwijding en -vernauwing besproken.

De principes van ultrageluid, of meer populair echo, en de techniek om shear stress non-invasief te bepalen worden in **Hoofdstuk 3** besproken. Daarnaast worden de procedures waarmee verschillende endotheelcelfunctiemarkers werden verkregen uit humaan bloed. Van belang is ook de bespreking over de relatie tussen volbloedviscositeit, m.a.w. de stroperigheid van bloed en shear stress. Het blijkt dat de viscositeit met name gedictieerd wordt door veranderingen in de hematocriet, het relatieve aandeel van bloedcomponenten zoals rode bloedcellen en eiwitten. In dit hoofdstuk wordt ook de studie-opzet uiteengezet.

Daar de studie toegespitst was op de adaptatie van de bovenarmsarterie (de a. brachialis), met een relatief kleine diameter van ongeveer 4 mm, was het van belang om te bestuderen of het gebruikte ultrageluidstelsel wel geschikt was voor deze opzet. Daartoe werden dertig gezonde proefpersonen, tussen de 30 en 60 jaar oud, geïncubeerd. Het bleek dat inderdaad zowel de hemodynamische als de vaatwandeigenschappen betrouwbaar gemeten konden worden. De shear rate, een indirecte maat van de shear stress, kon met een intrasubject variabiliteit, d.w.z. de variatie die een enkele observeerder waarneemt binnen één enkele proefpersoon, van ongeveer 22% worden bepaald. Dit stemt overeen met eerdere bevindingen in de halsslagader (a. carotis communis). Verder laat **Hoofdstuk 4** zien dat de gemiddelde shear stress in gezonde mannen en vrouwen, onafhankelijk van de leeftijd, ongeveer 0.5 Pa is. Dit is opvallend laag, aannemende dat het algemeen goed is dat in grote arteriën de shear stress ongeveer 1.5 Pa is.

In **Hoofdstuk 5** ligt de nadruk op mogelijke hemodynamische en vaatwand verschillen tussen gezonde individuen en patiënten met nierfalen, die nog geen niervervangende therapie ondergaan. Bij 29 patiënten met nierfalen en 16 gezonden controles viel op dat de patiëntengroep gemiddeld een dikkere vaatwand had, 433 vs. 386 μm . Dit was direct gerelateerd aan de kreatinine-waarde in het bloed, wat een indirecte maat is voor de ernst van het nierfalen. Hoe hoger het kreatinine was, des te dikker was de slagaderwand. Een andere observatie was dat de shear stress 50% lager was in patiënten (= 0.28 Pa), wat werd toegeschreven aan een hogere perifere weerstand.

Hoofdstuk 6 en **7** beschrijven de eigenlijke arteriële remodellering (rijping) na het aanleggen van een AVF voor hemodialyse. Bij 16 patiënten werden twee slagaders onderzocht, nl. de a. brachialis en de a. radialis, één van de onderarmsslagaders. Na het aanleggen van de AVF nam de diameter van beide slagaders drastisch toe, als antwoord op de chronische toename in bloedstroomsnelheid en daarmee shear stress. De mate waarin verschilden echter nogal. Al na 3 weken bereikt de a. radialis de maximum diameter (1.8x de beginwaarde), terwijl de a. brachialis na één jaar nog steeds toename vertoont tot 1.4x zijn beginwaarde. De shear stress nam maximaal 11 en 5x de beginwaarde toe in respectievelijk de a. radialis en brachialis. Toch bleek de diametertoename niet voldoende om de shear stress weer te doen dalen. Doordat de diameter van de a. radialis zo snel toenam, nam de wanddikte significant af (van 406 tot 354 μm). Later wordt dit gecompenseerd door "groei" van de vaatwand tot 487 μm na één jaar.

MMP-2, een eiwit dat in staat is om bestanddelen van de vaatwand af te breken, kon worden gekoppeld aan de diametertoename van de a. radialis. Dit versterkte het idee dat AVF rijping wordt gestimuleerd door MMP.

In **Hoofdstuk 8** blijkt voor het eerst dat de expressie van p21^{WAF1}, een celcyclus regulator, geassocieerd is met de mate van intima hyperplasie in vernauwde en dysfunctionele AVFs. De celcyclus remmer was aanzienlijk lager in 18 aangedane vaten (3%) vergeleken met 10 gezonde vaten (63%). Cdk2, een ander

eiwit, welke de celcyclus stimuleert bleek hoger te zijn in aangedane vaten (43%) dan in de gezonde bloedvaten (2%).

De resultaten van de verschillende hoofdstukken worden samengevat en bediscussieerd in **Hoofdstuk 9**. Omdat alle vorige hoofdstukken min of meer gerelateerd waren aan endotheelfunctie worden ook gangbare methoden besproken om deze functie te meten. Deze testen blijken echter niet geschikt te zijn voor het doel aangezien ze worden toegepast op een nogal stijf vat en bovendien wordt er geen aandacht geschonken aan de stimulus-response relatie. Een studie met 14 gezonde individuen, die zes herhaalde momenten werden geobserveerd, toonde een intrasubject variatie van 56%.

Gezien het feit dat slechts één enkele fistel gedurende de studieperiode niet matureerde, was het statistisch niet mogelijk om pre-operatieve hemodynamische en vaatwande eigenschappen te relateren aan functionele maturatie van individuele AVFs. Het huidige proefschrift heeft echter wel geresulteerd in nieuwe en innovatieve inzichten aangaande de maturatie van AVFs. Met name de rol van de shear stress, matrixdegraderende eiwitten, zoals MMP-2, en celcycluseiwitten bieden mogelijkheden om therapeutisch in te grijpen en aldus een adequate maturatie te garanderen en tevens intima hyperplasie te voorkomen.

DANKWOORD

Gratitude is not only the greatest of virtues, but the parent of all others
Cicero (106 BC – 43 BC)

Dankwoord

Hoewel ik voornemens was om een zekere opstandigheid te vertonen in dit dankwoord, wil ik me toch dapper aansluiten bij de schare van promovendi met een standaard opzet. Eén uitzondering wil ik toch maken; meestal is het namelijk de partner die de laatste plaats inneemt, maar ...

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Hoewel jullie, Miriam en Elian, mijn kleine zusjes, niet actief hebben bijgedragen aan de wording van dit proefschrift, kan ik me niet voorstellen hoe mijn leven anders zou zijn geweest zonder jullie. Alles heeft een doel.

Zonder al de patiënten die hun consent hebben gegeven aan ons onderzoek, was geen letter op papier gekomen. Ik wil jullie bedanken voor alle uren die we samen gespendeerd hebben. De contacten waren uitzonderlijk en jullie hebben mij geleerd wat in geen tekstboek te vinden is.

Prof. Kitslaar, beste Peter kon en kan ik nog steeds niet zeggen. Op de één of andere manier is het ontzag wat u inboezemde tijdens de sollicitatie voor het student-assistentschap nooit verdwenen. Niet dat er van een onpersoonlijke relatie sprake was, integendeel. Over uw klinische expertise hoeven we hier uiteraard niets te melden, spreekt voor zich. Ook wist u op de achtergrond de diplomatieke en logistieke zaken te dirigeren. Is natuurlijk allemaal erg vanzelfsprekend, maar op het moment dat ik bijna het "le!" voelde om u Peter te noemen, gebeurde er in mijn eigen leven iets absurds waar helaas een zeer geliefd familielid bij betrokken was. Toen leerde ik de andere kant van de onderzoeksbank kennen en ik moet zeggen dat me dit verschrikkelijk tegenviel. Vol bewondering heb ik gadegeslagen hoe u deze zaak ter harte nam en de nodige beslissingen doordrukte. Als "verdediging" kunt u natuurlijk aandragen dat dit bij de dagelijkse kost hoort, maar toch... u weet inmiddels ook dat het aan de andere kant van de onderzoeksbank anders voelt. Met ontzag en trots kijk ik aldus terug op de afgelopen 5 jaar waarin ik heb mogen groeien tot arts onder uw mentorschap. Peter, bedankt en heel veel liefde en gezondheid voor jou en je vrouw gewenst na je dagen als practiserend chirurg.

Dr. Tordoir, beste Jan, toen ik mijn eerste stapjes zette in de AiO-wereld voelde ik enige gereserveerdheid uit jouw hoek. Je wist niet precies wat je van die "Dammers" kon verwachten. Gelukkig kon ik bogen op een grote overmaat aan ADHD en kon ik je overtuigen van mijn enthousiasme, wat naast het onderzoek in dit proefschrift heeft geleid tot vele nevenproducties ("typiste"!!!!!!). Oninteressant, zeker als ik terugdenk aan de eerste trip die we samen maakten. Het was weer een "golf"-congres van ons aller Mitch, en wel in San Antonio, Texas. Tijdens de (vele) Margarita's leerde ik je pas echt kennen; toen was je geen chirurg meer, maar gewoon Jan. Jij kunt als geen ander vertellen dat het arts zijn toch absoluut geen roeping is (liever was je tuinman geworden), ... Ondanks je laconiek ogende (NB: ogende) instelling heb je je vol overgave gestort op de vaattoegang voor hemodialyse, waarmee je inmiddels mag ik wel zeggen wereldfaam hebt bereikt. Jan, mag ik je bedanken voor je altijd onleesbare kerstkaarten en verbeteringen

aan de manuscripten... Nee, Jan bedankt voor de steun en het vertrouwen die je me gegeven hebt om dit onderzoek tot een goed einde te brengen. Je was nogal verbaasd toen ik vertelde dat ik de neurochirurgie als professie ambieerde; toch heb je me ook hierin met raad en daad bijgestaan en teer ik nog op jouw adviezen.

Prof. Hoeks, Arnold, wat moet jij gedacht hebben toen die snotneus van een witjas kwam melden dat hij even jullie onderzoek kwam doen. Volgens mij in de strekking van: "Oeioeioei". Ik weet nog wel, die eerste maanden, verstoken van enige sociale contacten, zat ik de literatuur, die jij langzaam onder mijn neus opstapelde, door te ploegen. Maar ook jij had pech, deze AiO stond iedere dag wel 10x met een aantal (soms) relevante vragen voor je desk. Om deze ellende te voorkomen heb je me toen maar gedumpt in het AzM, naast het onderzoekslab, onder het mom van: "daar zijn de patiënten en bovendien kom je dan in contact met mede (klinische) AiO's". Niets was minder waar. Het vreemde was echter dat jij sindsdien wel minimaal 10x per dag op onze kamer te vinden was voor het één of ander (wetenschap en/of koffie). Jij hebt me leren lopen in de wetenschappelijke wereld en hoewel ik inmiddels los kan staan, zal ik nog regelmatig vallen of mijn evenwicht verliezen voordat ik net zo rennen kan als jij. Arnold, wat betreft wetenschap en onderwijs kan ik geen gelijke bedenken. Altijd weet je het probleem te benoemen (zelfs als je er geen verstand van hebt!) en kom je met een mogelijke oplossing. Lezen is voor jou helemaal een koud kunstje en het bij jouw afgeleverde artikel kwam binnen 24 uur (rood) weer terug op mijn bureau. Goed, om je nu niet helemaal af te schilderen als een workaholic moet ik ook zeggen dat niemand zo'n verscheidenheid aan onderwerpen kan aansnijden in een discussie. Je bent echt een belezen man en ik hoop dat iedereen, die met jou kennis heeft mogen maken, hiervan geproefd heeft en dit ook zal koesteren. Hey Arnold, als je nu weer boer wordt, krijgen we dan een adreswijziging?

Tryfona. Een speciale plaats moet toch wel mijn chaotische collega innemen. Einstein zei het al: "Nur ein Genie schafft Ordnung aus dem Chaos" Voor jou gaat dit zeker op. Jouw gedachtenvluchten zijn onnavolgbaar, maar na een paar glazen whiskey kon ook ik je nieuwe projecten zien opdoemen uit de mist van je ideeën. Maar veel leuker waren de talloze discussies over jeugd, oud worden, vrouwen, drank, sex, student-assistenten (wat heb jij er veel versleten zeg), de professie arts, de (on)zin van alles, ethiek en ga zo maar verder. Veel was gestoeld op je klassieke opvoeding in het Griekse. Onze auto-verhalen waren ook om in te lijsten, wat een AiO-malaise, maar dat is nu allemaal geschiedenis. Tryfon, ik geloof vast dat je een fantastisch chirurg wordt, met oog voor z'n patiënten, maar ook zul je de klinische wetenschap op z'n kop doen staan. Mag ik wel naar je Laureatoratie komen luisteren? Bedankt voor een onvergetelijke tijd.

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Zonder Yvonne en Johanna waren er vele zaken misgelopen. D.w.z. afspraken niet nagekomen, brieven niet gepost, artikelen ongeadresseerd ingestuurd, en ik kan zo nog wel een regel of wat volschrijven. Jullie ondersteuning vanuit het secretariaat Chirurgie is onontbeerlijk gebleken bij het tot stand komen van het proefschrift en bovendien kon je bij jullie zo lekker rodd... eh... kletsen over van allerlei zaken. Dames, ik heb genoten van de tijd samen, tot ziens.

Tja, ergens moet je leren echoën en waar kon dat beter dan op het Functioneel lab van de vaten in het AzM. Ook hier heb ik heel wat uurtjes doorgebracht, echoën, statussen doorploegen en natuurlijk rodd... eh... kletsen. Bij jullie was het altijd gezellig even een kopje thee te drinken. In een willekeurige volgorde wil ik jullie dan ook bedanken: Anja, Laura, Simone, Yvonne, Marjon, Evelien, Monique en Ineke.

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ABOUT THE AUTHOR

Curriculum vitae

The author was born May 23, 1975 in Heerlen, the Netherlands. He attended the "Sint-Janscollege" in Hoensbroek, where he graduated from in 1993.

The same year, he started his medical training at the medical faculty of the University of Maastricht. During this study, he served as a research assistant at the departments of Physiology, University of Maastricht, laboratory of Microcirculation, and Surgery, University Hospital Maastricht under the supervision of prof. dr. Kitslaar, prof. dr. ir. Slaaf, dr. oude Egbrink, and dr. Kurvers. He was a member of the founding committee of the "Maastricht Medical Students Research Conference Foundation". Its first conference was held in 1997 and has since then drawn great attention of many students with scientific interest, annually.

He received his doctoral degree in 1997 and after completion of the first year of clinical internships he enrolled in the MD/PhD program at the Cardiovascular Research Institute Maastricht, departments of Biophysics, University of Maastricht, and Surgery, University Hospital Maastricht. He served as a PhD student from 1998 until 2003, during which the work resulting in the present thesis was accomplished under the inspiring supervision of prof. dr. ir. Hoeks, prof. dr. Kitslaar, and dr. Tordoir. Simultaneously, he graduated as a Medical Doctor in 2001, the same year when he married miss Diana Thomissen. During his PhD training, he served as secretary for the committee of Advocacy of the Maastricht University PhD student Association (ProVUM). Furthermore, he became a full member of the Vascular Access Society in 1999 and presented original research at many (inter)national congresses.

Since May 1, 2003 he started as a junior house officer in neurosurgery at the department of Neurosurgery, Erasmus Medical Center Rotterdam, Rotterdam, the Netherlands, supervised by prof. dr. C.J.J. Avezaat.

LIST OF PUBLICATIONS

You must keep sending work out; you must never let a manuscript do nothing but eat its head off in a drawer. You send that work out again and again, while you're working on another one. If you have talent, you will receive some measure of success - but only if you persist.

Isaac Asimov (1920 - 1992)

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