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Blood Pressure Elevation: Impact on Cardiovascular Structure and Endogenous Fibrinolysis

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ABSTRACT

Blood pressure elevation is a major risk factor for cardiovascular events and the risk increases in a dose-dependant manner. It is of importance to identify subjects prone to develop hypertension and adverse cardiovascular remodeling in order to start treatment timely. The increased risk of myocardial infarction and ischemic stroke in hypertension suggests that the condition is associated with prothrombotic mechanisms. Our research group recently discovered that the capacity for activation of the endogenous fibrinolytic system by acute release of tissue plasminogen activator (t-PA) is markedly impaired in subjects with hypertension. This impairment could contribute to the increased risk for atherothrombotic events.

The predictive value of blood pressure elevation (SBP 140-160 and/or DBP 85-95 mmHg; BPE group) or normal blood pressure (SBP 110-130 and DBP 60-80 mmHg; NC group) was studied in a cohort of 20-year old men investigated in 1987. The prevalence of hypertension was 74.5% and 5.9% in the BPE and NC group, respectively, at 20 year follow-up. The difference in blood pressure level at baseline between the groups contrasted even more at follow-up. Further, the BPE group had significantly increased left ventricular mass index and intima-media thickness compared to the NC group.

We investigated if the impaired fibrinolytic capacity in untreated hypertension could be restored by chronic and acute blood pressure lowering. T-PA release was stimulated by infusion of substance P in the perfused-forearm model before and during chronic and acute blood pressure lowering. Chronic antihypertensive treatment with either the calcium antagonist felodipine or the ACE-inhibitor lisinopril, increased the amount of t-PA released and improved the rapidity of the t-PA response. Changes were similar in the two treatment groups, suggesting the improvement to be related to the blood pressure lowering *per se*. However, acute blood pressure lowering with intravenous sodium nitroprusside did not affect the stimulated t-PA release. The results of the two studies indicate that high blood pressure decreases the cellular content of t-PA, rather than interfering with the release mechanisms of the protein.

Further, we explored the impact of the tensile force component of blood pressure on the regulation of fibrinolytic proteins by studying cultured endothelial cells in an *in vitro* biomechanical experimental model. Prolonged cyclic strain, mimicking the hypertensive state, was found to suppress t-PA gene expression and protein secretion. In contrast, the main inhibitor of t-PA, plasminogen activator inhibitor-1 was induced, adding to the negative effects of elevated blood pressure on fibrinolysis.

In conclusion, blood pressure elevation in young age predicts adverse cardiovascular remodeling and hypertension twenty years later. Hypertension increases the risk of atherothrombotic events by impaired fibrinolysis, possibly through a direct inhibitory effect on t-PA expression by enhanced tensile stress. Chronic blood pressure lowering restores the endogenous fibrinolytic capacity, and this could contribute to the beneficial effect of antihypertensive therapy.

Key words: hypertension, left ventricular hypertrophy, intima-media thickness, fibrinolysis, endothelium, tissue plasminogen activator, antihypertensive agents, mechanical stress, plasminogen activator inhibitor-1

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LIST OF ORIGINAL PAPERS

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I Ridderstråle W, Saluveer O, Johansson M, Bergbrant A, Jern S, Hrafnkelsdóttir TJ. Consistency of Blood Pressure and Impact on Cardiovascular Structure over 20 Years in Young Men.
Manuscript.

II Ridderstråle W, Ulfhammer E, Jern S, Hrafnkelsdóttir T. Impaired Capacity for Stimulated Fibrinolysis in Primary Hypertension is Restored by Antihypertensive Therapy.
Hypertension 2006;47:686-91.

III Ridderstråle W, Saluveer O, Carlström M, Jern S, Hrafnkelsdóttir TJ. The Impaired Fibrinolytic Capacity in Hypertension is not Improved by Acute Blood Pressure Lowering.
Submitted.

IV Ulfhammer E, Ridderstråle W, Andersson M, Karlsson L, Hrafnkelsdóttir T, Jern S. Prolonged Cyclic Strain Impairs the Fibrinolytic System in Cultured Vascular Endothelial Cells.
Journal of Hypertension 2005;23:1551-7.

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PAPER I-IV

ABBREVIATIONS

ACE	angiotensin converting enzyme
AIX	augmented pressure/pulse pressure
ANOVA	analysis of variance
BMI	body mass index
BP	blood pressure
BPE	blood pressure elevation
cDNA	complementary deoxyribonucleic acid
CHD	coronary heart disease
C_T	cycle threshold
DBP	diastolic blood pressure
ECG	electrocardiogram
ELISA	enzyme-linked immunosorbent assay
FBF	forearm blood flow
FPF	forearm plasma flow
FVR	forearm vascular resistance
GAPDH	glyceraldehyd 3-phosphate dehydrogenase
HAEC	human aortic endothelial cells
hsCRP	high sensitivity C-reactive protein
IMT	intima-media thickness
IVSd	interventricular septum diameter in end diastole
LVDd	left ventricular diameter in end diastole
LVM	left ventricular mass
LVMI	left ventricular mass index
LVDs	left ventricular diameter in end systole
MAP	mean arterial pressure
mRNA	messenger ribonucleic acid
NC	normal control
NTproBNP	N-terminal pro-brain natriuretic peptide
PAI-1	plasminogen activator inhibitor-1
PWV	pulse wave velocity
RT-PCR	reverse transcriptase polymerase chain reaction
RWT	relative wall thickness
SBP	systolic blood pressure
SNP	sodium nitoprusside
t-PA	tissue plasminogen activator
u-PA	urokinase plasminogen activator
WH	waist hip ratio

INTRODUCTION

Blood pressure elevation

In 1827 Richard Bright described autopsy cases of contracted kidney that during life had "dropsy" (probably heart failure), hardness of the pulse (high blood pressure) and albuminuria [1]. He attributed the clinical findings to renal disease and he later classified the condition as "Bright's disease", also including left ventricular hypertrophy (LVH) [2]. However, some years later, Fredrick Akbar Mahomed could demonstrate that in three quarters of cases with high arterial pressure, LVH, and dropsy, there was no albuminuria or evidence of renal disease, thus in 1874 defining what later has been named essential hypertension [3, 4]. Still today in 90-95% of hypertension cases the etiological factors responsible for the blood pressure increase remain unknown [5]. Secondary forms of hypertension are rare and the causes disparate *i.e.* renal disease, endocrine disease, neurological disease, and aortic coarctation [5].

Blood pressure elevation is a major risk factor for cardiovascular events and the risk increases as a continuum of increased blood pressure [6, 7]. Hypertension is a diagnosis set at an arbitrarily defined blood pressure level that has been subject for change over the years. Today the cut-off for the hypertension diagnosis is systolic blood pressure (SBP) ≥140 mmHg or diastolic blood pressure (DBP) ≥90 mmHg at repeated office blood pressure measurements. Treatment of blood pressure is, however, recommended at lower levels in individuals at high cardiovascular risk, for example diabetics or with established cardiovascular disease [8]. The prevalence of hypertension is 25-30% in the adult population world-wide, and in the ages above 70 years about 60-70% [9]. It is of importance to detect harmful blood pressure levels early to timely hinder the adverse effects of this silent trait. As the evidence for the beneficial effects of treating blood pressure below today's threshold builds up, the diagnostic level will be adjusted accordingly.

Complications of blood pressure elevation

The major complications of high blood pressure are stroke, myocardial infarction, heart failure, peripheral arterial disease, renal failure, and cardiovascular death [8]. Paradoxically, the majority of clinical events in hypertension are ischemic rather than hemorrhagic, despite what one might have expected to be the result of high pressure load. This fact is partly explained by the association between hypertension and atherosclerosis, and subsequent atherothrombotic events. In a meta-analysis of 14 studies comparing antihypertensive treatment to placebo in hypertension with a minimum duration of treatment of one year, blood pressure treatment significantly reduced vascular mortality, stroke by 40% and coronary heart disease (CHD) by 14% during an average time period of 5 years [10]. When comparing these results to the expected reduction in events based on epidemiological data, the conclusion was that the reduction in events occurred surprisingly early after starting treatment [10]. Indeed, the beneficial effect of blood pressure treatment in reducing atherothrombotic events has been suggested to be greater than what would be expected from slowing the atherosclerotic process alone [11], and evolving clinical and laboratory evidence indicate that hypertension

confers a prothrombotic state [12]. Our research group has hypothesized that the increased pressure load on the vessel wall could impair a physiologically important defense against arterial thrombus formation *i.e.* the endogenous fibrinolytic system found in the vascular endothelium.

Cardiac and vascular remodeling in hypertension

Biomechanical stress of blood pressure

Hypertension results in alterations in two major biomechanical forces acting on the arterial vessel wall: a) increased cyclic strain, the elongation of endothelial cells during rhythmic distension of the vessel and, b) increased shear stress, the frictional force of blood flow on the endothelial surface [13, 14]. Mechanical forces acting on endothelial cells can initiate complex signal transduction cascades leading to functional changes within the cell by means of stimulation of integrins, G-protein receptors, tyrosine kinase receptors, ion channels or junction proteins [15-17]. Mechanosensors in the cell can also trigger second messengers that transduce signals into the nucleus and activate transcription factors [15-17]. During the rhythmic distension of the arterial wall in hypertension, the tensile force component can be assumed to be the main deformation force acting on the endothelium.

Organ damage

Increased pressure load on the heart and vessels results in structural alterations with a continuum of vascular damage, and are determinants of cardiovascular risk. Left ventricular hypertrophy (LVH) [18], concentric remodeling of the left ventricle [19], diastolic dysfunction [20], left atrial enlargement [21, 22], increased intima-media thickness (IMT) [23], arterial stiffness [24], microalbuminuria [25] and changes in the retinal arteries [26] are all signs of cardiovascular remodeling and increased risk of cardiovascular events [8]. The prevalence of LVH, increased IMT and their correlation has previously been reported in cross-sectional studies of mild untreated hypertension [27]. Hypertension is often associated with impaired endothelial vasodilatation capacity [28] probably due to lack of nitric oxide (NO), and this defect predicts future cardiovascular events [29]. The implications of blood pressure elevation on cardiovascular structure and function in middle-aged men have been studied to some extent in prospective studies [30-33]. However, prospective studies on blood pressure elevation in young subjects are lacking.

Twenty years ago, Bergbrant *et al* investigated a population of young subjects with blood pressure elevation assumed to be in an early phase of the hypertensive disease, before secondary pressure-induced cardiovascular changes had appeared. The cohort consisted of young men with blood pressure elevation (BPE) and normal controls (NC) [34-36], selected by blood pressure levels at military enlistment. This cohort of well-defined subjects offers now a unique opportunity to prospectively elucidate the significance of BPE in young age.

Normal vascular function

The endothelium

The luminal side of all blood vessels of the body is lined with a multifunctional monolayer of endothelial cells *i.e.* the endothelium. The total surface area of the endothelium has been reported to be 1000 m², and with a weight of 1.5 kg in a 70 kg adult subject [37, 38], constituting one of the largest organs in the body. Through nutritive blood flow on the luminal side and metabolically demanding tissue on the outside, the endothelium possesses a strategic position. It is sensitive to mechanical, chemical, and humoral stimuli, and synthesis and releases a vast number of biologically active mediators. By means of interplay with the underlying smooth muscle cell layer in the vessel the endothelium regulates vascular tone by release of vasoactive substances *i.e.* NO, prostacyclin (PGI_2), and endothelin-1 (ET-1). The endothelium also maintains blood fluidity by expressing anti-coagulating and anti-thrombotic compounds on the surface of the endothelial cells, and it expresses a potent fibrinolytic function.

Hemeostasis

In the event of vessel injury, the endothelial lining of the vessel is disrupted and collagen and subendothelial basement membranes in the vessel wall are exposed to the blood. This triggers the coagulation cascade and activates platelets that adhere to the vessel wall and aggregate, building a platelet plug. Ultimately, thrombin is generated leading to the conversion of fibrinogen to fibrin, that is further stabilized by polymerization, and a thrombus is formed. This sequence of actions is the result of evolution and has prevented fatal blood loss in case of injury.

The fibrinolytic system

Vessel patency

In the event of an atherosclerotic plaque rupture (Figure 1) the haemostatic system reacts as to a vessel injury. The lipid-rich content in the plaque activates the coagulation cascade with subsequent thrombus formation often leading to partial or complete occlusion of a vessel, in this case the evolutionary wound healing process potentially leads to a life-threatening condition. The fibrinolytic system is a counter-regulatory mechanism to the coagulation cascade as it protects the circulation from fibrin formation and thrombosis. This is illustrated by the observation that in about 30% of events with vessel occlusion in acute myocardial or cerebral infarction the infarct related artery spontaneously reperfuses [39, 40].

Regulation

The fibrinolytic system is regulated by circulating factors and factors released from the vascular endothelium. Fibrin is degraded to fibrin degradation products (FDPs) by the protease plasmin, which in turn requires plasminogen activators (PAs) for conversion from the proenzyme plasminogen (Figure 1). PAs and plasminogen binding sites

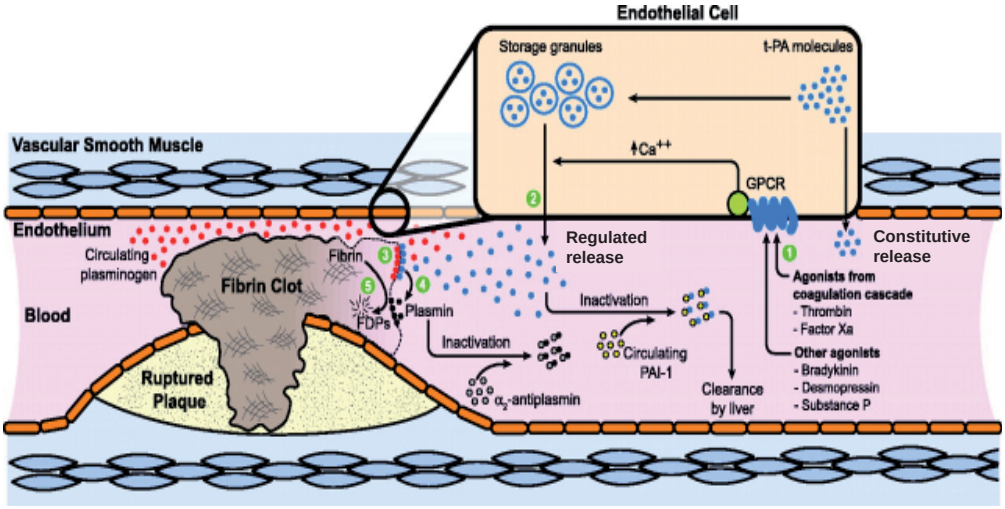

Figure 1. The endothelial fibrinolytic response to an evolving thrombus. Agonists generated from the coagulation cascade act on endothelial cell surface G-protein-coupled receptors (GPCRs) (1) to stimulate release of t-PA from storage granules, a step that requires an increase in intracellular calcium concentration (2). Free t-PA acts on thrombus-bound plasminogen (3) to produce plasmin (4) that, in turn, degrades cross-linked fibrin into fibrin degradation products (FDPs) (5), thus dissolving the thrombus. The fibrinolytic process is inhibited by inactivation of t-PA by PAI-1 and plasmin by α_2-antiplasmin.
(Partly modified from, Oliver JJ *et al*. ATVB 2005;25:2470-79. Reprinted with permission from Lippincott Williams & Wilkins.)

on the surface of a fibrin clot, platelets, and endothelial cells, concentrate the activator and the substrate, an important regulatory mechanism that will accelerate and focus fibrinolysis [41, 42]. There are two immunologically distinct plasminogen activators; tissue plasminogen activator (t-PA) and urokinase plasminogen activator (u-PA) [43]. In the vascular compartment, t-PA induced activation of plasminogen is the physiologically most important trigger of fibrinolysis [43, 44]. U-PA appears mainly to be involved in later stages of fibrin dissolution and processes involving cell movement and tissue remodeling [43]. The activity of the fibrinolytic enzymes is regulated by serine protease inhibitors (serpins). The main inhibitor of plasmin is α_2-antiplasmin [45], while plasminogen activator inhibitor-1 (PAI-1) is considered to be the physiologically most important inhibitor of t-PA and u-PA in plasma [46, 47]. The interaction between t-PA and PAI-1 is rapid [47], and in plasma there is molar abundance of PAI-1 compared to t-PA [48]. Although PAI-1 is synthesized in endothelial cells *in vitro* [49], there is no releasable pool in the endothelium [50]. Plasmin and PAs are protected from inhibition, thus sustaining their activity, as long as bound to fibrin or cell surfaces [51].

Tissue plasminogen activator

Synthesis and constitutive release of t-PA

Endothelial cells synthesize and store t-PA [52], and are considered to be the main source of circulating t-PA [53, 54]. T-PA is a 527-amino acid glycoprotein with a

molecular weight of 65-75 kD depending on its degree of glycosylation [54, 55]. The human gene coding for t-PA is localized on chromosome 8 [54]. T-PA is released from the endothelium into the circulation both through a constitutive and a regulated secretion pathway (Figure 1) [56]. In constitutive release, the newly synthesized protein continuously leaves the Golgi apparatus in transport vesicles to fuse with the cell membrane. During regulated release, large amounts of t-PA are released from an intracellular storage pool [53]. *In vivo* and *in vitro* data suggest that the amount of t-PA released by both these pathways is proportional to t-PA synthesis [57], and studies have shown that t-PA synthesis is principally regulated at the transcriptional level [58]. Hence, it is warranted to study t-PA gene expression by analyzing t-PA mRNA levels in endothelial cells to instantaneously monitor regulation of the protein in response to different stimulus.

Regulated release of t-PA

A key step in the activation of the endogenous fibrinolytic system is the immediate stimulated release of active t-PA from specialized intracellular storage granules [53]. This storage pool enables massive t-PA release upon stimulation with a several-fold increase in secretion rate when needed to stop an evolving thrombus [58]. This leads to very high concentration of t-PA locally in the vessel lumen and protects it from occlusion and flow arrest. T-PA is released in its active form and the presence of fibrin increases the activity of t-PA several hundred-fold [59, 60]. Also, t-PA already present during thrombus generation, before the network of fibrin is stabilized, is more effective in inducing fibrinolysis compared to when added afterwards [61, 62]. Most agonists that stimulate t-PA release activate endothelial surface bound G-protein coupled receptors resulting in enhanced cytoplasmic Ca^{2+} stimulating exocytosis of the storage granules [63, 64]. Importantly, products of the coagulation cascade *i.e.* thrombin and Factor Xa are potent triggers of regulated t-PA release [53, 57, 65]. Also, metabolites of tissue ischemia [66, 67], mental stress [68], and sympathoadrenal activation release t-PA [69]. *In vivo*, regulated release of t-PA can also be induced by a number of endogenous and exogenous receptor agonists such as norephinephrine [70], methacholine [70], acetylcholine [71], substance P [72], bradykinin [73], desmopressin [74], adenosine triphosphate (ATP) and uridine triphosphate (UTP) [66].

Plasma levels of t-PA and PAI-1

Prospective studies consistently show that an elevated systemic plasma level of t-PA antigen predicts both myocardial infarction and ischemic stroke [75-77]. This may seem paradoxical, given the thromboprotective role of t-PA. However, the level of plasma t-PA antigen represents the sum of all the different molecular forms of t-PA, *i.e.* t-PA in complex with inhibitors, mainly PAI-1, and uncomplexed, active t-PA. In plasma, approximately 80% of t-PA is in complex with inhibitors whereas about 20% is biologically active [78-80]. The plasma half-life of t-PA is short, only 3-5 minutes [81], due to receptor mediated clearance of the protein in the liver [82]. This makes the plasma level very sensitive to changes in liver blood flow [83]. In addition, active t-PA is cleared more rapidly than t-PA bound to PAI-1 [84]. Elevated plasma levels of PAI-1 are frequently seen in conjunction with the metabolic syndrome *i.e.* in obesity, hyperlipidemia, hyperinsulinemia, and hypertension [85]. An increased plasma level

of PAI-1 will be paralleled by an increase in t-PA antigen, despite unchanged t-PA release from the endothelium. More importantly, it has been shown that the plasma t-PA level does not predict the capacity for locally stimulated release of t-PA from the endothelium [80].

The crucial role of t-PA in fibrinolysis and vessel patency

Regulated t-PA release from the endothelium is pivotal for the endogenous fibrinolytic defense against intravascular thrombus formation and the threat of tissue infarction. In fact, recently, in a prospective observational cohort study, the capacity for stimulated t-PA release was a major determinant of future risk of cardiovascular events in patients with stable coronary heart disease [86]. Furthermore, a polymorphism in the t-PA gene associated with low basal secretion rate of t-PA was found to be associated with increased risk of future myocardial infarction [87, 88]. In clinical practice, treatment with recombinant t-PA to restore vessel patency in ischemic stroke improves prognosis [89], and reduces mortality in myocardial infarction [90].

Impaired capacity for endogenous fibrinolysis in hypertension

In vivo measurement of endogenous fibrinolysis

Given the central role of stimulated release of t-PA from the endothelium in preventing atherothrombotic events, it is of interest to measure this endogenous defense quality *in vivo* in humans. For this reason, in 1994, our research group developed a regional *in vivo* technique, based on the perfused-forearm model, which allowed direct measurement of basal and stimulated release of t-PA from the endothelium [70, 91]. Since the forearm vascular bed is studied, there is no confounding influence of central reflexes or liver clearance. In line with our hypothesis, studies in this model have shown markedly reduced capacity for stimulated release of t-PA in subjects with hypertension [92], and chronic renal impairment and hypertension [93].

Potential mechanisms

The mechanisms for the defective t-PA release in hypertension are not clear. In an *ex vivo* model, perfusion of human vessels at high intraluminal pressure was found to down-regulate the expression of t-PA and decrease t-PA secretion from the endothelium [94]. Interestingly, the decreased t-PA secretion during high pressure perfusion was present already after two hours [94], suggesting an altered release mechanism of the protein from the endothelium. Thus, facts at hand indicate that increased pressure load on the endothelium down-regulates t-PA expression in the long run, but also rapidly impairs the release of the protein from the endothelial cells. Some previous studies have looked at the impact of cyclic strain on the expression of fibrinolytic proteins in endothelial cells *in vitro*. The results of these studies have been conflicting and few studies have covered both long and short-term effects or the effect on gene expression [95-98].

How to go further?

Our research group has explored the hypothesis that the increased burden of atherothrombotic events in hypertension is aggravated by a defective fibrinolytic response that contributes to a prothrombotic state. A logical step to further understand this impairment is to study if blood pressure lowering, both long- and short-term, restores the impaired capacity for stimulated fibrinolysis *in vivo*. Since increased pressure seems to alter the fibrinolytic capacity in the endothelium, it is of interest to analyze the impact of the tensile biomechanical force on regulation of fibrinolytic proteins in endothelial cells.

AIMS

With this background the objective of the present work was:

- to investigate the implication of blood pressure elevation in young age on cardiovascular function and structure, and hypertension prevalence at 20 year follow-up

- to test the hypothesis that acute and chronic blood pressure lowering restores the impaired capacity for stimulated fibrinolysis in untreated hypertension

- to test the hypothesis that the fibrinolytic proteins, t-PA, u-PA, and the inhibitor PAI-1, are regulated by the biomechanical force cyclic strain in endothelial cells

METHODS

Subjects

Study I

Subjects were recruited from a large cohort of young men examined at a military enlistment center in Gothenburg 1987 [35]. Individuals with systolic >145 mmHg and/or diastolic blood pressure >84 mmHg, were informed by letter, and 1-3 years later a second and a third blood pressure recording was performed [35]. Blood pressure elevation (BPE) was defined as SBP 140-160 mmHg and/or DBP 85-95 mmHg in all of the three consecutive blood pressure readings. A random sample of individuals from the same enlistment with SBP 110-130 mmHg and DBP 60-80 mmHg both at enlistment and at a second blood pressure recording, was selected as normal control group (NC). Fifty-four and twenty individuals, in BPE and in NC groups, respectively, were included. At baseline, apart from blood pressure levels, the only variable differing between the two groups was s-insulin, which was higher in the BPE group [35].

The subjects were invited to a follow-up examination at the Clinical Experimental Research Laboratory during 2007, this was possible in 49 of 54, and in 17 of 20, subjects in the BPE and NC group, respectively. Out of the 49, 2 subjects had died (one cardiovascular death). Altogether, this gives a 20 year follow-up of 89% (66/74). In the BPE group 2 subjects were not reached and 3 declined participation. In the NC group 3 subjects declined participation. The 5+3 dropouts did not differ in terms of body mass index (BMI), systolic and diastolic blood pressure at enlistment, left ventricular mass, cholesterol, and 24 h systolic or diastolic blood pressure compared to the participants in the respective group (unpaired *t* test; *P*=ns). At follow-up, morbidity in the BPE group included diabetes, heart failure, osteitis, paroxysmal atrial fibrillation, previous myocardial infarction, and previous deep vein thrombosis (6 subjects). In the NC group no subject had suffered any of these diseases. Subject characteristics of the participants at follow-up are shown in Table 1.

Table 1. Subject characteristics, mean and (SEM)

	Study I		Study II		Study III	
Variables	BPE group (n=49)	Control group (n=17)	Felodipine group (n=10)	Lisinopril group (n=10)	Intervention group (n=9)	Reference group (n=3)
Age, years	40.1 (0.3)	39.4 (0.8)	62.4 (2.0)	60.5 (3.5)	50.2 (4.3)	48.7 (4.8)
Body mass index, kg/m²	28.5 (0.7)	27.0 (1.0)	25.7 (0.9)	26.4 (0.9)	31.6 (2.0)	27.1 (2.9)
S-cholesterol, mmol/L	5.2 (0.2)	5.2 (0.2)	5.2 (0.3)	5.6 (0.1)	5.7 (0.2)	5.6 (0.5)
S-triglycerides, mmol/L	1.6 (0.2)	1.4 (0.2)	1.4 (0.1)	1.5 (0.1)	1.7 (0.2)	1.8 (0.6)
P-glucose, mmol/L	5.4 (0.2)	5.0 (0.1)	5.5 (0.2)	5.5 (0.1)	5.4 (0.2)	5.0 (0.6)
S-insulin, mU	11.4 (1.1)	7.9 (1.3)	8.3 (1.3)	8.5 (1.4)	14.1 (2.6)	7.2 (2.7)
Systolic BP, mmHg	144.0 (1.9)[a*]	124.5 (1.7)[a]	172.0 (3.7)[b*]	158.9 (3.8)[b]	151.7 (3.0)[b]	148.3 (3.9)[b]
Diastolic BP, mmHg	91.2 (1.4)[a*]	81.2 (2.3)[a]	82.1 (2.2)[b]	81.8 (2.1)[b]	80.7 (2.5)[b]	83.2 (1.4)[b]

[a]Office blood pressure. [b]Untreated intraarterial blood pressure measured at the beginning of the experiment. *Significant difference between groups studied. Abbreviations: BPE=blood pressure elevation

Studies II-III

In Study II, 20 subjects (12 men and 8 women) and in Study III, 12 subjects (11 men and 1 woman) with documented primary hypertension (treated or untreated) were recruited through advertisement or in collaboration with a population screening study. All subjects were non-smokers without a history of major illness, and were on no other medical treatment than antihypertensive drugs. Subjects with blood lipid derangements or impaired fasting glucose were not included. All women were postmenopausal. Secondary hypertension was excluded by standard procedures. Subject characteristics are shown in Table 1.

The study protocols in Studies I-III were approved by the Ethics Committee of the University of Gothenburg and conducted according to the Declaration of Helsinki. The nature, purpose and potential risks were carefully explained to each subject before written informed consent was obtained.

Cell culture

In Study IV, human aortic endothelial cells (HAEC) from four individuals were used (Clonetics, USA) to represent vascular endothelial cells of adequate human origin. Cells were maintained in EGM-2 complete culture medium, consisting of EBM-2 basal medium (Clonetics) supplemented with 2% fetal bovine serum and growth factors (SingleQuots kit; Clonetics) and incubated at 37ºC in a humidified 5% carbon dioxide incubator. Cells were used for cyclic strain experiments at passages 4-6.

Study design and experimental protocols

An overview of the studies is shown in Table 2.

Study I

The prognostic implication of blood pressure elevation or normal blood pressure level in young age was studied in respect of blood pressure level, hypertension prevalence, and cardiovascular function and structure 20 years later. At baseline, the subjects were investigated with office blood pressure, 24 h ambulatory BP, intraarterial BP and invasive hemodynamics, blood pressure reaction to stress, echocardiography, anthropometrics, exercise ECG and blood was collected for hormonal and standard laboratory analysis. The findings have previously been described in detail [34-36]. At follow-up, a physical examination was performed and medical history recorded. Standard blood samples were collected including fasting blood glucose, insulin, lipid profile, N-terminal pro-brain natriuretic peptide (NTproBNP), and high sensitive C-reactive peptide (hsCRP). Anthropometric data and electrocardiography (ECG) were collected. Cardiovascular function and structure was assed by echocardiography, pulse wave analysis, IMT measurement, and 24 hour ambulatory blood pressure measurement.

Studies II-III

These studies were designed to investigate if chronic and acute blood pressure lowering, respectively, restores the impaired capacity for stimulated fibrinolysis in hyper-

Table 2. Overview of the studies

	Study I	Study II	Study III	Study IV
Study type	Explorative prospective	Hypothesis testing	Hypothesis testing	Hypothesis testing
Object studied	Population cohort	Vascular bed *in vivo*	Vascular bed *in vivo*	Human aortic endothelial cells
Subjects	BPE / NC	HT	HT	-
Number	49 / 17	10 + 10	9 + 3	4 (cell donors)
Intervention	-	Chronic blood pressure lowering	Acute blood pressure lowering	Cyclic strain; short term and long term
Major outcome variable	Hypertension, cardiovascular function and structure	Stimulated fibrinolytic capacity	Stimulated fibrinolytic capacity	Expression and release of fibrinolytic proteins

Abbreviations: BPE=blood pressure elevation group, NC=normal control group, HT=hypertension.

tension [92, 93]. Previously untreated or treated hypertensives (after approximately 4 weeks cessation of blood pressure treatment) were included if blood pressure levels were >140 mmHg systolic and >90 mmHg diastolic. Firstly, the capacity for stimulated fibrinolysis was investigated in the perfused-forearm model when the subjects had untreated high blood pressure (details about this model are given later in this section). Secondly, in Study II, the capacity for stimulated fibrinolysis was investigated again after 8-10 weeks of adequate blood pressure lowering (chronic BP lowering). To differentiate drug-specific effects from those of blood pressure reduction, as such, two drugs with different modes of action were used. Subjects were randomized to treatment with either the ACE-inhibitor lisinopril (Zestril®, AstraZeneca, Sweden) or the calcium antagonist felodipine (Plendil®, AstraZeneca, Sweden) in increasing doses, aiming at office BP <130/85 mmHg, or if this was not achieved, to a maximal dose of lisinopril at 20 mgx2 or felodipine 10 mgx2 daily.

Secondly, in Study III, the capacity for stimulated fibrinolysis was investigated again after acute lowering of blood pressure with intravenous sodium nitroprusside (SNP) (Nitropress®, Hospira Inc) (n=9). The SNP was dissolved and diluted in 5% dextrose to the concentration of 200 µg/mL and infused with a starting dose of 0.3 µg/kg/min which was raised every five minutes to lower the systolic BP to <120 mmHg or if this was not possible, to achieve a 25% reduction in mean arterial pressure (MAP). When on target blood pressure for 30 minutes (acute blood pressure lowering) the fibrinolytic capacity was assessed again. The SNP infusion was continued during this provocation. A small group was given saline instead of SNP and used as reference (n=3).

Study IV

This study was designed to examine the regulatory effects of the tensile force component of blood pressure on fibrinolytic proteins. In hypertension, the cyclic strain of

the endothelium is enhanced which possibly influences the expression and secretion of the fibrinolytic proteins t-PA, u-PA, and the inhibitor PAI-1 in vascular endothelial cells. HAECs were seeded at standard densities (2 x 10^5 cells per well) in six-well flexible-bottomed BioFlex plates precoated with collagen type I (Flexcell International Corporation, USA) and cells were grown to confluence. Thereafter identical cultures were exposed to either cyclic strain, in a Flexercell Tension Plus FX-4000T system, or served as static controls. Strain stimulation was set to 10% stretch at 60 cycles per minute (0.5 s elongation alternating with 0.5 s relaxation) and based on compliance values from medium-sized arteries this corresponds to an intraluminal pressure of 170 mmHg [99]. All cell batches were exposed to strain for 6, 24, 48, and 72 h and mechanically stimulated cells were compared with static control cells from the same individual, within the same experiment and from the same time-point.

Cardiovascular function and structure

Echocardiography

Echocardiography was performed by one echo technician (S.E.) both at baseline and at follow-up (Study I) without information on the clinical status of the subjects. Standard commercial equipments were used: At baseline ACUSON 128 Cardiovascular System (ACUSON Computed Sonography, USA) and at follow-up ACUSON Sequoia 256 (Siemens, Germany). Parasternal M-mode recordings were made and based on these measurements the left ventricular mass (LVM) was calculated according to the corrected formula of the American Society of Echocardiography and indexed for height [100]. Relative wall thickness (RWT) was calculated as interventricular septum plus posterior wall thickness divided by left ventricular diameter in diastole (IVSd+LVPWd)/LVDd. At follow-up a more extensive echocardiography examination was done. The transmitral, early diastolic and atrial peak filling velocity was assessed with pulsed wave doppler in the four chamber view. The longitudinal, myocardial, peak early diastolic velocity was assessed in the base of the left ventricular wall with spectral, pulsed-wave tissue poppler. Left and right atrial areas were measured in apical four chamber view in end-systole using two-dimensional echocardiography.

Pulse wave analysis and pulse wave velocity

The subjects were investigated by the author or a sonographic technician (H.K.) at the Clinical Experimental Research Laboratory. The brachial blood pressure was measured and registered immediately before applanation tonometry was performed. First radial and secondly carotid artery waveforms were recorded with a high-fidelity micro manometer (SPC-301, Millar Instruments, USA). Pulse wave analysis (SphygmoCor, AtCor Medical, Australia) was then used to generate central (aortic) pulse wave form using a transformation formula [101], which has been prospectively validated [102]. The recordings were ECG-gated and the SphygmoCor device automatically calculated the central blood pressures, central augmentation index (AIX) and pulse wave velocity (PWV).

Intima-media thickness

The subjects were investigated by the author or a sonographic technician (H.K.). Measurements were done with real time B-mode ultrasound, ACUSON 128XP/10c, using a 7 Mhz probe. Imaging of the IMT was done in the far wall of the common carotid artery 2 to 3 cm proximal of the bifurcation visualizing the luminal-intimal and medial-adventitial interfaces defining the IMT. The ultrasound picture was "frozen" and digitized on a Power Macintosh 7100/80. The IMT was then measured by an automated computerized program (Iôtec System, Iôdata Processing), average IMT was calculated as the mean value of a great number of local IMT measures performed every 100 μm along at least one cm of longitudinal length of the artery [103]. Total IMT was calculated as (left+right)/2.

Blood pressure measurements

Office blood pressure

At baseline (Study I) a mercury sphygmomanometer was used for office blood pressure measurements. After resting 15 minutes in the supine position blood pressure was recorded in the right arm with Korotkoff phase I used for systolic and Korotkoff phase V for the diastolic reading. Readings were made with 2 mmHg accuracy and an average of three blood pressure measurements was calculated. At follow-up (Study I) office blood pressure was measured in the morning in the sitting position after 5-10 minutes rest using the previously validated [104, 105] automatic Omron 705IT (Omron Healthcare Co., Ltd. Kyoto, Japan) device. One measurement was done in both arms followed by two additional measurements in the arm with the highest value. The size of the cuff was adjusted to the circumference of the upper arm. The blood pressure was calculated as the mean out of three readings one to two minutes apart. The methodological differences at baseline and follow-up were compared in a small separate study (n=14). Sitting BP measured by the Omron device was compared to BP in the supine position measured by the mercury sphygmomanometer. SBP was on the average 3.3 (1.38) ($P<0.05$) and DBP 1.9 (1.02) ($P=0.09$) mmHg higher, respectively, when measured by the Omron device in the sitting position.

Twenty-four hour ambulatory blood pressure

At baseline (Study I) the 24 h ambulatory blood pressure was measured with Spacelab 90202 (Redmond, Washington, USA) and at follow-up (Study I) with Spacelabs model 90217-6Q (Spacelabs Medical, Issaquah, WA, USA) in the none-dominant arm. Systolic, diastolic and mean arterial blood pressure and heart rate were recorded every 20 minutes throughout the 24 h. Mean values were calculated for wake periods and sleeping periods. A minimum number of 15 successful BP measurements, daytime (06.00-22.00) and nighttime (22.00-06.00), respectively, were set as lowest acceptable standard. Hypertension was defined as mean 24 h SBP >130 mmHg, and/or DBP >80 mmHg according to ESC/ESH 2007 Guidelines [8], or ongoing pharmacological treatment of hypertension (Study I).

Intraarterial blood pressure

As a vital part in the perfused-forearm model (Studies II and III) an intraarterial catheter was placed in the brachial artery in the non-dominant arm for blood sampling, intraarterial infusions and blood pressure monitoring. Intraarterial blood pressure was continuously monitored on a digital monitor (SC 9000, Siemens Medical Systems Inc) connected by pressure transducers to the catheter via a five-way stop-tap.

The perfused-forearm model

This model enables the study of vascular and endothelial function *in vivo* in humans without systemic interference and it is therefore well suited for studies on local release-rates of fibrinolytic proteins. The principle of the model is to asses the local release (or uptake) in the forearm vasculature by comparing the plasma concentration of a substance in simultaneously collected arterial and venous blood, and to correct the arteriovenous gradient for the current plasma flow in the forearm [68, 70, 91].

Catheterization procedure and experimental milieu

Under sterile conditions an 18 gauge intraarterial catheter (Hydrocath Arterial Catheter, Becton Dickinson) was placed in the brachial artery in the non-dominant arm using the Seldinger technique [106]. The catheter was advanced some 10 cm in the proximal direction and connected to a five-way stop-tap for arterial infusions, blood sampling, and blood pressure recordings. An intravenous cannula was placed in an antecubital vein in the same arm for venous blood sampling. In Study III an intravenous cannula was also inserted in the other arm for intravenous infusion of SNP or saline. All experiments were performed in a dimly lit, temperature-regulated, and soundproof room. Unnecessary communication and disturbance was avoided during the experiments and efforts were maid to maintain a comfortable and relaxing atmosphere. Calm music by choice of the subject was played on a low volume. After all catheters were in place, at least 45 minutes were allowed before starting the experiment by baseline recordings. When the experiment was completed the catheters were removed and at least 20 minutes of manual compression over the arterial wall perforation was performed to avoid hematoma.

Intraarterial infusion and hemodynamic recordings

The stimulated fibrinolytic capacity *i.e.* stimulated release rate of t-PA was assessed during intraarterial infusion of substance P (substance P; Clinalfa, Switzerland) by means of a syringe infusion pump (Alaris Asena GS; Cardinal Health, Switzerland) at a constant rate of 1 mL/min for 20 minutes. Substance P was dissolved in saline to a concentration of 8 pmol/mL. Forearm blood flow (FBF) was measured by venous occlusion pletysmography simultaneously in both arms. A mercury-in-silastic strain gauge was placed around the forearm recording the increase in circumference of the forearm during venous occlusion, achieved by rapid inflation of blood pressure cuffs applied proximal of the elbow on both arms, to 40 mmHg using the MAPPC® soft-

	Baseline		Substance P infusion								Baseline		
Time (min)	**5**	**10**	**1,5**	**3**	**6**	**9**	**12**	**15**	**18**	**20**	**2**	**10**	**20**
Venous	x	x	x	x	x	x	x	x	x		x	x	x
Arterial	O	O								O	O	O	O
Hematocrit	I	I											

Figure 2. Blood sampling protocol in the perfused forearm model (Studies II-III)

ware (Elektromedicin, Sweden). FBF was calculated and expressed as mL per minute and liter tissue. A mean of 3-5 recordings was calculated at each point of measure. Average intra- and inter-observer coefficients of variation were in our lab, 5.6% and 4.6%, respectively (Study II). FBF measurements were done immediately after every venous blood sampling (Figure 2). Forearm vascular resistance (FVR) was calculated as the ratio of MAP to FBF, and expressed as arbitrary resistance units.

Blood sampling

Blood sampling was performed according to a strict protocol (Figure 2). During baseline recordings, venous and arterial blood was collected simultaneously. To avoid interruption of the intraarterial infusion only venous blood was collected during the substance P infusion. Arterial values during the infusion were interpolated from values immediately before and after the end of infusion (Figure 2). At each sampling point the first 2 mL of blood were discarded, and subsequently 4 mL were drawn with ice chilled syringes and collected in ice chilled tubes containing 1/10 vol. 0.45 M sodium citrate buffer, pH 4.3, that stabilizes active t-PA [107] (Stabylite®, Biopool International, Sweden). Tubes were kept on ice until plasma was isolated by centrifugation at 4ºC and 2000 g for 20 minutes. Plasma aliquots were then transferred to plastic tubes and immediately frozen and stored at -70ºC until assay. Arterial hematocrit was determined in duplicate using micro-hematocrit centrifuge.

Calculation of local t-PA release

Net release or uptake of t-PA at every blood sampling point was calculated according to the formula:

$$\text{Net release} = (C_V - C_A) \times FPF$$

C_V denotes venous and C_A arterial concentration of t-PA [68, 70, 91]. Forearm plasma flow (FPF) was estimated from FBF and arterial hematocrit, corrected for 1% trapped plasma according to the formula: FPF = FBF x ((101-hematocrit)/100). The accumulated release of t-PA was calculated as the area under the curve from start of substance P provocation until 20 minutes after termination of the infusion, using the statistical software Prism 3.0 (GraphPad Inc.). In this analysis negative areas were ignored [66, 93].

Cell stretch device

The cyclic strain experiments were performed with a Flexercell Tension Plus FX-4000T system (Flexcell International Corporation) equipped with a 25 mm BioFlex loading station to provide a well-defined equibiaxial and circumferential strain across a membrane surface (Figure 3). BioFlex loading station is composed of a single plate with six planar 25 mm cylinders per plate centered beneath each well of the BioFlex plate, and the top surface is just below the BioFlex membrane surface. Each BioFlex membrane is stretched over the post when under vacuum pressure, creating a single-plane uniformly stretched circle. Both static cultures and cells exposed to cyclic strain were seeded onto identical collagen coated BioFlex plates to ensure standardized culture conditions.

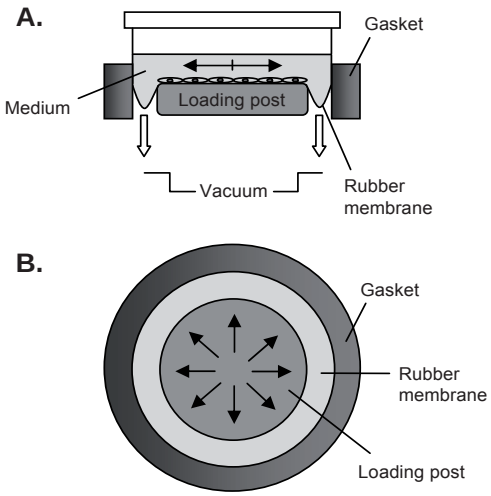

Figure 3. Schematic illustration of the strain device used in Study IV. The rubber membrane is stretched over the loading post when under vacuum suction. Cells covering the loading post are exposed to equibiaxial and circumferential strain.
A. Side view of the BioFlex well.
B. Shows the BioFlex well viewed from above.

Biochemical assays

Protein quantification

The concentration of t-PA and PAI-1 in plasma (Studies I, II and III) and the culture medium (Study IV) and intracellular content of t-PA and u-PA (Study IV) were determined by enzyme-linked immunosorbent assays (ELISA; TintElize t-PA, Biopool International; COALIZA PAI-1, Chromogenix; TintElize PAI-1, Biopool International; ZYMOTEST u-PA, Haemochrom Diagnostica). TintElize t-PA, and COALIZA PAI-1, detects the total amount of the respective protein (both free and complex bound) with equal efficiency [108, 109]. TintElize PAI-1 detects all forms of PAI-1, although the efficiency for detection of t-PA/PAI-1 complexes is higher than for active PAI-1, and the latent form of PAI-1 has been reported to be less well detected [110]. The principle of these assays is that the analyzed protein binds to an antibody in a pre-coated microtest well. After the protein has bound to its antibody new peroxidase labeled antibodies are added. The wells are then washed and peroxidase substrate added. Peroxidase converts the substrate to a yellow product directly proportional to the amount of protein in the sample. All samples were assayed in duplicate. In the TintElize PAI-1 kit the sample luminescence was corrected for background luminescence in a quenching well. Plasma t-PA and PAI-1 activity (Study II) were measured

by biofunctional immunosorbent assays (Chromolize t-PA and Chromolize PAI-1, Biopool International) and expressed in ng/mL using the specific activity of 0.60 and 0.75 IU/ng, respectively (data on file, Biopool International). Intraassay variation coefficients were <5%.

mRNA quantification

Real-time reverse transcriptase polymerase chain reaction (real-time RT PCR) performed on an ABI Prism 7700 Sequence Detection System (Applied Biosystems) was used to quantify the levels of t-PA, PAI-1 and u-PA mRNA in endothelial cells (Study IV). Firstly, RNA was isolated and purified with Trizol and mRNA was converted to complementary DNA with GeneAmp RNA PCR kit (Applied Biosystems). The principle of the real-time RT PCR is that when a fluorescently labeled probe is hybridized to its target sequence during PCR, the Taq polymerase cleaves the reporter dye. The reporter dye is then released to the solution and the increase in dye emission is monitored in real-time. When the reporter fluorescence reaches a preset level it is called the cycle threshold (C_T). There is a linear relationship between C_T and the log of initial copy numbers [111]. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal standard to correct for potential variation in RNA loading and cDNA synthesis. The relative expression value of the target gene is obtained by calculating the difference in threshold cycles for a target and a reference gene in a treated sample, and then comparing it to a control sample.

Other biochemical analyses

Serum-insulin (immunometric method), plasma-glucose, lipid profiles, N-terminal pro-brain natriuretic peptide (NTproBNP), and high sensitivity C-reactive peptide (hsCRP) were analyzed by standard methods at the Department of Clinical Chemistry at the Sahlgrenska University Hospital. Baseline serum insulin (Study I; 1987) was analyzed by radioimmunoassay (Diagnostic Products Corp. USA).

Statistics

Standard statistical methods were used. Unless otherwise stated, values are presented as mean and standard error of the mean (SEM). Between-group comparisons of single variables were performed by Student's two sample *t*-test. Paired *t*-test was done where applicable. Between-group changes over time were analyzed with two-way ANOVA for repeated measurements (Study I). Correlation analyses were done using Pearson correlation (Study I). Linear regression (continuous variables) and logistic regression (dichotomous variables) were used to compare predictors (Study I). Responses to substance P were evaluated by two-way (treatment/no treatment and time) and one-way (time) ANOVA for repeated measurements (Studies II and III). Expression of t-PA was evaluated on a log-transformed scale using one-way (treatment) or two-way (treatment and time) ANOVA for repeated measurements. When ANOVA indicated a significant treatment or treatment x time effect, responses at individual time points were evaluated by contrast analysis (Study IV). Proportions of categorical data were compared by chi-square (Studies I and II). Findings were considered significant at $P< 0.05$ (two tailed tests).

RESULTS

Study I

Hypertension

Hypertension was present in 35/47 (74.5%) of the BPE subjects and 1/17 (5.9%) of the NC subjects (Figure 4). Out of the total 36 subjects with hypertension at follow-up, only 4 were on pharmacological treatment. At baseline 27/44 (61.4%) in the BPE group and 2/17 (11.8%) in the control group had hypertension using the same definition.

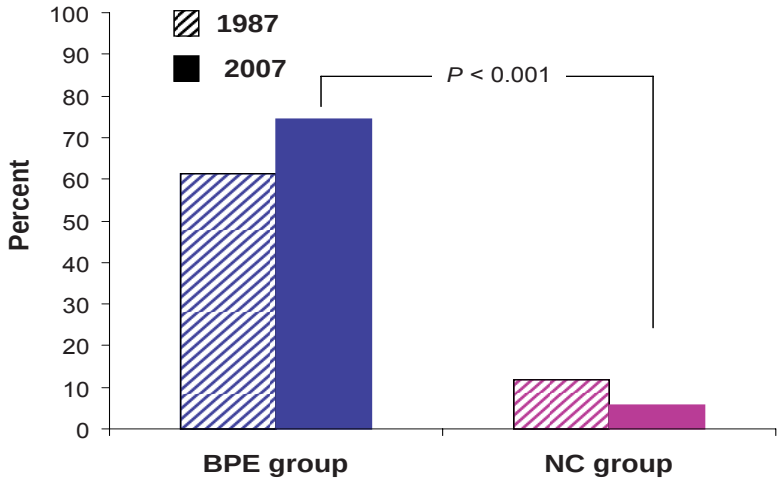

Figure 4. Prevalence of hypertension at follow-up. Between groups Chi-square.

Blood pressure

The 24 h ambulatory blood pressure for the two groups is shown in Figure 5. 24 h MAP had increased from 86.6 (0.8) to 97.2 (1.2) mmHg (paired *t* test; $P<0.0001$), and from 83.1 (1.5) to 88.1 (1.2) mmHg ($P<0.01$) at follow-up in the BPE and the NC group, respectively. The raise in MAP was significantly steeper in the BPE compared to the NC group (two-way ANOVA; $P=0.01$). Also, the 24 h DBP had increased from 72.3 (1.0) to 81.5 (1.2) mmHg ($P<0.0001$), and from 69.3 (1.5) to 73.5 (1.1) mmHg ($P<0.01$) at follow-up in the BPE and NC group, respectively. The increase in diastolic blood pressure was significantly steeper in the BPE compared the NC group ($P=0.01$). However, the 24 h SBP in the BPE group at baseline was 133.2 (1.3) mmHg and at follow-up 132.0 (1.4) mmHg (P=ns), and in the NC group 124.5 (2.3) at baseline and 118.9 (1.4) mmHg ($P<0.05$) at follow-up (Figure 5). The change in systolic blood pressure was similar in the two groups (P=ns). The office blood pressure recordings showed a similar pattern as the 24 h recordings, with unchanged SBP and significant increase in DBP in both groups at follow-up (Table 3).

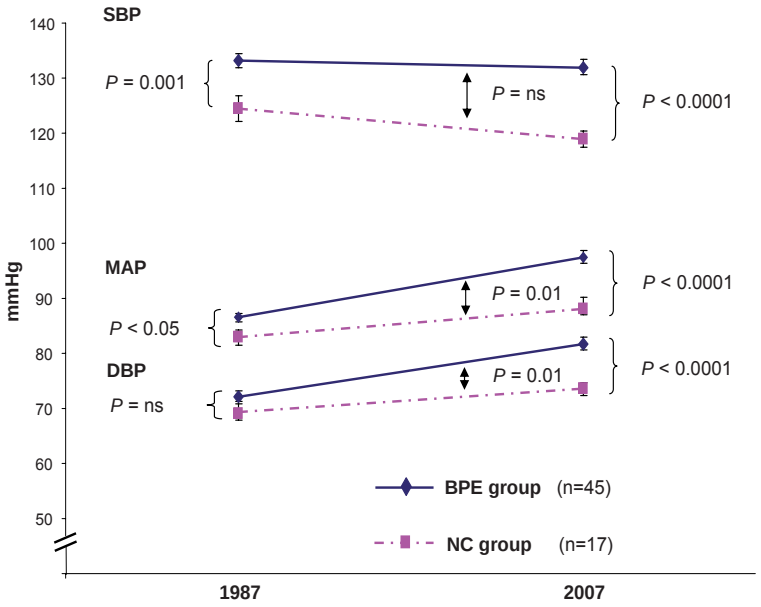

Figure 5. Twenty-four hour blood pressure at baseline and follow-up. Differences between groups at baseline and follow-up, unpaired *t* test (braces). Between-group difference in trajectory, two-way ANOVA (arrows), in this analysis n=42 in the BPE group.

Table 3. Population characteristics at follow-up. Mean (SEM)

Variable	Blood pressure elevation (n=47)	Controls (n=17)	*P* value
Age, years	40 (0.3)	39 (0.8)	ns
SBP office, mmHg	144.0 (1.9)	124.5 (1.7)	< 0.0001
DBP office, mmHg	91.2 (1.4)	81.2 (2.3)	< 0.01
Weight, kg	95.2 (2.7)	88.1 (3.6)	ns
BMI, kg/m^2	28.49 (0.74)	26.97 (0.97)	ns
Waist/hip ratio	0.93 (0.01)	0.89 (0.02)	< 0.05
Hemoglobin, g/L	152 (2)	152 (2)	ns
Creatinine, µmol/L	85 (1.6)	89 (2.5)	ns
Uric acid, µmol/L	345 (9)	313 (17)	0.072
S-Cholesterol, mmol/L	5.2 (0.19)	5.2 (0.23)	ns
S-Triglyceride, mmol/L	1.62 (0.15)	1.39 (0.18)	ns
S-LDL, mmol/L	3.25 (0.18)	3.08 (0.18)	ns
S-HDL, mmol/L	1.32 (0.5)	1.49 (0.09)	ns
S-ApoA1, g/L	1.30 (0.21)	1.38 (0.4)	ns
S-ApoB, g/L	1.14 (0.05)	1.07 (0.08)	ns
P-Glucose, mmol/L	5.4 (0.17)	5.0 (0.12)	ns
S-Insulin, mU/L	11.4 (1.13)	7.9 (1.29)	0.082
hsCRP, mg/L	1.21 (0.17)	1.27 (.21)	ns
S-NT-proBNP	39.7 (4.1)	27.7 (3.5)	0.09
PAI-1 antigen ng/mL	21.4 (1.7)	17.1 (3.6)	ns
t-PA antigen ng/mL	9.9 (0.52)	7.5 (0.82)	< 0.05

Abbreviations: SBP=systolic blood pressure, DBP=diastolic blood pressure, BMI=body mass index, LDL=low density lipoprotein, HDL=high density lipoprotein, ApoA1=Apo lipoprotein A1, ApoB=Apo lipoprotein B, hsCRP=high sensitivity C-reactive peptide, NT-proBNP=N-terminal pro brain natriuretic peptide, PAI-1=plasminogen activator inhibitor 1, t-PA=tissue plasminogen activator.

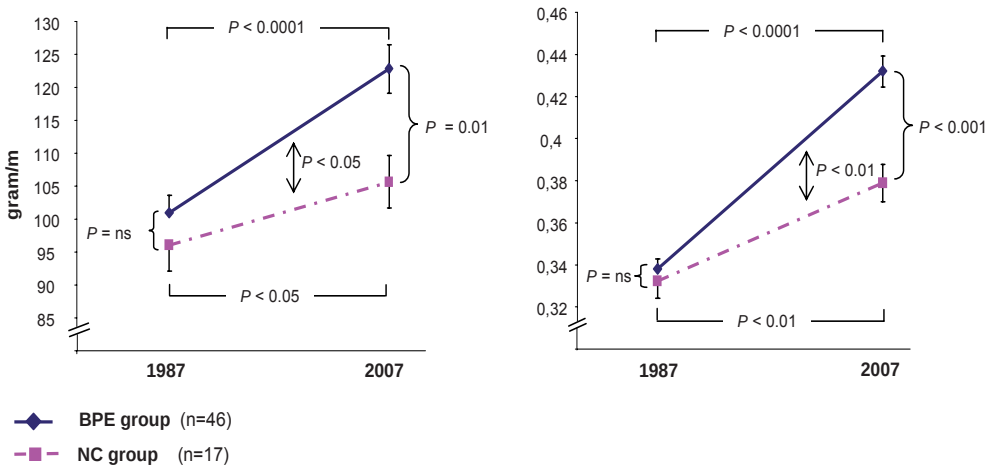

Figure 6. Left ventricular mass index at baseline and follow-up, left panel. Relative wall thickness at baseline and follow-up, right panel. Between-group difference in trajectory over time, two-way ANOVA (arrows). Differences between the groups at baseline and follow-up, unpaired *t*-test (braces). Differences within groups over time, paired *t*-test (brackets).

Cardiac structure and function

Left ventricular mass index (LVMI) and relative wall thickness (RWT) are shown in Figure 6. LVMI had increased from 100.0 (2.5) to 122.9 (3.7) g/m ($P<0.0001$) and from 96.1 (3.9) to 105.6 (4.0) g/m ($P<0.05$) at follow-up, in the BPE and the NC group, respectively. The increase of LVMI in the BPE group was significantly steeper compared to the NC group (two-way ANOVA; $P<0.05$). RWT increased from 0.34 (0.005) to 0.43 (0.007) ($P<0.0001$) and from 0.33 (0.008) to 0.38 (0.009) ($P<0.01$) at follow-up, in the BPE and the NC group, respectively (Figure 6). The increase of RWT was significantly steeper in the BPE compared to the NC group ($P<0.01$). Diastolic function measurements were similar in the two groups (Table 4). However,

Table 4. Echocardiography, AIX and PWV at follow-up. Mean (SEM)

Variable	Blood pressure elevation (n=46)	Controls (n=17)	Unpaired *t*-test *P*
LVDd, mm	51.7 (0.42)	52.0 (0.59)	ns
LVDs, mm	33.7 (0.48)	33.9 (0.45)	ns
IVSd mm	11.2 (0.2)	9.9 (0.2)	< 0.001
LVPWd mm	11.0 (0.2)	9.7 (0.2)	< 0.0001
LVM, gram	224.1 (6.8)	190.9 (7.6)	< 0.01
Ejection fraction, %	63.6 (0.7)	63.6 (0.6)	ns
E-wave, cm/s	73.9 (1.6)	71.2 (2.2)	ns
A-wave, cm/s	52.2 (1.3)	48.8 (1.7)	ns
DT, ms	176.3 (2.8)	170.5 (3.4)	ns
Em, cm/s	17.1 (0.38)	17.8 (0.57)	ns
E-wave/Em	4.4 (0.13)	4.0 (0.12)	ns
Left atrial area, cm²	20.9 (0.6)	18.3 (0.7)	<0.05
Atrial size inequality	2.46 (0.31)	1.00 (0.30)	=0.01
AIX	16.5 (1.75)	13.3 (2.51)	ns
Pulsewave velocity, m/s	8.79 (0.16)	8.30 (0.26)	ns

Abbreviations: LVDd=left ventricular diameter in end diastole, LVDs=left ventricular diameter in end systole, IVSd=inter ventricular septum diameter in end diastole, LVPWd=left ventricular posterior wall diameter in end diastole, LVM=left ventricular mass, LA-RA=left atrial minus right atrial area, E-wave=early peak diastolic filling velocity, A-wave=atrial peak filling velocity, DT=deceleration time, Em=early diastolic left ventricular lateral wall myocardial relaxation velocity, AIX=augmented pressure/pulse pressure.

the left atrial area was significantly larger in the BPE compared to the NC group, and this structural difference was confirmed by larger atrial size inequality (left atrial area minus right atrial area) in the BPE compared to the NC group (Table 4).

Vascular structure and function

The intima-media thickness was 0.61 (0.01) mm in the BPE group compared to 0.57 (0.01) mm in the NC group (unpaired *t* test; $P<0.05$). Central (aortic) pulse pressure was significantly higher in the BPE group or, 36.4 (1.3) mmHg compared to 29.2 (1.4) mmHg, in the NC group (unpaired *t* test; $P<0.01$), but AIX and PWV were comparable in the two groups (Table 4).

Anthropometric data and lipid profile

Subjects in both groups had increased significantly in weight and BMI at follow-up, but there was no significant difference between the groups (Table 3). Waist/hip ratio (WH) was similar in the two groups at baseline but had increased significantly only in the BPE group to 0.93 (0.01) while it was 0.89 (0.02) in the NC group, resulting in a significant difference between the groups at follow-up ($P<0.05$). The increase in WH was significantly steeper in the BPE compared to the NC group (two-way ANOVA; $P=0.05$). The groups had similar lipid and metabolic profiles at follow-up (Table 3).

Predictors of left ventricular mass and blood pressure

In a forward linear regression model of the combined groups, 56% of LVMI at follow-up was explained by the combination of office MAP at baseline, BMI at baseline, and insulin at baseline (adj. R^2 0.560; $P<0.0001$). In the same model, 33% of 24 h MAP at follow-up was explained by the combination of office MAP at baseline, and 24 h DBP at baseline (adj. R^2 0.330; $P<0.0001$). Using forward logistic regression, 33% of hypertension prevalence was explained by office MAP at baseline, and 24 h DBP at baseline (Cox and Snell R^2 0.333; $P<0.001$).

Study II

Hemodynamic responses

Baseline hemodynamic and fibrinolytic variables are shown in Table 5. After target blood pressure levels were reached, the patients were treated on average for 10 and 9 weeks in the lisinopril and felodipine groups, respectively, before the second study day was performed. Treatment lowered the intraarterial systolic and diastolic blood pressure on the average from 165 (3)/82 (2) to 140 (3)/71 (1) mmHg ($P<0.01$, throughout). Changes in blood pressure were similar in the lisinopril and felodipine groups, or 24/12 and 26/10 mmHg, respectively (P=ns). Baseline FBF and FVR were not affected by treatment. Also, baseline fibrinolytic protein concentrations were not changed in either group by treatment. Substance P induced highly significant decreases in FVR and increases in FBF, both when patients were untreated and on active treatment (ANOVA, $P<0.0001$ for all). The responses of FVR (Figure 7) and FBF to substance P stimulation were of the same magnitude on both treatment days (two-way ANOVA, ns).

Table 5. Baseline hemodynamic, fibrinolytic and inflammatory variables. Mean and (SEM)

Parameter	Untreated	Treated	*P* value
Systolic blood pressure, mmHg*	165.4 (3.0)	140.3 (3.4)	<0.00001
Diastolic blood pressure, mmHg*	81.9 (1.5)	71.1 (1.4)	<0.00001
Mean arterial pressure, mmHg*	115.3 (1.7)	98.7 (1.9)	<0.00001
Forearm blood flow, mL/L tissue	62.3 (7.2)	47.7 (4.8)	n.s.
FVR, arbitrary units	2.5 (0.3)	2.6 (0.3)	n.s.
Plasma t-PA antigen, ng/mL	8.9 (0.5)	8.6 (0.6)	n.s.
Plasma t-PA activity, IU/mL	0.66 (0.05)	0.68 (0.05)	n.s.
Plasma PAI-1 antigen, ng/mL	31.1 (3.2)	29.8 (6.2)	n.s.
Plasma PAI-1 activity, IU/mL	6.1 (1.1)	6.7 (2.5)	n.s.
hs-CRP, mg/L	3.6 (0.4)	2.7 (0.5)	n.s.

*Blood pressure measured intra arterially before the experiments. Abbreviations: FVR=forearm vascular resistance, t-PA=tissue plasminogen activator, PAI-1=plasminogen activator inhibitor 1, hs-CRP=high sensitivity C-reactive peptide.

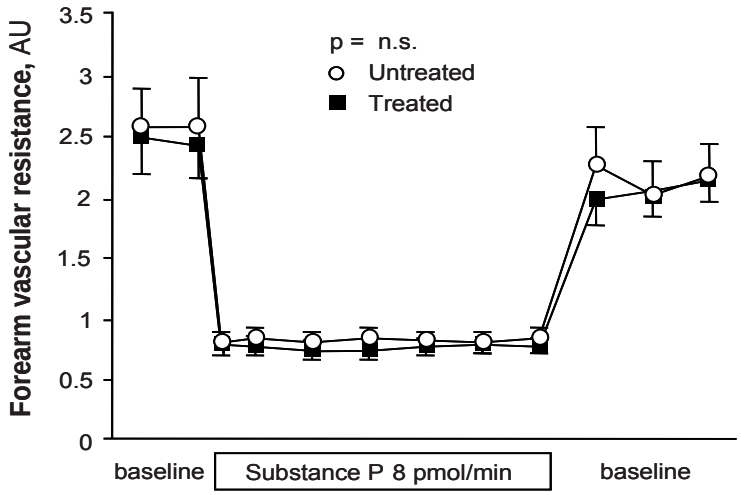

Figure 7. FVR (arbitrary units) during baseline and in response to 20 minutes of intraarterial infusion of substance P (8 pmol/min) in untreated (o) and treated (■) hypertensive patients (baseline measurements 15 minutes before and 20 minutes after the infusion). Two-way ANOVA, mean and SEM.

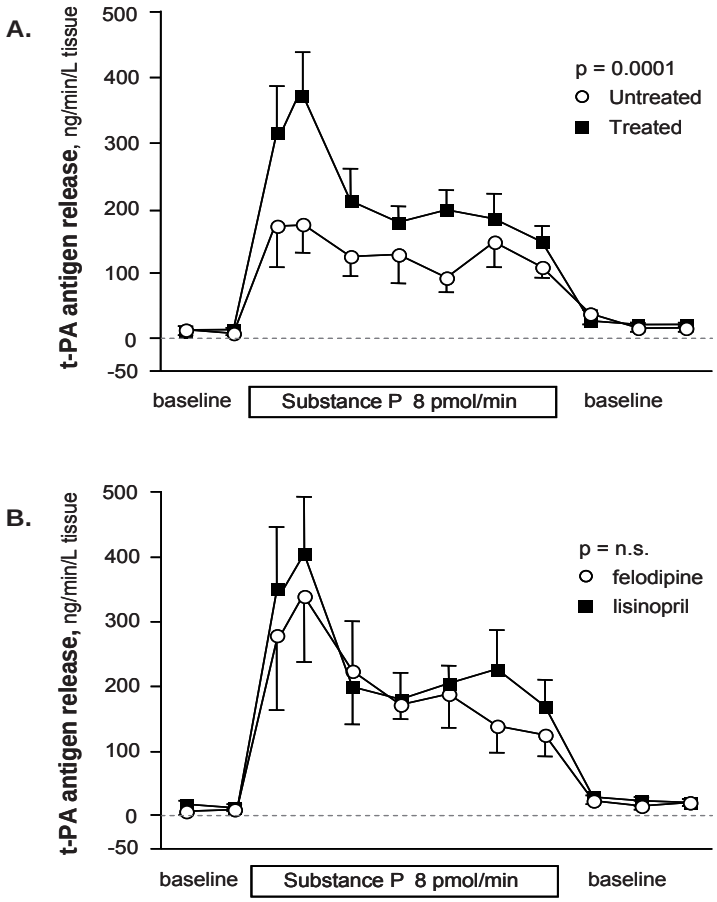

Figure 8. Chronic blood pressure lowering restores the defect t-PA response. Net forearm release rates of t-PA antigen during baseline and in response to 20 minutes of intraarterial infusion of substance P (8 pmol/min) (baseline measurements 15 minutes before and 20 minutes after the infusion). **A**. untreated compared to treated hypertensive patients. **B**. felodipine compared to lisinopril treated hypertensive patients. Two-way ANOVA, mean and SEM.

Chronic blood pressure lowering restores the defect t-PA response

Substance P induced highly significant t-PA secretory responses of the forearm both when patients were untreated and when they were on active antihypertensive treatment (ANOVA, $P<0.0001$). In line with the hypothesis, the t-PA antigen release response was significantly greater on treatment (Figure 8A; two-way ANOVA, $P=0.0001$). There were no significant differences in the t-PA release responses between the treatment groups (Figure 8B), although the increase in t-PA release was numerically larger in the lisinopril treated group (P=ns). The cumulated t-PA antigen release during substance P infusion increased from 3,000 (655) to 4,557 (701) ng/L tissue with treatment (t test, $P<0.05$). The release of active t-PA during the first 6 minutes of infusion was significantly improved by treatment (two-way ANOVA, $P=0.03$).

The t-PA antigen release, which was in the order of 9.5 and 11.8 ng/min and L tissue at baseline, increased significantly and peaked at 257 (58) and 445 (77) ng/min and L tissue during the substance P infusion, in untreated and treated patients, respectively (P<0.0001, for both). The peak t-PA release was significantly improved by treatment (t test, P=0.02) and was of almost identical magnitude in the lisinopril and felodipine groups (P=ns). On the whole, substance P induced a 27- and 38-fold increase in t-PA release in untreated and treated patients, respectively (P<0.05 for change in fold increase).

Antihypertensive treatment also altered the temporal response pattern to stimulation, Figure 9. When patients were untreated, one third of the patients had a delayed onset of the t-PA response; in six out of 20 patients the peak release rate occurred 9 minutes or later after initiating substance P stimulation. The response pattern was normalized with treatment, and on the second study day all patients had the peak release rate during the first 6 minutes of stimulation (chi^2-test, P=0.008). Thus, treatment improved the response pattern and shortened the average time to peak secretion from 6.7 (1.4) to 2.7 (0.3) minutes (t test, P=0.01). Again, the improvement of the temporal response pattern was similar in the two treatment groups.

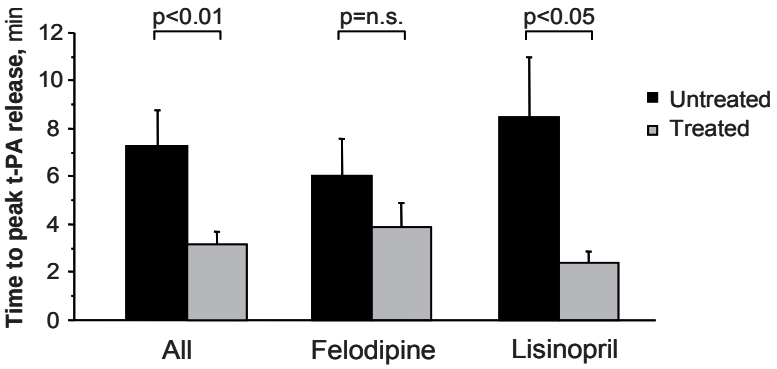

Figure 9. Histogram showing the time (in minutes) to peak t-PA release in untreated (black) and treated (gray) hypertensive patients. Left the whole group; middle, felodipine treated group and right, lisinopril treated group. Mean and SEM.

Study III

Hemodynamic responses

Baseline hemodynamic and fibrinolytic variables before the two infusions are shown in Table 6. As expected, the blood pressure was significantly lowered by SNP infusion with baseline MAP of 108.9 (3.9) mmHg and 82.4 (3.9) mmHg during high- and low-pressure conditions, respectively (t test, P<0.001). This blood pressure reduction

resulted in on the average 23% lower MAP during the low-pressure provocation (*t* test, *P*<0.001). Baseline FBF and FVR were similar during high- and low-pressure conditions (Table 6; *P*=ns). Intrabrachial substance P infusion resulted in a significant increase in FBF at all occasions (ANOVA, *P*<0.001). FVR and FBF responses to substance P infusion were similar during both pressure conditions (Figure 10; two-way ANOVA, *P*=ns for both).

Table 6. Baseline hemodynamic and fibrinolytic variables. Mean (SEM)

Parameter, Intervention group	**High pressure**	**Low pressure**	***P* value**
Systolic blood pressure, mmHg*	151.7 (3.0)	117.9 (3.5)	<0.001
Diastolic blood pressure, mmHg*	80.7 (2.5)	64.8 (2.2)	<0.001
Mean arterial blood pressure, mmHg*	108.6 (2.6)	83.0 (2.6)	<0.001
Forearm blood flow, mL/L tissue	36.7 (3.1)	33.7 (2.7)	ns
FVR, arbitrary units	3.29 (0.26)	2.85 (0.31)	ns
Plasma t-PA antigen, ng/mL	9.05 (0.40)	8.96 (0.52)	ns
t-PA release, ng/min/L tissue	12.4 (4.0)	15.8 (5.7)	ns
Parameter, Reference group	**Infusion 1**	**Infusion 2**	
Systolic blood pressure, mmHg*	148.3 (3.9)	150.3 (3.0)	ns
Diastolic blood pressure, mmHg*	83.2 (1.4)	85.8 (1.3)	<0.01
Mean arterial blood pressure, mmHg*	110.3 (2.1)	112.3 (1.8)	<0.05
Forearm blood flow, mL/L tissue	57.0 (4.5)	48.3 (4.2)	ns
FVR, arbitrary units	1.99 (0.15)	2.4 (0.22)	ns
Plasma t-PA antigen, ng/mL	9.46 (1.20)	8.34 (1.48)	<0.05
t-PA release, ng/min/L tissue	16.5 (7.1)	28 (9.7)	ns

*Blood pressure measured intraarterially before the experiments.
Abbreviations: FVR=forearm vascular resistance, t-PA=tissue plasminogen activator.

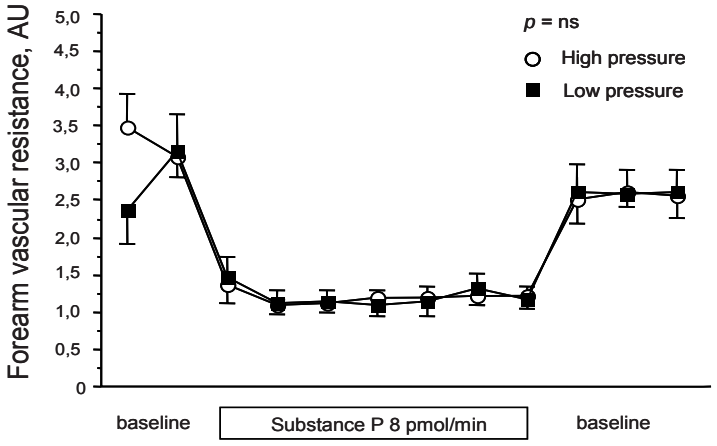

Figure 10. Forearm vascular resistance (arbitrary units) during baseline and in response to 20 minutes of intraarterial infusion of substance P (8 pmol/min). During High pressure (o) and during Low pressure (■) conditions (baseline measurements 15 minutes before and 20 minutes after the infusion). Two-way ANOVA, mean and SEM.

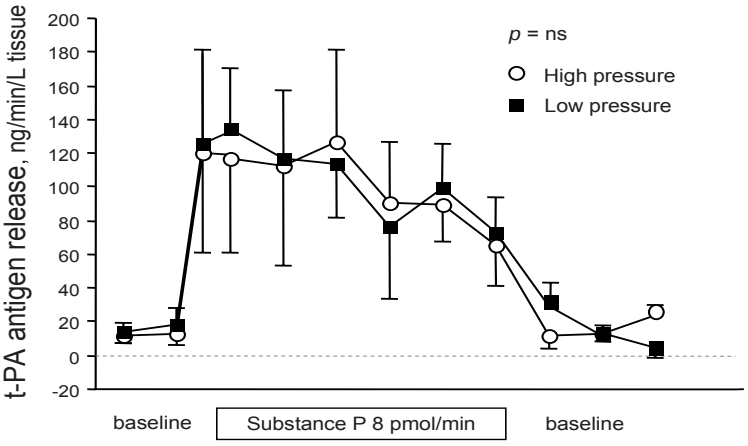

Figure 11. Net forearm release rates of t-PA antigen during baseline and in response to 20 minutes intraarterial infusion of substance P (8 pmol/min). During High pressure (o) and during Low pressure (■) conditions (baseline measurements 15 minutes before and 20 minutes after the infusion). Two-way ANOVA, mean and SEM.

Acute blood pressure lowering does not restore the defect t-PA response

Substance P induced a highly significant t-PA secretory response of the forearm, both during high blood pressure and during acutely lowered blood pressure (ANOVA, $P<0.01$ for both). The t-PA antigen release response to intrabrachial substance P was similar during both pressure conditions (Figure 11; two-way ANOVA, *P*=ns). Also, the accumulated release of t-PA, calculated as the area under the curve, was almost identical, or 2,395 (750) and 2,394 (473) ng/L tissue, during high- and low-pressure conditions, respectively (*t* test, *P*=ns). The t-PA release rate increased significantly in response to substance P and peaked at 199 (77) and 167 (41) ng/min/L tissue during high and low pressure ($P<0.05$ for both), respectively, and the responses were similar both times (*t* test, *P*=ns). Median time to peak release rate of t-PA was 3 minutes during both provocations, and the average time to peak secretion was 5.4 (1.9) and 5.8 (1.7) minutes during high and low pressure, respectively (*t* test, *P*=ns).

Reference group

Baseline hemodynamic and fibrinolytic variables are shown in Table 6. In this group, hemodynamic and t-PA responses were similar during both infusions (two-way ANOVA; *P*=ns for all).

Study IV

Cyclic strain suppresses the expression of t-PA

In response to cyclic strain, t-PA mRNA expression (Figure 12) showed a biphasic temporal response pattern, with an early transient inductive response at 6 h, which switched to a suppression during prolonged strain stimulation reaching a plateau phase at 48 and 72 h (two-way ANOVA; $P<0.001$). At 6 h, cyclic strain was associated with a 53% up-regulation of t-PA mRNA ($P<0.001$ compared to static cells), which thereafter declined with time. After 24 h, t-PA mRNA was slightly decreased in cells exposed to cyclic strain compared to static controls (13% reduction) and after 48 h the relative reduction in t-PA mRNA was 28% ($P<0.01$). T-PA mRNA expression remained at a suppressed level in HAECs strained for 72 h (29% reduction, $P<0.01$). Analyses of secreted t-PA antigen in conditioned medium from strained HAECs showed increased relative release rates, which peaked at 6 h, and then declined in parallel with the t-PA mRNA data. The t-PA secretion over 72 h showed a similar pattern as t-PA mRNA, with the exception that the strain effect was more delayed (two-way ANOVA; $P<0.01$). The average t-PA secretion from strained HAECs during the first 6 h increased with 60% compared to static control cells ($P<0.001$), then diminished with prolonged cyclic strain stimulation, and from 48 to 72 h switched from an induction to a 12% reduction. Interestingly, prolonged cyclic strain affected the mRNA expression of the other plasminogen activator, u-PA, in a similar way. The difference was that the strain mediated suppression of u-PA mRNA was present first after 72 h of stimulation (19% reduction, $P<0.01$).

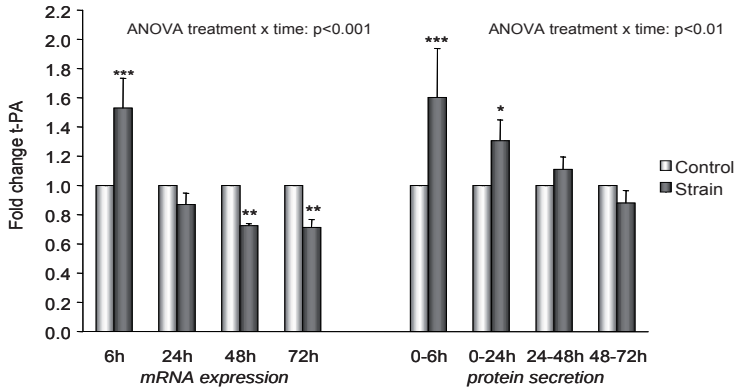

Figure 12. Relative mRNA expression and secretion of tissue-type plasminogen activator (t-PA) in HAECs exposed to 10% cyclic strain for 6-72 h. n=4, *p<0.05, **p<0.01, ***p<0.001, by contrast analysis.

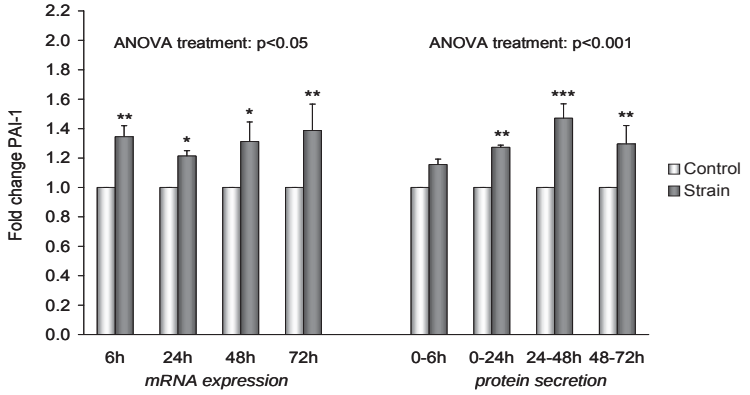

Figure 13. Relative mRNA expression and secretion of plasminogen activator inhibitor 1 (PAI-1) in HAECs exposed to 10% cyclic strain for 6-72 h. n=4, *p<0.05, **p<0.01, ***p<0.001, by contrast analysis.

Cyclic strain induces the expression of PAI-1

Analyses of PAI-1 mRNA (Figure 13) in the same samples showed increased expression in strained HAECs at all time-points (ANOVA; $P<0.05$). The magnitude of the relative increases in PAI-1 mRNA expression ranged between 21 and 39%, but there was no significant temporal pattern from 6 to 72 h. Similar to the induced expression of PAI-1 mRNA, cyclic strain also stimulated PAI-1 antigen secretion into the conditioned medium of HAECs at all time-points compared to static control cells (ANOVA; $P<0.001$). Relative strain-induced increases in PAI-1 secretion were in the order of 16 to 47%, and the release rates of PAI-1 were significantly increased from 24 - 72 h ($P<0.01$ throughout).

DISCUSSION

Globally, elevated blood pressure has a tremendous impact on the burden of morbidity and mortality in cardiovascular disease. As a risk factor, the effects of elevated blood pressure in young age may not become evident until later in life, and identification of individuals at increased risk of developing high blood pressure is of importance. Also, it is not clear which pathophysiological factors increase the atherothombotic risk connected to this trait, or how blood pressure lowering treatment affects the different vascular functions in this perspective. This thesis has focused on blood pressure elevation and the effects on the vascular structure and function. First, the significance of blood pressure elevation in young age, for future establishment of hypertension and secondary structural changes, was explored in a prospective study. More specifically, we wanted to further clarify the mechanisms impairing the endothelial t-PA release, which our group has previously found in hypertension. To this end, the effect of chronic and acute blood pressure lowering on t-PA release was studied invasively in hypertensive patients, and the effect of biomechanical strain on the t-PA production was studied in cultured endothelial cells.

Implication of blood pressure elevation in young age

The major findings of this study were that the single category variable of brachial blood pressure level separates the trajectory of the two groups not only regarding blood pressure, but also with respect to cardiovascular subclinical organ damage, known to be associated with cardiovascular risk. At follow-up, the prevalence of hypertension was strikingly high, and concomitant adverse development of cardiovascular structure was evident in the BPE group. This illustrates the valuable predictive information that an even mildly elevated blood pressure or normal blood pressure, carries over 20 years. To our knowledge, no study, has previously compared the development of cardiovascular structure in men with elevated blood pressure at such young age and normal controls over 20 years.

Blood pressure levels

The most apparent change in blood pressure was the increased DBP while SBP levels were similar over time in both groups. This pattern is in accordance with previous observations in both hypertensives and normotensives across this age span [112]. However, the BPE group, starting at a higher level, increased the DBP with a significantly steeper trajectory than the NC group, subsequently being at increasingly higher risk since elevated DBP is the strongest predictive brachial blood pressure of coronary heart disease in the ages below 50 years [113]. According to our results, a high blood pressure at the age of 20 year has an excellent predictive value for the prevalence of hypertension at age 40. The incidence of hypertension was 77% in a 10 year follow-up of 26 un-medicated middle aged men with borderline hypertension in a study from Finland [31]. In the study, when including both normotensives and hypertensives, the best predictor of blood pressure level and need for medication at follow-up was blood pressure level at baseline *i.e.* 24 h MAP [31]. This notion was confirmed in the present study as office MAP and 24 h DBP at baseline were the best predictors of 24 h MAP and hypertension at follow-up.

Left ventricular hypertrophy

The increased pressure load on the left ventricle was manifest in the distinct different developmental patterns of LVMI and RWT in the two groups. Importantly, both variables were independent predictors of cardiovascular events [19, 114, 115]. Linear regression showed that office MAP, BMI and s-insulin at baseline were the best predictors of LVMI at follow-up. This is logical since increased MAP reflects pressure load on the left ventricle and increased BMI results in a relative volume overload of the left ventricle. In addition, insulin may contribute as a growth hormone for cardiomyocytes and insulin resistance and the metabolic syndrome are correlated to LVH both in hypertension and normotension [116, 117]. In the present study, s-insulin levels at baseline were strongly correlated to LVMI at follow-up only in the BPE group.

Diastolic function

Echocardiographic diastolic heart function measurements were in the normal range in both groups. However, the best long-term estimate of average left ventricular filling pressure *i.e.* left atrial size [118] was significantly larger in the BPE compared to the NC group. Also, the more sensitive atrial size inequlity [119], using each heart as its own reference, showed a larger inequlity in the BPE compared to the NC group. Importantly, increased left atrial size is a predictor of cardiovascular death [22], stroke and death [21] partially mediated by left ventricular mass. The central pulse pressure and IMT were significantly higher in the BPE group compared to the NC group and these factors are strongly related to vascular disease [120, 121]. Clearly, even a mildly elevated blood pressure in young age indicates high risk of progressive adverse development of cardiovascular structure and function.

Chronic but not acute blood pressure lowering restores the impaired fibrinolytic capacity in hypertension

Importance of regulated t-PA release

In established hypertension the risk of atherothrombotic events is high, and previous studies have shown an impaired fibrinolytic defense against evolving arterial thrombus formation [92, 93]. A prompt onset of a regulated t-PA release response with a short delay to peak release is probably pivotal for its antithrombotic effect, since t-PA is about two orders of magnitude more effective when present during clot formation than in dissolving an existing thrombus [61, 62]. In healthy man, acute release of t-PA can be initiated within less than a minute and the release increases manifold with stimulation [72, 73, 91, 122]. There is evidence that a response of the same rapidity and magnitude can be elicited in the coronary circulation [69, 123, 124]. If the response is slow and defective, as it appears to be in patients with untreated hypertension [92], it may reduce the potential for a timely activation of the fibrinolysis in case of an atherothrombotic event. The findings in this thesis show that the capacity for stimulated t-PA release can be significantly improved by chronic antihypertensive therapy. In contrast, acute blood pressure lowering has no such effect.

Acute blood pressure lowering

The rationale for investigating the effect of acute blood pressure lowering was the findings in a previous *ex vivo* study using intact vessels indicating that high intraluminal pressure suppresses t-PA release into the perfusion medium within 1-2 h, suggesting receptor-related protein release mechanisms rather than transcriptional regulation to be responsible [94]. Both constitutive and regulated releases of t-PA from the endothelium are dependent on increases in intracellular calcium levels [63, 64]. Hoetzer *et al* found that acute administration of 17 β-estradiol locally in the forearm augmented bradykinin-induced t-PA release [125]. They postulated the effect to be mediated through activation of an endothelial cell-surface estrogen receptor pathway that has been shown to rapidly increase intracellular calcium concentrations [126]. Recently, acute administration of ascorbic acid was found to improve endothelial t-PA release in heavy smokers, suggesting the mechanism of recovery to be mediated through reduction in oxidative stress [127]. Hence, prompt modulators of the capacity to release t-PA upon stimulation exist, and altered mechanical forces acting on the endothelial cell in hypertension could be one of them, although in this study we were not able to discriminate such a quality. The number of subjects studied was rather small and it can not be excluded that the observed non-effect is a matter of sample size. However, as the t-PA responses during high and low blood pressure were almost identical, we find it rather unlikely that a larger sample size would lead to different results.

Chronic blood pressure lowering

In contrast to the lack of effect of acute blood pressure lowering on the fibrinolytic system, we were able to demonstrate that chronic pharmacological blood pressure lowering markedly improved the t-PA release from the vascular endothelium in hypertensive patients. This observation has potentially important clinical implications since blood pressure lowering reduces the risk of cardiovascular events, and restoration of the capacity for an effective fibrinolytic response could be an important contributing mechanism. More specifically, chronic blood pressure lowering resulted in increased total amount of t-PA released locally upon stimulation, and a significantly higher peak release rate of t-PA. Furthermore, the temporal pattern of the t-PA release response was significantly improved with an earlier onset of peak release. In all six patients with a delayed t-PA release response, the time-to-peak was normalized. Keeping in mind that t-PA is much more effective when added in the beginning of thrombosis than when added afterwards, this might be of pathophysiological importance.

The change in t-PA release was of similar magnitude in the lisinopril and felodipine groups, suggesting that the improvement was related to the blood pressure reduction *per se*. However, the observation that the improvement was numerically greater with the ACE inhibitor may indicate that there are additional, drug-specific effects. This important issue needs to be addressed in larger studies. In contrast to the improvement in t-PA release, we found no effect of chronic blood pressure treatment on the vasodilatory capacity of the endothelium. Previous studies on the effect of chronic blood pressure treatment have shown either no change [128, 129] or improvement [130-133]

in the vasodilation capacity. To our knowledge, the effects of blood pressure lowering on substance P-induced vasodilation have not been described previously and it is possible that these different aspects of endothelial functions *i.e.* vasodilation and fibrinolytic capacity, are regulated through different mechanisms.

Drug-specific effects

There are no previous studies on the specific effects of different antihypertensive drugs on the release of t-PA from the endothelium, but differential effects on the plasma levels of fibrinolytic proteins have been reported. Thus, in contrast to a calcium antagonist and an angiotensin II type 1 receptor blocker, two ACE inhibitors (enalapril and quinapril) improved the fibrinolytic balance in plasma [134, 135]. Unfortunately, endothelial t-PA release can not be extrapolated from these studies, since 1) mixed venous plasma t-PA represents the integrated release from the whole vasculature and 2) the rapid degradation of t-PA by the liver makes steady-state levels very sensitive even to minute changes in hepatic clearance [83]. Furthermore, as previously demonstrated by us, baseline plasma levels do not predict the individual's capacity for activation of the acute release response [80], which is likely to be the key determinant of a successful clot resolution.

Interpretation

The mechanisms by which chronic blood pressure lowering improves t-PA release in hypertension are not clear. The impaired endogenous fibrinolysis was restored by chronic, but not acute, blood pressure lowering. It is thus tempting to speculate that elevated pressure load on the endothelium in hypertension decreases the production of t-PA in the endothelial cells. Decreased expression leads to reduced cellular content of t-PA, consequently affecting the releasable pool of t-PA. It follows that lowering of blood pressure might reverse the inhibitory effects of increased biomechanical forces on the endothelium.

Prolonged cyclic strain impairs fibrinolysis

Hence, we hypothesized that hypertension results in down-regulation of t-PA expression, secondary to the increased pressure load on the endothelium. The regulatory effect of the tensile force component (cyclic strain) of blood pressure on fibrinolytic proteins was therefore studied in cultured endothelial cells. The main finding of this study was that endothelial cells exposed to prolonged cyclic strain responded by decreasing both gene and protein expression of t-PA. After 48 h of strain stimulation, t-PA mRNA was reduced by 28% and this effect was found to be sustained after 72 h. However, the temporal response pattern was biphasic and the late suppression phase was preceded by an early inductive response at 6 h after onset of stimulation. T-PA protein secretion showed a similar pattern as t-PA mRNA, although the suppressive response was more delayed. A similar response pattern was observed for u-PA mRNA, with a transient early up-regulation followed by a suppression of its expression after 72 h of cyclic strain stimulation. The expression of PAI-1 mRNA and secretion of PAI-1 protein was induced at all time-points compared to static control cells. This

observation is in agreement with previous studies [95, 96, 136]. However, the present study is the first to document the inductive response on the level of gene-expression. The different regulation patterns of the fibrinolytic proteins indicate that cultured endothelial cells can respond to strain stimulation in a distinct and selective way.

A few previous studies have explored the effect of cyclic strain on t-PA protein secretion from endothelial cells and reported somewhat inconsistent results. Two studies showed no change in t-PA secretion in human umbilical cord vein endothelial cells exposed to 10% cyclic strain for 24 h [95, 96]. However, Iba *et al* observed a significant increase in t-PA secretion and mRNA expression in human saphenous vein endothelial cells exposed to more than 7% cyclic strain in a previous version of the Flexercell strain device [97, 98]. These observed inconsistencies could possibly be due to use of different cell-types or different strain devices. When extrapolating observations from *in vitro* experiments to the *in vivo* situation, one must always take into account that the outcome might be quite diverse. On the other hand, scrutinizing cellular and gene responses is crucial for the overall pathophysiological understanding of a disease on the clinical level.

Concluding remarks

This thesis highlights the impact of a mild blood pressure elevation on structural changes related to increased cardiovascular risk in young subjects. Furthermore, it confirms the negative effect of blood pressure elevation on the endogenous fibrinolysis, and discovers a novel beneficial mechanism of blood pressure lowering as the fibrinolytic capacity was restored by antihypertensive treatment. Further, cyclic strain stimulation of the endothelium down-regulates t-PA expression, proposing a link between elevated blood pressure and reduced t-PA release on the cellular level. The findings support our hypothesis that pressure induced impairment of the endogenous fibrinolysis is an important promoter of the increased risk of atherothrombotic events in hypertension.

CONCLUSIONS

Blood pressure elevation in young age has a significant impact of future risk of developing hypertension and causes adverse cardiac and vascular remodeling present already at the age of 40 years (Paper I).

Chronic blood pressure lowering restores the impaired fibrinolytic capacity in hypertension, and represents a novel mechanism behind the preventive effect of blood pressure-lowering therapy on the risk for atherothrombotic events (Paper II).

Acute blood pressure lowering does not restore the impaired fibrinolytic capacity in hypertension (Paper III).

Prolonged cyclic strain suppresses t-PA and induces PAI-1 in vascular endothelial cells, suggesting enhanced tensile strain in hypertension to impair fibrinolytic capacity (Paper IV).

POPULÄRVETENSKAPLIG SAMMANFATTNING

Risken för hjärtinfarkt och slaganfall ökar kontinuerligt med stigande blodtrycksnivå. Det är viktigt att tidigt identifiera personer som kommer att utveckla hypertoni (högt blodtryck) innan ogynnsamma förändringar i uppbyggnaden av hjärta och kärl, sekundärt till det höga trycket, har hunnit ske. Dessa förändringar, som t ex förtjockad hjärtmuskelvägg och ökad tjocklek av innersta lagret i blodkärlsväggen, är kopplade till än högre risk att drabbas av hjärt-kärlsjukdom.

Hjärtinfarkt och slaganfall orsakas av blodproppar i blodkärlen i hjärtat och hjärnan, som gör att blodflödet stoppas. Blodets transport av syre till cellerna i organen hindras och cellerna dör på grund av syrebrist. Ofta uppstår blodpropp i kärlen i anslutning till att åderförkalkningsplack i kärlväggen spricker och blodet reagerar med att levra sig. För att motverka uppkomsten av blodproppar frisätter det innersta cellagret i kärlväggen ett protein, vävnadsplasminogen aktivator (t-PA), som är nyckelenzymet för aktiveringen av propplösande mekanismer i blodkärl. I cirka 30% av hjärtinfarkter och slaganfall lyckas kroppens egna försvar lösa upp proppen och därmed återställa blodflödet. Vår forskargrupp har nyligen visat att blodkärlens förmåga att frisätta t-PA är nedsatt vid högt blodtryck och detta skulle delvis kunna förklara den ökade risken för hjärtinfarkt och slaganfall vid hypertoni.

I avhandlingen undersökte vi hur en lätt blodtrycksförhöjning i ung ålder påverkar kärlen och hjärtat och risken att utveckla hypertoni tjugo år senare. År 1987 undersöktes 49 personer med en lätt blodtrycksförhöjning, systoliskt 140-160 och/eller diastoliskt 85-95 mmHg (blodtrycksgrupp), samt 17 personer med helt normala blodtryck, systoliskt 110-130 och diastoliskt 60-80 mmHg (kontrollgrupp). Dessa personer följdes upp under 2007 med 24 timmars blodtrycksmätning, ultraljud av hjärtat och mätning av halsblodkärlets väggtjocklek. Vi kunde konstatera att förekomsten av hypertoni var 74,5 % i blodtrycksgruppen och 5,9 % i kontrollgruppen och att den skillnad som förelåg i blodtrycksnivå 1987 alltså hade förstärkts 20 år senare. Blodtrycksgruppen hade också påtagligt ökad väggtjocklek i hjärtat och ökad tjocklek av halsblodkärlsväggen jämfört med kontrollgruppen.

Vidare undersökte vi om den nedsatta förmågan att frisätta t-PA, som vi tidigare hade sett vid obehandlad hypertoni, förbättrades av långvarig eller akut blodtryckssänkning. Stimulerad frisättning av t-PA mättes lokalt i underarmen vid två tillfällen, först med obehandlat högt blodtryck och sedan efter långvarig eller akut sänkning av blodtrycket. Underarmsmodellen är en metod som vidareutvecklats vid vårt forskningslaboratorium och gör det möjligt att mäta stimulerad t-PA frisättning direkt från kärlväggen i människor. Genom behandling med två principiellt olikverkande läkemedel (felodipin och lisinopril) erhöll vi ett välkontrollerat blodtryck i båda grupperna under ca två månader. Resultaten visade på kraftigt förbättrad förmåga att frisätta t-PA, både i form av ökad mängd frisatt t-PA samt en snabbare frisättning. Resultaten var likartade för de båda läkemedlen, vilket indikerar att den förbättrade t-PA frisättningen var en direkt följd av blodtryckssänkningen. Akut blodtryckssänkning inducerades med intravenös infusion av ett läkemedel som vidgar blodkärlen och på så vis minskar

blodtrycket omedelbart. Efter 30 minuters sänkt blodtryck mättes den stimulerade t-PA frisättningen igen. Resultaten visade att t-PA frisättningen var oförändrat låg efter akut blodtryckssänkning. Att långvarig, men inte akut blodtryckssänkning, förbättrar t-PA frisättningen kan tyda på att de förändrade tryckkrafterna, som verkar på blodkärlsväggen vid högt blodtryck, påverkar cellens produktion och innehåll av t-PA.

Vi gick därför vidare med att undersöka om cellens produktion av t-PA påverkas av högt blodtryck. För detta användes odlade celler från det innersta lagret av blodkärlsväggen. De utsattes för en sträckande kraft motsvarande högt blodtryck i ett experimentellt sträcksystem och produktionen av t-PA jämfördes med celler utan sträckning. Resultaten visade att långvarig upprepad sträckning av cellerna minskade produktionen och även frisättningen av t-PA.

Sammanfattningsvis visar resultaten att lätt förhöjt blodtryck i ung ålder förutsäger blodtrycksnivån 20 år senare och leder till ogynnsam utveckling av hjärtats och kärlens uppbyggnad. Den nedsatta förmågan att frisätta t-PA vid obehandlad hypertoni normaliserades av långvarig, men inte av akut blodtryckssänkning. Det talar för att produktionen och inte frisättningsmekanismen av t-PA från kärlväggen påverkas av det höga blodtrycket. Detta bekräftades av försöken med upprepad sträckning som påvisade minskad produktion och frisättning av t-PA.

Vi visar för första gången att blodtrycksbehandling förbättrar kroppens egna försvar mot blodproppar. Denna verkningsmekanism kan vara en förklaring till blodtrycksbehandlingens skyddande effekt mot hjärtinfarkt och slaganfall.

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