HAEMOSTATIC AND INFLAMMATORY ALTERATIONS IN HYPERTENSION AND HYPERLIPIDAEMIA, AND THE IMPACT OF ANGIOTENSIN II

Mikael Ekholm
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“To be is to do” – Socrates
“Do be do be do” – Frank Sinatra

“To do is to be” – Jean-Paul Sartre
ABSTRACT

The process of atherosclerosis is multifactorial, and endothelial dysfunction is considered to precede atherosclerosis. Angiotensin (Ang) II, the main effector of renin-angiotensin-alderosterone system (RAAS), is implicated in hypertension and has been shown to promote atherosclerosis.

Familial combined hyperlipidaemia (FCHL) and familial hypercholesterolemia (FH) have been identified as risk factors for increased risk of cardiovascular heart disease and premature death. FCHL has a different phenotype compared to FH, but both lipid disorders are accompanied by subclinical atherosclerosis and endothelial dysfunction. We speculated that patients with hypertension and hyperlipidaemia were more sensitive to the potential proinflammatory and procoagulatory effects of Ang II than healthy individuals. The present research program was set up to investigate the extent to which the RAAS affects the inflammatory and thrombotic properties of individuals with hypertension and hyperlipidaemia.

In Paper I we examined the impact of treatment with the ACE inhibitor ramipril on coagulation in patients with mild-to-moderate hypertension. We observed that ramipril attenuates thrombin generation in essential hypertension by reducing thrombin-antithrombin complex, and tended to reduce fibrinogen levels.

In Paper II we wanted to clarify the impact of antihypertensive treatment per se. Therefore, we examined the effects of long-term treatment of ramipril compared to the alpha 1-adrenoceptor blocker doxazosin on inflammation and haemostasis in patients with mild-to-moderate hypertension. We found that antihypertensive treatment seems to exert a minor impact on systemic inflammation. Treatment with ramipril, but not doxazosin, appeared to reduce thrombin generation. This extended our previous findings in paper I suggesting that treatment with ramipril reduces thrombin generation in addition to the effects on blood pressure reduction alone. Drugs blocking the renin-angiotensin-alderosterone system may reduce atherothrombotic complications beyond their effects of lowering blood pressure. We also observed a decrease in t-PA antigen and a tendency to decreased PAI-1 activity in the doxazosin treated group, which would implicate beneficial effects by treatment with doxazosin in hypertensive patients regarding fibrinolysis. This may be of benefit in the treatment of patients with hypo fibrinolysis, such as patients with FCHL.

In Paper III we examined how an intravenous infusion of Ang II affected inflammation and haemostasis in patients with FCHL and healthy control subjects. In Paper IV we characterized the studied the patients with FCHL, in paper III, with respect to insulin resistance and in more detail regarding fibrinolysis. We also performed placebo experiments to make it possible to assess the influence of diurnal variations and to verify the stability of the experimental design. We found that FCHL had an increased systolic blood pressure response during infusion of Ang II compared to controls, indicating an increased vascular
responsiveness in FCHL. Patients with FCHL exhibited a low-grade chronic inflammation, an impaired fibrinolysis, while the coagulation system seemed intact. FCHL shared several characteristics with the metabolic syndrome, including high triglycerides and low HDL cholesterol levels, insulin resistance and high body mass index. An infusion of Ang II increased systemic inflammation in a similar way in FCHL and controls. Ang II did not have any impact on thrombin generation, in either FCHL or controls. Ang II did not affect fibrinolysis in FCHL, whereas fibrinolysis was enhanced in healthy controls. The different responses to Ang II stimulation probably involved t-PA activity but not PAI-1 activity, and this suggests that patients with FCHL were incapable of increasing fibrinolysis in response to Ang II. We could not observe any short-term effects on PAI-1 activity, in either FCHL or controls. Our findings suggested that patients with FCHL had a low-grade chronic inflammation, impaired fibrinolysis and insulin resistance, contributing to the risk of cardiovascular heart disease and premature death in FCHL. We also suggested that Ang II acted as a proinflammatory and enhanced fibrinolysis, without impact on thrombin generation. However, taking the possible effects of diurnal variations of our coagulation markers, not taken into account in paper III, and analysing the impact of Ang II during the ongoing infusion time, post hoc analyses showed that thrombin generation instead increased, similarly in FCHL and controls. Hence, our new conclusion became that Ang II acts as a prothrombotic agent.

In Paper V we examined how an intravenous infusion of Ang II affected inflammation and haemostasis in patients with FH and healthy controls. We also performed placebo experiments to make it possible to assess the influence of diurnal variations and to verify the stability of the experimental design. We found that patients with FH had higher systolic blood pressure than controls at baseline, whereas blood pressure responses were equal in FH and controls. FH showed an intact fibrinolysis and an increased thrombin generation potential compared to controls, but did not show any convincing signs of an on-going low-grade inflammation. A systemic infusion of Ang II caused an increase in systemic inflammation, fibrinolysis and possibly also thrombin generation similar in FH and control subjects. During Ang II infusion FH exhibited possible signs of an activated anticoagulant system. Our findings suggested that patients with FH had an affected coagulation system, rather than altered fibrinolysis or inflammation, contributing to the increased risk of cardiovascular heart disease and premature death in FH.

Thus, blocking the renin-angiotensin-aldosterone system by an ACE inhibitor may prevent atherothrombotic complications in hypertensive patients beyond the effects on BP by reducing thrombin formation. Different mechanisms may contribute to the increased incidence of cardiovascular complications in patients with FCHL and FH. A beneficial effect of ACE inhibition in patients with FCHL might be to attenuate inflammation in combination with its documented positive influence on insulin resistance, while in patients with FH, may benefit be obtained mainly by reduced thrombin generation.

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<td>ACE</td>
<td>angiotensin converting enzyme</td>
</tr>
<tr>
<td>AKT</td>
<td>ak strain transforming or protein kinase B</td>
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<tr>
<td>Ang</td>
<td>angiotensin</td>
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<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
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<tr>
<td>APC</td>
<td>activated protein C</td>
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<td>ARB</td>
<td>angiotensin receptor blocker</td>
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<td>AT</td>
<td>antithrombin</td>
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<td>AT1R</td>
<td>angiotensin 1 receptor</td>
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<td>BP</td>
<td>blood pressure</td>
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<tr>
<td>CAT</td>
<td>calibrated automated thrombogram</td>
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<tr>
<td>CCB</td>
<td>calcium channel blocker</td>
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<tr>
<td>CD</td>
<td>cluster of differentiation</td>
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<tr>
<td>CHD</td>
<td>coronary heart disease</td>
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<td>COX</td>
<td>cyclooxygenase</td>
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<td>CRP</td>
<td>c-reactive protein</td>
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<tr>
<td>CVD</td>
<td>cardiovascular disease</td>
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<td>ECM</td>
<td>extracellular matrix</td>
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<td>ED</td>
<td>endothelial dysfunction</td>
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<tr>
<td>EDCF</td>
<td>endothelium-contracting factor</td>
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<td>EDRF</td>
<td>endothelium-derived relaxing factor</td>
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<tr>
<td>EPCR</td>
<td>endothelial protein C receptor</td>
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<td>ERK</td>
<td>extracellular signal regulated kinase</td>
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<td>F</td>
<td>coagulation factor or clotting factor</td>
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<td>F1+2</td>
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<td>FCHL</td>
<td>familial combined hyperlipidaemia</td>
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<td>FH</td>
<td>familial hypercholesterolemia</td>
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<tr>
<td>GAG</td>
<td>glycosaminoglycan</td>
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<td>GLUT-4</td>
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<td>G protein oestrogen receptor</td>
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<td>HDL</td>
<td>high-density lipoprotein</td>
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<td>HOMA-IR</td>
<td>homeostasis model assessment of insulin resistance</td>
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<tr>
<td>Hs</td>
<td>high sensitive</td>
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<tr>
<td>ICAM</td>
<td>intracellular adhesion molecule</td>
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<td>intermediate-density lipoprotein</td>
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<td>interleukin</td>
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1 INTRODUCTION
1.1 GENERAL BACKGROUND
The most common cause of death in economically developed countries is cardiovascular disease (CVD). During the period 1990 to 2010, deaths from CVD increased from 26% to 30% of all deaths globally. Death rate caused by CVD is expected to decrease to 24% of all deaths in 2030, but will still retain its leading position due to an increasing prevalence in developing countries (1). The expected decline globally is thought to be due to a dramatic shift in deaths from infectious diseases and malnutrition, with very short life expectancy, compared to CVD and cancer. Given the high mortality and morbidity burden in CVD, it is of great importance for societies and their health care systems to improve strategies to decrease the incidence of CVD in the future (2).

A well-known cause of death within CVD is coronary heart disease (CHD), and traditional risk factors for this disease are hypertension, hypercholesterolemia, diabetes mellitus, obesity, tobacco smoking, age, male sex and family history. In the EUROASPIRE III trial (figure 1), almost 9000 participants with CHD in 22 European countries were included. The study showed that more than 50% of the patients had hypertension or hypercholesterolemia and 35% had diabetes mellitus or were obese (3).
The INTERHEART study (5) studied incidence of acute myocardial infarction globally. This large-scale study enrolled around 15,000 cases and 15,000 controls. The association of nine modifiable risk factors to acute myocardial infarction and a risk referred to as the population attributable risk were calculated. The study showed that the incidence of an acute myocardial infarction was in more than 90% of the cases associated with the nine measured risk factors. The most important risk factor turned out to be abnormal lipids, apolipoprotein (Apo) B/ApoA1 ratio, in all geographic regions. Five of the traditionally most common and well-described risk factors, hyperlipidaemia, hypertension, tobacco smoking, diabetes mellitus and obesity, accounted for around 80% of the population attributable risk.

Several additional risk factors for CVD have been recognized: hypercoagulability, impaired fibrinolysis, hyperinsulinemia, physical inactivity, impaired high-density lipoprotein (HDL) cholesterol and psychosocial factors. Most of the risk factors are related to lifestyle and metabolic disorders, and often several of the risk factors are present in a cluster. The most common condition with several risk factors is the metabolic syndrome. Varying definitions are given by different organizations, but the most common risk factors referred to include hypertension, dyslipidaemia, insulin resistance and abdominal obesity, culminating in an elevated risk of CVD and type 2 diabetes mellitus (6).

Angiotensin (Ang) II has an important role for inflammation in the vessels. During the past decades Ang II has been shown to initiate and accelerate hypertension, endothelial dysfunction (ED) and atherosclerosis (7). Conversely, inhibition of the renin-angiotensin-aldosterone system (RAAS) reduces atherosclerosis in animal models (8), and death from CVD in humans (9).

Ang II may have an impact on thrombosis. The extrinsic coagulation pathway is of great importance in the initiation of blood coagulation, and tissue factor (TF, also known as thromboplastin or clotting factor (F) III) initiates this pathway. Ang II may increase TF expression in monocytes, endothelial cells (EC) and in vascular smooth muscle cells (VSMC) (10, 11), and conversely treatment with RAAS blockade can diminish TF in plasma and in monocytes (12). We have reported that infusion with Ang II induces thrombocyte activation acutely (13). Also platelets have been shown to possess receptors for Ang II, angiotensin type 1 receptor (AT1R) subtype, but the clinical importance of these receptors is not known. TF is accepted as the initiator of coagulation, and the amount of TF exposure will predict whether or not clotting will occur. It has been assumed that an increased expression of TF is an adaptive defence mechanism that aims to facilitate haemostasis at sites of injury, but these mechanisms can contribute to a prothrombotic state in a number of diseases.

Accumulating evidence indicates an association between hypercholesterolemia and activation of the RAAS in the progress of atherosclerosis (14). Exposure of Ang II to hypercholesterolemic animals has been reported to be potently proatherogenic, and data strongly suggest that Ang II potentiates atherosclerosis in experimental models of hyperlipidaemia (7, 15). Conversely, it seems that ACE inhibitors offer vasoprotective effects...
by reducing atherosclerosis, and this is well documented in animal experiments (16-18) and in large human trials (9, 19, 20). In addition, studies have shown that Ang II has important effects with regard to oxidation of low-density lipoprotein (LDL) cholesterol in the vessel wall (16), and on macrophage uptake of oxidized (ox) LDL cholesterol and entry into vessels (21).

A common primary lipid disorder in humans is FCHL (phenotype IB according to the Fredrickson classification) (22), defined by an elevation of triglycerides and cholesterol in combination with reduced plasma HDL cholesterol. Patients with FCHL also exhibit elevated very low density lipoprotein (VLDL) cholesterol and small dense LDL cholesterol. FCHL has an association with insulin resistance, impaired endothelial reactivity, hypercoagulability, impaired fibrinolysis and systemic inflammation (23-27). Familial hypercholesterolemia (FH) (phenotype IIa according to the Fredrickson classification) (22) is less common and is characterized mainly by elevated total and LDL cholesterol. FH exhibits impaired endothelial reactivity (28) and a prothrombotic state without influence on fibrinolysis (29, 30). Together, FCHL and FH phenotypes account for about half of the primary lipid disorders.

The present research program was set up to investigate whether activation of RAAS in hypertensive patients has an impact on inflammation and haemostasis. We also wanted to clarify if the potential effects blocking the RAAS on inflammation and haemostasis were due to the antihypertensive effect per se. Patients with FCHL have a different phenotype as compared to FH. Both conditions have a poor vascular outcome and are recognized by impaired endothelial reactivity, but otherwise different characteristics regarding inflammation and haemostasis. We therefore studied the inflammatory and haemostatic responses to Ang II stimulation in these subjects separately.

1.2 INFLAMMATION IN VESSELS

Atherosclerosis is nowadays considered a disease caused by a chronic inflammation, associated with ED (31). Low-grade inflammation contributes to atherosclerosis, and several mediators of inflammation are up-regulated in subjects with atherosclerotic disease (31). Among the markers of inflammation for diagnostic use, the cytokines interleukin (IL)-6, and in particular C-reactive protein (CRP), have generated considerable attention. CRP is generated by hepatic cells and is modulated by IL-6, but also by the cytokines tumor necrosis factor (TNF)-α and IL-1 (32), thereby contributing to the up-regulation of monocyte chemoattractant protein-1 (MCP-1) and selectins, such as P- and E-selectins and cell adhesion molecules, such as intracellular adhesion molecule -1 (ICAM-1) and cell adhesion molecule-1 (VCAM-1). CRP attenuates the synthesis of endothelial nitric oxide (NO) (33), and causes augmented plasminogen activator inhibitor-1 (PAI-1) (34). Increased concentrations of acute phase reactants like CRP, IL-6, leucocyte count and fibrinogen are all associated with an increased risk of CVD (35-37). Phospholipase A2, which is implicated in the oxidation of LDL and subsequent oxidative stress and inflammation, can
predict atherosclerotic disease (38). These data clearly support a pivotal role for cytokines in the inflammatory process in early stages of atherogenesis.

Inflammatory mediators have been associated with components of metabolic disturbances, and cytokines might be a link between dysregulated metabolism and inflammation, as CRP, fibrinogen and IL-6 are closely related to the metabolic syndrome (32).

1.3 ENDOTHELIAL DYSFUNCTION AND ATHEROSCLEROSIS

The endothelium has an important function in preserving a physiological structure and function. Healthy, intact endothelium exhibits a thromboreistant, protective surface between the vascular lumen and VSMCs in the vessel wall, with the lamina elastic interna in between. In particular, a normally functioning endothelium prevents platelet adhesion (39). ECs form a monolayer that produces factors that regulate vascular tone, inflammation, haemostasis, vascular cell growth and death, angiogenesis and the migration of leukocytes. Vascular tone is dependent on a delicate balance between vascular dilators, such as NO, and vascular constrictors, such as Ang II. Also, VSMCs are affected by ECs and other factors, and VSMCs can themselves release cytokines and growth-regulatory mediators, which in turn have an impact on vessel phenotype and growth.

1.3.1 Oxidative stress

In 1985, Sies described oxidative stress as an imbalance between anti- and prooxidants, with a subsequent increase of reactive oxygen species (ROS) bioavailability, leading to tissue damage (40). An important factor of the biology of ECs is the cell reduction–oxidation reaction (redox) state. A molecule of particular importance in endothelial function is NO. The traditional risk factors for CVD can initiate ED by changing the cell redox state and, consequently, the oxidative stress in the vascular wall.

The ratio between ROS and NO regulates the redox state and is of vital importance for proper function of the vascular endothelium. Increased generation of superoxide anion, •O2 and, subsequently, oxidative stress result in an enhanced catabolism of NO, which leads to ED and impaired vasodilatation. ROS also has the ability to reduce the activity of NO synthase (NOS) and to increase the breakdown of NO. Also, NO is a potent endogenous inhibitor of VSMC migration and growth (41) and impairs the up-regulation of adhesion molecules and cytokines (42). The transcription of the pleiotropic nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-κB) has a pivotal role in endothelial up-regulation of cytokines and adhesion molecules, and NO is a powerful inhibitor of activation of NF-κB (43). It is to be noted that NO and the superoxide anion react to form the powerful oxidant peroxynitrite, ONOO−, that can damage ECs. ROS also lowers the availability of tetrahydrobiopterin. If the latter occurs, the oxygenase function of NOS is replaced by its reductase function and ROS are produced instead of NO, which increases NF-κB activity, and the expression of cytokines (42). An imbalance between NO and ROS increases the risk for vasospasm, VSMC proliferation, proinflammatory and prooxidant states and ED predict atherosclerotic disease (38). These data clearly support a pivotal role for cytokines in the inflammatory process in early stages of atherogenesis.

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can create an imbalance between tissue plasminogen activator (t-PA) and PAI-1 and may predispose a prothrombotic state.

Oxidative stress up-regulates and activates redox-sensitive genes for chemokines (such as MCP-1) and vascular adhesion molecules (such as VCAM-1 and ICAM-1). The superoxide anion may mediate increased activity of NF-κB that has a vital function in up-regulating these proinflammatory genes (44). As a consequence of this activity, leukocytes interact with the endothelium, and subsequently transmigrate into the subendothelial layer of the vessel wall. After transmigration of the leukocytes, cytokines (such as IL-6) are released, resulting in recruitment of additional monocytes. In the vessel wall, monocytes subsequently differentiate into macrophages. Via the scavenger receptors, macrophages take up oxLDL and are then transformed into foam cells, present in the early stages of atherosclerosis. As the plaque progresses at inflammatory sites, macrophages and other migrating cells, as well as activated ECs, produce cytokines and matrix metalloproteinases, eventually causing the plaque to rupture.

### 1.3.2 Recruitment of leukocytes, platelet dependent

ED may be defined as an imbalance between, on the one hand vasodilating, and on the other hand vasoconstricting substances, produced by the endothelium (45). Current concepts of atherogenesis include involvement of platelets, the immune system and chronic inflammation (46).

The healthy endothelium controls platelet activity through inhibitory mechanisms, while a systemic inflammatory environment induces ECs to develop a phenotype that make them adhesive for platelets (47). Numerous studies have shown that platelets have the ability to adhere to the intact ECs and also to modulate their function. Thus, exposure of the subendothelial surface, as in plaque rupture, is not necessary for platelet adhesion to vascular cells. Activated platelets release mediators and growth hormones, which will induce up-regulation of adhesion molecules and the release of chemottractants that, in turn, regulate the adhesion and subsequent transmigration of leukocytes into the vessel wall. It is important to take into account when analysing platelets in animal models that human platelets differ from platelets in for example the mouse in many important ways (such as difference in expression of surface receptors, higher platelet count), and the results in animal experiments can not be uncritically applied to the human situation (39).

The contact between platelets and the inflamed ECs is accomplished by the binding of endothelial P-selectin to glycoprotein (GP) Ib/IX/V (also referred to as the von Willebrand factor (vWF) receptor complex). Platelet-selectin glycoprotein ligand-1 (PSGL-1), interacting with P-selectin, present on leukocytes and to a minor degree also on platelets, mediates rolling of platelets to ECs under high shear stress. However, the association between PSGL-1/GPib/IX/V and P-selectin is insufficient for a stable and durable adhesion. The firm and long-lasting binding of platelets to ECs is mediated through the β3 integrins (GPIIbIIIa) and αvβ3, the vitronectin receptor.
Adherent platelets undergo a series of alterations, both morphological and biochemical, leading to release of potent proinflammatory and mitogenic substances, such as IL-1β, IL-8, cluster of differentiation (CD) 40L, platelet factor 4, thromboxane A2 (TXA2), platelet-derived growth factor, platelet activating factor (PAF) and regulated on activation, normal T cell expressed and secreted (RANTES), thereby changing the phenotype of the ECs into a chemotactic, adhesive, and proteolytic state. The changes of the ECs, induced by platelets, will support chemotaxis, and subsequently adhesion of monocytes to the inflammatory sites (48). Activated platelets also secrete glycosaminoglycans (GAGs) that contribute to the immobilization of chemokines and the adhesion and transmigration of leukocytes (49).

The firm adhesion of neutrophils to platelets is facilitated by macrophage antigen-1 (MAC-1 or CD11b/CD18) activation, induced by P-selectin (PSGL-1) and augmented by platelet-endothelial cell adhesion molecule (PECAM-1), arachidonic acid metabolites and inflammatory lipids. The interaction between MAC-1 and platelet surface ligands (ICAM-2, GPlb/IIa/IIIa and GPlb/IIIa-bound fibrinogen) also elicits signalling to promote leukocyte activation and migration through the endothelium and extravasation (50). Binding of fibrinogen to MAC-1 also primes leukocyte release of the cytokines (IL-1β, IL-8, IL-6 and TNF-α), which potentiates the proinflammatory response. The binding of fibrinogen and fibrin on mononuclear cells are mediated by Toll-like receptor 4 (51). Lymphocyte function-associated antigen 1, LFA-1 or αLβ2, ligation with ICAM-2 is also present, but MAC-1 has been shown to have the dominant role in promoting stable leukocyte-platelet interaction. Figure 2 illustrates platelet-dependent recruitment of leukocytes.

1.3.3 Recruitment of leukocytes, platelet independent

During inflammation, ECs, leukocytes and platelets may release a variety of cytokines and chemokines. In the leukocyte adhesion cascade, leukocytes first roll on inflamed ECs. Rolling is initiated and mediated by the interaction of endothelial P- and E-selectin with their counterparts PSLG-1 and E-selectin ligand, respectively. Conversely, P- and E-selectins are not expressed at the cell surface in absence of inflammatory stimuli. The P-selectin molecule is stored in platelet n-granules (52), or in EC Weibel-Palade bodies, and may be released upon stimulation (53).

The firm attachment of circulating cells to the inflamed vasculature is mediated by the leukocytes-expressed β2-integrins. The most powerful activators of these integrins are chemokines, secreted by cytokine-activated ECs (54), stromal cells, platelets (55), or by leukocytes themselves. PAF is a chemotactic proinflammatory lipid, which acts in cooperation with P-selectin to cause integrin activation (56). In blood vessels, chemokines are sequestered by GAGs on the luminal surface of inflamed ECs to be ideally exposed to leukocytes (57).

Adherent platelets undergo a series of alterations, both morphological and biochemical, leading to release of potent proinflammatory and mitogenic substances, such as IL-1β, IL-8, cluster of differentiation (CD) 40L, platelet factor 4, thromboxane A2 (TXA2), platelet-derived growth factor, platelet activating factor (PAF) and regulated on activation, normal T cell expressed and secreted (RANTES), thereby changing the phenotype of the ECs into a chemotactic, adhesive, and proteolytic state. The changes of the ECs, induced by platelets, will support chemotaxis, and subsequently adhesion of monocytes to the inflammatory sites (48). Activated platelets also secrete glycosaminoglycans (GAGs) that contribute to the immobilization of chemokines and the adhesion and transmigration of leukocytes (49).

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Recruitment of leukocytes, platelet dependent. Leukocytes can be recruited to inflamed endothelial cells through interaction with platelets. The first contact by rolling of platelets on endothelial cells is mediated by interaction of endothelial P-selectin to platelet PSGL-1 or GPIb/IX/V, respectively. Firm binding is then mediated through the β3 integrins. Adherent platelets release inflammatory substances, which support chemotaxis and adhesion of monocytes. The β2 integrin, MAC-1 have a dominant role in promoting stable leukocyte-platelet interaction. GP, glycoprotein; ICAM, intracellular cell adhesion molecule; LFA, lymphocyte function-associated antigen; PSGL, platelet selectin glycoprotein ligand; MAC, macrophage antigen; vWF, von Willebrand factor; αβ3, the receptor for vitronectin and αβ3, also called GP IIbIIIa. Figure modified from Ghasemzadeh M, 2012 (58).

Following integrin priming by selectins, and subsequently slow rolling, chemokines on neutrophils induce a rapid activation of integrins (59). The key role of the β2-integrins is well established. Leukocyte adhesion and transmigration at inflammatory sites is dependent on the β2-integrin ligation of MAC-1 and LFA-1 on leukocytes, with their specific ECs ligands, ICAM-1 and ICAM-2 (60). Also, the β1-integrin very late antigen 4, VLA-4 or αβ1, and its major endothelial counter-receptor VCAM-1, have an important role in monocyte and lymphocyte arrest. Figure 3 illustrates platelet independent recruitment of leukocytes.
Figure 3. Recruitment of leukocytes, platelet independent. Leukocytes can adhere to inflamed endothelial cells through specific interaction with endothelial cells. At first, rolling is initiated by binding of selectins with their receptors, while firm attachment is mediated by the leukocytes-expressed MAC-1 and LFA-1 (β2 integrins) and by VLA-4 (αβ1 integrin). VCAM, vascular cellular adhesion molecule; ICAM, intracellular cell adhesion molecule; LFA, lymphocyte function-associated antigen; MAC, macrophage antigen; PSGL, platelet-selectin glycoprotein ligand and VLA, very late antigen. Figure modified from Ghasemzadeh M, 2012 (58).

1.4 THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM

The RAAS is a dynamic physiologic system and is central in regulating the balance of fluids and electrolytes and blood pressure (BP). All RAAS peptides are derived from angiotensinogen. Renin, the rate-limiting enzyme is produced in the kidney (juxtaglomerular cells), and is released in response to vasodilation or low sodium. Renin cleaves angiotensinogen into Ang I. ACE hereafter cleaves Ang I to generate Ang II, which is the predominant peptide of the RAAS (61).

Ang II primarily exerts its influence through the receptors AT1R and AT2R. A number of signalling pathways are activated when Ang II interacts with AT1R and AT2R. The main AT1R-mediated Ang II effects include vasoconstriction by VSMC stimulation, sodium retention in the kidneys, and aldosterone release from the adrenal cortex (62). AT2R-mediated effects generally oppose those effects mediated by AT1R, and include vasodilation and anti-inflammatory effects in VSMCs, but also anti-proliferative effects in the myocardium (63). Figure 4 summarizes the different RAAS components.

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Figure 4. The renin-angiotensin-aldosterone system. Renin is secreted due to various stimuli, and then cleaves angiotensinogen into the inactive decapeptide Ang I. Renin and prorenin can interact with (pro)renin receptors to activate the MAP kinases ERK1/2 and p38 pathways. ACE cleaves Ang I into the octapeptide Ang II, or to Ang-(1-7) by ACE2 and probably ACE. ACE2 may also produce Ang-(1-7) from Ang II. ACE also inactivates bradykinin into inactive fragments. Importantly, Ang II may be generated directly from angiotensinogen through non-ACE pathways. Ang II activates AT1R, a G protein-coupled receptor. Vasoconstriction and stimulation of aldosterone tend to elevate blood pressure.

Ang II also activates AT2R, a G protein-coupled receptor, which can antagonize the effects of activation of the AT1R. Ang, angiotensin; ACE, angiotensin converting enzyme; MAPK, mitogen-activated protein kinase; extracellular signal regulated kinase, ERK; angiotensin 1 type receptor, AT1R; angiotensin 2 type receptor, AT2R; (P)RR, (pro)renin receptor; c-Src, cellular Src kinase, a non-receptor tyrosine kinase; SHP-2, Src homology 2 domain-containing phosphatase 2; NO, nitric oxide; NEP, neutral endopeptidase and PEP, prolyl endopeptidase.

1.4.1 The ACE2-Ang-(1-7)-Mas axis
Studies have identified a number of angiotensinogen derived peptides and their receptors (Figure 4). Ang-(1-7) is derived from Ang II through the influence of ACE2. Then Ang-(1-7) exerts its effect via the Mas receptor (64). The axis of ACE2-Ang-(1-7)-Mas may lead to vasodilatation via activation of NO, and decreased fibrosis, thereby enhancing the effect of ACE inhibitor blockade of Ang II (65). Ang-(1-7) also mediates anti-inflammatory and anti-fibrotic effects.
anti-thrombotic effects (66) via activation of NO and inhibition of ROS, derived from nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase (Nox) (67). Increased Ang-(1-7) has been associated with a favourable phenotype, with attenuated inflammation in atherosclerotic plaques (68). In summary, the ACE-Ang-(1-7)-Mas axis appears to work as a system of opposite effects, that may have the ability to complement the ACE-Ang II-AT1R axis.

1.4.2 Aldosterone

Aldosterone is produced in the adrenal cortex and acts on sodium reabsorption in the kidney. Common stimuli are Ang II, high plasma levels of potassium and adrenocorticotropic hormone. Aldosterone is also implicated in vascular inflammation, oxidative stress, fibrosis, remodelled and ED, particularly in the presence of salt (69). Conversely, vascular remodelling effects are reduced by the use of mineralocorticoid receptor (MR) blockers (70). In VSMCs and in ECs, aldosterone exerts its effects via mitogen-activated protein (MAP) kinase (also known as extracellular signal regulated kinase (ERK)(c-Src (cellular Src kinase, a non-receptor tyrosine kinase), and participates in epidermal growth factor receptor transactivation (71, 72) (figure 5). Aldosterone induced increase in oxidative stress in VSMCs may also have an impact on ED through a reduction in NO bioavailability (72). Vascular inflammation in ECs is promoted by aldosterone induced expression of ICAM-1 and adhesion of leukocytes in an MR dependent manner (73).

There is cross-talk between aldosterone and Ang II in VSMCs. Aldosterone increases the expression of AT1R in vivo (74), and Ang II stimulates aldosterone synthesis by the adrenal gland.

There are synergetic effects between aldosterone and Ang II on VSMC proliferation (75), migration (76), constriction (77) and senescence (78). The synergetic effect of aldosterone and Ang II in VSMC proliferation is a very rapid response, already after 5 minutes. This response is compatible with a non-genomic and MR dependent pathway via activation of AT1R and transactivation of epidermal growth factor receptor. A second peak, between 2 and 4 h, is compatible with a genomic pathway. Aldosterone injections in healthy subjects induce a rapid (within 10 minutes) increase in vascular resistance (79).

The G protein oestrogen receptor (GPER) is the principal mediator of oestrogen effects, but studies have reported that aldosterone is a much more potent agonist at GPER than oestrogen (80). GPER is a widely expressed receptor in cardiovascular tissues, and is present in the heart, in the ECs (81) and in VSMCs. GPER has been shown to mediate vasodilation and to lower BP, and the vasodilator effects appears to be EC dependent, secondary to activation to phosphatidylinositol 3-kinase and NOS (80). The endothelial dependent vasodilation seems to be a function of gender, age, or both, with a strong impact in premenopausal women, and a weak influence in men. MR antagonists may also act as GPER antagonists (80).

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GPER is also expressed in normal hepatocytes and GPER activation increases LDL receptor expression and suppresses circulating LDL cholesterol, while estrogen deficiency...
increases LDL cholesterol. The clearance of LDL via GPER activation has been linked to down-regulated levels of proprotein convertase subtilisin kexin type 9 (84).

Taken together, GPER seems to influence two of the most important atherosclerotic risk factors, hypertension and dyslipidemia, which implies that GPER may influence the development of atherosclerotic complications.

In vivo data have shown that mutual influence between aldosterone-stimulated MR and Ang receptor pathways plays a role for vascular effects of aldosterone. The AT1R seems to be required for MR-induced ED and vascular remodelling, inflammation and oxidative stress (85). Aldosterone has been linked to the metabolic syndrome and obese patients, and in the Framingham offspring study aldosterone was a predictor for incidence in the metabolic syndrome (86). An association between body weight mass and plasma aldosterone concentration has been shown in normotensive overweight patients. (87). Also, aldosterone decreases after weight loss (88), and aldosterone might be considered a potential adipocyte-derived factor since adipocytes have been shown to synthesize aldosterone in an AT1R dependent manner (89).

1.4.3 Renin, prorenin and renin-prorenin receptor

The RAAS exert effects independent of Ang I and Ang II (figure 4). This includes the direct effect of renin and prorenin, its proenzyme inactive form (90). The use of both ACE inhibitors and AT1R blockers has been reported to result in an at least 3-fold increase in plasma renin activity (91). The (pro) renin receptor may bind both renin and prorenin. The active receptor activates the MAP kinases ERK1/2 and p38 pathways. This entails in turn cell growth and fibrosis in cardiomyocytes, ECs and VSMCs (92). Inhibition of renin with aliskiren (a renin inhibitor) led to reduction in BP, improvements in systemic insulin resistance, improved insulin signalling and glucose uptake (93). These improvements were also associated with decreased levels of Ang II, aldosterone, AT1R, and consequently attenuated oxidative stress and fibrosis. For this reason it is difficult to interpret whether these effects were due to direct renin blockade or through decreasing downstream components of the RAAS. The exact significance of the (pro)renin receptor still is unclear, and a role for this receptor is lacking (94).

1.4.4 Alternative enzymes that generate Ang II

The RAAS system is complicated by enzymes (besides renin and ACE) that can generate Ang II. Chymase, generated in mast cells, is supposed to be the main enzyme responsible for cleaving Ang I into Ang II (95). Ang II can also be formed by direct proteolysis of angiotensinogen by a number of other enzymes, such as cathepsin G, tonin and t-PA, but the contribution of these enzymes is controversial (96, 97). There are estimates that at least 40% of Ang II is formed by non-ACE pathways (98). This suggests that full-scale suppression of the RAAS is not possible by ACE inhibition alone.

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1.4.5 Atherosclerosis and the RAAS

The RAAS is of vital importance in the pathobiology of vascular disease, and convincing data indicate that Ang II promotes atherosclerosis (99). Thus, it has been proposed that RAAS inhibition may have anti-atherosclerotic effects beyond the effects of the BP reduction (9).

Ang II causes oxidative stress and impaired NO activity. A cascade of intracellular signalling responses is initiated when Ang II binds to the AT1R. Ang II is a powerful activator of vascular Nox (of which Nox 1, 2, 4 and 5 are present in arteries), that induces the production of ROS (superoxide anion and hydrogen peroxide, H2O2) from ECs and VSMCs (100), and activation of redox-sensitive kinases (101). Activation of the AT1R induces generation of Nox-derived ROS that act as a second messenger to stimulate multiple signalling molecules (102). ROS also increase intracellular calcium and activate NF-kB and activating protein-1. These molecules participate in migration and cell-growth, and also in the expression of inflammatory genes and extracellular matrix (ECM) modulation. Ang II also activates RhoA/Rho-kinase, important in vascular contraction and growth (103). A pivotal step in atherosclerosis is the attraction of leukocytes to the endothelium. One of the primary Ang II effects is to induce ED and to generate a proinflammatory phenotype in human VSMCs. Ang II activate NF-κB and stimulates the up-regulation of the adhesion molecule VCAM-1, the chemokine MCP-1 and the cytokine IL-6. Also, ICAM-1 and E-selectin may mediate Ang II-induced monocyte adhesion (104). Ang II is pivotal in vascular

Figure 6. Angiotensin II and its effects in the development of atherosclerosis. Nox, NADPH oxidase; ROS, reactive oxygen species; MMP, matrix metalloproteinase; VSMCs, vascular smooth muscle cells; NO, nitric oxide; PGII, prostaglandin II (also called prostacyclin); LDL, low-density lipoprotein and LOX, lectin-like oxidized low-density lipoprotein receptor. Figure modified from Volpe M, 2012 (4).
remodelling as it induces the expression of a number of growth factors in VSMCs (105). Ang II has been observed to induce a dose dependent increase in IL-6 in rat VSMCs (106) and to modulate vascular cell migration, decrease VSMC apoptosis (107) and alter ECM composition (108). Additionally, Ang II may increase TF expression in monocytes, ECs and VSMCs (10, 11), and conversely treatment with RAAS blockade can diminish TF in plasma and in monocytes (12). Figure 6 summarizes Ang II and its effects in the progression of atherosclerosis.

Some of the clinical effects of ACE inhibition therapy may possibly be caused by interrupting Nox-derived ROS. Studies have shown an antioxidant effect of AT1R blockers. Indeed, direct inhibition of Nox and other ROS modulators has emerged as an attractive strategy to improve ED and vascular damage in hypertensive patients (109).

1.4.5.1 Antihypertensive therapies addressing inhibition of the RAAS
Evidence supports a positive effect of RAAS inhibition (110). Some studies suggest that ACE inhibitors improve EC function, both in hypertensive patients and in patients with CHD (111, 112). Large scale studies of ACE inhibition post myocardial infarction (MI) and in heart failure demonstrated convincing evidence of reductions in the risk of recurrent MI (19, 20). Later, trials were designed to test the hypothesis that ACE inhibition in patients with CVD, but no heart failure, reduced the risk of atherosclerotic events. Indeed, the HOPE trial showed convincingly that treatment with ramipril in patients with high risk for vascular disease, without heart failure, reduced the risk of stroke, myocardial infarction and death (9). The EUROPA study demonstrated that treatment with perindopril in patients with stable CHD, reduced the risk for cardiovascular events (113). In contrast, the PEACE trial failed to confirm that patients with known CHD had a benefit of ACE inhibitors, in addition to modern conventional therapy (114). The results in that study might have been due to the low number of hard endpoint events (myocardial infarction or death). The lack of efficacy observed might simply have been affected by the enrolment of a low-risk population and by a high proportion of patients treated by statins (70%), compared to HOPE (29%) and EUROPA (56%). The QUIET study also failed to confirm a decrease in overall mortality rate after treatment with an ACE inhibitor (115). This outcome may partly have been due to enrolment of a low-risk population with both normal LDL cholesterol and body mass index. It is to be noted that, both the PEACE and QUIET trials were characterized by a very high proportion of patients previously subjected to at least one revascularization procedure (72% and 100%, respectively).

1.5 HYPERTENSION
Hypertension is globally the most common cause of morbidity and mortality. In the vascular system hypertension entails arterial remodelling and ED. Common to these processes are alterations in ECs and VSMCs to a vasoconstrictor, mitogenic, profibrotic, promigratory and proinflammatory phenotype influenced by oxidative stress.

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Convincing evidence indicates that patients with hypertension have ED, both in the macrocirculation (conduit arteries) and microcirculation (arterioles, 10-150 µm), and small arteries, 150-300 µm. The microvessels are known to be the main site of vascular resistance. Folkow described that already a small reduction in vessel diameter has a major effect on resistance, and that this was a result of the Poiseuille’s law (116). Blood vessels undergo structural alterations during long-term hypertension. This includes remodelling and rarefaction. Remodelling is known to be responsible for the increase in vascular resistance in hypertension (117). In small arteries and arterioles remodelling demonstrates a rearrangement of the vessel wall without growth that causes a narrowing of the lumen and an increased media-to-lumen ratio known as inward eutrophic remodelling. Rarefaction is defined as a decrease in the length or small vessel numbers in a defined volume.

Oxidative stress and ED are associated with both remodelling and rarefaction (117, 118). The endothelium may be activated by various stimuli, both chemical and physical. Hypertension in otherwise healthy subjects has been related to high levels of sICAM-1 and IL-6 (119), indicating that hypertension may contribute to an inflammatory state and to atherosclerosis. When stimulated the endothelium releases endothelium-contracting factors (EDCF) and endothelium-derived relaxing factors (EDRF), which exert effects on the underlying VSMCs. The balance between these factors determines the tone of the VSMCs. The most significant EDRF is NO, but also prostaglandin I2 (PGI2, or prostacyclin) and endothelium-derived hyperpolarizing factors are important vasodilator signals. The ECs secrete several EDCFs. One of the most important is Ang II, but also endothelin and cyclooxygenase (COX)-derived prostanooids are significant vasoconstrictors (120). In hypertension, oxidative stress induces increased production of the prostanooid TXA2 by constitutive (COX-1) and inducible (COX-2) cyclooxygenases, which leads to increased vasoconstriction and reduced endothelium-dependent vasodilation (121). It is to be noted that COX itself can produce ROS by oxidizing NADPH (121). Hence, ROS are upstream and downstream of the COX-prostanoid system.

During long-term BP elevation the ECs age prematurely and their turnover is accelerated. Eventually they are replaced by regenerated ECs (122). Importantly, the replaced endothelium has a reduced ability to release EDRFs, and in particular NO. The result of blunted NO release is a weakening of the brake to EDCFs and an endothelium-dependent contraction (120). This ED in micro- and macrocirculation also entails platelet aggregation, up-regulation of adhesion molecules, and growth of VSMCs (122, 123). These changes in the ECs contribute to thrombosis, inflammation, vascular remodelling and, in the end, atherosclerosis. 1.5.1 Shear stress and circumferential stretch

The pulsatile BP causes the blood vessels to be subject to a constant shear stress and circumferential stretch. The frictional force during the flow of blood in the vessels causes wall shear stress while the pulsation generates circumferential stretch, perpendicular to the blood flow. Under physiologic conditions, pulsatile forces with a clear direction (laminar...
An important effect of ECs and VSMCs is augmented resistance in small vessels. The cardiac hypertension induces vascular remodelling. This will lead to increased wall thickness and an increase of oxidative stress in ECs (135). Studies have also suggested that circumsferential shear stress and circumferential stretch) cause only a transient signalling of proinflammatory and proliferative pathways, and these pathways become down-regulated when such directed forces are maintained. On the other hand, pathologic conditions with forces that are either too high or too low or without a direction (such as disturbed flow and undirected stretch at branch points) induce sustained signalling of proinflammatory and proliferative pathways (124).

How the vessel wall responds to these forces is of vital importance, and a number of integrins, receptors and ECM components have been extensively studied (125).

1.5.1.1 Shear stress
In linear parts of the vessels the blood flow is laminar and shear stress is directed and high. In contrast, in curvatures and branches the blood flow is irregular and disturbed, which results in low shear stress. Persistent laminar blood flow and high shear stress protects against atherosclerosis by up-regulating the expressions of protective EC genes and proteins. In contrast, disturbed flow with low shear stress activates EC genes and proteins promoting atherosclerosis.

The most important flow mediated vasodilator is NO, which is generated from endothelial NO synthase; but also PGI2 is an important vasodilator (126). During steady laminar shear stress NO and PGI2 are up-regulated, inducing vasodilatation and inhibition of platelet aggregation, and prothrombotic molecules such as TF are down-regulated (127). The main inducer of fibrolysis, t-PA, may be shear stress regulated, and t-PA secretion by ECs increases with increasing shear stress (128). Also, thrombomodulin (TM) is up-regulated by high shear stress (129). Laminar steady high shear stress has been shown to down-regulate AT1R in an NO dependent manner (130). In addition, AT1R antagonists reduce oxidative stress in human hypertension (131).

Low or reversing shear stress predisposes to atherosclerosis. The low flow or flow reversal causes the production of superoxides by Nox, which scavenge NO, leading to a decrease in NO bioavailability, generation of peroxynitrite and ED (132). Peroxynitrite reduces vasodilatation by reducing NO and also by decreasing the bioavailability of PGI2 (by nitration of PGI2 synthase) (133). Peroxynitrite also causes endothelial NO synthase uncoupling (by oxidizing tetrahydrobiopterin, the cofactor of endothelial NO synthase) (134). Consequently, low shear stress induces a proinflammatory and prothrombotic state through several mechanisms.

1.5.1.2 Circumferential stretch
Elevated BP causes a pressure that is perpendicular to the flow direction, and long-term hypertension induces vascular remodelling. This will lead to increased wall thickness and an augmented resistance in small vessels. The cardiac pulsatile flow generates stretch on both ECs and VSMCs.

An important effect of the pulsatile character of BP is the release of Ang II and a subsequent increase of oxidative stress in ECs (135). Studies have also suggested that circumferential shear stress and circumferential stretch) cause only a transient signalling of proinflammatory and proliferative pathways, and these pathways become down-regulated when such directed forces are maintained. On the other hand, pathologic conditions with forces that are either too high or too low or without a direction (such as disturbed flow and undirected stretch at branch points) induce sustained signalling of proinflammatory and proliferative pathways (124).

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stress activates AT1R, without involvement of An II (136). In VSMCs, the mechanical stress by circumferential stretch activates integrins through interaction with ECM and results in actin polymerization (137).

### 1.5.2 Microvascular (capillary) rarefaction

During chronic hypertension, the endothelium loses its function, and the microvessels will be constricted, unperfused and eventually the vessels disappear. This phenomenon is known as microvascular rarefaction (138). Rarefaction exists in two subforms (139):

- **Functional rarefaction**: Refers to a reduction in the actual number of vessels perfused.
- **Structural rarefaction**: Refers to a decrease in the actual number of vessels.

ED has been shown to be associated with vessel rarefaction (140). During hypertension, peripheral resistance is increased by microvascular rarefaction and a significant component of the increase in total peripheral resistance may be due to vessel rarefaction (138).

Imbalanced angiogenesis (i.e. impaired formation of microvessels), contributes to rarefaction, and ageing has been shown to be associated with impaired angiogenesis (141). NO has been shown to be implicated in angiogenesis, and microvascular rarefaction in hypertension may partially be due to reduced angiogenesis because of impaired NO biosynthesis (142). In healthy conditions, hypoxia serves as the main trigger for angiogenesis, and chronic hypoxia may induce angiogenesis by vascular endothelial growth factor pathways (143). However, in CVD with associated decreased NO synthesis, ischemia-induced angiogenesis is generally impaired (144).

#### 1.5.2.1 Antihypertensive therapies addressing rarefaction

Few studies have focused on how antihypertensive drugs affect the microvasculature. Studies have shown that long-term treatment with various antihypertensive drugs normalize vascular structure and reverse rarefaction (145). One study compared the effect of treatment with an ACE inhibitor and a calcium channel blocker (CCB) on the retinal microvasculature. Treatment was associated with improvement in vessel narrowing and rarefaction, and no differences were observed between the treatment regimens (146). In another study, an ACE inhibitor restored the structure of arterioles and small arteries in hypertensive subjects, whereas a ß-blocker did not (147). The ability to normalize the structure in the microvessels might be restricted to drugs with vasodilator capacity, such as ACE inhibitors and angiotensin receptor blockers (ARBs), excluding drugs reducing cardiac output, such as ß-blockers and diuretics (145). In support for this assumption are two studies with increased vascular area and capillary density during treatment with prazosin, an alpha 1-adrenoceptor blocker (148, 149), but the results are inconclusive, since doxazosin, another alpha 1-adrenoceptor blocker inhibited EC adhesion, migration and invasion (150). The drug class ARB also has vasodilator capacity and can increase microvessel density. In a LIFE substudy, the ARB losartan reduced vascular rarefaction and hypertrophy, compared to a ß-blocker (151).
The proangiogenic contribution of ACE inhibitors on rarefaction has been examined in animal models, and drugs targeting the RAAS induce angiogenesis in most animal studies. These effects are probably mediated by bradykinin, and the generation of pathways including NO and vascular endothelial growth factor (139).

### 1.5.3 Hypertension and endothelial dysfunction and the RAAS

Derangements of the RAAS contribute to elevated BP and target-organ damage. Chronic inflammation in the vessel is considered to be the link between hypertension and atherosclerosis, but evidence of a causal link between oxidative stress and hypertension is not convincing, and definitive proof is still lacking.

Activation of the RAAS is a major cause of hypertension. Studies in essential hypertension have demonstrated that systolic and diastolic BP relate positively with biomarkers of oxidative stress and negatively with antioxidant levels (152). Vascular Nox activity is increased in hypertension and is highly sensitive to Ang II and aldosterone. In small arteries of hypertensive patients, the ECs and VSMCs expression of Nox 1, 2, 4 and 5 generate increased production of ROS (153). Measurements of ROS production in VSMCs resistance arteries of hypertensive patients have shown increased levels of superoxide anion and hydrogen peroxide and up-regulated Ang II-stimulated redox signalling, when compared to conditions in normotensive subjects (154).

### 1.5.3.1 Antihypertensive therapies addressing endothelial dysfunction

Interpretation of the effects on ED of ACE inhibitors and other antihypertensive drugs are confounded by the simultaneous reduction in BP as decreased BP per se may have the ability to reduce atherosclerotic events.

Intravenous administration of the ACE inhibitor perindopril restored normal coronary artery vascular response to endothelial stimuli in hypertensive patients (111). In agreement, the TREND study showed that the ACE inhibitor quinapril decreased vasoconstriction to acetylcholine in coronary arteries in subjects with known CHD (112). Perindopril increased FMD in hypertensive patients, while the other antihypertensive agents did not (CCBs, first and third generation β-blocker and ARB). Perindopril but also CCBs and ARB reduced oxidative stress and increased plasma antioxidant capacity (155). These findings are in accordance with the BANFF study. This study demonstrated that quinapril improved FMD in patients with CHD. On the other hand, no change was seen with the other antihypertensive drugs (156). In two studies comparing ARB to CCBs, the reduction in BP was equivalent, while the ARB reduced atherosclerosis, and the CCBs did not (157, 158).

In yet another study comparing an ARB with a CCB in hypertensive patients, the group receiving ARB demonstrated improved endothelial function and an associated reduction in oxidative stress. The CCB did not have this effect (159). One study examined the effects of inhibiting the RAAS by comparing ACE inhibition to ARB in hypertensive patients with no effects on markers for inflammation, coagulation, or endothelial function (160).

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1.6 HYPERLIPIDAEMIA

Hyperlipidaemia is known as an excess of lipoproteins in the blood, consisting of cholesterol, cholesterol esters, apolipoproteins, phospholipids, and triglycerides. Based on density, they are divided into five major classes. (Table 1).

<table>
<thead>
<tr>
<th>Major apolipoproteins</th>
<th>Density (g/mL)</th>
<th>Diameter (nm)</th>
<th>Free cholesterol (wt %)</th>
<th>Cholesterol esters (wt %)</th>
<th>Phospholipid (wt %)</th>
<th>Triglycerol (wt %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CM</td>
<td>&lt; 0.940</td>
<td>80-1200</td>
<td>1-3</td>
<td>2-4</td>
<td>3-6</td>
<td>80-95</td>
</tr>
<tr>
<td>VLDL</td>
<td>0.940-1.006</td>
<td>30-80</td>
<td>4-8</td>
<td>16-22</td>
<td>15-20</td>
<td>45-65</td>
</tr>
<tr>
<td>IDL</td>
<td>1.006-1.019</td>
<td>25-50</td>
<td>4-8</td>
<td>20-26</td>
<td>20-24</td>
<td>26-36</td>
</tr>
<tr>
<td>LDL</td>
<td>1.019-1.063</td>
<td>18-28</td>
<td>6-8</td>
<td>45-50</td>
<td>18-24</td>
<td>4-8</td>
</tr>
<tr>
<td>HDL</td>
<td>1.063-1.210</td>
<td>5-15</td>
<td>3-5</td>
<td>15-20</td>
<td>26-32</td>
<td>2-7</td>
</tr>
</tbody>
</table>

CM, chylomicrons; VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein and wt, weight.

Primary hyperlipidaemias are genetic disorders, while secondary hyperlipidaemias arise due to endogenous or exogenous causes. Endogenous causes can be disorders like diabetes mellitus, thyroid disease, kidney disease, liver disorders or Cushing’s disease. Exogenous causes are obesity, lack of exercise, unfavourable diet or excessive alcohol consumption.

Primary (or familial) hyperlipidaemias are classified into five different types according to the Fredrickson classification (22), which was later adopted by the WHO (Table 2).

1.6.1 Familial combined hyperlipidaemia

FCHL is a common inherited cause of hypercholesterolaemia caused by a number of gene polymorphisms (161). The prevalence of FCHL is estimated to be 0.5-2.0%, and the prevalence of CHD in patients less than 60 years old is estimated to be as high as 20% (162). FCHL is characterised by increased levels of apolipoprotein B100 containing lipoproteins:
develop CH
premature death
le
LDL
inherited by
FH is a
fibrinolysis and inflammation
reactivity and hypercoagulability
increasing platelet activation (25)
expression of NF
hyperlipidemia, and insulin resistance
VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein and HDL, high density lipoprotein.
VLDLs, intermediate-density lipoproteins (IDLs) and LDLs. FCHL is frequently associated with reduced HDL cholesterol, and insulin resistance (163, 164). Also, patients with FCHL have a predominance of small dense LDL cholesterol, which is a diagnostic indicator for CHD risk (165). VLDL activates NF-
receptor for LDL, and
coagulant phenotype mainly by
increasing platelet activation (166). FCHL is characterized by impaired endothelium reactivity and hypercoagulability (23, 167), increased PAI-1 levels and a chronic inflammation (25-27). Thus, patients with FCHL exhibit ED, disturbed coagulation, impaired fibrinolysis and on-going chronic inflammation. These characteristics can all be responsible for the increased incidence in cardiovascular events in FCHL subjects.

### 1.6.2 Familial hypercholesterolemia

FH is a disorder that is caused mainly by genetic disturbance in the receptor for LDL, and inherited by an autosomal dominant pattern. The LDL receptor (LDLR) function is disturbed and the levels of LDL are dramatically increased (168). The heterozygous condition affects at least 1 in 500 individuals (0.2%). Patients with FH are affected by increased CHD and premature death (168). If not treated, about 50% of men and 20% of women are expected to develop CHD before the age of 50 years (168).

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Lipoproteins elevated</th>
<th>Cholesterol concentration</th>
<th>Triglyceride concentration</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Chylomicrons</td>
<td>Normal to ↑</td>
<td>↑↑↑</td>
<td>&lt;1</td>
</tr>
<tr>
<td>IIIa</td>
<td>LDL</td>
<td>↑↑</td>
<td>Normal</td>
<td>10</td>
</tr>
<tr>
<td>IIIb</td>
<td>LDL and VLDL</td>
<td>↑↑</td>
<td>↑↑</td>
<td>40</td>
</tr>
<tr>
<td>III</td>
<td>IDL</td>
<td>↑↑</td>
<td>↑↑</td>
<td>&lt;1</td>
</tr>
<tr>
<td>IV</td>
<td>VLDL and chylomicrons</td>
<td>↑ to ↑↑</td>
<td>↑↑↑</td>
<td>5</td>
</tr>
</tbody>
</table>

VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein and HDL, high density lipoprotein.

Familial hypercholesterolemia (FH) is a genetic disorder characterized by elevated levels of LDL cholesterol, leading to an increased risk of cardiovascular diseases. FH occurs in two forms: familial hypercholesterolemia (FH) and very-familial hypercholesterolemia (VH). FH is caused by mutations in the LDL receptor (LDLR) gene, which results in decreased LDL receptor activity and reduced uptake of LDL cholesterol. This leads to accumulation of LDL cholesterol in the bloodstream, increasing the risk of atherosclerosis and premature cardiovascular disease.

### Table 2. Primary (or familial) hyperlipidaemias according to the Fredrickson classification.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Lipoproteins elevated</th>
<th>Cholesterol concentration</th>
<th>Triglyceride concentration</th>
<th>Frequency (%)</th>
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<td>↑↑↑</td>
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FH is associated with impaired endothelial reactivity (28), and hyperactive platelets in humans (169). Hypercholesterolemia has been shown to exhibit a prothrombotic state without any influence on fibrinolysis (28, 30). The cause of the procoagulant state is unknown, but one mechanism might be that elevated levels of oxLDL in FH have been shown to cause an increase in the expression of active TF in human monocytes (11, 170). In one of these studies the authors found that TF inhibition reduced the levels of IL-6, indicating crosstalk between inflammation and coagulation during FH. They proposed that oxLDL may lead to induced expression of both TF and IL-6, and the development of a prothrombotic and proinflammatory state. Consistent with these studies, patients with high LDL concentrations exhibited an increased TF plasma activity (171). Accumulating evidence demonstrates that hypercholesterolemia activates the RAAS, and one possible mechanism might be the observation that oxLDL up-regulates ACE and AT1Rs (172). Also, studies have shown that hypercholesterolemia is associated with hypertension (173). FH is not associated with insulin resistance (174).

1.6.3 Hyperlipidaemia and the RAAS

The first sign of atherosclerosis is the fatty streaks that appear during the first or second decade of life. The exact mechanisms are unclear, but according to the oxidation hypothesis of atherosclerosis, LDL translocates from the lumen into the vessel wall. The LDL particle is retained when it undergoes oxidative modification by ROS or enzymes (175). OxLDL has itself proinflammatory effects and stimulates proinflammatory signalling pathways (176). OxLDL may also activate ECs and stimulate the up-regulation of VWF and adhesion molecules. The presence of chemokines like MCP-1 attracts leukocytes into the intima that promotes an activation of the immune system. Leukocytes are continuously recruited into the subendothelial area, and monocytes then differentiate into macrophages. In the vessel wall, macrophages take up oxLDL via the scavenger receptors, a process that eventually leads to the transformation of macrophages into foam cells (177).

In VSMCs, oxLDL interacts with Toll-like receptors (TLR) 4 and CD36, which enhances the production of cytokines, chemokines and growth factors, resulting in recruitment of monocytes and differentiation of macrophages (178).

Lectin-like oxidized LDLR (Lox-1) is a receptor on inflamed ECs and VSMCs, promoting the uptake of oxLDL. The activation of Lox-1 is stimulated ROS, oxLDL and Ang II (179). Lox-1 also causes an up-regulation of the AT1R (180), demonstrating a potential cross-talk between Lox-1 and AT1R.

Both LDL and oxLDL can activate the RAAS and their receptors in human ECs (172), and hypercholesterolemia and elevated Ang II levels in hypertension can accelerate the development of ED and atherosclerosis (181). These data indicate a link between hypercholesterolemia, hypertension and atherosclerosis.

Other lipoproteins, such as the triglyceride-rich lipoprotein VLDL and IDL also have considerable atherogenic potential (166). These lipoproteins may undergo oxidative

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modification like that of LDL, and some evidence suggests that VLDL particles may have inflammatory effects in vascular cells (25).

1.7 INSULIN RESISTANCE

Insulin resistance is a disorder in which the body fails to respond to insulin and exhibits dysregulated insulin stimulated uptake of glucose in skeletal muscle and adipocytes, leading to high levels of insulin and blood glucose. Insulin resistance is a risk for type 2 diabetes. The prevalence of insulin resistance is increasing globally, in particular in hypertensive and obese subjects.

Insulin is secreted from pancreatic β-cells as a result of glucose stimulus. When insulin binds to the insulin receptor a series of intracellular events takes place, referred to as the insulin signalling cascade. It all culminates in glucose transporter type-4 (GLUT-4) translocation, from the cytoplasm to the cell membrane (figure 7). GLUT-4 translocation involves downstream signalling via the insulin receptor and insulin receptor substrate-1 pathway, but also through phosphatidylinositol 3-kinase and ak strain transforming (AKT, also referred to as protein kinase B) pathway (182).

Most insulin resistance states are a consequence of defects in the upstream signalling pathway. Insulin resistance seldom exists isolated; instead it often coexists with other cardiovascular risk factors, the metabolic syndrome in particular (183).

1.7.1 Insulin resistance and the RAAS

Clinical trials implicate that the RAAS has a potential role in insulin resistance as well as in type 2 diabetes, while blockade of the RAAS has been shown to prevent both states (9, 184, 185). A meta-analysis has shown that ARB and ACE inhibitors decrease the incidence of new onset diabetes by 23% and 27%, respectively (186). Ang II stimulation of ATIR results in vasoconstriction and a decrease in microvascular blood flow, and these hemodynamic changes attenuate the delivery of both glucose and insulin to skeletal muscle. Data also indicates that Ang II mediates its effects by direct, non-hemodynamic actions. The activation of ATIR by Ang II induces ROS by Nox. ROS on its own, or by activating MAP kinase or NF-xB signalling, then inhibits the insulin signalling pathway. The result is inhibition of the translocation of GLUT-4, which remains in the cytoplasm (figure 7).

1.7.1.1 The ACE2-Ang(1-7)-Mas axis

Studies have shown that Ang-(1–7), which mediates its effect by interacting with the Mas receptor, has a positive regulatory impact on insulin signalling via stimulation of AKT (187). Hence, the balance between the two axes ACE-Ang II-ATIR and ACE2-Ang-(1-7)-Mas is a crucial factor in insulin resistance, and a ratio of the peptides Ang II and Ang-(1-7) is likely to be a determinant of the overall activity.
Aldosterone has inhibitory effects on insulin signalling by inducing insulin resistance in VSMCs through insulin receptor substrate-1 expression and AKT signalling (191). In ECs, aldosterone may predict the development of insulin resistance. In the vasculature, MR activation reduces NO production and increases ROS, leading to ED, VSMC hypertrophy, fibrosis and inflammation (190).

Aldosterone interferes directly with insulin signalling. Indeed, studies have shown that aldosterone may predict the development of insulin resistance. In the vasculature, MRs have been described on both ECs and VSMCs (73). Activation of the AT1R is the primary stimulator of adrenocortical release of aldosterone, and inhibition of RAAS by ACE inhibitors or ARBs decrease plasma aldosterone levels, while dietary salt restriction increases aldosterone production and vascular insulin resistance (189). Excess aldosterone and consequently MR activation reduces NO production and increases ROS, leading to ED, VSMC hypertrophy, fibrosis and inflammation (190).

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aldosterone and MR activation increase expression of ICAM-1 and Nox-4, leading to oxidative stress and decreased NO bioavailability (73). Hypertension and obesity are associated with insulin resistance. Aldosterone has also been shown to reduce insulin secretion directly in pancreatic cells (192).

1.7.1.3 Renin, prorenin and renin-prorenin receptor
Blockade of renin improves insulin sensitivity. In subjects with the metabolic syndrome treatment with a renin inhibitor improved insulin sensitivity, as compared to an ARB (193). In a rat model of RAAS activation, a renin inhibitor led to improved insulin resistance and signalling, and also improved glucose uptake in skeletal muscle (93). Improvements in insulin signalling may also be due to decreased levels of Ang II and aldosterone, and this makes it hard to determine whether the effects of renin inhibition are through effects of renin blockade, or through the effects of decreasing downstream levels of Ang II or aldosterone.

1.8 HAEMOSTASIS
Haemostasis consists of the platelet system, the coagulation and the fibrinolytic system, and is often classified as:

- **Primary haemostasis:**
  - Vascular contraction.
  - Platelet activation and aggregation.

- **Secondary haemostasis:**
  - Coagulation. Consists of TF (extrinsic) and contact (intrinsic) pathways.
  - Fibrin formation and clot stabilization.

- **Fibrinolysis:**
  - Removing of the clot, once blood vessel integrity has been restored.

Haemostasis is a fine-tuned balanced system, and in normal conditions the factors for haemostasis are present in inactivated forms in circulating blood and can typically be activated upon vessel inflammation or damage. During inflammatory states involving ED disturbances at several levels of the haemostatic system, this may result in pathophysiological changes in primary and secondary haemostasis or fibrinolysis.

1.8.1 Coagulation
The TF (extrinsic) and contact (intrinsic) pathways divide the coagulation cascade into two parts.

1.8.1.1 Tissue factor pathway (extrinsic)
The TF (or extrinsic) pathway, the major inducer of the coagulation cascade, is initiated when TF is bound to FVIIa-FVIIa (see figure 8). The TF-FVIIa complex cleaves FX into Fxa, and FIX into FIxa, respectively. FXa then forms the prothrombinase complex when associated with FVa and Ca$^{2+}$ on activated platelets, resulting in thrombin formation. The reaction is
much faster (300,000-fold than baseline) in the presence of negatively charged surface phospholipids (i.e. phosphatidylserine) on activated platelet membranes. Microparticles (MPs) may also accelerate thrombin generation due to a procoagulant phosphatidylserine-rich surface that supports thrombin generation (194).

TF has for long been known to be present in the extravascular space (in subendothelial tissue) and not exposed to flowing blood, except during plaque rupture. TF is also present in circulating blood, mainly in three pools. The most important source of TF is monocytes, expressed as membrane-anchored and membrane-bound. There is no evidence that granulocytes express TF, rather they seem to acquire TF from monocytes (195). Controversial is the expression of TF in platelets. Platelets might store TF in α-granules or acquire TF from TF-containing MPs (196, 197). The second source of circulating TF is different cell-derived MPs, which are considered to be key players in atherothrombosis. The third pool, probably of minor importance, is a spliced form of TF, which is soluble and circulates in the blood (198).

The importance of the intravascular blood-borne TF is controversial. Blood-borne TF means that the coagulation cascade may be activated without contact between the blood and the extravascular space (i.e. plaque rupture). This appears to be an important contribution in intravascular thrombosis (199).

Blood-borne TF implicates that TF apparent on the surface of cells has a low activity, and under normal conditions TF is obviously in an inactive, cryptic, state. To be functionally active, and to exhibit a procoagulant activity, TF has to undergo an activation step, decryption. The molecular differences between these states are controversial and remain to be clarified. Several mechanisms have been suggested to explain the decryption step (200).

Many cytokines are responsible for expression of TF on mononuclear cells in vitro. Studies have reported that the TF expression is mostly dependent of IL-6. Inhibition of IL-6 has been shown to completely block thrombin generation, whereas other cytokines had no effect (201). IL-6 has been shown to be relevant for activation of coagulation (202). Also CRP has been shown to induce TF expression in both ECs and VSMCs (203). TF also possesses a function as a signalling receptor. TF binding of FVIIa has been shown to trigger VSMC proliferation (204).

### 1.8.1.2 Thrombin generation

Thrombin is generated in three phases: the initiation phase, the amplification phase and the final propagation phase. During the first phase, only small amounts of thrombin are generated and during the next two phases, and in particular in the final phase, large amounts of thrombin are formed (205):

- **Initiation phase:** Requires activated TF on ECs, monocytes, MPs or subendothelial TF after vascular injury. TF activates FVII and then TF-FVIIa activates FX and FIX. FXa activates FV. FXa complexes with FVa and if the stimulus is strong, enough thrombin
is formed to initiate the coagulation process. The amount of thrombin generated is under the influence of tissue factor pathway inhibitor (TFPI) activity as it rapidly inhibits FXa.

- Amplification phase: This response takes place on phospholipid surfaces, mostly on activated platelets. The stimulus is amplified as platelets are activated and accumulate cofactors on their surfaces. Activated platelets release FV, which is activated by thrombin or FXa into FVa. Circulating vWF-FVIII complexes bind to activated platelets and FVIII is released and activated into FVIIIa by thrombin.

- Propagation phase: Finally, the active proteases combine with their cofactors on phospholipid surfaces of activated platelets, but can also take place on MPs, activated ECs or VLDLs. The tenase complex, consisting of FIXa-FVIIIa and Ca2+ activates FX into FXa. Thereafter the prothrombinase complex, consisting of FXa-FVa and Ca2+, induces a thrombin burst that converts fibrinogen into fibrin. Thrombin activation of FXIII cross-links the fibrin monomers and stabilizes the platelet plug. Thrombin also stimulates on-going coagulation by feedback activation of FXIa.

1.8.1.3 Contact pathway (intrinsic)

Thrombin may also be activated by the contact (or intrinsic) pathway (see figure 8). The contact activation pathway is less prominent for haemostasis under healthy conditions than the TF pathway. However, states like hyperlipidaemia may lead to thrombosis via the contact pathway (206).

The contact pathway consists of FVIII to FXII. Also required are prekallikrein and the cofactor high-molecular-weight kininogen, and calcium ions. The contact pathway requires a surface with negatively charged phospholipids, often secreted from platelets. Interaction with phospholipids may also occur on certain lipoproteins, such as chylomicrons, VLDLs or oxLDLs. Thus hyperlipidaemia can promote a prothrombotic state via the contact pathway.

The contact phase is initiated as prekallikrein is converted to kallikrein, cleaving FXII to FXIIa. In turn, FXIIa then cleaves FXI to FXIIa. FXIIa will also convert prekallikrein to kallikrein, through mutual activation. Kallikrein can acts upon kininogens that leads to the release of the vasodilator bradykinin. FXIIa activates FIX to FIXa, in the presence of Ca2+. FIXa then cleaves FXA to active FXa. Activation of FXa needs the tenase complex on platelets or certain lipoproteins. FVIII is mainly produced by the liver, and circulates in an inactive state in blood in complex with vWF. The activation of FVIII to FVIIIa occurs with minute quantities of thrombin or FXa. During activation FVIII dissociates from vWF and becomes fully activated and then participates in the FX activating complex. But when thrombin concentration increases, FVIIIa is inactivated by thrombin. Hence, thrombin has a dual action upon FVIII, and regulates the extent of the amount of the tenase complex formed and consequently has an important impact on the activity of the coagulation cascade (207, 208).
Thrombin also promotes the release of EC MPs that seem to be critical in vascular
receptor for thrombin, and can activate platelets even at very low thrombin concentrations.

P selectin and CD40L, activation of adenosi

is the most effective platelet activator and causes

Procoagulant activity

Figure 8. Blood coagulation cascade. To clarify the figure zymogens are not depicted. F. coagulation factor; Xa-Va, Fxa-FVa complex or the prothrombinase complex; IXa-VIIia, FIIa-FVIIIa complex or the tenase complex; IIa, FIIa or thrombin; XIIia, FXIIia; PC, protein C; APC, activated protein C; PS, protein S; TF-VIIa, tissue factor-FVIIa complex; AT, antithrombin; TM, thrombomodulin; t-PA, tissue plasminogen activator; TFP, tissue factor pathway inhibitor; Xa, FXa; TAFII, thrombin activatable fibrinolysis inhibitor; α2-A2-antiplasmin; PAI, plasminogen activator inhibitor; FDP, fibrin degradation products and Ca2+, calcium ion. Figure modified from Bouma BN, 2006 (209).

1.8.1.4 The role of thrombin
Thrombin is of vital importance in the coagulation cascade. The main action is to act as a procoagulant by converting fibrinogen to fibrin monomers. Thrombin also exercises an anticoagulant role by binding to TM at the intact endothelium and to promote activation of the protein C (PC) pathway. APC then inactivates FVa and FVIIIa, two essential cofactors for FXa and FIXa, thereby down-regulating thrombin generation (210). Thrombin also has a central role in inflammatory response and stimulates different types of cells in the vasculature and in blood, including ECs, VSMCs and platelets. Effects of thrombin are mediated by their counter-receptors termed protease-activated receptors (PARs). PARs are G protein-coupled receptors, and four receptors have been identified (PAR 1-4), and PAR-1 has been identified as the main thrombin receptor in vessels and on platelets.

Thrombin signalling in platelets contributes significantly to haemostasis and thrombosis. It is the most effective platelet activator and causes the platelets to change shape and secrete adenosine diphosphate, serotonin, TXA2, chemokines, growth factors, mobilization of P-selectin and CD40L, activation of αIIbβ3 and, ultimately, platelet aggregation (211). Platelets in humans express PAR-1 and PAR-4. PAR-1 seems to be the main platelet receptor for thrombin, and can activate platelets even at very low thrombin concentrations.

Thrombin also promotes the release of EC MPs that seem to be critical in vascular pathophysiology (212). Once trace amounts of thrombin have been generated this is then able to activate FV, FVIII, FXI and FXIII.
In the healthy endothelium, thrombin activates PAR-1 and stimulates the production of prostacyclin and NO, leading to vasodilatation and activation of fibrinolysis by the release of t-PA (213). During inflammation and dysfunction of the endothelium PAR expression is increased in the endothelium, priming the response of ECs to thrombin and shifting ECs to a proinflammatory phenotype, inducing synthesis and release of PAI-1, contributing to impaired fibrinolysis (214). In pathophysiological conditions PAR-1 activation causes morphological changes, an increased vascular leakage and the release of proinflammatory cytokines, as well as up-regulation of adhesion molecules (215). In particular the synthesis of IL-6 seems to mediate the transition of the inflammatory process in the vessels from an acute to a chronic phase (216). Activation of PAR-1 also mobilizes p-selectin and vWF from Weibel-Palade bodies, promoting EC rolling and subsequently firm adhesion of both platelets and leukocytes.

In healthy arteries PARs are preferably expressed in ECs, while their expression in VSMCs is limited. In hypertension and atherosclerotic vessels PAR-1 is up-regulated in VSMCs (214). This implies that PARs on VSMCs have a more prominent role under pathological conditions. In states associated with ED, PARs in VSMCs mediate contraction, proliferation, migration, hypertrophy and the production of ECM (214).

Thrombin activatable fibrinolysis inhibitor (TAFI) originates from the liver, and is transported in the plasma as a zymogen, bound to plasminogen. The thrombin-TM complex activates TAFI preventing up-regulation of plasminogen binding and activation and reduces the binding of plasminogen, plasmin and t-PA to fibrin and thus slowing clot lysis (217).

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Figure 9. Role of thrombin in the coagulation system. ICAM, intracellular cell adhesion molecule; PAI, plasminogen activator inhibitor; vWF, von Willebrand factor; IL, interleukin; TAFI, thrombin fibrinolysis activatable inhibitor; TM, thrombomodulin; APC, activated protein C and F, coagulation factor. Figure modified from Davidson SJ, 2013 (218).
Altogether, in healthy conditions thrombin and PAR-1 activation causes endothelium dependent vasodilation. On the other hand, in pathological conditions, thrombin regulates several components, which induces a procoagulatory and proinflammatory state (figure 9).

1.8.2 Fibrinolysis
The fibrinolytic system aims to dissolve and remove clots within the circulation. The zymogen plasminogen is released from the liver and is accumulated in fibrin-rich clots. Upon activation plasminogen is cleaved to plasmin by a variety of enzymes. The main regulator of fibrinolysis is t-PA released from ECs. In endotoxemia and cytokinemia the fibrinolytic system is transiently activated but thereafter depressed due to elevated PAI-1 levels. PAI-1 appears in human blood in 3 different forms, active, latent (representing an inactive form) and complexed to t-PA or urokinase-PA. Concentration of PAI-1 exceeds t-PA by a 4:1 ratio and PAI-1 binding to t-PA or urokinase-PA occurs in a ratio of 1:1, thereby effectively limiting fibrinolysis. When PAI-1 levels are increased the t-PA half-life is considerably shortened, and a negative correlation is present between PAI-1 and plasmin-antiplasmin (PAP) complexes. The complex of t-PA/PAI-1 is stable, and eliminated from the circulation by the liver. PAI-1 half-life is around 2-4 h, and has a diurnal variation with a peak at 3 a.m. and falls during morning to a lowest level in the afternoon (219). TNF-α is important in the induction of the fibrinolytic responses, while IL-6 have been shown to be most relevant for coagulation activation (202), but also enzymes like kallikrein, FXIa and FXIIa may convert plasminogen into plasmin. Plasmin acts by degrading fibrin into d-dimers. The main inhibitor of fibrinolysis and t-PA is PAI-1, while α2-antiplasmin and α2-macroglobulin inhibits plasmin.

1.8.3 Fibrinolysis and the RAAS
The RAAS may influence the fibrinolytic system since Ang II have been reported to stimulate the production of PAI-1 by ECs and VSMCs, and bradykinin (which is degraded by the ACE) to stimulate the production of t-PA (220). Ang II can increase protein activity of TF and PAI-1 activities and the activities were increased already after 4 h (10). Ang II has also been observed to increase PAI-1 and t-PA activator messenger RNA in rat ECs and aortic VSMCs (221). Additional experiments have demonstrated that Ang IV caused an increased Ang II expression and the response exhibited a fast time and a dose-dependence, while the induction of PAI-1 messenger RNA expression produced by Ang II was evident within 1 h and is maximal at 4 h (222). Blocking the RAAS by ACE inhibition after myocardial infarction has been shown to improve the fibrinolytic balance (223).

Ang II infusion also increases the expression of messenger RNA PAI-1 in the rat (224). In normotensive subjects and in hypertensive patients, Ang II infusion resulted in an increase in PAI-1 antigen, whereas no changes occurred regarding t-PA antigen (225). In conflict, Ang II did not involve any changes in PAI-1 antigen or activity in healthy subjects in other studies (226, 227). Thus, a link between PAI-1 and Ang II is at present unclear.

Altogether, in healthy conditions thrombin and PAR-1 activation causes endothelium dependent vasodilation. On the other hand, in pathological conditions, thrombin regulates several components, which induces a procoagulatory and proinflammatory state (figure 9).

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1.9 CROSSTALK BETWEEN INFLAMMATION AND COAGULATION

Evidence suggests that inflammation and haemostasis are tightly interrelated and that these two systems affect each other in a bidirectional way, i.e. inflammation may lead to activation of the haemostatic system, while the haemostatic system has an impact on the inflammatory activity (228).

Cross-talk between inflammation and haemostasis helps to explain prothrombotic states in inflammatory environments, such as the marked inflammatory response following cardiac surgery, when an uncontrolled inflammatory response leads to a profound disturbance in the coagulation system. In contrast, activated coagulation factors can directly stimulate an inflammatory response. In particular thrombin can activate receptors on mononuclear cells or ECs, which may induce cytokines and cause an inflammatory response.

1.9.1 Inflammation induced coagulation activation

Cytokines are responsible for inflammatory induced activation of haemostasis. A number of trials show the vital importance of the cytokines IL-6 in the activation of coagulation, and also important roles of IL-1, IL-8, TNF-α, and MCP-1 (201, 229). The inflammatory mediators trigger disturbance of the haemostatic system through different mechanisms:

- Endothelial dysfunction
- Activation of platelets
- Activation of coagulation cascade through TF
- Suppressed anticoagulant pathways
- Impaired fibrinolytic activity

1.9.1.1 Endothelial dysfunction

Intact endothelium offers an anti-thrombotic surface, thereby preventing coagulation activation. Under healthy conditions ECs produce components with anti-inflammatory, anticoagulant and profibrinolytic properties, but in inflammatory conditions, ECs release procoagulant and anti-fibrinolytic mediators (like vWF, TXA2 and PAI-1). Activated ECs express TF and adhesion molecules, which are of vital importance in mediating the interaction of leukocytes and platelets with the ECs, and thus in activating the coagulant system and in promoting an inflammatory response. The cytokines IL-1, IL-6, IL-8, TNF-α and the chemokine MCP-1 have a key role in mediating the procoagulant changes in ED (201, 229).

1.9.1.2 Activation of platelets

In healthy conditions platelets circulate in a resting state. Intact ECs release NO and PGII to maintain disc-shape resting platelets. Several factors contribute to the activation of platelets. EC dysfunction activates platelets via the imbalance of increased production of vWF and TXA2 and the simultaneous decrease of NO and PGII. Platelets can also be directly activated by endotoxins, cytokines such as IL-6 or PAF and VLDL (166, 230, 231). One of the important roles of IL-1, IL-8, TNF-α, and MCP-1 (201, 229). The inflammatory mediators trigger disturbance of the haemostatic system through different mechanisms:

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strongest platelet activator is thrombin, by binding on platelet receptors for thrombin (in particular PAR-1, but also PAR-4) (232).

When activated, platelets undergo a substantial shape change, and immediately express at their surface or secrete, from alpha or dense granules, proinflammatory and procoagulant substances (39). Several hundreds of different platelet derived mediators have been identified. Activated platelets synthesize TXA2, while adenosine diphosphate and ATP are released from dense granules. These mediators amplify activation of platelets and recruit circulating platelets. Activated platelets also express receptors for β1 and β3 integrins, mediating platelet adhesion and aggregation.

When platelets and ECs are activated, P-selectin is translocated to the cell surface where it functions as a PSGL-1 receptor, expressed on leukocytes (and in small amounts on platelets). The subsequent P-selectin and PSGL-1 interaction in turn increases the release of cytokines and chemokines from neutrophils and monocytes. The interaction also stimulates the up-regulation of adhesion molecules and TF on ECs and leukocytes (233).

IL-1β is a pivotal mediator in the cytokine cascade and also an important activator of ECs, by inducing the cytokines IL-6, IL-8 and chemokine MCP-1 (234). Platelet IL-1β also induces up-regulation of adhesion molecules like ICAM-1 and the vitronectin receptor (αvβ3), contributing to adhesion of neutrophils and monocytes to ECs (235).

CD40L is also a key player in platelet activation. CD40L and its receptor CD40 are expressed in cells including platelets, ECs, VSMCs, T lymphocytes and macrophages (236). When activated, platelets rapidly (within seconds) express CD40L, and the interaction of CD40L and CD40 on ECs up-regulate the cytokines IL-8 and IL-6, MCP-1 and adhesion molecules, as well as increasing TF expression (237).

Human platelets appear not to express TF when activated (238). On the other hand, activated platelets generate MPs that seem to express TF on their surface and contain negatively charged phospholipids, two factors of major importance for coagulation reactions (239). Figure 10 summarizes the most common proteins released upon platelet activation.

### 1.9.1.3 Activation of coagulation cascade through TF

The coagulation cascade in inflammatory states is mainly mediated by TF (240). Under physiological conditions TF is not expressed or is in an inactive state in circulating cells or ECs. However, various inflammatory signals, like the cytokines IL-6, IL-1 or TNF-α, can induce TF activation in leukocytes (i.e. monocytes and macrophages) and ECs. Also CRP seems to have a role in coagulation as CRP can induces TF activation via a NF-κB pathway in both ECs and VSMCs (203). Other mediators like oxLDL (241) and oxygen free radicals (242) can enhance TF activation. This enables active TF to be exposed to blood and bind to FVIII. The complex of TF and FVIII then contributes to the conversion of FIX and FX into the active proteases FXa and FXa, thereby generating thrombin.

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Figure 10. Activation of platelets. MPs have negatively charged phospholipids on their surfaces and expresses TF, αIIbβ3, also known as glycoprotein IIbIIa, the fibronogen and vWF receptor; GP Ib-IX-V, the vWF receptor; αvβ3, the vitronectin receptor; αvβ1, the fibronectin receptor; α6β1, laminin receptor; α2β1 and GPVI are the receptors for collagen; GP, glycoprotein; β-TG, beta-thromboglobulin; PAF, platelet activating factor; CD, cluster of differentiation; MCP, monocyte chemoattractant protein; IL, interleukin; PDGF, platelet-derived growth factor; EGF, epidermal growth factor; VEGF, vascular endothelium growth factor; IGF, insulin growth factor; TGF-β, transforming growth factor beta and FGF, fibroblast growth factor.

Under physiological circumstances, the presence of negatively charged membrane surfaces is limited, so even if activated coagulation factors are generated, the propagation of the coagulation cascade is limited. During inflammation activated platelets and ECs express negatively charged phospholipids on their surfaces, which is a prerequisite for assembly of activated coagulation factor complexes.

### 1.9.1.4 Suppressed anticoagulant pathways

The mechanisms that regulate coagulation activation are antithrombin (AT), the PC system and TFPI. The purpose of these systems is to prevent clotting of blood under healthy conditions.
conditions and also to dampen the activity of the coagulation system during vascular inflammation. Figure 8 summarizes the anticoagulant pathways.

In addition to their effects on coagulation they also offer anti-inflammatory properties (243). The function of these pathways is impaired during inflammatory states and represents a mechanism in which inflammation leads to a procoagulant state (244).

1.9.1.4.1 Antithrombin
AT is produced by the liver and is the most important inhibitor of thrombin and FxAs, but also FIIa, FIXa and FXIIa. Besides the anticoagulant properties AT seems to exert anti-inflammatory properties. By binding of AT to thrombin, the levels of free, active thrombin is reduced, and this contributes per se to reduced activation of platelets, leukocytes and ECs. AT also seems to have direct interaction with leukocytes by blocking interaction with ECs, i.e. blocking leukocyte adhesion and migration on ECs (245).

In inflammation, the AT pathway is impaired as a consequence of increased consumption, decreased synthesis in the liver and degradation by neutrophil elastase. Proinflammatory cytokines also cause reduced synthesis of GAGs, such as heparin sulphate, which may contribute to impaired AT function (246).

1.9.1.4.2 Protein C system
PC is a zymogen synthesized in the liver, and PC is activated when thrombin binds to TM, generating APC. This activation preferably occurs on the surface of intact endothelium. TM not only accelerates PC activation, by binding thrombin, but also directly impairs most of the procoagulant and proinflammatory effects of thrombin. The activation rate of PC is estimated to be increased 10-fold when it is adheres to the endothelial protein C receptor (EPCR) (247). APC binds reversibly to EPCR and must dissociate from the receptor before it can bind to its cofactor protein S (PS) to form the PS-APC complex that in turn inactivates FVa and FVIIa.

The PC system is the most complex anticoagulant pathway and appears to be the most important in affecting inflammation. It is considered that the APC-EPCR complex has anti-inflammatory effects (248) by blocking the NF-κB translocation to the nucleus, resulting in decreased expression of cytokines and adhesion molecules in ECs and thereby inhibits the chemotaxis and adhesion of leukocytes to ECs. APC also stimulates fibrinolysis by forming a complex with PAI-1 and this reaction increases dramatically by vitronectin (249). TM has direct anti-inflammatory effects as TM inhibits leukocyte adhesion to the endothelium by attenuating the adhesion molecule expression via NF-κB and MAPK pathways (250). EPCR may be released as a circulating, soluble protein by IL-1, thrombin or inducible metalloproteinase in the ECs. This soluble EPCR then binds to a β2-integrin on neutrophils, and probably inhibits neutrophil trafficking (251). Additionally, the thrombin-TM complex activates TAFI, and thereby inhibits fibrinolysis. The function of the PC system is impaired during inflammatory states as both TM and EPCR have been shown to be down-regulated by inflammatory cytokines (252). Also, leukocyte activation and oxidant stress decrease the conditions and also to dampen the activity of the coagulation system during vascular inflammation. Figure 8 summarizes the anticoagulant pathways.

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activity of TM and neutrophil elastase may cleave TM rapidly, leading to a decreased PC activation (253).

1.9.1.4.3 Tissue factor pathway inhibitor

TFPI can reversibly inhibit FXa, and the FXa-TFPI complex subsequently inhibits the FVIIa-TF complex. Importantly, the complex of FXa-TFPI has been shown to be a more potent inhibitor of the FVIIa-TF than TFPI itself. TFPI is secreted by ECs and attached to the endothelium via GAGs. During inflammation and exposure of proinflammatory cytokines the synthesis of GAGs is reduced and this may affect the function of TFPI. Also ROS formation inhibits TFPI binding to FXa and loss of activity (254).

1.9.1.5 Impaired fibrinolytic activity

The fibrinolytic system controls haemostasis, and the pivotal enzyme, plasmin degrades the fibrin clot. The plasminogen activators generate plasmin from plasminogen. The major inhibitor of t-PA and urokinase-Pa, is PAI-1. When PAI-1 binds to the plasminogen activators, inactivation of these plasminogen activators takes place, and consequently the activity of the fibrinolysis is suppressed.

The ratio between t-PA and PAI-1 modulates the fibrinolytic activity (255). The immediate response during inflammatory stimuli is a transient increase in the secretion of t-PA from Weibel-Palade bodies in ECs (256). This increase in the fibrinolytic activation is followed by a delayed suppression of the t-PA production and a sustained increase in PAI-1, resulting in a suppression of the fibrinolytic activity. The major regulators of PAI-1 activity at inflammatory sites seem to be cytokines, such as IL-6, IL-1β and TNF-α (219, 257). CRP has also been shown to stimulate the expression of PAI-1 (34). Alpha granules in platelets also contain PAI-1 that can be released upon activation, which increases PAI-1 and thereby contributes to suppression of fibrinolysis in inflammatory states. High levels of PAI-1 and t-PA have been shown to predict development of a first cardiovascular event (258).

1.9.2 Coagulation induced inflammatory activation via PARs

Inflammation induces activation of the coagulation cascade, and the coagulation pathway triggers an intracellular inflammatory pathway. This cross-talk causes a positive feedback loop which amplifies an inflammation-thrombosis circuit (259).

The coagulation factors can induce vascular inflammation by their binding to PARs (260). PARs are present in ECs, leukocytes, platelets, fibroblasts and VSMCs (260). An exceptional characteristic of PARs is that these receptors carry their own ligand, which is unmasked until the receptor is cleaved. Thrombin is known to be the most essential player in activation of PARs, and can activate PAR-1, PAR-3 as well as PAR-4. FXa transmits activation of PAR-1 to PAR-3, while TF-FVIIa transmits activation of PAR-2 (260).

PAR activation causes an up-regulation of inflammatory molecules, like cytokines, chemokines, growth factors and adhesion molecules. Experiments in healthy human subjects activity of TM and neutrophil elastase may cleave TM rapidly, leading to a decreased PC activation (253).

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TFPI can reversibly inhibit FXa, and the FXa-TFPI complex subsequently inhibits the FVIIa-TF complex. Importantly, the complex of FXa-TFPI has been shown to be a more potent inhibitor of the FVIIa-TF than TFPI itself. TFPI is secreted by ECs and attached to the endothelium via GAGs. During inflammation and exposure of proinflammatory cytokines the synthesis of GAGs is reduced and this may affect the function of TFPI. Also ROS formation inhibits TFPI binding to FXa and loss of activity (254).

1.9.1.5 Impaired fibrinolytic activity

The fibrinolytic system controls haemostasis, and the pivotal enzyme, plasmin degrades the fibrin clot. The plasminogen activators generate plasmin from plasminogen. The major inhibitor of t-PA and urokinase-Pa, is PAI-1. When PAI-1 binds to the plasminogen activators, inactivation of these plasminogen activators takes place, and consequently the activity of the fibrinolysis is suppressed.

The ratio between t-PA and PAI-1 modulates the fibrinolytic activity (255). The immediate response during inflammatory stimuli is a transient increase in the secretion of t-PA from Weibel-Palade bodies in ECs (256). This increase in the fibrinolytic activation is followed by a delayed suppression of the t-PA production and a sustained increase in PAI-1, resulting in a suppression of the fibrinolytic activity. The major regulators of PAI-1 activity at inflammatory sites seem to be cytokines, such as IL-6, IL-1β and TNF-α (219, 257). CRP has also been shown to stimulate the expression of PAI-1 (34). Alpha granules in platelets also contain PAI-1 that can be released upon activation, which increases PAI-1 and thereby contributes to suppression of fibrinolysis in inflammatory states. High levels of PAI-1 and t-PA have been shown to predict development of a first cardiovascular event (258).

1.9.2 Coagulation induced inflammatory activation via PARs

Inflammation induces activation of the coagulation cascade, and the coagulation pathway triggers an intracellular inflammatory pathway. This cross-talk causes a positive feedback loop which amplifies an inflammation-thrombosis circuit (259).

The coagulation factors can induce vascular inflammation by their binding to PARs (260). PARs are present in ECs, leukocytes, platelets, fibroblasts and VSMCs (260). An exceptional characteristic of PARs is that these receptors carry their own ligand, which is unmasked until the receptor is cleaved. Thrombin is known to be the most essential player in activation of PARs, and can activate PAR-1, PAR-3 as well as PAR-4. FXa transmits activation of PAR-1 to PAR-3, while TF-FVIIa transmits activation of PAR-2 (260).

PAR activation causes an up-regulation of inflammatory molecules, like cytokines, chemokines, growth factors and adhesion molecules. Experiments in healthy human subjects
have shown that recombinant FVIIa results in a 4-fold increase in the concentrations of IL-8 and IL-6 in plasma (261). Activation of PARs transforms ECs into a proinflammatory phenotype, causing vascular permeability and local accumulation of platelets and leucocytes. In VSMCs, PAR activation mediates contraction, proliferation, migration, hypertrophy and modulation of the ECM, thereby contributing to atherosclerosis and hypertension. Platelets exhibit a pivotal role in the cross-talk between coagulation and inflammation, and are activated directly by thrombin or by proinflammatory mediators, such as PAF (230). The platelet P-selectins not only mediate adherence to leukocytes and ECs, but also enhance monocyte TF expression (262). Figure 11 summarizes PAR activation.

Figure 11. PAR activation. Exposure of TF-bearing cells results in generation of thrombin. Activation of platelets plays a pivotal role. Binding of TF-FVIIa complex or thrombin to specific PARs may affect inflammation by inducing release of cytokines, which modulates coagulation and fibrinolysis. PAR, protease-activated receptors; IL, interleukin; TLR, Toll-like receptor; CD, cluster of differentiation; PF, platelet factor and RANTES, regulated on activation, normal T-cell expressed and secreted. Figure modified from Levi M, 2012 (263).
2 AIMS

Hypertension, FCHL and FH have an increased risk of CVD and premature death. Ang II is implicated in hypertension and promotes atherosclerosis, and patients with hyperlipidaemia may be more sensitive to the potential proinflammatory and procoagulatory effects of Ang II. Antihypertensive treatment may reduce thromboembolic events in hypertension. Thus, the overall aims were investigate the potential importance of Ang II for haemostatic and inflammatory alterations in hypertension and hyperlipidaemia.

The specific aims of this project were as follows:

- To investigate the effects of blocking the RAAS with an ACE inhibitor on blood coagulation in subjects with hypertension (paper I).
- To clarify the impact of antihypertensive treatment by blocking the RAAS on inflammation and haemostasis beyond the effects of BP by comparing the effects of an ACE inhibitor to treatment with an alpha 1-adrenoceptor blocker in subjects with hypertension (paper II).
- To investigate the potential proinflammatory and prothrombotic effects of short-term stimulation by circulating Ang II in healthy individuals (papers III-V).
- To study the potential differences in the effects of circulating Ang II on inflammation and haemostasis in patients with FCHL and FH compared to healthy subjects (papers III-V).
3 MATERIALS AND METHODS

3.1 PATIENTS AND HEALTHY CONTROLS

None of the patients in paper I-V had any history or evidence of CVD, congestive heart failure, diabetes mellitus, atrial fibrillation/flutter, autoimmune disorder, liver or chronic kidney disease.

3.1.1 Papers I and II

3.1.1.1 Study population and procedures, paper I

Subjects with hypertension were included if their diastolic BP was 95-115 mmHg without antihypertensive treatment the previous 4 weeks. Details about the study, presenting the result that ACE inhibitors reduced cardiac workload during stressful situations, have been presented previously (264). In brief, 6 women and 10 men with mean age 51 years and mean body mass index 26 kg/m² (range 21-35 kg/m²) were included. During the study, medication that might affect the coagulation or BP was not allowed. The participants had stage I hypertension according to the WHO classification, with no signs of target organ damage or characteristics that could indicate secondary hypertension. The duration of the hypertension was on average 10 years (range 6-13 years).

The participants were given 5 mg ramipril or placebo once daily, during 6 weeks, in a double-blind cross-over design. All participants then continued into an open treatment with ramipril during a 6-month period. During this phase, dose titration of ramipril was allowed once daily. The goal was to achieve a diastolic BP lower than 95 mmHg, and the mean dose of ramipril was finally 7.5 mg once daily. Testing in the laboratory was carried out at 3 times: at 6 and 12 weeks, and after ramipril treatment at 6 months. The participants arrived to the laboratory at 12 AM. They were instructed to take their study medication at 08-09 AM in order to reach an estimated peak plasma drug concentration during testing. They were instructed to avoid smoking and refrain from caffeine products during the morning.

Blood was collected from antecubital veins into test tubes containing 0.13 mmol/L sodium citrate immediately before and after each mental stress test. The blood samples were then without delay centrifuged at 2000g for 20 minutes at 20°C, and stored at -80°C until analysis.

3.1.1.2 The mental stress test

In paper I patients underwent a mental stress test that was a modified video version of Stroop’s colour word conflict test during 20 minutes (264). During this test colour words were rapidly shown in incongruent colours, at the same time, a third incongruent colour word was presented orally. The participants were instructed to mark the colour on a protocol with randomly listed colour words and to ignore the other two conflicting information pieces. In this test a hemodynamic response will reach a steady state within 8-10 minutes and the responses are reproducible. After the mental stress test followed a strict 30 minutes of quiet
seated rest. Heart rate and BP were measured by a noninvasive technique (Dinamap Exercise Monitor, Critikon Inc., Tampa, FL).

3.1.1.3 Study population and procedures, paper II
Patients were included if their office systolic BP was >140 mm Hg and/or their diastolic BP was >90 mm Hg. They were randomized to double-blind treatment (stratified by sex) with ramipril 5 mg once daily or doxazosin 4 mg once daily for 2 weeks. During the initial phase, the dose was titrated to ramipril 10 mg or doxazosin 8 mg once daily. Thereafter the participants were treated during an additional 10 weeks. Prior treatment with RAAS blockade was not allowed. We ruled out secondary hypertension by routine biochemical and physical examinations. Patients with WH0 stage III hypertension were not included.

We randomized 71 patients (63 were previously never treated for hypertension). In all, 5 women and 5 men discontinued due to reported side effects (8 on doxazosin and 2 on ramipril). One female patient in the ramipril group and one male patient in the doxazosin group were excluded because of difficulties to obtain blood samples. Thus, 59 patients, achieved the targeted 10 weeks of treatment with 8 mg doxazosin once daily or 10 mg ramipril once daily (table 3).

Table 3. Patient characteristics and demographic information in paper II.

<table>
<thead>
<tr>
<th></th>
<th>ramipril</th>
<th>doxazosin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female (n)</td>
<td>20/12</td>
<td>19/8</td>
</tr>
<tr>
<td>Age (years)</td>
<td>54 ± 13</td>
<td>53 ± 11</td>
</tr>
<tr>
<td>Smokers (n)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26 ± 4</td>
<td>27 ± 5</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.2 ± 0.5</td>
<td>5.5 ± 0.4</td>
</tr>
<tr>
<td>Office systolic BP (mm Hg)</td>
<td>155 ± 9</td>
<td>151 ± 8</td>
</tr>
<tr>
<td>Office systolic BP (mm Hg)</td>
<td>93 ± 7</td>
<td>93 ± 10</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>75 ± 15</td>
<td>77 ± 12</td>
</tr>
<tr>
<td>eGFR (mL/minute/1.73m²)</td>
<td>93 ± 15</td>
<td>92 ± 13</td>
</tr>
<tr>
<td>Leucocyte count (10⁹/L)</td>
<td>5.2 ± 1.4</td>
<td>5.0 ± 1.0</td>
</tr>
<tr>
<td>Platelet count (10⁹/L)</td>
<td>214 ± 43</td>
<td>219 ± 50</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.3 ± 0.8</td>
<td>5.5 ± 1.3</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.3 ± 0.4</td>
<td>1.4 ± 0.4</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>3.5 ± 0.8</td>
<td>3.5 ± 1.1</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.1 ± 1.0</td>
<td>1.1 ± 0.8</td>
</tr>
</tbody>
</table>

Data are presented as mean ± values SD. Blood samples were taken fasting. No significant differences existed between the ramipril and doxazosin groups. BP, blood
pressure values obtained in the office on inclusion; eGFR, estimated glomerular filtration rate, which was calculated using the chronic kidney disease epidemiology collaboration formula; HDL, high density lipoproteins; and LDL, low density lipoproteins

After fasting overnight the participants arrived in the morning for the examinations at baseline and at week 12. The patients were asked to take their study medication 2 h before they arrived to the laboratory, to achieve peak plasma concentrations. They were instructed not to smoke and to refrain from caffeine-containing beverages, fruit juices or vitamin C during the morning, and to refrain from any other medication (including thrombocyte inhibitory drugs for 7 days and non-steroid anti-inflammatory drugs for 2 days) prior to the examinations. Fasting blood samples were obtained by blood collection needles (Eclipse, 21G x 1-1/4”) after 20 minutes of supine rest into Vacutainer tubes (Becton Dickinson Co. Cedex, Meylan, France).

### 3.1.2 Paper III-V

#### 3.1.2.1 Study population

In papers III and IV, FCHL was thought to exist if the lipoprotein phenotypes IIa, IIb, or IV, according to the Fredrickson classification (22), were found in the family or in the patient at several different times. There were 5 patients on lipid lowering therapy with statins and 1 was on antihypertensive treatment with an ACE inhibitor (table 4).

<table>
<thead>
<tr>
<th>FCHL</th>
<th>ControlECDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female (n)</td>
<td>11/5</td>
</tr>
<tr>
<td>Age (years)</td>
<td>47 ± 6</td>
</tr>
<tr>
<td>Smokers (n)</td>
<td>5</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27 ± 3*</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>130 ± 13</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>84 ± 8</td>
</tr>
<tr>
<td>Pulse pressure (mm Hg)</td>
<td>46 ± 13</td>
</tr>
<tr>
<td>Heart rate (beats per minute)</td>
<td>64 ± 9</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.2 ± 0.9</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>8.4 ± 2.3****</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>0.8 ± 0.2**</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>3.4 ± 1.8</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>7.4 ± 5.7****</td>
</tr>
</tbody>
</table>

Data are presented as mean ± values SD. Statistical evaluation was performed by Student’s t-test. Significant differences between FCHL and controlECDL are denoted as: *P

Table 4. Patient characteristics of participants in paper III and IV.

<table>
<thead>
<tr>
<th>FCHL</th>
<th>ControlECDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female (n)</td>
<td>11/5</td>
</tr>
<tr>
<td>Age (years)</td>
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</tr>
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</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
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</tr>
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Data are presented as mean ± values SD. Statistical evaluation was performed by Student’s t-test. Significant differences between FCHL and controlECDL are denoted as: *P

After fasting overnight the participants arrived in the morning for the examinations at baseline and at week 12. The patients were asked to take their study medication 2 h before they arrived to the laboratory, to achieve peak plasma concentrations. They were instructed not to smoke and to refrain from caffeine-containing beverages, fruit juices or vitamin C during the morning, and to refrain from any other medication (including thrombocyte inhibitory drugs for 7 days and non-steroid anti-inflammatory drugs for 2 days) prior to the examinations. Fasting blood samples were obtained by blood collection needles (Eclipse, 21G x 1-1/4”) after 20 minutes of supine rest into Vacutainer tubes (Becton Dickinson Co. Cedex, Meylan, France).

### 3.1.2 Paper III-V

#### 3.1.2.1 Study population

In papers III and IV, FCHL was thought to exist if the lipoprotein phenotypes IIa, IIb, or IV, according to the Fredrickson classification (22), were found in the family or in the patient at several different times. There were 5 patients on lipid lowering therapy with statins and 1 was on antihypertensive treatment with an ACE inhibitor (table 4).

<table>
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<tr>
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</thead>
<tbody>
<tr>
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<td>11/5</td>
</tr>
<tr>
<td>Age (years)</td>
<td>47 ± 6</td>
</tr>
<tr>
<td>Smokers (n)</td>
<td>5</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27 ± 3*</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>130 ± 13</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>84 ± 8</td>
</tr>
<tr>
<td>Pulse pressure (mm Hg)</td>
<td>46 ± 13</td>
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<tr>
<td>Heart rate (beats per minute)</td>
<td>64 ± 9</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
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<tr>
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</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
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</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>3.4 ± 1.8</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>7.4 ± 5.7****</td>
</tr>
</tbody>
</table>

Data are presented as mean ± values SD. Statistical evaluation was performed by Student’s t-test. Significant differences between FCHL and controlECDL are denoted as: *P
**Table 5.** Patient characteristics in paper IV.

<table>
<thead>
<tr>
<th></th>
<th>FCHL</th>
<th>Control/ONAL</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female (n)</td>
<td>11/5</td>
<td>9/7</td>
<td>4/4</td>
</tr>
<tr>
<td>Insulin (mU/L)</td>
<td>10.0  (5.9-12.2)***</td>
<td>3.6 (3.0-4.4)</td>
<td>3.9 (3.1-6.6)</td>
</tr>
<tr>
<td>Glucose (mM/L)</td>
<td>5.2 ± 0.9</td>
<td>4.8 ± 0.4</td>
<td>5.0 ± 0.3</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.2 (1.3-3.0)***</td>
<td>0.7 (0.6-0.9)</td>
<td>0.8 (0.6-1.4)</td>
</tr>
</tbody>
</table>

Data are presented as mean values ± SD or as median and interquartile ranges. Statistical evaluation was performed by Student’s t-test or Mann-Whitney non-parametric test. Significant differences between FCHL and control/ONAL are denoted as: *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001. FCHL, familial combined hyperlipidaemia, HDL and LDL, high and low density lipoproteins.

In paper IV, we characterized the FCHL patients in paper III with respect to insulin resistance by HOMA-IR (calculated as fasting insulin in mU/L x glucose in mM/L/22.5). Placebo experiments with saline infusion were added to verify the stability of the experimental design and to assess the potential influence of diurnal variations (table 5).

3.1.2.2 Healthy controls

Age-matched healthy control subjects were recruited from the local area by advertisements and from hospital staff.

In paper III and IV the healthy subjects (9 males and 7 females) had fasting plasma triglyceride levels less than 2.3 mM/L and total cholesterol levels less than 6.0 mmol/L. Systolic and diastolic BPs were less than 130 and 80 mm Hg, respectively. All healthy controls were free of medicines, including oral contraceptives.

In paper V the healthy subjects (8 males and 8 females) had fasting plasma triglyceride levels less than 1.6 mmol/L and total cholesterol levels less than 5.6 mmol/L. Systolic and diastolic BPs were less than 140 and 85 mm Hg, respectively. All healthy controls were free of medicines, including oral contraceptives.
**Table 6. Patient characteristics in paper V.**

<table>
<thead>
<tr>
<th></th>
<th>FH</th>
<th>Control_1</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female (n)</td>
<td>8/8</td>
<td>8/8</td>
<td>4/4</td>
</tr>
<tr>
<td>Age (years)</td>
<td>43 ± 8</td>
<td>39 ± 10</td>
<td>40 ± 9</td>
</tr>
<tr>
<td>Current/former smokers (n)</td>
<td>0/5</td>
<td>0/7</td>
<td>0/3</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27 (24-29)</td>
<td>24 (21-27)</td>
<td>24 (22-26)</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>127 ± 14*</td>
<td>116 ± 12</td>
<td>116 ± 13</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>75 ± 8</td>
<td>73 ± 8</td>
<td>69 ± 7</td>
</tr>
<tr>
<td>Pulse pressure (mm Hg)</td>
<td>48 (44-64)**</td>
<td>42 (36-48)</td>
<td>41 (40-52)</td>
</tr>
<tr>
<td>Heart rate (beats/minute)</td>
<td>64 ± 11</td>
<td>63 ± 13</td>
<td>56 ± 7</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.0 ± 0.4</td>
<td>5.2 ± 0.4</td>
<td>5.0 ± 0.3</td>
</tr>
<tr>
<td>Insulin (mU/L)</td>
<td>6.4 (5.2-7.6)</td>
<td>4.6 (2.8-6.6)</td>
<td>3.9 (3.1-6.6)</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.5 (1.1-1.7)</td>
<td>1.0 (0.6-1.6)</td>
<td>0.8 (0.6-1.4)</td>
</tr>
<tr>
<td>Total chol (mmol/L)</td>
<td>8.6 ± 1.8****</td>
<td>4.4 ± 0.7</td>
<td>5.1 ± 0.6</td>
</tr>
<tr>
<td>HDL chol (mmol/L)</td>
<td>1.0 ± 0.3</td>
<td>1.2 ± 0.4</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>Non-HDL chol (mmol/L)</td>
<td>7.5 ± 1.9****</td>
<td>3.2 ± 0.8</td>
<td>3.9 ± 1.4</td>
</tr>
<tr>
<td>LDL chol (mmol/L)</td>
<td>6.8 ± 1.8****</td>
<td>2.8 ± 0.8</td>
<td>3.4 ± 1.1</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.3 (0.9-1.6)***</td>
<td>0.7 (0.5-1.0)</td>
<td>0.9 (0.6-1.2)</td>
</tr>
</tbody>
</table>

Data are presented as mean values ± SD or as median and interquartile ranges. Statistical evaluation was performed by Student’s t test or Mann-Whitney non-parametric test. Significant differences between FH and control_1 are denoted as: *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001. BP, blood pressure; HOMA-IR, Homeostasis model assessment of insulin resistance; chol, cholesterol; HDL, High-density lipoprotein and LDL, low-density lipoprotein.

### 3.1.2.3 Study procedures and blood sampling

All participants on antihypertensive or lipid lowering therapy were instructed to quit their medication 4 weeks before the start of the study. The subjects were asked to avoid smoking on the day of the investigation and avoid taking non-steroid anti-inflammatory or aspirin drugs at least 7 days prior to the investigation. After an overnight fast, the participants arrived at the Cardiovascular Research Laboratory between 07.00 and 08.00 a.m. For the Ang II infusion, an indwelling catheter was applied in supine position in a vein of the left arm. Blood was collected by Vacutainer technique, using blood collection needles (Eclipse, 21G x 1-1/4") inserted in a vein of the right arm. The participants rested in the supine position during 20-30 minutes before the investigations started. BP was measured using a mercury sphygmomanometer technique. BP values are presented as the calculated mean of two separate measurements, taken about one minute apart.

---

**Table 6. Patient characteristics in paper V.**

<table>
<thead>
<tr>
<th></th>
<th>FH</th>
<th>Control_1</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female (n)</td>
<td>8/8</td>
<td>8/8</td>
<td>4/4</td>
</tr>
<tr>
<td>Age (years)</td>
<td>43 ± 8</td>
<td>39 ± 10</td>
<td>40 ± 9</td>
</tr>
<tr>
<td>Current/former smokers (n)</td>
<td>0/5</td>
<td>0/7</td>
<td>0/3</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27 (24-29)</td>
<td>24 (21-27)</td>
<td>24 (22-26)</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>127 ± 14*</td>
<td>116 ± 12</td>
<td>116 ± 13</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>75 ± 8</td>
<td>73 ± 8</td>
<td>69 ± 7</td>
</tr>
<tr>
<td>Pulse pressure (mm Hg)</td>
<td>48 (44-64)**</td>
<td>42 (36-48)</td>
<td>41 (40-52)</td>
</tr>
<tr>
<td>Heart rate (beats/minute)</td>
<td>64 ± 11</td>
<td>63 ± 13</td>
<td>56 ± 7</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.0 ± 0.4</td>
<td>5.2 ± 0.4</td>
<td>5.0 ± 0.3</td>
</tr>
<tr>
<td>Insulin (mU/L)</td>
<td>6.4 (5.2-7.6)</td>
<td>4.6 (2.8-6.6)</td>
<td>3.9 (3.1-6.6)</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.5 (1.1-1.7)</td>
<td>1.0 (0.6-1.6)</td>
<td>0.8 (0.6-1.4)</td>
</tr>
<tr>
<td>Total chol (mmol/L)</td>
<td>8.6 ± 1.8****</td>
<td>4.4 ± 0.7</td>
<td>5.1 ± 0.6</td>
</tr>
<tr>
<td>HDL chol (mmol/L)</td>
<td>1.0 ± 0.3</td>
<td>1.2 ± 0.4</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>Non-HDL chol (mmol/L)</td>
<td>7.5 ± 1.9****</td>
<td>3.2 ± 0.8</td>
<td>3.9 ± 1.4</td>
</tr>
<tr>
<td>LDL chol (mmol/L)</td>
<td>6.8 ± 1.8****</td>
<td>2.8 ± 0.8</td>
<td>3.4 ± 1.1</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.3 (0.9-1.6)***</td>
<td>0.7 (0.5-1.0)</td>
<td>0.9 (0.6-1.2)</td>
</tr>
</tbody>
</table>

Data are presented as mean values ± SD or as median and interquartile ranges. Statistical evaluation was performed by Student’s t test or Mann-Whitney non-parametric test. Significant differences between FH and control_1 are denoted as: *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001. BP, blood pressure; HOMA-IR, Homeostasis model assessment of insulin resistance; chol, cholesterol; HDL, High-density lipoprotein and LDL, low-density lipoprotein.

### 3.1.2.3 Study procedures and blood sampling

All participants on antihypertensive or lipid lowering therapy were instructed to quit their medication 4 weeks before the start of the study. The subjects were asked to avoid smoking on the day of the investigation and avoid taking non-steroid anti-inflammatory or aspirin drugs at least 7 days prior to the investigation. After an overnight fast, the participants arrived at the Cardiovascular Research Laboratory between 07.00 and 08.00 a.m. For the Ang II infusion, an indwelling catheter was applied in supine position in a vein of the left arm. Blood was collected by Vacutainer technique, using blood collection needles (Eclipse, 21G x 1-1/4") inserted in a vein of the right arm. The participants rested in the supine position during 20-30 minutes before the investigations started. BP was measured using a mercury sphygmomanometer technique. BP values are presented as the calculated mean of two separate measurements, taken about one minute apart.

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Blood samples were taken at baseline, at 1 and/or 3 h, and 1 h after the infusion of Ang II. Blood was collected into Vacutainer tubes (Becton Dickinson, Meylan, France) that contained EDTA or sodium citrate (3.8%), as appropriate. The blood samples were immediately centrifuged at 2000g, at 20°C for 20 minutes, and then separated and administered in aliquots (0.5 mL) and stored in -80°C until analysis.

3.1.2.4 Ang II infusion

In paper III and IV we used Ang II (Angiotensin amid, Apoteksbolaget, Umeå, Sweden) while we used Ang II acetate (Clinalfa basic, Bachem AG, Basel, Switzerland) in paper V.

Ang II was dissolved in physiological saline. The starting dose was 2 ng/kg/minute and every 5 minutes the dose was incremented with 2 ng/kg/minute up to 10 ng/kg/minute. This dose was then maintained for 3 h. We have used the dose of 10 ng/kg/minute earlier (13) and this dose has been shown to increase plasma Ang II concentrations to about 50 pmol/L, which has been estimated to be equal to 5 to 10 times basal levels in healthy subjects (265).

If mean arterial pressure levels increased from the levels before the infusion began by more than 25 mm Hg, the infusion rate was reduced in steps of 2 ng/kg/minute due to a decision from the Regional Ethics Committee.

3.1.2.5 Placebo infusion

In paper IV and V we added separate experiments with placebo infusion (physiological saline) in eight subjects (4 women) to check the stability of the experimental design and to assess the potential influence of diurnal variations. The placebo participants had fasting triglyceride levels less than 2.2 mmol/L and total cholesterol levels less than 7.1 mmol/L, respectively, and their systolic BP and diastolic BP levels were less than 120 and 80 mm Hg, respectively.

3.2 METHODS

3.2.1 Calibrated automated thrombogram

In paper II and V thrombin generation potential was measured according to the method calibrated automated thrombogram (CAT), reported by Hemker et al., and in accordance to the manufacturer instructions (Thrombinoscope BV, Maastricht, the Netherlands) (266). We used Thrombinoscope BV reagents.

The reactions were carried out in 96-well microtiter plates (Immulon 2HB transparent U-bottom from Thermo Electron, Denmark). Two wells were needed for each experiment, one well to measure thrombin generation of a plasma sample and another for calibration. Briefly, 80 μL platelet-poor plasma was mixed with 20 μL of a platelet poor-plasma reagent containing TF and phospholipids reaching final concentrations of TF of 5 pM and phospholipids of 4 μM. In addition, 80 μL of the same platelet-poor plasma reagent was mixed with a thrombin calibrator. To start the reactions in wells for calibration and measurement, a fluorogenic...
substance, specifically cleaved by thrombin, was then added together with CaCl₂ in Hepes buffer.

The fluorescence was analysed every ½ minute during 1 h by a Fluoroscan Ascent fluorometer (Thermo Scientific Vanta, Finland). A commercial software by the manufacturer (Thrombinoscope version 2007) calculated and presented five variables:

- Peak thrombin concentration. The peak concentration of thrombin generated
- Endogenous thrombin potential. The area under the curve that corresponds to the total amount of thrombin generated over 1 h.
- Lag-time. The time interval until the beginning of thrombin generation. Corresponds to the clotting time
- Time to peak thrombin concentration. The time interval until the peak thrombin concentration. Takes into account the amplification and propagation phases
- Time to tail. Time that elapsed until the end of the thrombin generation, i.e. inhibition of thrombin generation by various anticoagulants.

Figure 12. The five variables calculated in calibrated automated thrombogram, ETP, endogenous thrombin potential and AUC, area under curve.

For example, a hypocoagulability state is characterized by a prolonged lag-time, and reductions in both peak thrombin and endogenous thrombin potential. On the other hand, a hypercoagulability state is characterized by a reduced lag-time and increased peak thrombin and endogenous thrombin potential values.

### 3.2.2 Other laboratory methods

We used commercially kits and calibrators to analyse quantities of inflammatory and haemostatic markers.
3.2.2.1 Paper I
Fibrinogen was measured by a polymerization time method. TAT complex was analysed by using an enzyme immunoassay (Enzygnost TAT Micro, Behringwerke AG, Marburg, Germany). FVII was detected by an amidolytic method measuring all FVII activity generated after thromboplastin activator addition to the test tubes.

3.2.2.2 Paper II
TAT complex was determined by using an enzyme immunoassay (Enzygnost TAT Micro; Behringwerke AG, Marburg, Germany). PAI-1 activity was analysed by using an enzyme immunoassay (TriniLIZE PAI-1 activity, Teaghlaidheagair Ltd., Ireland). T-PA antigen was determined by using assays from R&D Systems (Abingdon, UK). IL-6, IL-8, IL-68, high sensitive (hs) CRP, TNF-α and MCP-1 were analysed by using assays from MesoScale Diagnostic (Human Cytokine Assay, Ultra-Sensitive Kit, MSD, Bethesda, USA).

3.2.2.3 Paper III
hsIL-6, TNF-α and t-PA/PAI-1 complexes were assessed by using assays from R&D Systems (Abingdon, UK). F1+2 and TAT complex concentrations were determined by using enzyme assays from Behring-Werke AG (Marburg, Germany).

3.2.2.4 Paper IV
PAI-1 activity was analysed by an assay from Hyphen BioMed (Neuville-sur-Oise, France). PAP complex was measured by a classical two-site ELISA (267).

3.2.2.5 Paper V
PAI-1 activity was analysed by using an assay from Hyphen BioMed (Neuville sur Oise, France). PAP complex was determined by a classical two-site ELISA (267). F1+2 concentrations were analysed by using assays from Siemens Healthcare (Marburg, Germany). hsIL-6 was analysed by using assays from R&D Systems (Abingdon, UK). Fibrinogen levels were determined by means of a Fibri-Prest Automate method (von Clauss method) from Diagnostica Stago (Asnieres, France).

3.2.2.6 Routine analyses
3.2.2.6.1 Paper II
Leukocyte counts were analysed by using an automated blood cell counter (Technicon H1, Hematology System, Technicon Instruments Corp. Tarrytown, NY, USA). Plasma glucose was determined by an automated routine method (Synchrom LX, Beckman Coulter, Inc., Fullerton, CA, USA). The cholesterol and triglyceride levels were analysed by standard enzymatic techniques (Boehringer-Mannheim, Mannheim, Germany).
Leukocyte counts were determined by using an automated blood cell counter (Technicon H1, Hematology System, Technicon Instruments Corp., Tarrytown, NY, USA). Plasma glucose was determined by an automated routine method (Synchro TDX, Beckman Coulter, Inc., Fullerton, CA, USA). The various lipoprotein particles were analysed by precipitation and centrifugation steps. The cholesterol and triglyceride levels were analysed by standard enzymatic techniques (Boehringer-Mannheim, Mannheim, Germany).

HsCRP was determined by turbidimetry (Beckman Coulter, Fullerton, California, USA). Insulin levels were determined by an automated immunometric sandwich method (Modular E170, Roche Diagnostics GmbH, Mannheim, Germany) and detection with electrochemiluminescence immunoassay. Insulin resistance was assessed by the homeostasis model assessment of insulin resistance (HOMA-IR, by the formula; insulin in mU/L x glucose in mmol/L/22.5).

Leukocyte counts and hsCRP count were analysed by using an automated blood cell counter (Technicon H1, Hematology System, Technicon Instruments Corp., Tarrytown, NY, USA). Insulin resistance was assessed by HOMA-IR. Plasma creatinine, glucose, cholesterol and triglyceride contents of the various lipoprotein fractions were assessed by automated standard methods.

3.3 STATISTICAL ANALYSES

Statistical calculations in papers I-V were performed using Statistica/99 software, version 7.7, series: 1205 (Statsoft Inc., Tulsa, Oklahoma, USA). Normality was considered to be present if skewness was more than -1 and less than 1. Normally distributed data are presented as mean ± standard deviation (SD), whereas skewed data are presented as median values and interquartile ranges. Variables with skewed distribution were logarithmically transformed. A probability (P) less than 0.05 was considered statistically significant.

3.3.1 Paper I

We used non-parametric tests, by analysis of variance (ANOVA, Friedman test) with appropriate post hoc testing or by paired comparisons (Wilcoxon signed rank test), as appropriate. We estimated that the study, with 2 alpha 0.05, would require 15 participants to offer a power of 0.80 to detect a TAT complex difference of 0.4 µg/L by treatment, with a SD 0.5 µg/L.

3.3.1.2 Paper II

Graphing techniques were used to assess outliers. Extreme values were considered invalid if exceeding 2 SD beyond mean values for the given time and treatment group, as proposed by
Vaughan et al (91), also after logarithmic transformation. For TAT complex, extreme outlier values were present in 5/27 on doxazosin and in 4/32 on ramipril; these values were replaced by the mean for the given time and treatment group. As for the other markers of inflammation and coagulation, only single outliers were identified and excluded. Comparison between study groups and the effects of treatment were made by repeated measures multivariable analysis of variance (MANOVA), accounting for the potential confounding of smoking. Analysis of changes in TAT complex levels at baseline and after 3 months of treatment with ramipril and doxazosin were made by using non-parametric tests by paired comparisons (Wilcoxon signed rank test). Assuming 2 alpha 0.05 and a power of 0.80, we estimated a study population of 2 x 26 subjects in order to detect a 0.4 µg/L difference in TAT complex by treatment, with a SD 0.5 µg/L.

3.3.3.3 Paper III-IV
Changes within and between the groups at baseline were analysed by Student’s t-test or Mann-Whitney non-parametric test, as appropriate. The responses of Ang II infusion on BP, heart rate, markers of inflammation and haemostasis were determined by repeated measures ANOVA. We estimated that these studies, with 2 alpha 0.05, would require 15 subjects to have a power of 0.80 to detect a TAT complex difference of 0.4 µg/L, by infusion of Ang II, with a SD 0.5 µg/L.

3.3.3.4 Paper V
Changes within and between groups at baseline were investigated by Student’s t-test or Mann-Whitney non-parametric test, as appropriate. The responses of Ang II infusion on BP, heart rate, inflammatory and haemostatic markers were analysed by repeated measures ANOVA. Post hoc calculation indicated that the study, with 2 alpha 0.05, would require 14 subjects to have a power of 0.80 to detect a PAI-1 activity difference of 1.0 ng/mL by Ang II infusion, with a SD 1.3 ng/mL.

3.4 ETHICAL CONSIDERATIONS
The studies were performed in accordance with the Declaration of Helsinki (1989) of the World Medical Association. All studies were approved by the Regional Ethics Committee of Karolinska University Hospital in Stockholm. We received informed consent from all participants.
4 RESULTS

4.1 PAPER I

4.1.1 Effects on blood pressure and heart rate

The results are presented in table 7. Systolic and diastolic BPs were reduced by treatment with ramipril, no effects were observed on heart rate. Placebo during 6 weeks did not affect BP or heart rate (data not shown). Systolic BP and heart rate responses during mental stress were attenuated by ramipril, for details, see (264).

Table 7. Effects of ramipril treatment at rest and during mental stress.

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<td></td>
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<td>Systolic BP</td>
<td>154 (138-165)</td>
<td>146 (128-154)*</td>
<td>140 (131-165)</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>106 (94-110)</td>
<td>98 (86-109)***</td>
<td>94 (87-103)**</td>
</tr>
<tr>
<td>Heart rate</td>
<td>71 (66-73)</td>
<td>72 (66-80)</td>
<td>73 (67-78)</td>
</tr>
</tbody>
</table>

Data are presented as median values and interquartile ranges during rest and following 20 minutes of mental stress (n=15-16). Statistical evaluation was made by using non-parametric tests by paired comparisons (Wilcoxon signed rank test). Significant differences are given as: *P < 0.05, **P < 0.01, ***P < 0.001 compared to placebo. W, weeks; m, months and BP, blood pressure.

4.1.2 Effects on coagulation

The results are presented in table 8 and figure 13. Ramipril treatment reduced TAT complex after 6 weeks and 6 months, while ramipril tended to reduce fibrinogen levels at 6 months (P=0.06). Ramipril did not affect Factor VII.

Table 8. Effects of ramipril treatment at rest and during mental stress.

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<td>TAT complex (µg/L)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>2.30 (1.65-3.78)</td>
<td>2.00 (1.38-2.15)*</td>
<td>1.80 (1.62-1.96)*</td>
</tr>
<tr>
<td>Stress</td>
<td>2.05 (1.30-2.80)</td>
<td>1.90 (1.50-2.55)</td>
<td>1.80 (1.60-2.55)</td>
</tr>
<tr>
<td>Fibrinogen (g/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>3.20 (2.85-4.10)</td>
<td>2.95 (2.80-4.05)</td>
<td>3.00 (2.65-3.42)</td>
</tr>
<tr>
<td>Stress</td>
<td>3.45 (3.00-4.10)*</td>
<td>3.50 (3.05-3.90)*</td>
<td>3.10 (2.80-3.35)</td>
</tr>
<tr>
<td>Factor VII (mg/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>1.24 (1.08-1.34)</td>
<td>1.24 (1.02-1.48)</td>
<td>1.33 (1.07-1.46)</td>
</tr>
<tr>
<td>Stress</td>
<td>1.17 (1.02-1.40)</td>
<td>1.25 (1.10-1.50)</td>
<td>1.14 (1.06-1.40)</td>
</tr>
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4 RESULTS

4.1 PAPER I

4.1.1 Effects on blood pressure and heart rate

The results are presented in table 7. Systolic and diastolic BPs were reduced by treatment with ramipril, no effects were observed on heart rate. Placebo during 6 weeks did not affect BP or heart rate (data not shown). Systolic BP and heart rate responses during mental stress were attenuated by ramipril, for details, see (264).

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<td>2.00 (1.38-2.15)*</td>
<td>1.80 (1.62-1.96)*</td>
</tr>
<tr>
<td>Stress</td>
<td>2.05 (1.30-2.80)</td>
<td>1.90 (1.50-2.55)</td>
<td>1.80 (1.60-2.55)</td>
</tr>
<tr>
<td>Fibrinogen (g/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>3.20 (2.85-4.10)</td>
<td>2.95 (2.80-4.05)</td>
<td>3.00 (2.65-3.42)</td>
</tr>
<tr>
<td>Stress</td>
<td>3.45 (3.00-4.10)*</td>
<td>3.50 (3.05-3.90)*</td>
<td>3.10 (2.80-3.35)</td>
</tr>
<tr>
<td>Factor VII (mg/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>1.24 (1.08-1.34)</td>
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<td>1.33 (1.07-1.46)</td>
</tr>
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Mental stress did not affect TAT or Factor VII. Fibrinogen increased during stress following both placebo and ramipril treatment for 6 weeks, but no effects after 6 months of ramipril treatment.

Figure 13 illustrates changes in TAT complex levels for placebo and after ramipril therapy during 6 weeks and 6 months.

![Figure 13](image-url)
4.2 PAPER II

4.2.1 Effects on blood pressure and heart rate

The results are presented in table 9. Antihypertensive treatment reduced systolic and diastolic BP in both study groups.

Table 9. Treatment effects on blood pressure and heart rate.

<table>
<thead>
<tr>
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<th>Ramipril</th>
<th>Doxazosin</th>
<th>MANOVA</th>
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<tbody>
<tr>
<td><strong>Systolic blood pressure (mm Hg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>155 ± 9</td>
<td>151 ± 8</td>
<td>P group=0.38</td>
</tr>
<tr>
<td>12 weeks</td>
<td>135 ± 12</td>
<td>142 ± 12</td>
<td>P time&lt;0.001</td>
</tr>
<tr>
<td>$\Delta$ 0 to 12 weeks</td>
<td>-19 ± 12</td>
<td>-11 ± 12</td>
<td>P group x time=0.23</td>
</tr>
<tr>
<td><strong>Diastolic blood pressure (mm Hg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>93 ± 7</td>
<td>93 ± 10</td>
<td>P group=0.88</td>
</tr>
<tr>
<td>12 weeks</td>
<td>79 ± 6</td>
<td>83 ± 10</td>
<td>P time&lt;0.001</td>
</tr>
<tr>
<td>$\Delta$ 0 to 12 weeks</td>
<td>-15 ± 8</td>
<td>-10 ± 7</td>
<td>P group x time=0.085</td>
</tr>
<tr>
<td><strong>Heart rate (beats per minute)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>62 ± 8</td>
<td>59 ± 8</td>
<td>P group=0.65</td>
</tr>
<tr>
<td>12 weeks</td>
<td>62 ± 7</td>
<td>59 ± 10</td>
<td>P time=0.74</td>
</tr>
<tr>
<td>$\Delta$ 0 to 12 weeks</td>
<td>0 ± 5</td>
<td>0 ± 7</td>
<td>P group x time=0.98</td>
</tr>
</tbody>
</table>

Data are given mean values ± SD for 27-32 participants in each treatment group at week 0 or 12. $\Delta$ represents absolute changes by treatment given as mean values ± SD.

4.2.2 Effects on inflammation

The results are presented in table 10. Although antihypertensive treatment overall did not affect hsIL-6, there was a difference between treatment with ramipril (increase) and with doxazosin (decrease). There were no changes in the soluble receptor for IL-6, soluble IL-6R, and there were no changes in for the other markers of inflammation, either in treatment with ramipril or doxazosin.

Table 10. Treatment effects on inflammatory markers.

<table>
<thead>
<tr>
<th>Week</th>
<th>Ramipril</th>
<th>Doxazosin</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>HsIL-6 (pg/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.28 (0.21-0.38)</td>
<td>0.29 (0.20-0.39)</td>
<td>P group=0.78</td>
</tr>
<tr>
<td>12</td>
<td>0.37 (0.24-0.48)</td>
<td>0.26 (0.20-0.43)</td>
<td>P time=0.37</td>
</tr>
<tr>
<td>$\Delta$ 0 to 12</td>
<td>0.13 ± 0.27</td>
<td>-0.01 ± 0.21</td>
<td>P group x time=0.012</td>
</tr>
</tbody>
</table>

4.2 PAPER II

4.2.1 Effects on blood pressure and heart rate

The results are presented in table 9. Antihypertensive treatment reduced systolic and diastolic BP in both study groups.

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<td>-15 ± 8</td>
<td>-10 ± 7</td>
<td>P group x time=0.085</td>
</tr>
<tr>
<td><strong>Heart rate (beats per minute)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>62 ± 8</td>
<td>59 ± 8</td>
<td>P group=0.65</td>
</tr>
<tr>
<td>12 weeks</td>
<td>62 ± 7</td>
<td>59 ± 10</td>
<td>P time=0.74</td>
</tr>
<tr>
<td>$\Delta$ 0 to 12 weeks</td>
<td>0 ± 5</td>
<td>0 ± 7</td>
<td>P group x time=0.98</td>
</tr>
</tbody>
</table>

Data are given mean values ± SD for 27-32 participants in each treatment group at week 0 or 12. $\Delta$ represents absolute changes by treatment given as mean values ± SD.

4.2.2 Effects on inflammation

The results are presented in table 10. Although antihypertensive treatment overall did not affect hsIL-6, there was a difference between treatment with ramipril (increase) and with doxazosin (decrease). There were no changes in the soluble receptor for IL-6, soluble IL-6R, and there were no changes in for the other markers of inflammation, either in treatment with ramipril or doxazosin.

Table 10. Treatment effects on inflammatory markers.

<table>
<thead>
<tr>
<th>Week</th>
<th>Ramipril</th>
<th>Doxazosin</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>HsIL-6 (pg/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.28 (0.21-0.38)</td>
<td>0.29 (0.20-0.39)</td>
<td>P group=0.78</td>
</tr>
<tr>
<td>12</td>
<td>0.37 (0.24-0.48)</td>
<td>0.26 (0.20-0.43)</td>
<td>P time=0.37</td>
</tr>
<tr>
<td>$\Delta$ 0 to 12</td>
<td>0.13 ± 0.27</td>
<td>-0.01 ± 0.21</td>
<td>P group x time=0.012</td>
</tr>
</tbody>
</table>
4.2.3 Effects on fibrinolysis

The results are presented in table 11. There were no changes in PAI-1 activity by treatment. Concentrations of t-PA antigen increased by ramipril, while it decreased by doxazosin.

Table 11. Treatment effects on fibrinolytic markers.

<table>
<thead>
<tr>
<th>Week</th>
<th>Ramipril (IU/mL)</th>
<th>Doxazosin (IU/mL)</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAI-1 activity</td>
<td>4.6 (1.2-10.1)</td>
<td>7.1 (2.3-16.1)</td>
<td>$P_{group}=0.54$</td>
</tr>
<tr>
<td>0</td>
<td>6.1 (3.0-17.4)</td>
<td>7.2 (2.9-15.1)</td>
<td>$P_{time}=0.93$</td>
</tr>
<tr>
<td>$\Delta$ 0 to 12</td>
<td>5.3 ± 9.2</td>
<td>-3.2 ± 13.1</td>
<td>$P_{group x time}=0.89$</td>
</tr>
</tbody>
</table>
Data are given as mean values ± SD or median values and interquartile ranges for 25-29 participants in each treatment group at week 0 and 12. \( \Delta \) represents absolute changes by treatment given as mean values ± SD. PAI, plasminogen activator inhibitor and t-PA, tissue plasminogen activator.

4.2.4 Effects on coagulation

The results are presented in figure 14 and table 1. TAT complex decreased by treatment, and this was dependent on a reduction in TAT complex in the ramipril group alone. There were no changes in the CAT data by treatment.

Figure 14 illustrates the changes in TAT complex after 3 months of therapy with ramipril and doxazosin.

![Figure 14](image_url)

**Figure 14.** TAT complex levels at baseline and after 3 months of treatment with ramipril and doxazosin. Data are presented as median values and interquartile ranges, \( n = 28 \) for the ramipril group and \( n = 22 \) for the doxazosin group. Statistical evaluation was made by using non-parametric tests by paired comparisons (Wilcoxon signed rank test). Significant differences are given as; \( ^* P < 0.05 \).
Table 12. Treatment effects on markers of coagulation.

<table>
<thead>
<tr>
<th>Week</th>
<th>Ramipril</th>
<th>Doxazosin</th>
<th>MANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAT complex (µg/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>3.17 (2.15-3.78)</td>
<td>2.61 (2.14-3.23)</td>
<td>P group=0.14</td>
</tr>
<tr>
<td>12</td>
<td>2.69 (2.14-3.00)</td>
<td>2.75 (2.03-3.29)</td>
<td>P time=0.025</td>
</tr>
<tr>
<td>Δ 0 to 12</td>
<td>-0.47 ± 1.21</td>
<td>-0.05 ± 0.73</td>
<td>P group x time=0.014</td>
</tr>
<tr>
<td>CAT, peak thrombin (nM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>321.51</td>
<td>322.46</td>
<td>P group=0.11</td>
</tr>
<tr>
<td>12</td>
<td>313.61</td>
<td>337.29</td>
<td>P time=0.92</td>
</tr>
<tr>
<td>Δ 0 to 12</td>
<td>-8 ± 71</td>
<td>15 ± 52</td>
<td>P group x time=0.45</td>
</tr>
<tr>
<td>CAT, endogenous thrombin potential (nM x minutes)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1938 ± 298</td>
<td>2071 ± 270</td>
<td>P group=0.12</td>
</tr>
<tr>
<td>12</td>
<td>1975 ± 396</td>
<td>2068 ± 386</td>
<td>P time=0.93</td>
</tr>
<tr>
<td>Δ 0 to 12</td>
<td>37 ± 380</td>
<td>-3 ± 323</td>
<td>P group x time=0.50</td>
</tr>
<tr>
<td>CAT, lag-time (minutes)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>3.7 ± 0.9</td>
<td>3.8 ± 0.6</td>
<td>P group=0.68</td>
</tr>
<tr>
<td>12</td>
<td>3.8 ± 0.8</td>
<td>3.5 ± 0.6</td>
<td>P time=0.27</td>
</tr>
<tr>
<td>Δ 0 to 12</td>
<td>0.1 ± 1.1</td>
<td>-0.3 ± 0.7</td>
<td>P group x time=0.14</td>
</tr>
<tr>
<td>CAT, time to peak (minutes)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>6.7 ± 1.4</td>
<td>6.9 ± 1.1</td>
<td>P group=0.26</td>
</tr>
<tr>
<td>12</td>
<td>6.8 ± 1.2</td>
<td>6.6 ± 1.0</td>
<td>P time=0.53</td>
</tr>
<tr>
<td>Δ 0 to 12</td>
<td>0.1 ± 1.6</td>
<td>-0.4 ± 1.3</td>
<td>P group x time=0.44</td>
</tr>
</tbody>
</table>

Data are given as mean values ± SD or as median values and interquartile ranges for 22-31 participants in each treatment group at week 0 or 12. Δ represents absolute changes by treatment given as mean values ± SD. TAT, thrombin-antithrombin and CAT, calibrated automated thrombogram.

Changes in TAT by treatment did not relate significantly to changes in BP from baseline to week 12, i.e., r = -0.01 (p = 0.98) for systolic BP, r = -0.08 (p = 0.60) for diastolic BP and r = 0.04 (p = 0.76) for pulse pressure (n=50); similar relations were obtained when the two study groups were analysed separately.
4.3 PAPER III-V

4.3.1 Effects on blood pressure and heart rate

4.3.1.1 Baseline

The results are presented in tables 4 and 6. PAPER III and IV: FCHL had similar BP and heart rate compared to controlFH. PAPER V: In FH, systolic BP and pulse pressure were higher compared to controlFH (P < 0.05 and P < 0.01, respectively).

4.3.1.2 Ang II infusion

The results are presented in table 13 and 14. Ang II infusion caused a marked and rapid increase in BP in all groups. PAPER III and IV: In FCHL, the systolic BP increase was greater compared to controlFH. The diastolic BP increased similarly in FCHL and controlFH. After the end of Ang II infusion, BP returned rapidly to baseline levels. The heart rate decreased in both groups and returned to baseline levels after the end of the infusion. The heart rate responses were similar in FCHL and controlFH.

Table 13. Systolic and diastolic blood pressure and heart rate before, during and after angiotensin II infusion in FCHL and controlFH.

<table>
<thead>
<tr>
<th></th>
<th>0 h</th>
<th>0.5 h</th>
<th>2.5 h</th>
<th>+0.5 h</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Systolic blood pressure (mm Hg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FCHL</td>
<td>130 ± 13</td>
<td>161 ± 19</td>
<td>160 ± 11</td>
<td>131 ± 13</td>
<td>P group=0.002</td>
</tr>
<tr>
<td>ControlFH</td>
<td>123 ± 12</td>
<td>143 ± 10</td>
<td>142 ± 11</td>
<td>119 ± 11</td>
<td>P time&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>(P group x time=0.03</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Diastolic blood pressure (mm Hg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FCHL</td>
<td>84 ± 8</td>
<td>103 ± 7</td>
<td>102 ± 9</td>
<td>82 ± 8</td>
<td>P group=0.028</td>
</tr>
<tr>
<td>ControlFH</td>
<td>81 ± 8</td>
<td>96 ± 9</td>
<td>95 ± 8</td>
<td>76 ± 9</td>
<td>P time&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>(P group x time=0.136</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Heart rate (beat per minute)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FCHL</td>
<td>64 ± 9</td>
<td>62 ± 7</td>
<td>61 ± 7</td>
<td>67 ± 10</td>
<td>P group=0.043</td>
</tr>
<tr>
<td>ControlFH</td>
<td>58 ± 10</td>
<td>56 ± 8</td>
<td>56 ± 8</td>
<td>61 ± 9</td>
<td>P time&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>(P group x time=0.944</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean values ± SD, n=16 for each group. Comparisons over time were evaluated by repeated measures ANOVA. FH: familial combined hyperlipidaemia.

PAPER V: In FH, systolic and diastolic BP increased during Ang II infusion, and the responses were similar in FH compared to controlFH. After the end of Ang II infusion, BP returned rapidly to baseline levels. The heart rate decreased in both groups, and returned
to baseline levels after the infusion. The heart rate responses were similar in FH and control.

Table 14. Systolic and diastolic blood pressure and heart rate before, during and after angiotensin II infusion in FH, control, and saline (placebo).

<table>
<thead>
<tr>
<th></th>
<th>0 h</th>
<th>0.3 h</th>
<th>3 h</th>
<th>&gt;0.3 h</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Systolic blood pressure (mm Hg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FH</td>
<td>127 ± 14</td>
<td>152 ± 18</td>
<td>152 ± 13</td>
<td>127 ± 14</td>
<td>*P group&lt;0.01</td>
</tr>
<tr>
<td>Control</td>
<td>116 ± 12*</td>
<td>139 ± 16</td>
<td>138 ± 14</td>
<td>117 ± 10</td>
<td>P time&lt;0.0001</td>
</tr>
<tr>
<td>Placebo</td>
<td>112 ± 9</td>
<td>107 ± 11</td>
<td>111 ± 10</td>
<td>111 ± 8</td>
<td>P time=0.60</td>
</tr>
<tr>
<td><strong>Diastolic blood pressure (mm Hg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FH</td>
<td>74 ± 8</td>
<td>99 ± 7</td>
<td>93 ± 8</td>
<td>77 ± 10</td>
<td>*P group&lt;0.11</td>
</tr>
<tr>
<td>Control</td>
<td>73 ± 8</td>
<td>90 ± 11</td>
<td>88 ± 10</td>
<td>70 ± 10</td>
<td>P time=0.0001</td>
</tr>
<tr>
<td>Placebo</td>
<td>69 ± 7</td>
<td>71 ± 12</td>
<td>73 ± 13</td>
<td>69 ± 7</td>
<td>P time=0.70</td>
</tr>
<tr>
<td><strong>Heart rate (beat per minute)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FH</td>
<td>64 ± 11</td>
<td>61 ± 14</td>
<td>62 ± 11</td>
<td>66 ± 11</td>
<td>*P group&lt;0.75</td>
</tr>
<tr>
<td>Control</td>
<td>63 ± 13</td>
<td>59 ± 12</td>
<td>60 ± 11</td>
<td>67 ± 11</td>
<td>P time=0.0001</td>
</tr>
<tr>
<td>Placebo</td>
<td>56 ± 7</td>
<td>56 ± 5</td>
<td>57 ± 7</td>
<td>59 ± 7</td>
<td>P time=0.60</td>
</tr>
</tbody>
</table>

Data are presented as mean values ± SD, n=16 for FH and n=8 for control and placebo. At baseline, statistical evaluation was performed by Student’s t-test. Significant differences are given as: *P < 0.05. Comparisons over time were evaluated by repeated measures ANOVA. FH, familial hypercholesterolemia.

Due to a limitation from the Regional Ethics Committee, the mean arterial pressure was not allowed to increase by more than 25 mm Hg. Therefore, the Ang II infusion rate was reduced in 6 FCHL and in 2 control, and the maintenance dose of Ang II was lower in FCHL, as compared to control (3 ±1.0 vs. 9.8±0.7 ng/kg/minute, P < 0.05) (paper III and IV). In paper V, Ang II infusion rate was reduced in 7 FH and in 3 controls. The maintenance doses of Ang II were similar in both FH and controls (7.5±2.8 vs. 8.7±2.3 ng/kg/minute, P=0.18).

Table 14. Systolic and diastolic blood pressure and heart rate before, during and after angiotensin II infusion in FH, control, and saline (placebo).

<table>
<thead>
<tr>
<th></th>
<th>0 h</th>
<th>0.3 h</th>
<th>3 h</th>
<th>&gt;0.3 h</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Systolic blood pressure (mm Hg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FH</td>
<td>127 ± 14</td>
<td>152 ± 18</td>
<td>152 ± 13</td>
<td>127 ± 14</td>
<td>*P group&lt;0.01</td>
</tr>
<tr>
<td>Control</td>
<td>116 ± 12*</td>
<td>139 ± 16</td>
<td>138 ± 14</td>
<td>117 ± 10</td>
<td>P time&lt;0.0001</td>
</tr>
<tr>
<td>Placebo</td>
<td>112 ± 9</td>
<td>107 ± 11</td>
<td>111 ± 10</td>
<td>111 ± 8</td>
<td>P time=0.60</td>
</tr>
<tr>
<td><strong>Diastolic blood pressure (mm Hg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FH</td>
<td>74 ± 8</td>
<td>99 ± 7</td>
<td>93 ± 8</td>
<td>77 ± 10</td>
<td>*P group&lt;0.11</td>
</tr>
<tr>
<td>Control</td>
<td>73 ± 8</td>
<td>90 ± 11</td>
<td>88 ± 10</td>
<td>70 ± 10</td>
<td>P time=0.0001</td>
</tr>
<tr>
<td>Placebo</td>
<td>69 ± 7</td>
<td>71 ± 12</td>
<td>73 ± 13</td>
<td>69 ± 7</td>
<td>P time=0.70</td>
</tr>
<tr>
<td><strong>Heart rate (beat per minute)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FH</td>
<td>64 ± 11</td>
<td>61 ± 14</td>
<td>62 ± 11</td>
<td>66 ± 11</td>
<td>*P group&lt;0.75</td>
</tr>
<tr>
<td>Control</td>
<td>63 ± 13</td>
<td>59 ± 12</td>
<td>60 ± 11</td>
<td>67 ± 11</td>
<td>P time=0.0001</td>
</tr>
<tr>
<td>Placebo</td>
<td>56 ± 7</td>
<td>56 ± 5</td>
<td>57 ± 7</td>
<td>59 ± 7</td>
<td>P time=0.60</td>
</tr>
</tbody>
</table>

Data are presented as mean values ± SD, n=16 for FH and n=8 for control and placebo. At baseline, statistical evaluation was performed by Student’s t-test. Significant differences are given as: *P < 0.05. Comparisons over time were evaluated by repeated measures ANOVA. FH, familial hypercholesterolemia.

Due to a limitation from the Regional Ethics Committee, the mean arterial pressure was not allowed to increase by more than 25 mm Hg. Therefore, the Ang II infusion rate was reduced in 6 FCHL and in 2 control, and the maintenance dose of Ang II was lower in FCHL, as compared to control (3 ±1.0 vs. 9.8±0.7 ng/kg/minute, P < 0.05) (paper III and IV). In paper V, Ang II infusion rate was reduced in 7 FH and in 3 controls. The maintenance doses of Ang II were similar in both FH and controls (7.5±2.8 vs. 8.7±2.3 ng/kg/minute, P=0.18).
4.3.2 Effects on inflammation

4.3.2.1 Baseline

The results are presented in table 15. Paper III and IV: FCHL had higher levels of IL-6 (P < 0.05), leukocyte counts (P < 0.001) and hsCRP (P < 0.05) as compared to controlFH and controlFCHL, while TNF-α was on the same levels in both groups. Paper V: FH had the same levels of IL-6, leukocyte counts, TNF-α and hsCRP compared to controlFH.

However, the fibrinogen level was higher in FH (P < 0.05) compared to controlFH.

Table 15. Markers of Inflammation before, during and after angiotensin II infusion in FCHL, controlFH, FH, controlFCHL and saline (placebo).

<table>
<thead>
<tr>
<th></th>
<th>0 h</th>
<th>3 h</th>
<th>+1 h</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HsIL-6 (pg/mL)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FCHL</td>
<td>1.1(1.0-1.8)</td>
<td>2.3(1.6-2.7)</td>
<td>2.9(1.9-3.6)</td>
<td>P group=0.035</td>
</tr>
<tr>
<td>ControlFCHL</td>
<td>0.7(0.5-1.2)*</td>
<td>1.5(1.1-2.1)</td>
<td>2.4(1.5-3.4)</td>
<td>P group x time=0.19</td>
</tr>
<tr>
<td>FH</td>
<td>0.4(0.4-0.9)</td>
<td>1.1(0.8-1.5)</td>
<td>2.0(0.8-4.3)</td>
<td>P group=0.21</td>
</tr>
<tr>
<td>ControlFH</td>
<td>0.6(0.3-1.0)*</td>
<td>1.9(0.9-2.8)</td>
<td>2.5(1.5-4.1)</td>
<td>P group x time=0.0001</td>
</tr>
<tr>
<td>Placebo</td>
<td>0.8(0.7-1.4)</td>
<td>1.0(0.6-2.3)</td>
<td>1.2(0.7-2.6)</td>
<td>P group x time=0.10</td>
</tr>
<tr>
<td>Leukocyte count (x 10⁶)</td>
<td>5.9(5.5-6.9)</td>
<td>6.4(5.9-8.0)</td>
<td>6.1(5.6-7.3)</td>
<td>P group=0.004</td>
</tr>
<tr>
<td>FCHL</td>
<td>5.7(4.3-7.3)</td>
<td>5.4(4.1-6.6)</td>
<td>5.4(4.4-6.8)</td>
<td>P group x time=0.0001</td>
</tr>
<tr>
<td>ControlFCHL</td>
<td>5.2 ± 1.2</td>
<td>5.7 ± 1.4</td>
<td>5.8 ± 1.5</td>
<td>P group=0.38</td>
</tr>
<tr>
<td>FH</td>
<td>5.6 ± 1.3</td>
<td>6.4 ± 1.7</td>
<td>6.1 ± 1.6</td>
<td>P group x time=0.0001</td>
</tr>
<tr>
<td>Placebo</td>
<td>5.5 ± 0.6</td>
<td>5.6 ± 0.8</td>
<td>5.8 ± 1.1</td>
<td>P group x time=0.47</td>
</tr>
<tr>
<td><strong>TNF-α (pg/mL)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FCHL</td>
<td>1.0 ± 0.3</td>
<td>1.1 ± 0.2</td>
<td>1.1 ± 0.2</td>
<td>P group=0.90</td>
</tr>
<tr>
<td>ControlFCHL</td>
<td>1.0 ± 0.4</td>
<td>1.0 ± 0.3</td>
<td>1.1 ± 0.3</td>
<td>P group x time=0.17</td>
</tr>
<tr>
<td><strong>HsCRP (mg/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FCHL</td>
<td>1.7(0.8-3.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ControlFCHL</td>
<td>0.6(0.3-2.1)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FH</td>
<td>1.5(0.9-2.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ControlFH</td>
<td>0.5(0.2-2.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>0.6(0.3-1.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.3.2.2 Ang II infusion

The results are presented in table 15 and figure 15. **Paper III-V.** Ang II infusion caused a similar increase in IL-6 in all four groups. The leukocyte count increased in a similar manner in all six groups during Ang II infusion. TNF-α did not change either in FCHL or controlFH. Fibrinogen increased in controlFH (P time < 0.05) and tended to increase in FH (P time=0.07). We could not observe any changes in placebo.

Figure 15 illustrates the changes in IL-6 in all groups during Ang II infusion.

**Figure 15.** IL-6 in plasma before, during and after angiotensin II infusion. Data are presented as median values and interquartile ranges. PAI-1, plasminogen activator inhibitor-1; FCHL, familial combined hyperlipidaemia and FH, familial hypercholesterolemia.
At baseline, data are presented as mean values ± SD or as median values and interquartile ranges. At baseline, statistical evaluation was performed by Student’s t-test or Mann-Whitney non-parametric test. Significant differences are given as: *P<0.05, **P<0.01 or ***P<0.001. Comparisons over time were evaluated by repeated measures ANOVA. FCHL, familial combined hypercholesterolemia; FH, familial hypercholesterolemia; ContrFH, the effect of physiological saline infusion in placebo (Δt) is also shown.

### 4.3.3 Effects on fibrinolyis

#### 4.3.3.1 Baseline

The results are presented in table 16. Paper III and IV: FCHL had 3-fold higher levels of both t-PA/PAI-1 complexes and PAI-1 activity compared to controlFCHL, (both P < 0.001). Also PAP complex were higher in FCHL compared to controlFCHL (P < 0.001). Paper V: In FH, PAI-1 activity and PAP complex levels were similar to controlFH.

| Table 16. Markers of fibrinolysis before, during and after angiotensin II infusion in FCHL, controlFCHL, FH, controlFH and saline (placebo). |
|---|---|---|---|---|---|
| | 0 h | 1h | 3h | +1h | ANOVA |
| **PAI-1 activity (ng/mL)** | | | | | |
| FCHL | 3.4 (1.0-7.0) | 2.3 (0.8-5.9) | 1.0 (0.3-2.3) | 0.9 (0.3-1.8) | P group=0.0001 |
| ControlFCHL | 0.6 (0.2-1.1)*** | 0.5 (0.2-0.8) | 0.2 (0.1-0.3) | 0.2 (0.1-0.3) | P time=0.0001 |
| FH | 1.1 (0.6-1.7) | 0.5 (0.3-1.3) | 0.2 (0.1-0.5) | 0.2 (0.1-0.6) | P group=0.44 |
| ControlFH | 0.9 (0.5-2.9) | 0.6 (0.4-1.5) | 0.4 (0.2-0.6) | 0.3 (0.3-0.5) | P time=0.0001 |
| Placebo | 0.7 (0.5-1.7) | 0.4 (0.3-1.1) | 0.3 (0.2-0.5) | 0.2 (0.1-0.2) | P time=0.0001 |
| **PAP complex (μg/mL)** | | | | | |
| FCHL | 157 ± 37 | 147 ± 34 | 146 ± 32 | 143 ± 29 | P group=0.0001 |
| ControlFCHL | 103 ± 8*** | 105 ± 8 | 112 ± 12 | 127 ± 26 | P time=0.08 |
| FH | 99 ± 13 | 95 ± 14 | 106 ± 14 | 109 ± 15 | P group=0.99 |
| ControlFH | 94 ± 27 | 96 ± 27 | 110 ± 44 | 109 ± 41 | P time=0.0001 |
| Placebo | 83 ± 23 | 85 ± 20 | 88 ± 13 | 92 ± 20 | P time=0.21 |
| **t-PA/PAI-1 complex (ng/mL)** | | | | | |
| FCHL | 3.0 ± 0.8 | 2.7 ± 0.7 | 2.6 ± 0.8 | 2.6 ± 0.8 | P group=0.0001 |
| ControlFCHL | 1.0 ± 0.8*** | 1.0 ± 0.7 | 0.9 ± 0.7 | 0.7 ± 0.6 | P time=0.0001 |

Data are presented as mean values ± SD or as median values and interquartile ranges. At baseline, statistical evaluation was performed by Student’s t-test or Mann-Whitney non-parametric test. Significant differences are given as: *P<0.05, **P<0.01 or ***P<0.001. Comparisons over time were evaluated by repeated measures ANOVA. FCHL, familial combined hypercholesterolemia; FH, familial hypercholesterolemia; ContrFH, the effect of physiological saline infusion in placebo (Δt) is also shown.

### 4.3.3 Effects on fibrinolyis

#### 4.3.3.1 Baseline

The results are presented in table 16. Paper III and IV: FCHL had 3-fold higher levels of both t-PA/PAI-1 complexes and PAI-1 activity compared to controlFCHL, (both P < 0.001). Also PAP complex were higher in FCHL compared to controlFCHL (P < 0.001). Paper V: In FH, PAI-1 activity and PAP complex levels were similar to controlFH.

| Table 16. Markers of fibrinolysis before, during and after angiotensin II infusion in FCHL, controlFCHL, FH, controlFH and saline (placebo). |
|---|---|---|---|---|---|
| | 0 h | 1h | 3h | +1h | ANOVA |
| **PAI-1 activity (ng/mL)** | | | | | |
| FCHL | 3.4 (1.0-7.0) | 2.3 (0.8-5.9) | 1.0 (0.3-2.3) | 0.9 (0.3-1.8) | P group=0.0001 |
| ControlFCHL | 0.6 (0.2-1.1)*** | 0.5 (0.2-0.8) | 0.2 (0.1-0.3) | 0.2 (0.1-0.3) | P time=0.0001 |
| FH | 1.1 (0.6-1.7) | 0.5 (0.3-1.3) | 0.2 (0.1-0.5) | 0.2 (0.1-0.6) | P group=0.44 |
| ControlFH | 0.9 (0.5-2.9) | 0.6 (0.4-1.5) | 0.4 (0.2-0.6) | 0.3 (0.3-0.5) | P time=0.0001 |
| Placebo | 0.7 (0.5-1.7) | 0.4 (0.3-1.1) | 0.3 (0.2-0.5) | 0.2 (0.1-0.2) | P time=0.0001 |
| **PAP complex (μg/mL)** | | | | | |
| FCHL | 157 ± 37 | 147 ± 34 | 146 ± 32 | 143 ± 29 | P group=0.0001 |
| ControlFCHL | 103 ± 8*** | 105 ± 8 | 112 ± 12 | 127 ± 26 | P time=0.08 |
| FH | 99 ± 13 | 95 ± 14 | 106 ± 14 | 109 ± 15 | P group=0.99 |
| ControlFH | 94 ± 27 | 96 ± 27 | 110 ± 44 | 109 ± 41 | P time=0.0001 |
| Placebo | 83 ± 23 | 85 ± 20 | 88 ± 13 | 92 ± 20 | P time=0.21 |
| **t-PA/PAI-1 complex (ng/mL)** | | | | | |
| FCHL | 3.0 ± 0.8 | 2.7 ± 0.7 | 2.6 ± 0.8 | 2.6 ± 0.8 | P group=0.0001 |
| ControlFCHL | 1.0 ± 0.8*** | 1.0 ± 0.7 | 0.9 ± 0.7 | 0.7 ± 0.6 | P time=0.0001 |

Data are presented as mean values ± SD or as median values and interquartile ranges. At baseline, statistical evaluation was performed by Student’s t-test or Mann-Whitney non-parametric test. Significant differences are given as: *P<0.05, **P<0.01 or ***P<0.001. Comparisons over time were evaluated by repeated measures ANOVA. FCHL, familial combined hypercholesterolemia; FH, familial hypercholesterolemia; ContrFH, the effect of physiological saline infusion in placebo (Δt) is also shown.
control FCHL: control FH; t-PA, tissue plasminogen activator; PAI-1, plasminogen activator inhibitor-1 and PAP, plasmin-antiplasmin.

4.3.3.2 Ang II infusion

The results are presented in table 16 and figures 16-18. Paper III and IV: FCHL and controlFH had similar decreases in t-PA/PAI-1 complex (both P < 0.0001) and PAI-1 activity (both P < 0.0001) during Ang II infusion. PAP complex levels remained unchanged in FCHL during infusion of Ang II (P time=0.36) and during physiological saline infusion (P time=0.21). PAP complex levels increased in controlFHc during Ang II (P time < 0.0001). Paper V: In FH and controlFH, we observed a decrease in PAI-1 activity (both, P time < 0.0001) and an increase in PAP complex (both, P time < 0.0001) during Ang II infusion. During saline infusion caused a decrease in PAI-1 activity (P time < 0.0001), reflecting a diurnal variation in fibrinolysis.

Figure 16 and 17 illustrates the changes in PAI-1 activity and PAP complex in FCHL, controlFHc, FH, controlFH and placebo during Ang II infusion, and figure 18 illustrates the changes in t-PA/PAI-1 complex in FCHL and controlFHc during Ang II infusion.

Figure 16. PAI-1 activity in plasma before, during and after angiotensin II infusion. Data are presented as median values and interquartile ranges. PAI-1, plasminogen activator inhibitor-1; FCHL, familial combined hyperlipidaemia; FH, familial hypercholesterolaemia. ControlFHc, FH, controlFHc and controlFHc. The effect of physiological saline infusion in placebo experiments (Δ) is also shown.
Figure 1. PAP complex in plasma before, during and after angiotensin II infusion. Data are presented as mean values ± SD. PAP, plasmin-antiplasmin; FCHL, familial combined hyperlipidaemia and FH, familial hypercholesterolemia. FCHL, □; control FCHL, ■; FH, ○ and control FH, ●. The effect of physiological saline infusion in placebo experiments (Δ) is also shown.
4.3.4 Effects on coagulation

4.3.4.1 Baseline

The results are presented in Table 17. *Paper III:* FCHL and control_FCHL had the same concentrations of F1+2 and TAT complexes. *Paper V:* FH and control_FH had the same F1+2 levels.

**Table 17.** Coagulation markers before, during, and after angiotensin II infusion in FCHL, control_FCHL, FH, control_FH, and saline (placebo).

<table>
<thead>
<tr>
<th>TAT complex (μg/L)</th>
<th>0 h</th>
<th>1 h</th>
<th>3 h</th>
<th>+1 h</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>FCHL</td>
<td>1.5 (1.3-1.8)</td>
<td>1.8 (1.6-2.5)</td>
<td>2.3 (1.8-3.0)</td>
<td>2.1 (1.5-4.2)</td>
<td>P = 0.94</td>
</tr>
<tr>
<td>C_FCHL</td>
<td>1.7 (1.6-2.6)</td>
<td>1.8 (1.4-2.3)</td>
<td>2.5 (1.6-3.1)</td>
<td>1.7 (1.4-3.4)</td>
<td>P = 0.14</td>
</tr>
<tr>
<td>FCHL</td>
<td>1.5 (1.3-1.8)</td>
<td>1.8 (1.6-2.5)</td>
<td>2.3 (1.8-3.0)</td>
<td>P = 0.77</td>
<td></td>
</tr>
<tr>
<td>C_FCHL</td>
<td>1.7 (1.6-2.6)</td>
<td>1.8 (1.4-2.3)</td>
<td>2.5 (1.6-3.1)</td>
<td>P = 0.03</td>
<td></td>
</tr>
<tr>
<td>F1+2 (pmol/mL)</td>
<td>135 ± 36</td>
<td>134 ± 44</td>
<td>130 ± 37</td>
<td>P = 0.20</td>
<td></td>
</tr>
<tr>
<td>C_FCHL</td>
<td>122 ± 29</td>
<td>121 ± 36</td>
<td>110 ± 31</td>
<td>P = 0.09</td>
<td></td>
</tr>
<tr>
<td>FH</td>
<td>189 (152-229)</td>
<td>174 (139-201)</td>
<td>183 (139-216)</td>
<td>197 (137-222)</td>
<td>P = 0.14</td>
</tr>
<tr>
<td>C_FH</td>
<td>157 (130-226)</td>
<td>169 (97-196)</td>
<td>144 (112-192)</td>
<td>162 (105-187)</td>
<td>P = 0.29</td>
</tr>
<tr>
<td>Placebo</td>
<td>148 (135-167)</td>
<td>119 (118-133)</td>
<td>122 (112-133)</td>
<td>111 (105-132)</td>
<td>P = 0.05</td>
</tr>
</tbody>
</table>

Data are presented as mean values ± SD. At baseline, statistical evaluation was performed by Student’s t-test. Comparisons over time were evaluated by repeated measures ANOVA. FCHL, familial combined hypercholesterolemia; FH, familial hypercholesterolemia; C_FCHL, control FCHL and C_FH, control FH.
4.3.4.2 Ang II infusion

The results are presented in table 17 and figure 19. **Paper III**: FCHL and controlFH had similar, not significant, increases in TAT complex during Ang II infusion when analysing the response in TAT complex during the infusion time, and including 1 h after the end of the infusion. On the other hand, if we analyse TAT complex during the *ongoing* infusion (0 h to 3 h), we instead get a significant increase in TAT complex, similar in FCHL and controlFH. **Paper III-V**: F1+2 did not change in FCHL, controlFH, FH or controlHL during Ang II infusion. In placebo, we observed a decrease in F1+2 during saline infusion.

Figure 19 illustrates the changes in TAT complex in FCHL and controlFH, during the *ongoing* Ang II infusion.

![Figure 19. TAT complex in plasma before and during the ongoing angiotensin II infusion. Data are presented as median values and interquartile ranges. TAT, thrombin-antithrombin and FCHL, familial combined hyperlipidaemia. FH and controlHL.](image)

4.3.4.3 Calibrated automated thrombogram

4.3.4.3.1 Baseline

The results are presented in table 18. **Paper V**: In FH subjects, we observed a higher peak concentration of thrombin generated and a higher endogenous thrombin potential (i.e. total amount of thrombin generated) compared to findings from controlHL. FH and controlHL had similar time dependent parameters of thrombin generation; lag-time, time to peak thrombin and time to tail.
Table 18. Calibrated automated thrombogram parameters before, during and after angiotensin II infusion in FH, controlFH, and saline (placebo).

<table>
<thead>
<tr>
<th></th>
<th>0 h</th>
<th>1 h</th>
<th>3 h</th>
<th>+1 h</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to tail (minutes)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FH</td>
<td>367 ± 47</td>
<td>367 ± 61</td>
<td>368 ± 56</td>
<td>364 ± 64</td>
<td>P group=0.05</td>
</tr>
<tr>
<td>Csal</td>
<td>311 ± 57**</td>
<td>304 ± 65</td>
<td>303 ± 68</td>
<td>312 ± 56</td>
<td>P t ime=0.73</td>
</tr>
<tr>
<td>Pbo</td>
<td>325 ± 40</td>
<td>318 ± 43</td>
<td>314 ± 38</td>
<td>306 ± 48</td>
<td>P t ime=0.59</td>
</tr>
<tr>
<td>Time to peak thrombin (minutes)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FH</td>
<td>6.6 (5.7)</td>
<td>6.8 (5.6)</td>
<td>6.8 (5.7)</td>
<td>6.9 (6.4)</td>
<td>P group=0.33</td>
</tr>
<tr>
<td>Csal</td>
<td>6.6 (5.7-7.0)</td>
<td>6.6 (6.0-7.2)</td>
<td>6.6 (5.7-7.0)</td>
<td>6.5 (5.7-6.9)</td>
<td>P t ime=0.01</td>
</tr>
<tr>
<td>Pbo</td>
<td>6.8 (6.6-8.2)</td>
<td>7.0 (6.5-8.3)</td>
<td>7.0 (6.3-8.1)</td>
<td>6.7 (6.5-8.1)</td>
<td>P t ime=0.69</td>
</tr>
<tr>
<td>Lag-time (minutes)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FH</td>
<td>3.7 ± 0.4</td>
<td>3.8 ± 0.5</td>
<td>3.9 ± 0.4</td>
<td>3.8 ± 0.4</td>
<td>P group=0.08</td>
</tr>
<tr>
<td>Csal</td>
<td>3.4 ± 0.6</td>
<td>3.5 ± 0.7</td>
<td>3.5 ± 0.7</td>
<td>3.5 ± 0.6</td>
<td>P t ime=0.001</td>
</tr>
<tr>
<td>Pbo</td>
<td>4.0 ± 0.6</td>
<td>4.1 ± 0.6</td>
<td>4.1 ± 0.6</td>
<td>4.2 ± 0.6</td>
<td>P t ime=0.16</td>
</tr>
<tr>
<td>Time to peak thrombin (minutes)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FH</td>
<td>6.5 (6.2-7.3)</td>
<td>6.8 (6.2-7.6)</td>
<td>6.8 (5.5-7.7)</td>
<td>6.9 (6.4-7.4)</td>
<td>P group=0.33</td>
</tr>
<tr>
<td>Csal</td>
<td>6.6 (5.7-7.0)</td>
<td>6.6 (6.0-7.2)</td>
<td>6.6 (6.0-7.2)</td>
<td>6.5 (5.7-6.9)</td>
<td>P t ime=0.01</td>
</tr>
<tr>
<td>Pbo</td>
<td>6.8 (6.6-8.2)</td>
<td>7.0 (6.5-8.3)</td>
<td>7.0 (6.3-8.1)</td>
<td>6.7 (6.5-8.1)</td>
<td>P t ime=0.69</td>
</tr>
<tr>
<td>Endogenous thrombin potential (nM x minutes)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FH</td>
<td>2452 (2065-2766)</td>
<td>2494 (2264-2771)</td>
<td>2478 (2241-2813)</td>
<td>2423 (2151-2790)</td>
<td>P group=0.05</td>
</tr>
<tr>
<td>Csal</td>
<td>2000 (1821-2194)**</td>
<td>2019 (1776-2176)</td>
<td>1961 (1747-2248)</td>
<td>2028 (1761-2104)</td>
<td>P t ime=0.08</td>
</tr>
<tr>
<td>Pbo</td>
<td>2011 (1878-2034)</td>
<td>1957 (1860-2230)</td>
<td>1690 (1867-2312)</td>
<td>1838 (1738-2233)</td>
<td>P t ime=0.37</td>
</tr>
<tr>
<td>Peak thrombin (nM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FH</td>
<td>367 ± 47</td>
<td>367 ± 61</td>
<td>368 ± 56</td>
<td>364 ± 64</td>
<td>P group=0.05</td>
</tr>
<tr>
<td>Csal</td>
<td>311 ± 57**</td>
<td>304 ± 65</td>
<td>303 ± 68</td>
<td>312 ± 56</td>
<td>P t ime=0.73</td>
</tr>
<tr>
<td>Pbo</td>
<td>325 ± 40</td>
<td>318 ± 43</td>
<td>314 ± 38</td>
<td>306 ± 48</td>
<td>P t ime=0.59</td>
</tr>
</tbody>
</table>

FH: Familial hypercholesterolemia. Data are presented as mean values ± SD or median values and interquartile ranges; n=16 for each FH and controlFH, and n=8 for placebo. At
baseline, statistical evaluation was performed by Student’s t-test or Mann-Whitney non-parametric test. Significant differences are given as: *P < 0.05 or **P < 0.01. Comparisons over time were evaluated by repeated measures ANOVA. FH, familial hypercholesterolemia, C
FH, control FH; Pbo, placebo and g x t, group x time.

4.3.4.3.2  Ang II infusion
The results are presented in table 18. Paper V: Neither, peak concentration of thrombin generated or endogenous thrombin potential (i.e. total amount of thrombin generated) did change during infusion of Ang II. We could observe that the thrombin generation was slightly delayed as reflected by increases in the time dependent parameters; lag-time, time to peak and time to tail, and this was due to an increase in FH alone during Ang II infusion, *P time < 0.01, **P time < 0.01 and *P time < 0.05, respectively. No changes were observed in controlFH or placebo.
5 GENERAL DISCUSSION

5.1 STUDIES IN HYPERTENSION

5.1.1 Paper I

The main finding in paper I was that treatment with the ACE inhibitor ramipril, compared to placebo, decreased thrombin generation in essential hypertension. TAT complex levels, a marker of thrombin generation in vivo, were attenuated after 6 weeks of treatment, and the effects were retained after 6 months of therapy. FVII activity levels were unaffected, indicating that other mechanisms than reduced availability of FVII were more likely to be operating. Fibrinogen levels tended to decrease during ramipril treatment at 6 months (P = 0.06). These findings are in agreement with findings in overweight hypertensive patients treated with perindopril (268).

Fibrinogen is an acute phase reactant, as well as a marker of the activity in the coagulation system. Fibrinogen is an independent risk factor of future CHD (269, 270), and high levels of fibrinogen and LDL cholesterol have been reported to be present in plaque formation in vessels (271). Furthermore, VSMC migration and proliferation can be stimulated by fibrinogen suggesting that fibrinogen might be involved in early plaque formation (271-273). Fibrinogen also binds to ICAM-1 and can stimulate gene expression of this adhesion molecule, promoting the adhesion of platelets and leukocytes to ECs (274). Hence, fibrinogen mediates inflammation in addition to its central importance in the coagulation cascade.

The attenuated thrombin generation and fibrinogen levels during ramipril treatment could be of importance for the reduction of thromboembolic events seen during treatment with ramipril in patients at high cardiovascular risk (9). However, the anti-thrombotic effects of ramipril might have been due to the reduction in BP per se. To further assess the contribution of BP decrease to the anti-thrombotic effects we compared ramipril treatment with the alpha 1-adrenoceptor blocker doxazosin in paper II.

5.1.2 Paper II

5.1.2.1 Coagulation

Confirming our findings in paper I, plasma levels of TAT complex decreased by ramipril therapy in paper II. As TAT complex was unaffected in the doxazosin group, and the reductions in BP between the groups were comparable and there was no relation between changes in TAT complex and changes in BP by treatment, the anti-thrombin effects of ramipril are not likely to be related to reduction in BP.

Ang II has been reported to up-regulate expression of the key initiator of coagulation, tissue factor (11), and tissue factor is elevated in hypertensive patients, as compared with normotensive controls (275). Studies in vitro and in vivo have shown that Ang II stimulates formation of procoagulant MPs from ECs and mononuclear cells (276, 277), and that...
ACE inhibition reduces monocyte tissue factor expression and activity in plasma in hypertensive patients (12, 278). Our findings of reduced plasma levels of TAT complex following treatment with ramipril is in line with a decreased expression and activity of tissue factor by ACE inhibition, resulting in an attenuated thrombin generation in plasma. This confirms our previous findings (12) and clearly provides one possible mechanism by which ACE inhibition may reduce the risk of atherothrombotic complications in high-risk cardiovascular patients (279, 280).

We observed no changes in CAT data over time in the ramipril or the doxazosin treated groups. CAT measures thrombin generation potential in plasma ex vivo, while TAT complex measures thrombin generation in vivo including effects exerted by the vascular endothelium and blood flow (281). In the present study thrombin generation potential in plasma ex vivo was unaltered. However, as we added exogenous tissue factor and phospholipids to trigger thrombin generation in the plasma samples, the contribution of tissue factor exposing MPs in the patient sample cannot be properly evaluated. Thus, we cannot exclude the possibility that the reduced levels of TAT complex in the ramipril treated group reflect an attenuated thrombin generation which is, at least partly, due to reduced expression of tissue factor on MPs originating from e.g. ECs and/or monocytes.

To get a better understanding of such mechanisms, one should measure plasma concentrations of MPs exposing tissue factor, as well as its origins, which could be the focus of future studies.

It is to be noted that other mechanisms may operate to reduce the levels of thrombin generation by ramipril that we observed in papers I and II. Natural inhibitors such as AT, the PC system or TFPI may contribute to the attenuated levels of TAT complex observed.

In conclusion, in papers I and II antihypertensive treatment with ramipril suggest reduced thrombin generation beyond the effects on BP reduction alone. Thus, drugs blocking the RAAS may reduce atherothrombotic complications beyond their effects to reduce BP. Whether these effects are present also with ARB drugs remain, however, to be studied.

5.1.2.2 Fibrinolysis

We observed no significant changes in PAI-1 activity by ramipril or doxazosin treatment. Levels of t-PA antigen tended to increase during ramipril treatment, while it was decreased by doxazosin. ACE inhibitor treatment increase the levels of bradykinin due to a reduced degradation (282), which entails anti-ischemic and antihypertensive effects due to vasodilatation, and also increased fibrinolysis (283). The tendency to increased t-PA antigen concentrations by ramipril might be due to increased levels of bradykinin. Hypertension has been associated with hypofibrinolysis with elevated of PAI-1 activity and decreased t-PA activity (284-286), and PAI-1 is related with insulin resistance, glucose intolerance, hyperinsulinemia and dyslipidemia (287). The alpha 1-adrenoceptor blocker doxazosin has been shown to improve insulin resistance, glucose intolerance, hyperinsulinemia and to improve the lipid profile (288, 289), features often present in hypertensive patients (12, 278). Our findings of reduced plasma levels of TAT complex following treatment with ramipril is in line with a decreased expression and activity of tissue factor by ACE inhibition, resulting in an attenuated thrombin generation in plasma. This confirms our previous findings (12) and clearly provides one possible mechanism by which ACE inhibition may reduce the risk of atherothrombotic complications in high-risk cardiovascular patients (279, 280).

We observed no changes in CAT data over time in the ramipril or the doxazosin treated groups. CAT measures thrombin generation potential in plasma ex vivo, while TAT complex measures thrombin generation in vivo including effects exerted by the vascular endothelium and blood flow (281). In the present study thrombin generation potential in plasma ex vivo was unaltered. However, as we added exogenous tissue factor and phospholipids to trigger thrombin generation in the plasma samples, the contribution of tissue factor exposing MPs in the patient sample cannot be properly evaluated. Thus, we cannot exclude the possibility that the reduced levels of TAT complex in the ramipril treated group reflect an attenuated thrombin generation which is, at least partly, due to reduced expression of tissue factor on MPs originating from e.g. ECs and/or monocytes.

To get a better understanding of such mechanisms, one should measure plasma concentrations of MPs exposing tissue factor, as well as its origins, which could be the focus of future studies.

It is to be noted that other mechanisms may operate to reduce the levels of thrombin generation by ramipril that we observed in papers I and II. Natural inhibitors such as AT, the PC system or TFPI may contribute to the attenuated levels of TAT complex observed.

In conclusion, in papers I and II antihypertensive treatment with ramipril suggest reduced thrombin generation beyond the effects on BP reduction alone. Thus, drugs blocking the RAAS may reduce atherothrombotic complications beyond their effects to reduce BP. Whether these effects are present also with ARB drugs remain, however, to be studied.

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hypertensive patients (290). Therefore alpha 1-adrenoceptor blockers have been associated with positive effects on the fibrinolytic system, and it has been suggested that alpha 1-adrenoceptor blockers preferentially should be used in the treatment of hypertensive patients with insulin resistance (291). Our observation with a decrease in t-PA antigen and a tendency to decreased PAI-1 activity in the doxazosin treated group would implicate beneficial effects by treatment with doxazosin in hypertensive patients regarding fibrinolysis. This may be of benefit in the treatment of patients with hypofibrinolysis, such as patients with FCHL.

5.1.2.3 Inflammation

We measured several markers of inflammation (hsIL-6, IL-6R, IL-8, TNFα, MCP-1, and hsCRP). Antihypertensive treatment overall did not affect hsIL-6, although there were directional differences between ramipril (increase) and doxazosin (decrease); and IL-6R did not change in either group. The pleiotropic cytokine IL-6 exerts proinflammatory and anti-inflammatory properties and is produced by various cells in the cardiovascular system (292). IL-6 stimulates signalling pathways in the cell when it binds to its receptor, membrane-bound IL-6 R, named classic-signalling. Signal-transduction is mediated when the IL-6/membrane-bound IL-6R complex associates with the membrane-bound receptor gp130, which dimerizes and then initiates intracellular signalling pathways. Membrane-bound IL-6R is present in few cells in the human body, especially in some leukocytes and hepatocytes. In the other pathway, named trans-signalling, circulating IL-6 binds to a soluble form of IL-6R, and this complex of IL-6/soluble IL-6R can activate most cells as gp130 is expressed uniformly in the human body. The soluble form of gp130 in human blood is considered a natural inhibitor of IL-6 trans-signalling, when binding to circulating IL-6/soluble IL-6R. The proinflammatory influences of IL-6 are associated with trans-signalling, whereas regenerative signalling and anti-inflammatory effects have been attributed to IL-6 classic-signalling (293). The concentration of soluble IL-6R and soluble gp130 in human serum is normally 1000-fold compared to IL-6, and if the increase in IL-6 is modest, the cytokine will consequently be neutralized (294). In our study the change in IL-6 was modest, while soluble IL-6R did not change. Thus, our observed increase in IL-6 in the ramipril treated group might suggest an increased classic-signalling (i.e. anti-inflammatory) cytokine action. However, IL-6 most probably acts in a paracrine fashion, and IL-6 might reach much higher concentrations in the local inflammatory areas than what we are able to measure in peripheral veins.

IL-8 is a chemotactic cytokine released by macrophages and ECs, attracting neutrophils and monocytes to the site of inflamed ECs (295). IL-8 is related with an increased incidence of future CHD (296). In our study we did not see any difference in IL-8 levels due to treatment with ramipril or doxazosin. TNF-α is a pleiotropic cytokine primarily released by monocytes and macrophages, essential in the initial activation of vascular inflammation, and is like IL-6 central in the acute phase reaction. TNF-α plays a fundamental role in endothelium dependent vasodilatation (297) and in insulin resistance (298), and is elevated hypertensive patients (290). Therefore alpha 1-adrenoceptor blockers have been associated with positive effects on the fibrinolytic system, and it has been suggested that alpha 1-adrenoceptor blockers preferentially should be used in the treatment of hypertensive patients with insulin resistance (291). Our observation with a decrease in t-PA antigen and a tendency to decreased PAI-1 activity in the doxazosin treated group would implicate beneficial effects by treatment with doxazosin in hypertensive patients regarding fibrinolysis. This may be of benefit in the treatment of patients with hypofibrinolysis, such as patients with FCHL.
CRP has by itself proatherogenic and proinflammatory properties and CRP may directly in obese subjects (299). Ang II may be one of the factors that regulate TNF-α (300). We did not observe any changes in TNF-α during ramipril or doxazosin treatment. MCP-1 is a potent chemotactic factor that regulates migration and infiltration of leukocytes across the vascular endothelium, and MCP-1 has been associated with early development of atherosclerosis (301). MCP-1 is increased by oxidative stress and by stimulating factors such as cytokines and growth factors, and the major source of MCP-1 is monocyte and macrophages (302). Ang II can induce oxidative stress and leukocyte adhesion to human ECs (303, 304). Similar to our findings with TNF-α, we observed no changes in MCP-1 levels during treatment with ramipril or doxazosin. Others have found decreased levels of MCP-1 during ACE inhibitor treatment in hypertension and in CHD (278, 305), but little has been reported on the effect of doxazosin treatment. One study has reported inhibition by doxazosin on MCP-1 directed migration of human monocytes (306). CRP is an acute-phase protein of hepatic origin, regulated by cytokines, mostly by IL-6 and TNF-α (32). In contrast with the minor effects in IL-6 and TNF-α we observed no changes in hsCRP by ramipril or doxazosin. This is in line with findings in studies in hypertension with ACE inhibitors or angiotensin receptor blockers (160, 307), while doxazosin has been reported to decrease CRP, increase nitric oxide, and to decrease oxidative stress in hypertensive patients (308, 309).

In conclusion, circulating IL-6 may not be a sensitive marker to assess the potential influence of RAAS blockade on paracrine effect of IL-6 trans-signalling effects at inflammatory sites in human vessels in the clinical setting. Our results with IL-6, TNF-α, MCP-1, and hsCRP suggest that otherwise healthy patients with mild-to-moderate hypertension represent a population with a low activity of systemic inflammation, and detectable effects of antihypertensive treatment may require more advanced atherosclerotic disease.

5.2 STUDIES IN FAMILIAR HYPERLIPIDAEMIA

5.2.1 Papers III and IV

5.2.1.1 Characteristics of FCHL

FCHL exhibited higher IL-6, CRP and leukocyte counts compared to controls, whereas TNF-α level were similar in both groups. Hyperinsulinaemia may influence the release of IL-6 from adipocytes, and the inflammatory mediators IL-6, CRP, fibrinogen and leucocyte count are all associated to the metabolic syndrome, without dependence of obesity (32, 310, 311). Others have calculated insulin resistance by HOMA-IR in FCHL and confirmed that patients with FCHL are associated with insulin resistance (167, 312, 313). In visceral obesity, adipocyte tissue secretes several inflammatory adipokines, such as CRP, IL-6, fibrinogen and angiotensinogen. This indicates the importance of adipocytes in inflammatory states, contributing to vasculopathy and cardiovascular risk (314). IL-6, CRP and leukocyte count are all associated with increased incidence of CVD (35-37, 315). CRP has by itself proatherogenic and proinflammatory properties and CRP may directly in obese subjects (299). Ang II may be one of the factors that regulate TNF-α (300). We did not observe any changes in TNF-α during ramipril or doxazosin treatment. MCP-1 is a potent chemotactic factor that regulates migration and infiltration of leukocytes across the vascular endothelium, and MCP-1 has been associated with early development of atherosclerosis (301). MCP-1 is increased by oxidative stress and by stimulating factors such as cytokines and growth factors, and the major source of MCP-1 is monocyte and macrophages (302). Ang II can induce oxidative stress and leukocyte adhesion to human ECs (303, 304). Similar to our findings with TNF-α, we observed no changes in MCP-1 levels during treatment with ramipril or doxazosin. Others have found decreased levels of MCP-1 during ACE inhibitor treatment in hypertension and in CHD (278, 305), but little has been reported on the effect of doxazosin treatment. One study has reported inhibition by doxazosin on MCP-1 directed migration of human monocytes (306). CRP is an acute-phase protein of hepatic origin, regulated by cytokines, mostly by IL-6 and TNF-α (32). In contrast with the minor effects in IL-6 and TNF-α we observed no changes in hsCRP by ramipril or doxazosin. This is in line with findings in studies in hypertension with ACE inhibitors or angiotensin receptor blockers (160, 307), while doxazosin has been reported to decrease CRP, increase nitric oxide, and to decrease oxidative stress in hypertensive patients (308, 309).

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induce activation of ECs (316), and increased levels of CRP is closely associated with CHD risk factors, especially obesity and deposition of visceral distribution, in individuals with or without CHD (317). Hypertriglyceridemia, but not hypercholesterolemia is associated with increased leukocyte count (318). The FCHL patients had indeed very high concentrations of triglycerides (7.4 ± 5.7 mmol/L), which possibly has an impact of our results, as triglyceride levels above 1.7 mmol/L have been reported as an independent risk factor for CHD (319). Thus, our present findings prove additional evidence for a low-grade chronic inflammation in FCHL, and also suggest that patients with FCHL, to some extent, suffer from the metabolic syndrome.

The patients with FCHL exhibited an almost 6-fold higher PAI-1 activity and a 3-fold higher t-PA/PAI-1 complex level, when compared to control subjects, in agreement with previously reported studies and by us and others (27, 167, 313). PAP complex is a measure of the generation of plasmin and a marker for in vivo fibrinolysis, and increasing concentrations probably reflects increased t-PA activity (320). We observed higher PAP complex concentrations in FCHL compared to controls, which suggest that FCHL exhibited increased activities in both the activating and inhibitory proteins. F1+2 is a in vivo thrombin generation marker, and may predict CHD (321). In our study patients with FCHL and controls had similar concentrations of F1+2 and TAT complexes, i.e. no evidence of increased thrombin generation in vivo. This is somewhat unexpected since FCHL showed a low-grade inflammatory state, and inflammation may induce coagulation through several mechanisms (induced ED, activation of platelets and the coagulation cascade through TF, suppression of anticoagulant pathways and impaired fibrinolytic activity), and others have reported that FCHL is characterized by impaired endothelium reactivity and hypercoagulability (167). One explanation could be that the FCHL participants in that study (167) had a high prevalence of CHD, which in most studies is associated with high levels of F1+2 and TAT complex (322). In contrast, our patients were otherwise healthy, not obese, and were relatively young.

Thus, patients with FCHL exhibited a low-grade chronic inflammation, and signs of impaired fibrinolysis. They also suffered from insulin resistance and showed signs of the metabolic syndrome.

5.2.1.2 Blood pressure effect during Ang II infusion
The systolic BP response to Ang II was increased in FCHL, as compared to controls, indicating that FCHL exhibited increased vascular reactivity. This is possibly a sign of ED in patients with FCHL.

5.2.1.3 Coagulation during Ang II infusion
In paper III, F1+2 and TAT complex values remained unchanged in FCHL and controls during infusion of Ang II. This is in contrast to a previously reported increase in TAT complex and a tendency to increased F1+2 during short-time Ang II infusion in healthy
males (13). However F1+2 exhibits a diurnal decrease during morning hours (323, 324) but the possible effects of diurnal variations of these markers were not taken into account in paper III. Our post hoc analyses show that TAT complex actually increased in a similar way in controls and FCHL during the ongoing Ang II infusion (table 17 and figure 19). This is likely to be due to the short half-life of Ang II (about 15 seconds) (325), and TAT complex (5-15 minutes) (326). When the Ang II infusion ceased, the short half-life of TAT complex resulted in a decrease in TAT levels 1 h after the end of the infusion. F1+2 has a longer half-life (90 minutes) (326), which means that the cessation of Ang II infusion did not markedly affect the concentration of F1+2 60 minutes later. We did not perform placebo infusion experiments in this study, but others have shown that TAT complex has no diurnal variation during daytime (327). Taken together, circulating Ang II seems to increase thrombin generation. The cause of the increased thrombin generation is unclear. Inflammatory stimuli can through several mechanisms prime the coagulation system, and a proposed mechanism is cytokine caused TF expression by the cytokines IL-6 (201). During Ang II the levels of IL-6 increased. Possibly Ang II may stimulate activation of TF, at least in part due to increased concentrations of IL-6. Platelet activation by circulating Ang II (13) may have an important contributing role in the generation of thrombin.

5.2.1.4 Fibrinolysis during Ang II infusion

In paper III we observed that t-PA/PAI-1 complex levels decreased in the same way in FCHL and controls during infusion of Ang II, indicating an increase in fibrinolytic activity. This suggests that Ang II stimulates fibrinolysis, rather than attenuating it, in accordance with previous results in healthy volunteers (227). Others have reported that inflammatory stimulation caused a rapid t-PA release from storage sites in ECs in chimpanzees (326). In contrast, increasing doses of Ang II, during 45 min at 3 different dose levels in subjects with normal BP, and a steady dose during 45 minutes in hypertensive subjects, resulted in increased PAI-1 antigen, while the levels of t-PA antigen remained unchanged (225). However, others have reported that increasing doses with Ang II during 45 min at 3 different dose levels affected neither PAI-1 antigen nor activity levels in normotensive, otherwise healthy subjects (226). A possible explanation for our results may be that t-PA antigen/activity and PAI-1 antigen/activity do not equal changes in t-PA/PAI-1 complexes. The doses and duration of Ang II infusions were not the same, which probably caused different hemodynamic responses. Diurnal variations of PAI-1 and t-PA during the stimulation by Ang II may also have impacted on the results (219). Finally, the increase in PAI-1 antigen seen in one of the studies (225) might have been due to an Ang II induced stimulation of t-PA activity. To note is that t-PA antigen has a diurnal decrease during morning (329), and this decrease was absent in that study, which further suggests increased t-PA levels during Ang II.

In paper IV PAI-1 activity decreased similarly in healthy controls and FH during Ang II infusion, which most likely reflects a diurnal decrease in PAI-1 activity (330). Others have reported falling levels of PAI-1 activity during morning hours (219), which is confirmed by males (13). However F1+2 exhibits a diurnal decrease during morning hours (323, 324) but the possible effects of diurnal variations of these markers were not taken into account in paper III. Our post hoc analyses show that TAT complex actually increased in a similar way in controls and FCHL during the ongoing Ang II infusion (table 17 and figure 19). This is likely to be due to the short half-life of Ang II (about 15 seconds) (325), and TAT complex (5-15 minutes) (326). When the Ang II infusion ceased, the short half-life of TAT complex resulted in a decrease in TAT levels 1 h after the end of the infusion. F1+2 has a longer half-life (90 minutes) (326), which means that the cessation of Ang II infusion did not markedly affect the concentration of F1+2 60 minutes later. We did not perform placebo infusion experiments in this study, but others have shown that TAT complex has no diurnal variation during daytime (327). Taken together, circulating Ang II seems to increase thrombin generation. The cause of the increased thrombin generation is unclear. Inflammatory stimuli can through several mechanisms prime the coagulation system, and a proposed mechanism is cytokine caused TF expression by the cytokines IL-6 (201). During Ang II the levels of IL-6 increased. Possibly Ang II may stimulate activation of TF, at least in part due to increased concentrations of IL-6. Platelet activation by circulating Ang II (13) may have an important contributing role in the generation of thrombin.

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our placebo infusion study. Ang II has been shown to stimulate cultured human coronary ECs in a time dependent manner, to release PAI-1 protein and even very low levels of Ang II induced PAI-1 protein, which could be detected already after 6 h (331). Others have reported that Ang II increased PAI-1 messenger RNA expression in cultured human adipocytes after 3 h and PAI-1 protein release after 12 h (332). Hence, it has been argued that increased levels of Ang II are closely associated with elevated levels of PAI-1. On the contrary, studies by us and others show that PAI-1 is unaffected by Ang II infusion (226, 227). Ang II may induce PAI-1 release in the long-term setting, but we could not find any short-term impact of Ang II on the concentrations of PAI-1.

PAP complex concentrations remained unchanged during infusion of Ang II in FCHL, while these levels increased in controls. PAP complex levels remained unchanged with placebo infusion, suggesting no diurnal variation in PAP complex during morning hours. It has also been reported that the major plasmin inhibitor alpha-2 antiplasmin lacks diurnal variation (330). As PAP complex is a measure of the generation of plasmin and a marker for in vivo fibrinolysis, we propose that the increased PAP complex levels seen in the controls during stimulation by Ang II us caused by an increase in t-PA activity, in accordance with previous findings in vitro and in healthy volunteers (227, 331). The “non-response” of PAP complex concentrations during infusion of Ang II in FCHL, reflecting unchanged t-PA activity, may suggest incapacity of the ECs to release t-PA in response to Ang II, compatible with ED in FCHL. Taken together, Ang II seems to increase the fibrinolytic capacity by an increased t-PA activity concentration, at least in the short run, and argue against the claim that a brief stimulation by Ang II impairs fibrinolysis. Contrary to previous claims, we could not observe any short-term impact on PAI-1 activity by Ang II stimulation.

5.2.1.5 Inflammation during Ang II infusion

Ang II caused a 2- to 3-fold increase in IL-6, similar in FCHL and in healthy controls. This is in accordance with findings in rat VSMCs in vitro and in healthy controls showing a dose dependent release of IL-6 (106, 332). IL-6 may be produced by a number of cells and the origin of IL-6 by Ang II stimulation cannot be determined from our study. Unfortunately, we did not measure soluble IL-6R, and therefore it is doubtful to estimate the proinflammatory or anti-inflammatory effects due to the increase of IL-6 concentrations. The possible paracrine increase of IL-6 levels at inflammatory sites is generally difficult to measure. The marked increase in IL-6 in our study could suggest that IL-6 may have reached even higher levels at local areas in vessels, which may represent a local increase of trans-signalling, and a proinflammatory effect. Due to the substantial increase in IL-6 levels during Ang II infusion we suggest that Ang II may have proinflammatory effects.

The leukocyte count increased in a similar way in FCHL and controls during infusion of Ang II, and this is in line with results reported by others (334). The mechanism for this our placebo infusion study. Ang II has been shown to stimulate cultured human coronary ECs in a time dependent manner, to release PAI-1 protein and even very low levels of Ang II induced PAI-1 protein, which could be detected already after 6 h (331). Others have reported that Ang II increased PAI-1 messenger RNA expression in cultured human adipocytes after 3 h and PAI-1 protein release after 12 h (332). Hence, it has been argued that increased levels of Ang II are closely associated with elevated levels of PAI-1. On the contrary, studies by us and others show that PAI-1 is unaffected by Ang II infusion (226, 227). Ang II may induce PAI-1 release in the long-term setting, but we could not find any short-term impact of Ang II on the concentrations of PAI-1.

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The leukocyte count increased in a similar way in FCHL and controls during infusion of Ang II, and this is in line with results reported by others (334). The mechanism for this
increase is unknown, but the Ang II stimulated response in leukocyte count supports the assumption that Ang II may exert proinflammatory effects.

TNF-α concentration remained unchanged by the infusion of Ang II, and this extends observations reported by others (335). Probably stimulation during longer periods of time by Ang II may be necessary to stimulate TNF-α synthesis and release, as indicated by animal experiments (336). In any case we can conclude that TNF-α was not the cause for the observed increase in IL-6 in our study.

5.2.2 Paper V

5.2.2.1 Characteristics of FH

IL-6, leukocyte count and CRP concentrations remained similar in FH and controls, suggesting no convincing signs of systemic inflammation in FH.

FH and controls had comparable levels of PAI-1 activity. Furthermore, PAP complex levels were equal in FH and control subjects. Thus, FH patients exhibit an intact and unaffected fibrinolytic system.

An association between F1+2 levels and total cholesterol and LDL cholesterol levels has been reported in hypercholesterolemic patients in advance of atherosclerotic CVD events (337). In our study the F1+2 concentrations were not different in FH patients and in controls. However using the CAT method, the thrombin generation potential was higher in FH compared to controls, in accordance with the assumption that FH is a prothrombotic state. We observed higher fibrinogen concentrations in FH compared to controls, in agreement with others (338, 339). As data has been presented indicating that fibrinogen mediates inflammation beyond its central importance in the coagulation cascade, we speculate that the observed high fibrinogen levels in FH subjects imply activation of the coagulation system and represent signs of early, silent atherosclerosis.

In conclusion, FH showed an intact fibrinolysis, while the thrombin generation potential, i.e. significantly higher levels of the CAT parameters peak thrombin concentration and endogenous thrombin potential, was increased compared to controls.

5.2.2.2 Blood pressure effects during Ang II infusion

FH did not show any signs of increased vascular responsiveness to Ang II.

5.2.2.3 Coagulation during Ang II infusion

F1+2 levels remained unchanged in FH and controls during Ang II infusion, in spite of the elevated IL-6 levels. The absence of the expected diurnal decrease in F1+2 concentrations during morning hours, during stimulation by Ang II, may be considered as a relative increase in thrombin generation, similar to findings in healthy subjects (13) and in FCHL patients (340). Regarding CAT analyses, Ang II did not affect the concentration dependent thrombin generation data, peak thrombin concentrations and endogenous thrombin

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In conclusion, FH showed an intact fibrinolysis, while the thrombin generation potential, i.e. significantly higher levels of the CAT parameters peak thrombin concentration and endogenous thrombin potential, was increased compared to controls.
potential. Instead we observed an effect on the time dependent parameters in FH patients, i.e. slightly prolonged lag-time, time to peak thrombin concentration, and time to tail. How to interpret these results is somewhat unclear. Increased time to peak thrombin has been reported in acute coronary syndromes (341, 342), possibly due to increased TFPI plasma levels (341). One may speculate that the putative endothelial perturbation in patients with FH may cause EC release of TFPI during infusion of Ang II, which binds to TF added in the CAT analysis and leads to the delay of thrombin generation. This might be taken to suggest that patients with FH exhibit an activation of the anticoagulant system. We conclude that Ang II affects thrombin generation in the short run. The increase in fibrinogen concentrations were modest during infusion of Ang II, while the most important regulator of fibrinogen, IL-6, increased. We did not measure changes in fibrinogen during placebo infusion, but others have shown that fibrinogen does not have a diurnal variation during morning hours (329). Others have reported that fibrinogen responds to stimulation by IL-6 with a long delay of time (266). Thus, the duration of our experiments was probably too short to cause a substantial change in fibrinogen levels.

5.2.2.4 Fibrinolysis during Ang II infusion
PAI-1 activity decreased during infusion of Ang II, similarly in FH and controls. A similar decrease occurred during placebo infusion. Our results thus reflect the diurnal variation in PAI-1 activity (330). PAP complex increased similarly in FH and controls during Ang II. As alpha-2 antiplasmin lacks diurnal variation (330), and PAP complex did not show any diurnal variation in our placebo infusion group, we conclude that the increase in PAP complex levels were caused by an increase in t-PA activity, in accordance with our previous results (227). Thus, Ang II causes an acute increase in fibrinolysis.

5.2.2.5 Inflammation during Ang II infusion
Ang II stimulation caused a marked increase in both IL-6 and leukocyte count, similarly in both groups, extending reports in healthy subjects (13, 333, 334) and in accordance with our finding in FCHL and healthy control subjects. Although the origin of IL-6 or leukocyte count cannot be determined, our results indicate that Ang II may have proinflammatory properties.

Thus, we suggest that patients with FH have an increased thrombin generation, rather than alterations in the fibrinolytic activity or in inflammation, and this contributes to the increased risk of future CHD and premature death in FH. We also suggest that Ang II in FH, in the short run, acts as a proinflammatory, profibrinolytic and a prothrombotic agent.

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6 CONCLUSIONS

- Antihypertensive treatment with the ACE inhibitor ramipril reduces thrombin generation and tends to lower fibrinogen concentrations. This effect of ramipril on thrombin generation goes beyond the effects on BP reduction alone. Thus, drugs blocking the renin-angiotensin-aldosterone system may reduce atherothrombotic complications beyond their effects to reduce BP.

- Our observation that doxazosin decreased t-PA antigen and tended to decrease PAI-1 activity would implicate beneficial effects by treatment with doxazosin in hypertensive patients regarding fibrinolysis. This may be of value in the treatment of patients with hypofibrinolysis, such as patients with FCHL.

- Patients with FCHL have signs of an ongoing low-grade systemic inflammation and impaired fibrinolysis, while the coagulation system is intact. In contrast, patients with FH have an activated coagulation, intact fibrinolysis but no convincing signs of systemic inflammation.

- In contrast to FH, patients with FCHL share several characteristics with the metabolic syndrome, including high triglyceride and low HDL cholesterol levels, insulin resistance and high body mass index.

- In healthy individuals circulating Ang II acts as a proinflammatory agent and may increase thrombin generation and fibrinolysis. Since inflammation participates in vascular remodelling and atherosclerosis, Ang II may contribute to vascular dysfunction in hypertension.

- In patients with FCHL and FH, Ang II acts as a proinflammatory agent and may increase thrombin generation. Patients with FCHL appear to be incapable of increasing fibrinolysis in response to circulating Ang II, while patients with FH, on the contrary, seem to increase their fibrinolysis.

- In contrast to previous claims, we observed no effects of short term circulating Ang II on PAI-1 activity in healthy individuals or in patients with familial hyperlipidaemia.

- Thus, blocking the renin-angiotensin-aldosterone system by an ACE inhibitor may prevent atherothrombotic complications in hypertensive patients by reducing thrombin formation beyond the effects on BP. Different mechanisms may contribute to the increased incidence of cardiovascular complications in patients with FCHL and FH. ACE inhibitor treatment might be of benefit in patients with FCHL by reducing inflammation and insulin resistance, and in patients with FH mainly by reducing thrombin generation.
7 FUTURE PERSPECTIVES

Treatment with the ACE inhibitor ramipril reduced thrombin generation beyond the effects on BP reduction alone (Paper II). Possibly the reduced levels of TAT complex reflect an attenuated thrombin generation which is, at least partly, due to a reduced expression of tissue factor on MPs originating from ECs and/or blood cells. To get a better understanding of such mechanisms, one should measure plasma concentrations of MPs exposing tissue factor, as well as its origins.

FH showed an intact fibrinolysis while the thrombin generation potential, i.e. significantly higher levels of the CAT parameters peak thrombin concentration and endogenous thrombin potential were increased, as compared to controls (paper V). Possibly the increased thrombin generation, measured by TAT complex, in FH may be due to an increased expression of tissue factor on MPs originating from ECs and/or blood cells. To study the mechanisms involved, it would be worthwhile to examine plasma concentrations of MPs exposing tissue factor, as well as its origins.

ARB also inhibits RAAS, but through a different mechanism compared to ACE inhibitors. Blocking AT1R with an ARB will inhibit the negative feed-back on renin with increased renin concentrations and subsequently an increase in Ang II, while bradykinin levels are unaffected. Thus, ARB treatment may activate AT2R, which mediates effects generally opposite to the effects mediated by AT1R. Hence, several different mechanisms are in action when using an ARB, compared to the use of an ACE inhibitor. Therefore, it would be interesting to study the effects on inflammation and haemostasis in hypertensive patients and to compare treatment of an ARB to treatment with doxazosin, i.e. a similar design as in paper II, but changing the ACE inhibitor to an ARB.

Oxidized LDL induces TF expression in ECs and monocytes, and patients with raised LDL cholesterol levels exhibit increased TF plasma activity. Statins have been shown to reduce TF expression in ECs, monocytes and VSMCs. It would be interesting to examine the origin of TF expression in combination therapy with an ACE inhibitor and a statin and to compare with statin treatment alone in patients with high concentrations of cholesterol, such as FH.

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8 SVENSK SAMMANFATTNING

Åderförkalkning är multifaktiell och endoteldysfunktion (begynnande funktionsskada i kärlväggen) anses föregå åderförkalkning. Angiotensin (Ang) II, den huvudsakliga effekten av renin-angiotensin-aldosteronsystemet (RAAS), är inblandad i hypertoni och har visat sig bidra till åderförkalkning. Likemedlet “angiotensin converting enzyme” (ACE) hämmer minskar bildningen av Ang II.

Två vanliga blodfettsrubningar; familjär kombinerad hyperlipidemi (FCHL) och familjär hyperkolesterolem (FH), har båda identifierats som riskfaktorer för hjärt-karşijskador och för tidig död. FCHL har en annan fenotyp (observerbara egenskaper) jämfört med FH, men båda associeras med endoteldysfunktion och en subklinisk åderförkalkning. Vi speckulerade i att patienter med hypertoni och blodfettsrubningar och akut periodisk rekombinant angiotensin II genom infusionsberäkning. Vi undersökte de potentiella proinflammatoriska och prokoagulatoriska effekterna av Ang II jämfört med friska individer. Vi undersökte därför hur RAAS påverkar inflammation och koagulation hos friska kontroller samt hos individer med hypertoni och FH.

I delarbete I undersökte vi effekten av behandling med ACE hämman ramipril avseende koagulation hos patienter med mild- till-mäktig hypertoni. Vi observerade att ramipril dämpade bildningen av trombin (förorsakar blodkoagulation) hos individer med essentiell (utan känd orsak) hypertoni. Vi såg också en tendens till minskade nivåer av fibrinogen. Detta utökade våra tidigare resultat från delarbete I genom att vi här visade att behandling med ramipril minskade trombin bildningen, utöver de blodtryckssänkande effekterna. Läkemedel som blockera RAAS kan därmed minska aterotrombotiska komplikationer utöver de blodtryckssänkande effekterna. Vi observerade också fördelaktiga effekter på fibrinolysen (system som löser upp blodkoagler), som skulle unna tala för positiva effekter av behandling med doxazosin hos hypertensiva patienter avseende fibrinolys. Detta ska vara ett nytta vid behandling av patienter med nedsatt fibrinolys, såsom patienter med FCHL.

I delarbete II undersökte vi hur en intravenös infusion av Ang II under 3 timmar påverkade inflammation och koagulation hos patienter med FCHL och friska kontrollpersoner. I delarbete IV beskriver vi patienterna med FCHL i delarbete III, ytterligare med avseende på deras insulinresistens och fibrinolys. Vi genomförde också placebo experiment för att göra det möjligt att bedöma påverkan av de dagliga variationerna och kontrollera stabilitet i vår experimentella design. Vi fann att FCHL hade ett förhöjt systoliskt blodtryckssvar under infusionen av Ang II jämfört med

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kontroller, vilket skulle kunna tala för en ökad endoteldysfunktion hos FCHL. Patienter med FCHL uppsvisade en läggradig kronisk inflammation och en nedsatt fibrinolys, medan koagulationsystemet däremot förekom inte. FCHL hade en faktiskt ökad fibrinolys, medan fibrinolysen däremot ökade hos de friska kontrollerna. De olika svaren för Ang II stimuleringen berodde på att patienter med FCHL är oförmodgna att öka sin fibrinolys som var på Ang II. Vi kunde inte observera några kortslutande effekter på PAI-1-aktiviteten. Vår nya slutsats blev att fibrinolys eller inflammation, med FCHL uppvisade en ökad fibrinolys, men fibrinolysen däremot ökade hos de friska kontrollerna. I orsakade en ökning i fibrinolys, och hos patienter med FH förefaller den huvudsakliga effekten vara att minska bildningen av trombin.

I delarbete V undersökte vi hur en intravenös infusion av Ang II under 3 timmar påverkade inflammation och koagulation hos patienter med FH och friska kontroller. Vi genomförde också placeboexperiment för att göra det möjligt att bedöma påverkan av de dagliga variationerna och koagulationssystemet i vår experimentella design. Vi fann att patienter med FH hade högre systolisk blodtryck än kontrollerna, medan blodtryckssvaren var lika hos FH och kontrollerna. FH uppsvisade en intakt fibrinolys, men en ökad potential att bilda trombin jämfört med kontrollerna. FH hade inte några övertrygande tecken på en pågående läggradig inflammation. Infusion av Ang II orsakade en ökning i inflammation, fibrinolys och troligen bildandet av trombin, på samma sätt hos både FH och kontrollerna. Under Ang II infusionen visade FH tecken på ett aktiverat antikoagulatoriskt system. Vi föreslog att patienter med FH har ett aktiverat koagulationsystem i stället för en förändrad fibrinolys eller inflammation, vilket kan bidra till den ökade risken för hjärt-kärlsjukdomar och för tidig död hos FH.

Vår slutsats blev att blockerings av RAAS genom att använda en ACE hämman kan förhindra aterotrombotiska komplikationer hos patienter med hypertoni, utöver effekterna av blodtryckssänkningen framför allt genom att minska bildandet av trombin. Olika mekanismer kan bidra till ökningen av kardiovaskulära komplikationer hos patienter med FH och FH. Hos patienter med FCHL kan en effekt av ACE hämning vara att dämpa inflammation, i kombination med deras kända positiva inverkan på insulinresistens, och hos patienter med FH förefaller den huvudsakliga effekten vara att minska bildningen av trombin.
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