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Vascular Dysfunction in Stroke and CADASIL

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Abstract

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Cerebrovascular disease (CVD) is strongly linked to hypertension and generally occurs later in life than coronary artery disease (CAD). Three quarters of the patients with symptomatic CVD are above 65 years of age. The risk factors are the same for CVD and CAD, but the relative importance of the vascular risk factors differs greatly.

Genetic causes of stroke are relatively rare. Cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL) is a hereditary disease which causes CVD in young adults and middle-aged people, with migraine, stroke, psychiatric illness and dementia as clinical manifestations.

The subject of this thesis is vascular function in stroke and CADASIL. Endothelium-dependent vasodilation (EDV) and arterial stiffness were investigated by different methods in stroke patients and CADASIL patients compared with healthy controls. Venous occlusion plethysmography with intra-arterial acetylcholine was used to evaluate EDV in the forearm resistance vessels. Flow-mediated vasodilation of the brachial artery was used to evaluate EDV in a conduit artery. Stroke patients displayed reduced EDV in resistance vessels compared with a healthy control group, but this reduction was not significant when, in a larger group of stroke patients, adjustments were made for blood pressure, antihypertensive treatment and other risk factors. Flow mediated vasodilation of the brachial artery was reduced in the stroke patients even after adjustment for risk factors.

Compared with controls, the CADASIL patients showed similar EDV in the conduit artery, but reduced EDV in resistance vessels.

Arterial compliance was evaluated by augmentation index from pulse wave analysis, by a ratio of cardiac stroke volume and pulse pressure, and by the distensibility of the carotid artery in relation to pulse pressure. Stroke patients and CADASIL patients did not display any significant increase in arterial stiffness when evaluated by these methods.

Keywords: stroke, CADASIL, endothelium, vasodilation

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List of Papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals.

- I. Stenborg, A., Terent, A., Lind, L. (2006) Endothelium-Dependent Vasodilatation in Forearm is Impaired in Stroke Patients. *Journal of Internal Medicine*(259): 569-575
- II. Stenborg, A., Kalimo, H., Viitanen, M., Terent, A., Lind, L. Impaired Endothelial Function of Forearm Resistance Arteries in CADASIL Patients. *Stroke*(38): 2692-2697
- III. Stenborg, A., Terent, A., Lind, L. Endothelium-Dependent Vasodilation in Peripheral Conduit and Resistance Arteries in Stroke Patients. (manuscript)
- IV. Stenborg, A., Terent, A., Lind, L. Arterial Stiffness in Stroke Patients. (submitted)

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ABBREVIATIONS

Ach	acetylcholine
ADMA	asymmetric dimethylarginine
ANOVA	analysis of variables
ANCOVA	analysis of covariance
BMI	body mass index
CAD	coronary artery disease
CADASIL	cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy
CCA	common carotid artery
CO ₂	carbon dioxide
CT	computed tomography
EDV	endothelium-dependent vasodilation
EIDV	endothelium-independent vasodilation
eNOS	endothelium nitric oxide synthase
FBF	forearm blood flow
FMD	flow-mediated dilation
HDL	high density lipoprotein
iNOS	inducible nitric oxide synthase
LV	left ventricular
MELAS	mitochondrial myopathy, encephalopathy, lactic acidosis and stroke like episodes
MRI	magnetic resonance imaging
MRT	magnetic resonance tomography
MSNA	muscle sympathetic nervous activity
NIH	national institute of health
nNOS	neuronal nitric oxide synthase
NO	nitric oxide
NOS	nitric oxide synthase
PET	positron emission tomography
PIVUS	prospective investigation of the vasculature in Uppsala seniors
PP	pulse pressure
PWV	pulse wave velocity
RI	reflectance index
SD	standard deviation
SEM	standard error of the mean
SNP	sodium nitroprusside

SV	stroke volume
TIA	transient ischaemic attack
TOAST	trial of Org 10172 in Acute Stroke Treatment
VSMC	vascular smooth muscle cells

INTRODUCTION

Nitric oxide (NO), basal mechanisms

Three isoenzymes of nitric oxide synthase (NOS) convert the amino acid L-arginine to NO. The endothelial isoform, endothelium nitric oxide synthase (eNOS), is also expressed in platelets and in the endocardium.¹ The two other isoforms are neuronal nitric oxide synthase (nNOS) and the inducible nitric oxide synthase (iNOS). Endothelial cells synthesise NO at a basal level continuously, but synthesis is also dynamically increased in response to different stimuli. Being a lipid soluble molecule, it directly diffuses through the cell membranes of the producer cell into adjacent vascular smooth muscle cells (VSMC) and other target cells. The range of action is limited due to its rapid inactivation. In the target cell, guanylate cyclase activity is stimulated, increasing the level of cyclic guanosine monophosphate which reduces the intracellular calcium levels in VSMC, causing relaxation. Other effects of intravascular NO release are decreased adhesion of leukocytes and monocytes, and inhibition of platelet aggregation.²

Methods

A common way to evaluate endothelial function is to test endothelium-mediated vasodilation (EDV), which is regarded as a barometer for vascular health.³ EDV has been tested in several vascular beds: the coronary arteries, the cerebral circulation, the forearm and the cutaneous circulation. Coronary endothelial function has been proved to be closely related to endothelial function in the forearm.⁴ Acetylcholine (Ach) is a common stimulant of endothelial NO-release, but other options include metacholine and terbutaline. Shear stress, which is the main physiological stimulus to NO release in endothelial cells, is also used in clinical research.

The invasive method

In the invasive method, Ach is infused in the brachial artery, stimulating the eNOS, and the resulting NO-mediated increase in blood flow is measured by venous occlusion plethysmography. For comparison, sodium nitroprusside (SNP) is also given as an intra-arterial infusion in the same way as Ach. A

reduction in Ach-induced vasodilation, but normal SNP-induced vasodilation, is typical of endothelial dysfunction. In situations with a large difference in basal blood flow between groups, the endothelial function index, a ratio between Ach-induced vasodilation and SNP-induced vasodilation, may be used instead. The invasive method evaluates EDV in resistance vessels which are the small arteries distal to the conduit arteries that contribute most to the precapillary resistance, with diameters $< 500\mu\text{m}$.⁵

The ultrasound method

The ultrasound method for flow-mediated vasodilation (FMD) is non-invasive, and therefore widely used.^{6, 7} It is used to evaluate EDV in conduit arteries. A blood pressure cuff is placed below the elbow and inflated above systolic blood pressure for 4.5 minutes. Release of the blood pressure cuff causes a reactive hyperaemia in the forearm, and the resulting increase in shear stress in the brachial artery stimulates NO production and dilation of the brachial artery. The relative increase in diameter of the brachial artery is used as an indication of endothelial function. FMD is reduced in old age^{8, 9}, hypertension, diabetes⁷, high cholesterol¹⁰, obesity¹¹, hyperhomocysteinaemia⁷, low birth weight, smoking¹², coronary artery disease (CAD)¹³, and family history of diabetes and CAD. A problem with the FMD method is that the stimulus to NO production is not the same for all investigated individuals, because the levels of shear stress differ due to factors such as haematocrit and basal diameter. Part of the reduction in FMD with increasing age can be accounted for by the increase in diameter of the brachial artery that occurs with age and which reduces shear stress, the vasodilator stimulus.⁷ The same aspect is relevant for evaluating the impact of gender on FMD. Women seem to have a better FMD by direct comparison, but after correction for baseline diameter, women have a slightly worse FMD.⁹ For the FMD to reflect NO production, the stimulus must be of short duration only to avoid evaluating other mediators of flow-mediated vasodilation.¹⁴ The time of investigation needs to be standardised due to diurnal variations in FMD (low in the morning)¹⁵. FMD is also reduced after a high-fat meal¹⁶, which is the rationale behind 3–4 hours fasting before the investigation.

The pulse wave method

The pulse wave method is the most recently developed method.¹⁷⁻¹⁹ The pulse wave is recorded at the radial artery and shows the relationship between systolic and diastolic blood pressure as well as several reflection waves. The relative height of the first reflected diastolic wave compared with the systolic peak is recorded: the reflectance index (RI). Endothelial release of NO reduces the reflectance index. Terbutaline is administered

subcutaneously to stimulate endothelial NO-release. The relative decrease in the RI after terbutaline is used as an indicator of endothelial function.

Correlation with cardiovascular risk factors and disease

Most of the major cardiovascular risk factors are associated with endothelial dysfunction: high age²⁰, smoking¹², hypercholesterolemia²¹, hypertension²² and diabetes.²³ Optimal lipid levels are associated with preserved endothelial function.²⁴ Endothelial dysfunction is present in all of the different stages of cardiovascular disease. Subjects with a genetic predisposition to develop cardiovascular disease already display endothelial dysfunction at a young age.²⁵ Subsequently, endothelial dysfunction contributes to the development of hypertension²⁵, and hypertension contributes to endothelial dysfunction.²⁶ Endothelial dysfunction contributes to atherosclerosis²⁷, and in acute coronary syndromes there is severe endothelial dysfunction, which is also linked to inflammatory markers.²⁸ Recovery of endothelial function after acute coronary syndrome is associated with a good prognosis compared with sustained endothelial dysfunction.²⁸ Subsequent heart failure is associated with reduced endothelial function, but also with reduced vasodilation to exogenous NO.²⁹

Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of the eNOS by competing for the binding site of the substrate (L-arginine). Patients with hypertension, chronic renal disease and familial hypercholesterolemia have high levels of ADMA.³⁰ The measurement of endothelium-mediated vasodilation can be used in combination with other factors, such as smoking, to estimate cardiovascular risk in individuals. For example, the combination of LV hypertrophy and endothelial dysfunction increases the risk of cardiovascular events.³¹

A fundamental problem concerning the value of endothelial function measurements is the presumption that transiently evoked NO release is related to long-term basal NO release. This issue has not been answered so far, but in a study of diabetic patients, basal release of NO was not correlated with endothelial function.³² Many other aspects of NO in vascular regulation are difficult to comprehend; for example, inhibition of eNOS abolishes both expansive and constrictive arterial remodeling.³³

Endothelial function and stroke

The promotion of endothelial health has been suggested to be of importance in stroke prevention.³⁴ On the other hand, relatively few studies consider the role of endothelial dysfunction in stroke. Hypertensive relatives of stroke patients display reduced EDV compared with other hypertensive patients.³⁵ The follow-up of an evaluation of EDV in the coronary arteries in patients

undergoing coronary angiograms showed a four-fold higher risk of stroke or transient ischaemic attack (TIA) in subjects with reduced coronary EDV.³⁶ Carotid dissection, more prevalent in young stroke patients, is associated with reduced EDV, compared with patients with stroke of unknown origin.³⁷ Patients with acute symptomatic carotid stenosis have reduced EDV compared with patients with asymptomatic carotid stenosis.³⁸ In old adults with cardiovascular disease, EDV was inversely related to the volume of white matter hyperintensities.³⁹

The relationship between stroke and endothelial dysfunction also works the other way around; the largest possible brain damage, brain death, causes a severe endothelial dysfunction, which can be reversed by intravenous L-arginine.⁴⁰

The concept of endothelial dysfunction contributing to stroke development is mainly focused on the influence of NO on the vascular system. But it is also possible that NO may be neuroprotective during acute ischaemia. In the very early stage of ischaemic stroke, NO from endothelial seems to be protective, but subsequently, when it is synthesised in high levels from neurons and astrocytes (iNOS), it contributes to cellular damage.⁴¹

Strategies for improving EDV

The problem of reduced endothelial NO production or bioavailability cannot be solved by exogenous NO donors, since continuous administration of long-acting nitrates induces tolerance to the vasodilatory effect of NO and, paradoxically, actually reduce EDV.^{42, 43} A high intake of nitrite-containing food may increase NO generation without induction of tolerance.^{44, 45} EDV is also enhanced by exercise^{46, 47}, even in old age.⁴⁸ Exercise increases the expression of eNOS and of the extracellular super-oxide dismutase, which, located between the endothelial cells and the VSMC, preserves NO bioactivity.⁴⁹ Some of the commonly used anti-hypertensive drugs improve EDV.⁵⁰ Other ways of improving EDV in selected groups include treatment with low dose aspirin^{51, 52}, statins⁵³, estrogen⁵⁴ or antioxidants such as vitamin C and E.^{55, 56} Dietary supplementation by administration of L-arginine has not been proved valuable for improving EDV.⁵⁷ It may also be harmful in inflammatory conditions by the activation of iNOS.⁵⁸ Bilirubin is an endogenous factor which enhances EDV⁵⁹, and subjects with Gilbert's syndrome have less cardiovascular disease.⁶⁰

Shear stress and remodelling

Vascular remodelling may be constrictive or expansive, and occurs in both small and large arteries. In a healthy condition, vascular remodelling in re-

sponse to chronic alterations in blood flow is a mechanism that serves to adapt the vasculature to changes in tissue demand. An obvious example of this phenomenon is the remodelling of the vasculature of the uterus before, during and after pregnancy.³³ Shear stress, the main stimulus for vascular remodelling, is the friction produced by flowing blood on the endothelial surface of blood vessels. It is proportional to blood flow and viscosity, and is inversely proportional to the arterial radius. Local pulse pressure also contributes to expansive remodelling.⁶¹

Expansive remodelling of large arteries is seen in hypertension, obesity, diabetes, and with atherosclerosis, but is also seen in aging.⁶² Blood lipids seem to play an important role as a healthy blood lipid profile is associated with smaller vessel diameters.^{24, 63} Antihypertensive drugs may reverse some of the structural changes induced by hypertension, such as LV hypertrophy, but arterial remodelling of the carotid artery is not reversed by blood pressure reduction.⁶⁴ A reduced local pulse pressure seem to be required for regression of expansive remodelling of the CCA.⁶⁵

One of the negative consequences of an (inappropriately) increased arterial diameter is reduced shear stress and NO availability.⁶² Increased diameters of the carotid arteries are associated with stroke.⁶⁶ Moreover, wall shear stress was lower in the carotid artery responsible for a unilateral ischaemic stroke.⁶⁷

NO and the regulation of cerebral blood flow

Autoregulation is not an exclusive feature of the cerebral circulation, but the brain has the greatest “autonomy” in this respect. It ensures sufficient blood flow to the brain when there are changes in the systemic circulation. In many other parts of the body, innervation from the sympathetic nervous system has a considerable influence on vascular tone. Under normal circumstances, however, the vascular tone of cerebral vessels is not influenced by the sympathetic nervous system in spite of a dense innervation of the vessels.⁶⁸ Inhibition of NO production has no effect on cerebral blood flow in young healthy individuals, but reduces basal cerebral blood flow in old healthy individuals.⁶⁹ In the brain, the blood-brain barrier reduces vascular response to most humoral stimuli.⁷⁰ Hypoxia and hypercapnia, are, however, very potent vasodilators in the cerebral circulation, and the response to hypercapnia and hypoxia are mediated via NO.^{71, 72}

The mechanical forces involved in the regulation of cerebral blood flow are transmural pressure and flow-mediated vasodilation. Flow-mediated dilation of larger arteries is more prominent in the cerebral circulation compared to other vascular beds, increasing the risk of “steal” of blood flow from one territory to another. Protection from this “steal” phenomenon is mediated by dilation of vessels downstream.⁷³ In the cerebral circulation, NO is not the

only mediator of flow-mediated vasodilation, but multiple mechanisms are involved.⁷⁴ Exogenous NO donors, such as SNP, reduce basal cerebrovascular tone, but do not affect cerebral blood flow. SNP infusion also reduces cerebral hyperventilation-induced vasoconstriction, and increases hypoventilation-induced vasodilation.⁷¹

Hypertension and stroke

Reduction of blood pressure seems fundamental in stroke prevention. Every 10 mm of reduction in systolic blood pressure reduces the risk of stroke by a third, at least down to 115/75 in subjects aged 60-79 years.⁷⁵ As a general rule, hypertension, because of autoregulation, does not reduce cerebral blood flow, but may cause hypoperfusion of some areas of the brain, which can be reversed by antihypertensive medication.⁷⁶ In old hypertensive subjects, however, antihypertensive medication may even increase cerebral blood flow.⁷⁷

A high degree of blood pressure fluctuations is also harmful,^{78, 79} and reduced baroreflex sensitivity is associated with a worse outcome after a stroke.⁸⁰ Failure of blood pressure to be reduced at night-time also increases stroke risk as does too large a drop in blood pressure during the night.⁸¹ Stroke in humans is associated with a reduced autoregulation of cerebral blood flow⁸² and stroke prone rats loose autoregulation prior to stroke development.⁸³

The treatment of hypertension is guided by conventional blood pressure measurements. A role for pulse wave analysis in the treatment of hypertension has recently been suggested, to obtain estimations of the more relevant central blood pressure. Antihypertensive drugs differ in their capacity to lower central blood pressure, and this has been suggested as an explanation for their different capacity to prevent stroke.⁸⁴

Hypertension causes structural changes in cerebral blood vessels including fibrinoid degeneration of the intima, increased thickness of the arterial wall in small arteries, and atherosclerosis of large vessels.⁸⁵

ARTERIAL STIFFNESS

Advancing age is the major risk factor for vascular disease, and one of the main features of vascular ageing is a progressive increase in arterial stiffness, which can even occur without atherosclerosis.⁸⁶

The normal function of the elastic aorta is to transform the pulsatile blood flow from the heart into a less pulsatile flow in distal vessels and a non-pulsatile blood flow in capillaries.⁸⁷ This function depends on a normal arterial stiffness gradient, meaning that the central arteries are most elastic, and elasticity decreases gradually with increasing distance from the heart.

The reflection of the initial blood pressure wave after each heartbeat may have beneficial effects depending partly on the timing of this reflected pressure wave. In healthy vasculature, the reflection waves limit the transmission of pulsatile energy to the microcirculation. Furthermore, when the reflected wave returns to the heart in diastole, it increases the diastolic pressure, thus increasing the perfusion of the myocardium of the left ventricle, which is perfused only during diastole. With increasing arterial stiffness, the velocity of the pulse wave is increased, and the reflected pulse wave returns earlier to the heart. When, eventually, the return of the pulse wave to the heart comes as early as during systole, it will augment the systolic blood pressure, whereas the diastolic blood pressure is reduced.⁸⁷ During age-related gradual loss of arterial compliance, the most pronounced increase in stiffness is seen in the proximal arteries, eventually resulting in a reversal of the arterial stiffness gradient, which facilitates for pulsatile energy to be transmitted to the microvasculature.⁸⁸

Arterial stiffness is not a constant feature of an artery as tension of VSMC also affects stiffness. Arterial stiffness increases with the distending pressure (mean arterial pressure).⁸⁹ As arterial stiffness is only a descriptive term, it cannot be measured. Arterial compliance and distensibility are the terms used for quantification.⁹⁰

Methods

Pulse pressure (PP) is a simple surrogate measure of arterial stiffness in subjects over 40 years old⁹¹, and PP has been associated with cardiovascular events in several studies.⁸⁷ PP seems to be a less important risk factor for stroke than for coronary events.⁹²

The ratio of stroke volume/-PP (SV/PP) was developed as a better measure of total arterial compliance, and even though it has not been widely used, it proved useful for predicting cardiovascular events in hypertensive patients⁹³ and in a population based study.⁹⁴

Methods based on pulse wave analysis include augmentation index and pulse wave velocity. Pulse wave velocity (PWV) is now regarded as the golden standard method for arterial stiffness.⁹⁵ To obtain the velocity of the pulse wave in an arterial segment, time is measured by pulse wave recordings at two locations. The distance between the two locations is usually measured with a tape measure over the body surface.⁸⁷

For the evaluation of focal distensibility of an artery, ultrasound may be used, and the maximum change in diameter during the cardiac cycle in relation to the distending pressure is used as a measure of compliance.

STROKE

Stroke is a common and dreaded disease. Heart disease and stroke together are the two leading causes of mortality in adults over 15 years. The incidence of stroke is increasing in low- and medium-income countries, and remains high in high-income countries,⁹⁶ whereas stroke mortality is declining.⁹⁷ Both long-term survival and functional outcome are improved if patients are treated in specialised stroke wards.⁹⁸ Because stroke is so difficult to treat once it has occurred, prevention is fundamental, especially considering that as many as 25% of stroke survivors develop dementia as a result of the stroke.⁹⁹

Different types of stroke, such as brain haemorrhage and cerebral infarction, may give a similar clinical picture, but would be expected to have different pathophysiology and risk factor profiles. The classification of different types of ischaemic stroke has also been developed in order to reflect the pathophysiology.¹⁰⁰ Differences, however, are not as large as would be expected.¹⁰¹ Hypertension is the main risk factor for both ischaemic and haemorrhagic stroke. Siblings with ischaemic stroke have different subtypes of cerebrovascular disease.¹⁰² Smoking gives the same increase in the risk of haemorrhagic stroke as ischaemic.¹⁰³ Chronic kidney disease is, however, a risk factor only for haemorrhagic stroke.¹⁰⁴

Over 60% of stroke mortality relates to a few well-known modifiable risk factors: hypertension, smoking and poor diet.⁹⁶ Socioeconomic factors contribute significantly.¹⁰⁵ There are also some non-modifiable risk factors such as age, African and Asian race, and male gender. Genetic factors play a smaller role. The concordance for ischaemic stroke is 65% higher in monozygotic than in dizygotic twins. Among the known hereditary diseases which cause ischemic stroke are sickle-cell disease, Fabry's disease, Homocystinuria, Marfan syndrome, Ehlers-Danlos syndrome type IV, Pseudoxantoma elasticum, MELAS, and CADASIL.¹⁰⁶

Sub-classification of ischemic stroke

Classification into subtypes of ischaemic stroke is a frequently used tool in stroke research. The subtypes of ischaemic stroke have different prognosis and treatments.¹⁰⁰ The most important distinction is made between cardioembolic stroke and other ischaemic stroke as anticoagulation is useful in

the first group only.¹⁰⁷ The remaining group of non-cardioembolic stroke is usually further divided into large and small vessel disease with respect to the stroke mechanism. The tools for subdivision are imaging of the brain with computed tomography or magnetic resonance tomography (MRT), which may give information concerning the size, location, and age of the lesion. Carotid artery examination by ultrasound is used for information on carotid artery atherosclerosis and stenosis. The result from investigations and from the clinical examination of the stroke patient gives the stroke subtype. Deciding between small vessel and large vessel disease in (presumed) non-cardioembolic ischaemic stroke may be complicated. Even if the patient has a lacunar syndrome together with a lacunar stroke on MRT, 25% have a non-lacunar stroke mechanism.¹⁰⁸

Risk factors used to be a part of classification of stroke mechanism, as described, for example in the TOAST criteria.¹⁰⁰ However, a more recent study has shown that the difference in risk factor profile is very small in the different ischaemic stroke subtypes, which means that risk factors should not be used for evaluating stroke mechanism.¹⁰¹ Even when the stroke mechanism is defined, it is important to realise that patients with cerebrovascular disease often also have vascular disease in other vascular territories, and often have a combination of small and large vessel disease.¹⁰⁹

CADASIL

Cerebral Autosomal Dominant Arteriopathy (CADASIL) is one of the few hereditary cerebrovascular diseases found so far, genetically described first in 1993.¹¹⁰ It is a non-atherosclerotic, non-amyloid microangiopathy caused by mutations of the *NOTCH3* gene, which codes for a transmembrane receptor. During development, *NOTCH3* is required for the arterial differentiation of VSMC.¹¹¹ The site of the CADASIL-causing mutations in *NOTCH3* is the extracellular domain, which is cleaved on ligand binding. The mutation causes accumulation of cleaved ligand but does not seem to interfere with the signalling of the receptor.¹¹² The pathogenesis of CADASIL has not yet been elucidated. *NOTCH3* is expressed throughout the whole body, but the severity of the angiopathy varies greatly in different parts of the body. The cerebral vasculature is severely affected, whereas there are hardly any detectable changes in the renal vasculature, for example.¹¹³ Age of onset of clinical symptoms is highly variable. Migraine with aura is the most common first symptom. TIA and stroke are also common debut symptoms, but there are patients whose first symptom is reduced cognitive function. Most patients eventually develop dementia. Reduced executive capacity is a typical feature of early cognitive dysfunction in CADASIL^{114, 115}, as well as in other patients with extensive white matter hyperintensities, leukoaraiosis.¹¹⁶ Hypertension, although relatively uncommon, is associated with the prevalence of cerebral microbleeds, which are associated with disability.¹¹⁷ Smoking increases the risk of stroke in CADASIL.¹¹⁸ When CADASIL is suspected, magnetic resonance imaging (MRI) is very useful. T2 weighted sequences may display hyperintensities in the white matter of anterior temporal lobes, periventricular regions and external capsule.¹¹⁴ Studies examining the dynamics of cerebral blood flow in CADASIL have shown divergent results. Reduced basal blood flow is consistently found, but a study with phase-contrast MRI showed preserved cerebrovascular reactivity¹¹⁹, whereas a study using the MRI bolus tracking method found decreased reactivity in the patients. Studies using transcranial Doppler showed normal vasoreactivity¹²⁰ or reduced vasoreactivity¹²¹. The peripheral circulation in CADASIL is characterised by an increased resting heart rate¹²², reduced blood pressure¹²³, and reduced nocturnal dipping of blood pressure.¹²⁴ The discovery that CADASIL diagnosis can be made by skin biopsy led to the finding of systemic vascular changes in other vascular dementias as well.¹²⁵

AIMS OF THE PRESENT STUDIES

Study I

To investigate endothelium-mediated vasodilation in stroke patients in relation to healthy controls and in relation to controls with similar risk factors and medication.

Study II

To investigate endothelium-mediated vasodilation in CADASIL patients compared to matched controls.

Study III

To investigate endothelium-mediated vasodilation in stroke patients in relation to an age-matched population sample free from stroke.

Study IV

To investigate arterial stiffness in stroke patients in relation to an age-matched population sample free from stroke.

METHODS

Subjects

Study I

Stroke patients aged 65-75 years, living in the Uppsala area, Sweden, were identified from the Swedish National Stroke Register (www.riks-stroke.org). They were invited to participate in the study unless they had atrial fibrillation, CT findings of cerebral infarctions in different vascular territories indicating cardioembolic stroke, or were treated with warfarin. Of the 23 stroke patients included, 4 had had a haemorrhagic stroke, and the remainder had suffered a cerebral infarct. Time from stroke to examination varied between 3 and 25 months (median 11 months).

Controls were selected from a cohort of 70-year-old men and women from Uppsala who were invited to participate in a cardiovascular health investigation; The Prospective Investigation of the Vasculature in Uppsala Seniors (the PIVUS study: www.medsci.uu.se-pivus).¹²⁶ The healthy control group (n = 46) included subjects without history of cardiovascular disease. They had no regular medication and were non-smokers. The hypertension-matched control group (n = 32) consisted of subjects free from a history of stroke, but was frequency-matched regarding hypertension and antihypertensive medication, history of myocardial infarction, and smoking compared with the stroke patient group.

Study II

Ten CADASIL patients (6 men and 4 women, mean age 50.6 years, range 23.5 to 70) and twenty age- and gender-matched, healthy, non-smoking control subjects without regular medication were investigated. One patient also suffered from type 1 diabetes mellitus, and another patient had a substituted hypothyroidism. The 10 patients came from 7 families. Their *NOTCH3* mutations were R133C (n=1), R169C (n=3), C174R (n=1), R182C (n=1), C251Y (n=2), C435R (n=1). The mutation has not been identified for one patient, but the diagnosis was confirmed by the unequivocal presence of granular osmiophilic material in the skin biopsy. Furthermore, this patient's sister had characteristic CADASIL brain pathology at autopsy, and autosomal dominant inheritance has been verified in the family. The clinical

symptoms of the nine symptomatic patients were stroke (n=7), TIA (n=6), depression (n=2), acute encephalopathy (n=1), migraineous headache (n=3), and cognitive impairment (n=3). One of the patients was a current smoker. Five patients had treatment with low dose aspirin, and two patients were treated with statins.

Studies III and IV

Stroke patients aged 65 to 75 years old living in Uppsala, Sweden were identified from the Swedish National Stroke Register (www.riks-stroke.org). Clinically stroke-free subjects participating in the PIVUS study (www.medsci.uu.se/pivus/pivus.htm)¹²⁶ were used as controls. Medical charts of all the invited stroke patients and of all stroke patients in the PIVUS-study were reviewed to obtain information on symptoms, on findings from carotid duplex, CT or MRI in order to confirm the diagnosis of ischaemic stroke or primary intracerebral haemorrhage. Patients with paroxysmal or persistent atrial fibrillation or subarachnoid bleedings were not included. The study included 104 stroke patients and 973 controls. Of the stroke patients, 88 had suffered a cerebral infarction and 14 had suffered a intracerebral haemorrhage. Two patients had suffered both a haemorrhagic and an ischaemic stroke and were therefore not included in the comparison between the stroke subtypes. The mean time between stroke and the vascular examination was 2.4 years (range 3 months to 10 years). Examination of the carotid artery at the time of the (ischaemic) stroke, showed carotid stenosis in 20% of the patients, plaques only in 48%, and 32% of patients had no plaques. Furthermore, 44% of the ischaemic stroke patients had had a lacunar syndrome. In addition, 84% of the stroke patients were on antihypertensive treatment at the time of the vascular examination, with a median duration of treatment of 5 years (range 0.5–33)

Study protocols

Participants were investigated in the morning or at midday after > 4 hours of fasting. All medication except insulin was withheld the day of examination. A cannula was inserted in the brachial artery for regional infusions. Arterial blood was taken for analyses of fasting blood glucose and lipids.

The invasive forearm technique, studies I-III

Forearm blood flow (FBF) was measured by venous occlusion plethysmography before and at the end of the infusion of different dosages of the two vasodilators. A mercury in silastic strain gauge was placed at the upper third of the forearm, which rested comfortably slightly above the level of the

heart. The strain gauge was connected to a calibrated plethysmograph. Venous occlusion was achieved by a blood pressure cuff applied proximal to the elbow and inflated to 50 mm Hg by a rapid cuff inflator. Evaluations of FBF were made by calculations of the mean of at least five consecutive recordings. After evaluation of resting FBF, local intra-arterial drug infusions were given during 5 minutes for each dose with a 20-minute washout period between the drugs. The infused dosages were 25 and 50 $\mu\text{g}/\text{minute}$ for Ach (Clin-Alpha, Switzerland) to evaluate EDV, and 5 and 10 $\mu\text{g}/\text{minute}$ for SNP, (Nitropress, Abbot, UK) to evaluate endothelium-independent vasodilation (EIDV). The dosages of these drugs have been chosen to result in augmentation of FBF on the steep part of the dose-response curve. The drugs were given in a random order at a maximum rate of 1 ml/min.

Study I

As the primary measure of endothelium-dependent vasodilation, EDV was defined in this study as FBF during infusion of 50 $\mu\text{g}/\text{min}$ of acetylcholine minus resting FBF divided by resting FBF, and EIDV was defined as FBF during infusion of 10 $\mu\text{g}/\text{min}$ of SNP minus resting FBF divided by resting FBF. As a secondary analysis, FBF during the vasodilations were also given as absolute values.

Study II

The evaluation of FBF at baseline and during vasodilation with SNP and Ach is given in absolute numbers. Two ratios were formed. The first as FBF (in absolute numbers) during Ach infusion at 25 $\mu\text{g}/\text{min}$ divided by FBF during SNP infusion at 5 $\mu\text{g}/\text{min}$. The second ratio was defined as FBF during Ach infusion at 50 $\mu\text{g}/\text{min}$ divided by FBF during 10 $\mu\text{g}/\text{min}$. The endothelial function index was defined as the mean of these ratios in order to use information from both dosage steps.

The brachial artery ultrasound technique, studies II-III

The brachial artery was assessed by external B-mode ultrasound imaging 2 – 3 cm above the elbow (Acumen XP128 with a 10 MHz linear transducer, Acumen, Mountain View, CA, USA). A blood flow increase was induced by inflation of a pneumatic cuff placed around the forearm to a pressure at least 50 mm Hg above systolic blood pressure for 5 min. Flow-mediated vasodilation (FMD) was defined as the maximum brachial artery diameter recorded between 30 and 90 seconds following cuff release minus diameter at rest and divided by diameter at rest. Blood flow velocity in the brachial artery was measured by Doppler before cuff occlusion and immediately following cuff release.

Pulse wave analysis

Studies I-, II and IV

In the assessment of the pulse wave, a micromanometer-tipped probe (Sphygmocor, Pulse Wave Medical Ltd, Australia) was applied to the surface of the skin overlying the radial artery, and the peripheral radial pulse wave was continuously recorded. For accurate recordings the micromanometer must be applied with a light pressure to flatten the vessel wall so that the transmural forces within the vessel are perpendicular to the arterial surface. The mean values of around 10 pulse waves were used for analyses. The maximum systolic peak and the reflected waves were identified by the calculations of first and second derivative of the different parts of the pulse curve by the commercial software supplied by the manufacturer.

Studies I and II

The relative height of the first diastolic reflected wave, the reflectance index (RI), was used as an index of EDV. After a baseline recording, 0.25mg terbutaline was subcutaneously administered and a re-evaluation of the pulse wave was performed after 15 and 20 minutes. EDV evaluated by this technique was defined as the lowest RI value obtained after 15-20 min following terbutaline minus the baseline value in relation to the baseline value.

Studies II and IV

Based on transfer functions, the aortic systolic and diastolic blood pressures were calculated from the radial recordings with the Sphygmocor software.¹²⁷ The aortic augmentation index is calculated as the ratio between the amplitude of the first reflected wave divided by the amplitude of the first systolic peak.

Carotid artery compliance, study II and IV

The diameter of the common carotid artery of the right side, 1–2 cm proximal of the bifurcation, was measured at its maximum diameter in systole and at its minimal diameter in diastole. The distensibility of the common carotid artery was calculated as the change in diameter maximum to minimum in relation to the minimum diameter in diastole divided by the central pulse pressure obtained by pulse wave analysis.

Echocardiography and Doppler, Studies II and IV

Left ventricular dimensions were measured with M-mode online from the parasternal projections. Measurements included left atrial diameter, interventricular septal thickness, posterior wall thickness and left ventricular diameter in end diastole and end systole. The left ventricular diastolic filling pat-

tern of the mitral inflow was obtained from the apical transducer position with the pulsed Doppler sample volume between the tips of the mitral leaflets during diastole. The peak velocity of the early rapid filling wave (E wave) and the peak velocity of atrial filling (A wave) were recorded and the E to A ratio (E/A) was calculated. Left ventricular isovolumetric relaxation time was measured as the time between aortic valve closure and the start of mitral flow using the Doppler signal from the area between the left ventricular outflow tract and mitral flow. Left ventricular volumes were calculated according to Teichholz formula ($7 \cdot D^3 / (2.4 + D)$), from which stroke volume and ejection fraction were calculated.

Statistics

The statistical program package Stat View (SAS Inc) was used for calculations. Two-tailed P values are given, with $P < 0.05$ considered significant. Non-normally distributed variables were transformed before evaluation to achieve a normal distribution.

Between-groups differences in mean values for the different variables were evaluated by factorial ANOVA. ANCOVA was used for adjustments for risk factors.

Post-hoc analyses with the Bonferroni test were performed in Study I to test the ANOVA results. Spearman's correlation coefficient was used to relate time from the stroke event to the examination.

RESULTS AND DISCUSSION

STUDY I

Results

Basal FBF was similar in the three groups, but FBF during Ach and SNP was reduced both in the stroke patient group and in the hypertension-matched group (Figure 1). EDV evaluated by the invasive forearm technique differed between the groups ($P < 0.001$) by ANOVA analysis. Stroke patients showed a reduced EDV compared with healthy controls ($P < 0.001$), but not in comparison with hypertension-matched controls ($P = 0.27$). Furthermore, the hypertension-matched control group showed reduced EDV when compared with the control group ($P < 0.01$).

The difference between stroke patients and healthy controls was still significant after multiple ANCOVA analysis with the potential confounding variables of fasting blood glucose, serum cholesterol, smoking, BMI, gender, and blood pressure ($P < 0.001$). The difference between the hypertension-matched group and healthy controls also persisted after these adjustments ($P = 0.004$). For EIDV, there was a difference between the three groups ($P = 0.02$) by ANOVA analysis. In post-hoc analysis, there was a significant difference between stroke patients and healthy controls ($P = 0.006$), but not between stroke patients and hypertension-matched controls ($P = 0.15$). The hypertension-matched control group was not different in EIDV compared with the healthy control group ($P = 0.16$). In multiple ANCOVA analysis with EIDV as the dependent variable and fasting blood glucose, serum cholesterol, smoking, BMI and gender, and blood pressure as the potential confounding variables, the difference in EIDV between stroke patients and healthy controls was still significant ($P < 0.01$).

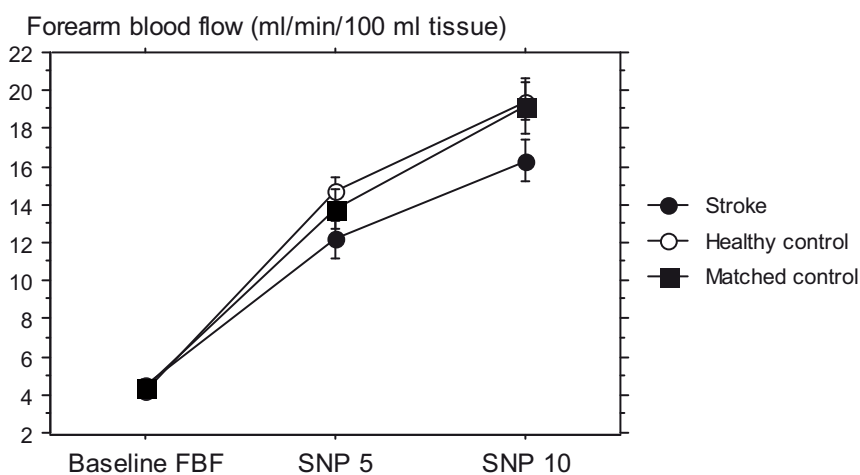
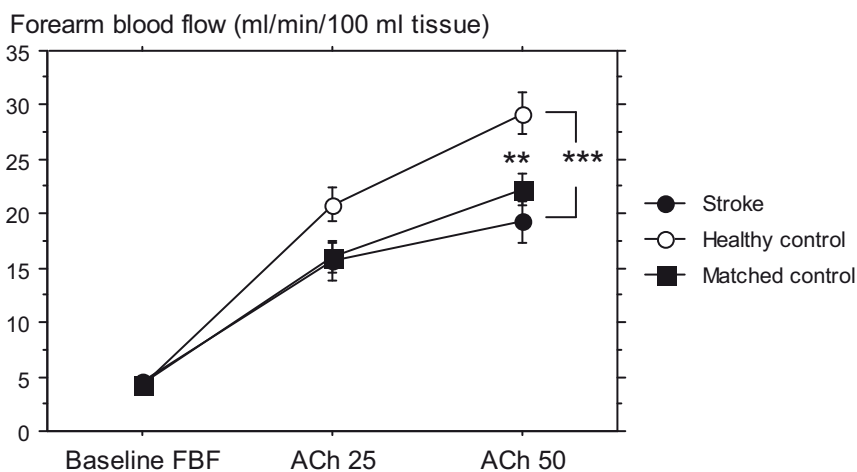


Figure 1 Forearm blood flow in mL/min/100 ml tissue. Top panel shows forearm blood flow during infusion of acetylcholine 25 and 50 μ g/min. Lower panel shows forearm blood flow during infusion of sodium nitroprusside 5 and 10 μ g/min. Mean and SEM are given.

For the pulse wave analyses, there was also a difference between groups regarding the change in RI following terbutaline ($P = 0.015$) by ANOVA analysis. There was a significant difference between stroke patients and healthy controls ($P = 0.007$) in post hoc analysis, but not between stroke patients and hypertension-matched controls ($P = 0.33$) or between hypertension-matched controls and healthy controls ($P = 0.051$). In multiple ANCOVA analysis with the change in RI as the potential confounding variables of fasting blood glucose, serum cholesterol, smoking, BMI and gender, and blood pressure as independent variables, the difference was no longer significant ($P = 0.09$). A significant relation between the time from the stroke

event and the examination was found regarding EDV ($r = 0.47$, $P = 0.046$), but not regarding EIDV or the change in RI ($P = 0.82$ and $P = 0.48$, respectively).

Discussion

In this study, stroke patients showed impaired acetylcholine-mediated vasodilation compared with age- and sex-matched healthy controls. This difference in EDV was still significant after adjustments for fasting blood glucose, serum cholesterol, BMI and blood pressure. However, no significant difference in EDV was found between stroke patients and a second control group, matched for blood pressure, antihypertensive treatment and myocardial infarction.

The main explanation is most likely the high prevalence of hypertension in the stroke group and the hypertension-matched group. Hypertension induces a profound impairment in EDV^{22, 128, 129}, which makes it difficult to detect the effects of additional risk factors or diseases such as stroke. It is therefore likely that most of the difference in EDV found between the stroke patients and the healthy controls is caused by hypertension.

Furthermore, we were unable to demonstrate a selective impairment of EDV in stroke patients as the stroke group also showed impaired EIDV compared with healthy controls. Thus, the vascular impairment seen in stroke patients is not only endothelium-dependent, but also includes the vasodilatory process induced by exogenously donated nitric oxide.

A limitation of the study is that motor function was not evaluated in detail in the arm in which plethysmography was performed, although no arm with severe paresis was investigated.

In conclusion, EDV, and EIDV were impaired in stroke patients. A high prevalence of hypertension may explain most of the impaired vasorelaxation in the stroke patients.

STUDY II

Results

Patients and controls had similar serum cholesterol levels and fasting blood glucose, but the patients had a higher body mass index. Manually measured systolic and diastolic blood pressure did not differ significantly between the groups. Intra-arterially measured pulse pressure was lower in the patients than in the controls (51 ± 7 SD vs. 58 ± 8 mm Hg, $P = 0.04$). None of the indices of arterial compliance differed between the groups.

FMD was similar in patients and controls. Maximum blood flow velocity in the brachial artery was reduced both at baseline and during hyperaemia in the CADASIL patients.

Forearm blood flow measured by venous occlusive plethysmography was reduced in the CADASIL patients, both at rest ($P = 0.03$) and following stimulation with high-dose Ach ($P = 0.02$).

The difference in hyperaemic blood flow was significant only for the highest dose of Ach. In order to further evaluate the endothelial vasodilatory function in this setting, we used the endothelial function index, which relates endothelium-dependent vasodilation to endothelium-independent vasodilation. The result was a reduced endothelial function index in the patients ($P = 0.019$, see Figure 2).

Discussion

The finding of similar FMD in patients and controls is interesting, as reduced basal blood flow in the patients and the tendency towards an increased basal brachial artery diameter would be expected to reduce FMD in the patients.

The reduced endothelial function index in the forearm resistance vessels of CADASIL patients indicates an impairment in endothelium-mediated vasodilation that cannot be explained merely by the structural changes in the small arteries as previously described.¹³⁰ Transgenic mice expressing mutant *NOTCH3* show early impairment in mechanotransduction, including flow-mediated vasodilation in resistance vessels.¹³¹

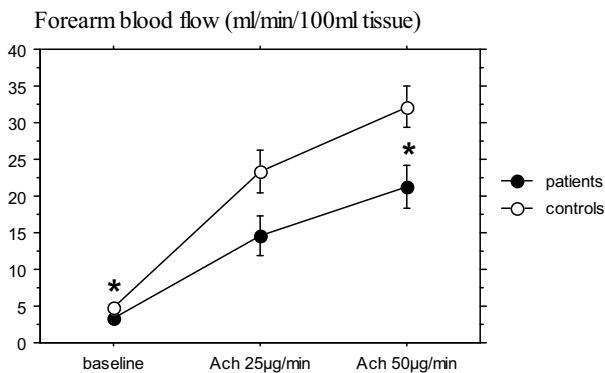


Figure 2 Forearm blood flow (ml/min/100ml tissue) in CADASIL patients and controls during infusion of acetylcholine. Mean value \pm SEM. * $P < 0.05$

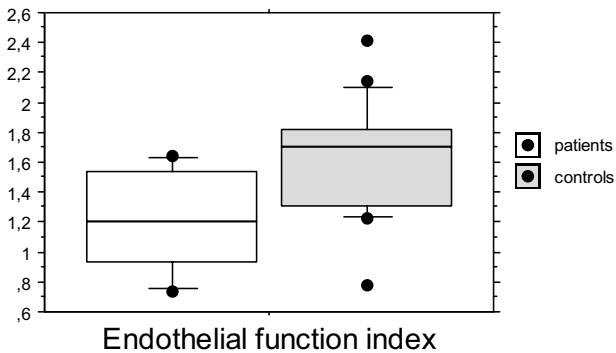


Figure 3 Endothelial function index in a box and whisker plot showing median value, 25th and 75th percentiles, 10th and 90th percentiles, and individual subjects outside the 10th and 90th percentiles. P = 0.019 for difference between patients and controls.

The results of the present study seem to indicate that reduced NO bioavailability may be a problem in CADASIL. Smoking, particularly harmful in CADASIL¹¹⁸, reduces both EDV and basal NO-production.¹³²

The reduced EDV in smaller resistance arteries in CADASIL patients that we showed in this study may be relevant to the disease process if partial failure of vasodilation in resistance arteries occurs early in CADASIL. The lack of collaterals would make the subcortical area in the brain vulnerable to a "steal" effect, directing blood flow from subcortical to cortical and extracranial vessels. In addition, the marked thickening and fibrosis in the arterial walls in the cerebral white matter and basal ganglia^{130, 133} might prevent normal flow-mediated vasodilation in the affected arteries.

The reduction of PP seen in our patients is in agreement with another study showing reduced systolic blood pressure in CADASIL patients.¹²³ The degree of reduction in blood pressure correlates with the degree of cognitive decline.

As this is a rare patient group, the sample size is small, and thereby the power to detect significant differences between patients and controls is very limited. False negative results could be produced.

In conclusion, this study showed normal flow-mediated vasodilation in a conduit artery, but reduction in basal blood flow and indications of reduced EDV in forearm resistance arteries in CADASIL patients.

STUDY III

Results

Stroke patients versus controls

Basal FBF measured by plethysmography was similar in patients and controls. FBF during Ach and SNP infusions were significantly lower in stroke patients following adjustment for gender only (SNP; $P = 0.005$ for $10\mu\text{g}/\text{min}$ -dose, Ach $50\mu\text{g}/\text{min}$ -dose, $P = 0.043$), but not significantly after adjustment for other cardiovascular risk factors ($P = 0.103$ for SNP $10\mu\text{g}/\text{min}$ and $P = 0.659$ for Ach $50\mu\text{g}/\text{min}$).

The distribution of FMD was similar but skewed in both stroke patients and controls. In-transformation was, however not performed before ANOVA as the zero value was relatively frequent in both patients and controls.

FMD was significantly lower in patients than in controls even after adjustment for risk factors and basal brachial diameter ($P = 0.005$). After multiple adjustments (also for aspirin use, statin use, body mass index and myocardial infarction), FMD was significantly reduced in the stroke patients ($P = 0.010$). FMD was not correlated with the values for FBF during Ach or SNP (data not shown).

Ischaemic versus haemorrhagic stroke

In the present sample of stroke patients, 14% had suffered a haemorrhagic stroke. No differences regarding blood pressure or vasodilatory indices were seen between those with ischaemic and haemorrhagic stroke, although there was a tendency towards reduced FMD in the latter group. More hemorrhagic stroke patients should have been recruited to permit analysis of differences between the stroke subtypes.

Discussion

We had previously shown in a pilot study that the vasodilatory responses to both Ach and SNP were impaired in the forearm resistance vessels in stroke patients compared with normotensive controls, but not when compared with controls matched for hypertension.¹³⁴ In the present study, including a substantially larger number of stroke patients, a similar picture appears. The normal increase in FBF induced by both Ach and SNP was reduced in our stroke patients compared with controls when adjusted for gender, but not when adjusted for major risk factors for cardiovascular disease, such as hypertension.

It should be noted that the response to SNP was blunted to a similar degree to that for Ach, so it could not be said that the vasodilatory function noted in resistance vessels in our stroke patients is endothelium-dependent. The dysfunction may well be at some step distal to the endothelium in the

vasodilatory chain, for example in the response of the VSMC to the endothelium-produced NO. There may be some support for this in the different location of atherosclerosis in extracranial versus intracranial vessels. Whereas arteriosclerosis in extracranial vessels primarily affects the vascular intima, the media is affected in intracranial vessels.¹⁰⁹

The present study confirms FMD impairment in stroke patients.^{38, 135, 136} A limitation of the study the cross-sectional design, which makes it difficult to draw conclusions concerning the predictive role of endothelial dysfunction in the pathogenesis of stroke. It is possible that FMD-reduction in stroke patients is lower after the stroke, as physical activity improves FMD.⁴⁶ Prospective studies, such as the PIVUS, will be able to evaluate the contribution of systemic endothelial dysfunction to cerebrovascular disease.

Choice of control group

One of the difficulties in the study of vasodilation in stroke patients is the choice of controls, as several of the risk factors identified for stroke also influence vasodilation indices.^{12, 20, 22, 23} One approach is to select controls that are pairwise matched for all of these risk factors. The data in stroke patients in the present study was collected in the same laboratory, and during the same time as the data collection in the PIVUS cohort, with a similar age as the stroke patients. We therefore used all the participants in the PIVUS study who lacked a history of stroke to serve as controls (n = 973) in order to allow adjustment for multiple confounding factors such as gender, blood pressure, diabetes, serum cholesterol, current smoking, obesity, a history of myocardial infarction, and the use of statins, aspirin and antihypertensive medication.

Asymmetry

The stroke patients in this study were investigated with the same protocol as the subjects in the PIVUS study, using one arm for the invasive method and the other arm for the ultrasound method without especially considering present/previous arm paresis, though avoiding any testing in an arm with dense paresis (one patient).

Asymmetry in vascular function of conduit or resistance vessels due to asymmetry in neural signalling after stroke is unlikely for several reasons. The sympathetic nervous system's regulation of vascular tension in the skeletal muscle serves to buffer blood pressure changes, and although blood flow may be differentially directed to forelimbs or hind limbs to prevent ortostatism¹³⁷, no published reports seem to treat centrally mediated lateralisation of sympathetic nervous discharge to skeletal muscle. The increased muscle sympathetic nerve activity (MSNA), which is present during skeletal muscle activity, is the same in working and resting muscle.¹³⁸ Furthermore, EDV of the forearm skeletal muscle circulation was not altered after chronic sympathectomy in humans.¹³⁹ Similar recordings of MSNA was made in

paretic and non-paretic limbs in hemiplegic stroke patients.¹⁴⁰ Blockade of the brachial plexus did not affect EDV measured by the invasive method.¹⁴¹

Impairment of cutaneous microcirculation and reduced cutaneous EDV in patients with hemiparesis and immobility may contribute to the development of oedema of the paretic limb, and may be reversed with neuromuscular electric stimulation.¹⁴² Another manifestation of skin vasomotor changes after stroke-induced hemiparesis is reduced skin temperature in paretic limbs.¹⁴³

Paresis in itself may be important, as FMD is correlated with baseline shear stress level.¹⁴⁴ However, of the stroke patients investigated in the present study who had had some degree of arm weakness and were investigated by FMD in the previously weak arm, there was no significant difference in FMD compared with the patients who were not examined in a previously weak arm ($P = 0.145$). Moreover, in seven patients in whom FMD was evaluated in both arms on a separate occasion, no consistent pattern of a difference between the arms was seen.

Asymmetry in sympathetic nervous system activity as a consequence of stroke has been documented in the regulation of the cardiac autonomic tone.^{145 146} Lesions in the right insular cortex are associated with reduced heart rate variability and with increased incidence of sudden death.¹⁴⁷

Silent brain infarcts

A limitation of this study is that brain MRI was not performed. This makes it difficult to evaluate the role of endothelial dysfunction in relation to cerebrovascular disease, as the prevalence of silent brain infarcts is high in this age group. The prevalence of silent brain infarcts varies in different studies.¹⁴⁸ In the cardiovascular health study, it was as high as 22% in the 65-69-year age group.¹⁴⁹

Conclusion

Stroke patients in this age group have a reduced flow-mediated vasodilation in conduit arteries compared to controls.

STUDY IV

Results

The aortic augmentation index was normally distributed and was similar in stroke patients and controls ($P = 0.54$). There was no significant difference between patients with ischaemic and haemorrhagic stroke ($P = 0.14$)

The distribution of carotid distensibility values was skewed and was ln-transformed before comparison. Stroke patients and controls showed similar values ($P = 0.76$). There was no significant difference between patients with

ischaemic and haemorrhagic stroke ($P = 0.48$). Furthermore, the ln-transformed values for the SV/PP ratio was not different in stroke patients compared to controls ($P = 0.92$), and there was no difference between ischaemic and hemorrhagic stroke patients ($P = 0.50$).

The diastolic diameter of the common carotid artery (CCA) was larger in the stroke patients, (67 ± 9 mm vs 64 ± 8 mm, $P = 0.003$, gender-adjusted), but there was no difference between ischaemic and haemorrhagic stroke patients ($P = 0.418$, gender-adjusted).

Discussion

Contrary to the hypothesis, this study could not demonstrate any difference in arterial stiffness between a fairly large group of stroke patients and control subjects. There are several possible explanations for this unexpected result.

First, the choice of methods may be criticised. The three techniques used in the study were chosen to suit a large epidemiological study, and the simultaneous measurement of pressure, diameter and flow obtained at the same point, required by the definition of compliance, was not achieved with these methods. Furthermore, pulse pressure is directly or indirectly used in the calculations of the data for all three indices, which could induce erroneous relationships. In this study, the stroke patients did not show an increased pulse pressure in the aorta or in the periphery. This may reflect the high prevalence of antihypertensive treatment in the patient group in addition to a high number of untreated hypertensives in the control group.

The aortic augmentation index may be a less valid method for arterial stiffness in this age group due to variations induced by medication, heart rate and LV dysfunction.⁸⁷ Several studies concerning arterial stiffness and stroke have used the pulse wave velocity method (PWV). In a study using both PWV and carotid distensibility, only the former was associated with risk of stroke.¹⁵⁰ A high PWV was associated with an increased risk of stroke in subjects with an elevated pulse wave velocity also in a longitudinal study of a community-based population.¹⁵¹ High PWV was associated with increased risk of fatal stroke in patients with hypertension.¹⁵² Thus, PWV might be the best method of evaluating arterial stiffness in stroke patients.

Another factor to be considered is the composition of the control group. It was not a healthy control group, but a population-based control group, with different medical conditions and medications and, perhaps most important, included a considerable number of subjects with previously undetected hypertension. We chose this large control group from the population to provide ourselves with the opportunity to adjust for multiple cardiovascular risk factors.

A possible interpretation of the findings of this study is that, after a stroke, antihypertensive drugs may reduce arterial stiffness by reducing its

functional component, the wall tension. The structural component, including an increased lumen might not be reversed by medication.

A more likely factor to be considered, however, is the fundamental influence of aging on arterial stiffness. In the report on arterial stiffness from the PIVUS study¹⁵³, values for the three measures of arterial stiffness were compared between the whole group and a group of healthy controls, as well as a young healthy control group. The carotid distensibility index in the young controls was almost three times the value for the 70 year-olds, but the old healthy control group had only a slightly better index than the entire group.

We can conclude that arterial stiffness and aortic pulse pressure were not increased in stroke patients in this cross-sectional study. This indicates that arterial stiffness, evaluated by the techniques used in the present study, is not a valid surrogate marker for stroke in this age group.

General discussion

Vascular function in mainly two types of cerebrovascular diseases is studied in this dissertation. The findings from these and other studies indicate that they are different in their vascular characteristics. The majority of stroke patients are characterised by hypertension, but CADASIL patients most often have reduced blood pressure. Stroke patients have enlarged carotid arteries and normal-sized brachial arteries, but CADASIL patients have normal-sized carotid arteries and a tendency towards enlarged brachial arteries ($P = 0.051$ adjusted for gender). Reduced large artery vasodilation was seen in the stroke patients, but in the CADASIL patients, vasodilation of the small arteries was reduced. Whereas the acute stroke event in common stroke may be due to macrovascular or microvascular disease, the cerebral injury from CADASIL is almost exclusively a result of microvascular pathology.

Pathological remodelling of arteries is a common feature in the two diseases. In CADASIL, stenosis of the smallest arteries in the cerebral white matter is combined with dilation in more proximal cerebral arteries.¹³⁰ In stroke, remodelling may be seen as stenosis or dilation of the carotid arteries or of the intracranial arteries.

Also similar in the two diseases is the need to focus not only on cerebral vascular changes, but to consider systemic pathological changes, such as hypertension/hypotension.

The microvasculature of the main organs is normally protected from pulsatile energy by a high resistance in prearteriolar vessels. The microvascular pathology described in CADASIL may reflect a partial loss of such protection resulting from the degeneration of VSMC that is typical of CADASIL. A gradual loss of VSMC in cerebral arteries is also seen in normal aging and in hypertension,¹⁵⁴ the two most important risk factors for stroke. Research into CADASIL and stroke ought to focus on finding ways to promote the survival of cerebral VSMC.

Brief summary

- Vasodilation in response to acetylcholine and nitroprusside was reduced in resistance vessels in stroke patients compared with healthy controls. Hypertension seems to be the main cause of this disturbance.
- Flow-mediated vasodilation of the brachial artery is reduced in stroke patients independently of hypertension, indicating reduced endothelium-mediated vasodilation or reduced response of vascular smooth muscle cells to nitric oxide
- The stroke patients had similar diameter of the brachial artery compared with controls, but the diameter of the carotid artery was increased.
- Basal and hyperemic blood flow in the forearm was reduced in the CADASIL patients
- Flow-mediated vasodilation was not reduced in the brachial artery in the CADASIL patients.
- Acetylcholine-mediated vasodilation was reduced in the CADASIL patients, reflecting a reduced endothelium-mediated vasodilation in resistance arteries.
- Arterial stiffness evaluated by augmentation index, carotid distensibility index and the SV/PP ratio was not increased in stroke patients or in CADASIL patients compared with controls.

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Stroke medicine could be compared to a vast ocean. During my journey on this ocean I received help with navigation from several persons. I now take the opportunity to express my gratitude to all who contributed to the thesis, and to mention some of the major contributions.

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In portu navigo – in calmer waters

Uppsala, January, 2008

Anna Stenborg

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