Hyperemic Brachial Artery Blood Flow Velocity

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Abstract

This thesis aims to evaluate the blood flow velocity in the Brachial artery during reactive hyperemia. Primarily to appraise the information it might contain regarding cardiovascular function and cardiovascular risk.

Ultrasonographic doppler measurements of the Brachial artery were made on the 1016 men and women aged 70 included in the prospective investigation of the vasculature in Uppsala seniors (PIVUS) study. Analysis of the blood flow velocity in the forearm was made in comparison to established methods of estimating endothelial function, clinical markers of cardiovascular risk, the Framingham risk score and global atherosclerosis determined by whole body magnetic resonance angiography.

Systolic blood flow velocity was positively related to cardiovascular risk whereas the diastolic velocity was inversely correlated. However, the systolic to diastolic blood flow velocity (SDFV) ratio was more closely associated with cardiovascular risk than its components apart.

Ultrasonographic markers of Carotid atherosclerosis were related to the SDFV ratio. Concentric left ventricular remodeling and left ventricular mass index were also associated with the SDFV ratio, but not to its numerator or denominator separately. A similar pattern was found when assessing SDFV ratio in relation to global atherosclerosis, as well as to established markers of arterial compliance and vasodilation.

In conclusion, during reactive hyperemia of the Brachial artery, the systolic to diastolic blood flow velocity ratio appears to contain information of additional value than its components separately, independently of established cardiovascular risk factors. Possibly, the SDFV ratio could offer a promising means to estimate cardiovascular risk in aging populations.

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Dedication:

To my parents for all Your love, care and support!
This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


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Abbreviations

Ach  Acetylcholine
Aix  Augmentation index
AMI  Acute myocardial infarction
BFI  Blood flow increase
BP  Blood pressure
CCAD  Common carotid artery distensibility
CRP  C-reactive protein
CV  Cardiovascular
C.V  Coefficient of variation
CVD  Cardiovascular disease
CT  Computed tomography
DBP  Diastolic blood pressure
ECG  Echocardiogram
EDV  Endothelium dependent vasodilatation
EIDV  Endothelium independent vasodilatation
FMD  Flow mediated vasodilation
FRS  Framingham Risk Score
GSM  Grey scale median
GWAS  Whole genome association studies
HDL  High density lipoproteins
IDF  International Diabetes Foundation
IM  Intima media
IMT  Intima media thickness
IVRT  Isovolumetric relaxation time
IVS  Interventricular septum
LDL  Low density lipoproteins
LV  Left ventricle
LVH  Left ventricular hypertrophy
MetS  Metabolic syndrome
MRI  Magnetic resonance imaging
NCEP/ATPIII  National Cholesterol Education program’s Adult Treatment Panel III report, USA
NO  Nitric oxide
PET  Positron emission tomography
PIVUS  The Prospective Investigation of the Vasculature in Uppsala Seniors
PP    Pulse pressure
PWV   Pulse wave velocity
RAAS  Renin Angiotensin Aldosterone system
RWT   Relative wall thickness
SBP   Systolic blood pressure
SDFV ratio  The systolic to diastolic blood flow velocity in the Brachial artery during reactive hyperemia
SV/PP ratio Stroke volume to pulse pressure ratio
VSMC  Vascular smooth muscle cells
Introduction

The most common cause of death worldwide is cardiovascular disease (CVD). It is mainly caused by atherosclerosis. Men and women are almost equally affected. 82% of all CVD occurs in low- and middle-income countries. High exposure to behavioral risk factors, less availability of healthcare, and inadequate preventive efforts are contributing factors. In affluent societies an exponential increase of CVD is seen after age 55. Roughly one third of the Swedish adult population has hypertension.

The consequences of arteriosclerosis in the forms of stroke, heart failure, myocardial infarction, claudicatio, and other conditions place a heavy cost on individuals and society. Even though we know of many avoidable cardiovascular (CV) risk factors such as smoking, inactivity, unhealthy diet, stress, and obesity, the global incidence of CVD is increasing. Early detection and intervention of subclinical atherosclerosis can slow down or reverse the process.

In the present thesis, we introduce a novel marker of arterial function, related to CV risk: the systolic to diastolic blood flow velocity ratio during hyperemia of the Brachial artery (SDFV ratio). The SDFV ratio takes into account both arterial stiffness and small vessel disease. Inexpensive, non-invasive, and possibly useful in assessing CV function in large aging populations, the SDFV ratio might offer a promising means to use our resources prudently.
The cardiovascular system

Structure, function and main regulation

The cardiovascular system is a functionally fine-tuned organ. Its functions are distribution of oxygen and nutrients and maintenance of homeostasis. By complex regulatory feedback mechanisms the blood flow is adjusted according to the metabolic requirements of various tissues at each moment. The venous side of the vascular system functions as a blood reserve (capacitance vessels) and transports metabolic waste products from tissues. By definition it is not affected by atherosclerosis and is thus not further discussed here.

The heart, essential as the pump in the CV system, has multiple ways to influence blood flow. Gradual pathological processes of the arterial tree may force the heart to adapt to the higher workload by increasing the size of the myocardial cells. A long-standing, increased peripheral resistance (i.e. an increased afterload) can lead to ventricular remodeling and hypertrophy.

The arteries have a common basic structure; (1) an inner lining by a single endothelial cell layer, subendothelial connective tissue and an elastic lamina (tunica intima), (2) a medial layer of mainly smooth muscle cells (tunica media), and (3) an outer layer of mainly fibrous connective tissue (tunica adventitia).

![Figure 1. Scheme of the common, basic structure of an artery. Ill: SJJ](image)

The arterial system is classified by the dimension and function of the vessel: large, (elastic) and medium-sized (muscular) conducting arteries, and the small resistance arteries.
Elastic arteries smoothen the fluctuations in pressure created by each cardiac cycle. A large content of elastin fibers in the walls of the Aorta and its large branches enable distension as an increased amount of blood passes during the systolic phase. When the vessel wall rebounds, the energy absorbed is released, propelling blood to maintain a forward flow during diastole. The pulsatile flow through the proximal Aorta gradually turns into a continuous flow through the capillaries. Along the arteries there is a gradual decrease of elastin content and arterial diameter but a significant increase of summed cross-sectional area.

In the course of a lifetime slow changes in the arterial tree appear. Within the arterial wall a fragmentation of elastin fibers and a progressive deposition of rigid collagen fibers take place. Interaction of the vascular smooth muscle cells (VSMC) and extracellular matrix through adhesion proteins and their membrane receptors (integrins) gradually lead to reduced viscoelastic properties and remodeling of the arterial wall.

Muscular arteries, such as the Brachial artery, have up to 40 layers of VSMC. The basal tonus of these vessel walls is largely influenced by the sympathetic nervous system. Alterations in diameter are also an effect of hormonal stimulation, including the Renin Angiotensin Aldosteron System (RAAS) and endothelial NO release. The systemic regulatory effect on blood pressure by these arteries is not as prominent as the large elastic arteries or the smaller ones. Their tonus mainly affects the systolic blood flow.

Resistance arteries: arterioles, metarterioles and the pre-capillary sphincters, form a widespread network governing peripheral resistance by local stimuli and sympathetic influence. Hypoxia and hypercapnea are the strongest stimuli for dilation, yielding an increased diastolic blood flow through a decreased resistance. This explains the phenomenon of reactive hyperemia, presented in this material. The basic structure of resistance vessels is an endothelial lining, 1—5 layers of VSMC and a thin adventitia. Although less than 0.5mm in diameter, their large summed cross-sectional area is instrumental in the regulation of blood flow.

The immense capillary network governs gas and nutrient exchange before redirecting blood into the veins.
Figure 2. The large cross sectional area of the resistance vessels makes it a powerful regulator of the blood flow velocity by small alterations in diameter. Ill:SJJ

Figure 3. Variability scheme of parameters steering blood flow through the arterial system. BP = blood pressure, \( V_{bf} \) = blood flow velocity, Diameter = vessel diameter. At rest, the velocity of blood through the aorta is approximately 1 m/s. In the capillaries the velocity of blood averages 0.3—1 mm/s. The lower velocity in the capillaries is compensated for by a larger total common cross-sectional area which is highly variable at the precapillary level. Ill:SJJ
Atherosclerosis

The atherosclerotic process (Greek; athera=porridge, sclerosis=stiffening) is initiated as a reparative response to mechanical tear on the vessels and toxic influences of oxidized low density lipoproteins (LDL) trapped in the vascular wall.

Microangiopathy (i.e. dysfunction of the small vessels and capillaries) develops faster in diabetic and hyperlipidemic subjects. Progressing over decades, macroangiopathy is unevenly distributed among the large and medium-sized (conduit) arteries, often at locations with turbulent or slower blood flow such as branching sites or curvatures.

Endothelial dysfunction is an early finding in atherosclerosis [1]. From childhood, “fatty streaks” are visible by microscope in the intima media of large and mid-size arteries [2, 3]. These represent clusters of immunocompetent cells (“foamcells” by histologic appearance, i.e. macrophages and T-lymphocytes) caught in the vascular wall after being attracted from the bloodstream, mainly by oxidized LDL. Engulfing lipids, these cells cause a local inflammatory response. VSMC are drawn to the site and start producing rigid collagen fibers replacing defective elastin causing fibromuscular atherosclerotic plaques to develop.

A fibrous cap stabilizes the plaque from rupture and protects it from contact with thrombogenic factors in circulating blood. Calcium and lipids are deposited, and the foam cells eventually burst. Released cytokines and other immunoattractants promote the inflammatory process. Apoptosis of nearby VSMC, tissue ischemia, and bleeding can follow. When the plaque finally ruptures, oxidized lipids and digestive enzymes are released in the vicinity. As the lipid necrotic core content comes in contact with the blood, precipitation of thrombosis and clot formation may cause irreversible damage due to vessel occlusion.
The atherosclerotic process. The intima layer is the site of the drama, infiltrated by low density lipoproteins. Macrophages on reparative mission are attracted to the area, cross the endothelium and engulf the lipids and become “foam cells”. “Fatty streaks” of foam cells are formed. Local inflammation and secretion of cytokines leads vascular smooth muscle cells (VSMC) to sacrifice their contractile ability and migrate into the area. Aiming to stabilize the forming plaque and prevent further invasion, they place themselves beneath the endothelium and produce a “fibrous cap” of collagen. Contact with the thrombogenic factors circulating in the blood is thus prevented but the arterial wall gradually stiffens. The foam cells finally burst and release oxidised lipids and digestive enzymes in the vicinity. Degeneration of the collagenous coat and induction of apoptosis in nearby VSMC. Eventually the fibrous cap, ruptures. Clot formation and thrombosis results when the lipid necrotic core comes in contact with the blood. III:SJJ
Risk factors for cardiovascular disease

A risk factor is something that increases the probability of developing a certain condition or disease. Risk factors include features which we are exposed to through our lifestyle and age. Age is the most powerful of them. In women clinical signs of atherosclerosis appear approximately ten years later than in men of the same age, possibly due to protective hormonal effects on the endothelium [4, 5]. Lately, the genetic impact on CVD has been elucidated by whole genome association studies (GWAS).

Other important CV risk factors can largely be avoided. Smoking, by itself, increases CVD 2–4 times [6]. In those who give up smoking, a prompt halving of the risk for coronary heart disease has been shown compared to those who continue [7]. The “metabolic syndrome” (MetS = obesity, hypertension, insulin resistance, and hyperlipidemia) has spread epidemically over the last few decades and carries a definite association to CVD. Persons with MetS [8] and diabetics [9] [10] are at 2–4 times increased risk of CVD. Other lifestyle factors with reported association to CVD include: inactivity and low socio-economic status [11, 12], high intake of (polyunsaturated) fat [13], [14] and salt [15, 16] psychosocial stress at home or at work [17], depression [18, 19] and excessive alcohol consumption [20] [21, 22]. Persons under CV treatment or those who have had a previous CV event also carry an elevated risk [23]. In addition, risk factors worsen the prognosis for recovery after a CV insult [24].

Pharmacological options are widely available for treatment of medical conditions known to be associated with an elevated risk of CVD (clinical risk markers) such as LVH and carotid plaques. Healthcare and information on beneficial life-style factors to improve health have never been more accessible than now (www.gapminder.org). However, CVD incidence is increasing globally. To reverse this trend we need better ways to detect individuals at risk for CVD. Earlier identification enables prevention of premature morbidity and death by medication and lifestyle changes.
Scoring systems

To assess the relative risk of an individual developing a cardiovascular event several scoring systems have been developed, based on the follow-up of large study populations.

The Framingham Risk Score (FRS)

The FRS is the first and perhaps most widespread scoring system. It is based on data from over 5,000 men and women recruited from 1948 at 30-60 years of age in Framingham, Massachusetts, USA. Coronary disease risk prediction of the FRS is adjusted for gender and based on age, blood pressure, diabetes, smoking, and cholesterol levels. Due to their different relation to CV risk, high density lipoproteins (HDL, associated with lower risk) and LDL (associated with high risk) are scored separately. By adding points given according to risk factors mentioned above [25] the calculated risk score gives an indication of the 10-year risk percentage of having coronary heart disease.

The SCORE-CARD evaluation system

The SCORE-CARD evaluation system was introduced in 2003. It is adjusted for European countries, where it is mainly used. It is based on study data from more than 200,000 subjects. Age, gender, smoking, total cholesterol, and systolic blood pressure are considered, and the only endpoint is CV death.
CV risk markers

With age and exposure to risk factors of CVD the rate of atherosclerosis increases. Established organ damage is frequently present in persons with MetS or diabetes years before clinical symptoms appear.

Measuring blood pressure, detecting left ventricular hypertrophy, and evaluating kidney function are some ways to clinically assess the CV system. If abnormalities are detected, intervention can reduce the risk and postpone further CVD. Such markers of increased CV risk are subclinical findings of known relation to atherosclerosis along the causal pathway from endothelial dysfunction to CV death.

The kidneys play an important role in the CV system. They respond to various stimuli, such as small changes in blood pressure and heart rate due to different types of stress. Furthermore, they produce potent hormones involved in the Renin Angiotensin Aldosterone System and use salt and water regulatory mechanisms to control blood pressure. As the arterial tree stiffens the fine capillaries and glomeruli of the kidneys suffer from diminished function, glomerulosclerosis, and protein leakage. Especially in persons with diabetes mellitus or inflammatory diseases, renal dysfunction often occurs earlier than normal.

In recent decades, the presence of several substances circulating in the blood (e.g. CRP, homocystein) have been shown to be associated with CV risk and are used as risk markers in clinical practice [26] [27, 28].

Carotid artery

The Carotid arteries have both elastic and conductive arterial properties and are ideal for studying atherosclerosis. They are easily accessible for assessment. Due to the turbulent blood flow at the division site and the short distance from the heart to resistance reflection sites, they are a frequent site for atherosclerotic plaques.

Carotid compliance

has been shown to be related to CV risk [29].
Carotid plaques
are associated with an increased risk of stroke [30]. The number [31] and size [32] of plaques have been related to CV risk.

Carotid plaque echogenicity
is the ultrasonographic grey-level of a certain region of interest (ROI) in the arterial segment. Grading pixels between 0 and 256, the Grey Scale Median (GSM) is calculated. If the number is low, a dark, echolucent plaque appears on the screen and is associated with a high lipid content. Echogenic areas appear white and indicate a high collagen content and possible calcification.

Echogenic plaques have been shown to be of lower risk [33, 34] than echolucent plaques, which seem more prone to rupture. Furthermore, the increased risk of echolucency seems to be valid also for the intima media (IM) complex of the carotid artery [35].

Carotid intima media thickness (IMT)
has been shown to be associated with the occurrence of Carotid plaques [36] and with Brachial artery IMT (Safar, Laurent, structural changes of large conduit arteries in hypertension). No significant correlation between Carotid IMT and Brachial artery flow-mediated vasodilation (FMD) has been found [37].
In the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) population, the IMT of the Carotid artery was positively related to the thickness and the echogenicity of the Brachial artery IM [38].

Left ventricular hypertrophy (LVH)
Remodeling and hypertrophy of the left ventricle occurs in response to a sustained increase of peripheral resistance (afterload). To overcome the resistance, myocardial cells increase in size, thickening the left ventricular (LV) wall. The remodeling is gradual and in early stages concentric. Further progress leads to concentric hypertrophy.

Remodeling and hypertrophy of the myocardium increases its oxygen demand. Coronary perfusion occurs during diastole, which is shortened as arteries stiffen. Insufficient coronary perfusion may lead to myocyte degeneration, myocardial stiffening, and arrhythmia by interruptions in the electric conductive pathway of the heart.

In later stages, eccentric remodeling with an increased LV lumen and regression of LV wall thickness indicates insufficiency of the myocardium to withstand the afterload. The LV filling increases, but the ejection fraction
(EF) decreases. This vicious cycle, if uninhibited, leads to heart failure and death.

LV remodeling in young individuals is mostly seen as a reversible, physiological response to vigorous exercise or pregnancy. In older subjects with hypertension, the various stages of LV remodeling or LVH are pathological. Patients with diabetes and/or kidney failure are especially prone to develop LV remodeling. Depending on the population studied, methods used, and the definition, 10—80% had LV concentric remodeling or hypertrophy. LVH is a well-known clinical marker of increased CV risk and sudden death [39].

![Figure 5](image)

*Figure 5.* Schematic illustration of the remodeling stages of the left ventricle in response to increased afterload i.e. peripheral resistance. Some extent of reversibility of concentric remodeling is seen in response to lowered afterload III:SJJ
The endothelium

In recent decades the endothelium has been revealed to be central in regulating vascular function. The endothelium is vulnerably exposed in the interface between tissues and the forces and contents of the bloodstream. It is involved in regulation of vascular tone, platelet and leukocyte interactions, thrombogenicity, and cell growth by cell membrane receptors and signal transduction mechanisms. Responding with synthesis and release of a variety of substances modulating vasodilatory, anti-inflammatory, or antithrombotic pathways, it is likely that subtle early injury to its properties alters endothelial function and hastens atherosclerotic progression.

Endothelial dysfunction is believed to be a major initial event in atherosclerosis and is an independent predictor of vascular disease events [40].

It has been hypothesized that endothelial function may serve as a clinical marker of CVD [41]. Subclinical detection of CVD provides maximal opportunity for prevention [42]. Recent studies have shown that by medical treatment some extent of reversibility both of endothelial dysfunction and of organ damage with a consequent reduction in CV risk can be achieved [43, 44].

Nitric Oxide (NO) in endothelial function

NO was formerly known as the endothelial derived relaxing factor (EDRF). Lipid-soluble and therefore cell membrane-diffusible, this molecule with a half life of a few seconds readily diffuses to the VSMC [45]. Their subsequent relaxation causes vasodilation.

NO has also proven to be central in platelet inhibition and leukocyte activation. Since NO can be released in different amounts, and given its short range of action, vessel diameter and thereby blood flow can be rapidly altered in response to local needs.

Evaluation of endothelial function

Two frequently used methods aiming to evaluate endothelial function are (1) the invasive forearm model, evaluating endothelium-dependent vasodilation
(EDV) and endothelium-independent vasodilation (EIDV) mainly in resistance arteries and (2) the non-invasive flow-mediated vasodilation (FMD), evaluating the function of the conduit artery endothelium.

EDV and EIDV

EDV reflects the ability of the endothelium in resistance vascular beds to release NO. NO release is triggered by the intravasal administration of an active substance, e.g. Acetylcholine (Ach). Endothelium-independent vasodilation (EIDV) is assessed in a similar way, but instead of an NO-triggering substance, an exogenous NO donor, such as nitroglycerin (NTG), or the NO analogue Sodium Nitroprusside (SNP) is administered. SNP acts directly on VSMC and causes relaxation. EIDV thus reflects vascular smooth cell function.

A reduced EDV compared to EIDV indicates impaired endothelial function of resistance vessels.

EDV, tested in coronary, cerebral, and cutaneous vascular beds, relates positively to endothelial function in the forearm [46].

Flow Mediated Dilation

reflects NO release in conduit arteries in response to reactive hyperemia. It is measured ultrasonographically. “Shear stress” and “reactive hyperemia” are discussed in detail below. Since it is non-invasive, there is no risk of the thrombosis, bleeding, vascular damage, or vasospasm sometimes seen with invasive methods. It is also significantly cheaper and therefore a useful tool for assessing endothelial function in large populations.

The endothelial function of the Brachial artery assessed by this technique closely correlates with that of the coronary arteries in the same patients [47]. Furthermore, FMD correlates to CV risk measured as events of stroke or myocardial infarction [36] as well as to prognosis and future cardiac events in patients with chest pain [48].
Arterial stiffness

Arterial stiffening is seen with aging and changes the characteristics of the pulsatile blood flow seen in young healthy subjects.

Methods aiming to assess arterial stiffness

The most commonly used methods are pulse wave velocity (PWV), pulse wave reflection and central pulse wave analysis.

Pulse Wave Velocity

is considered the “gold standard” to measure arterial stiffness. It is a direct method, transcutaneously registering the difference in time it takes for a pulse wave to reach the Carotid and the Femoral artery respectively (Laurent S, Cockroft European Heart Journal 2006 Expert consensus on arterial stiffness). The pulse wave is transmitted along the arterial wall, initiated as the stroke volume of each heartbeat distends the artery. Applanation tonometry measures the transmural pressure of an artery when flattened against a bony surface. This pressure is known to equal the endovascular pressure. In the Sphygmocor device a computer programs is synchronized with the echocardiogram and used to determine PWV. The speed at which the pulse wave travels is higher the stiffer the artery.

Pulse Wave Reflection

At division sites, the most prominent being the Aortic (Iliac) and when reaching the resistance vessels, the pulse-wave is reflected. The returning wave is superimposed upon the following forward travelling waves (Laurent S, Cockroft J 2008. Central Aortic Blood Pressure, Elsevier).

In young subjects this reflected wave occurs during diastole, increasing left ventricular (LV) filling and thereby contractile power and stroke volume.

As transmission velocity of these opposed waves increases with age, they merge earlier, during systole, leading to an increment of the systolic blood pressure (SBP) and a relative decrease in diastolic pressure (DBP). Distance to reflection, the elastic characteristics of the arteries and the heart rate all influence the retrograde wave enhancement of the SBP.
Central Pulse Wave analysis

enables indirect estimation of the retrograde enhancement by the augmentation index (AIx). The AIx is the percentage of the reflected pulse wave to the pulse pressure (PP). AIx is determined by the amplitude of the waves and their timing, which are influenced by heart rate and body height.

PP, the difference between SBP and DBP is not constant through the arterial tree, but decreases peripherally. In arteries closer to the heart, more time is needed for the reflected wave to travel back to the measuring point. This way the compliance of the large vessels can be calculated non-invasively.

Stroke volume to pulse pressure (SV/PP) ratio

The SV/PP ratio describes increase in blood pressure in response to the pressure exerted by the ejection of blood from the left ventricle with each heartbeat. The stroke volume of the LV, dependent on LV filling and the internal diameter and distensibility in response to peripheral resistance, is calculated from echocardiographic imaging.

Carotid artery distensibility

Using ultrasound, the diameter of the Common Carotid artery could be measured in systole and diastole. Expressed as the percentage increase from diastole, the distensibility is given by forming a ratio with pulse pressure.

Imaging methods

Estimation of atherosclerosis can be done by visualizing arterial structure and blood flow. Aiming thereby to assess CV risk, imaging methods are also used in follow-up of subjects in the clinical or scientific setting.

Ultrasonographic imaging

is used extensively in modern healthcare. It is based on the ability of a piezoelectric crystal to transform electric impulses into sound waves and also the reverse, to transform a sound wave into an electric current. Ultrasound signals are reflected to a varying degree depending on the various densities of different tissues.

A dense tissue is more echogenic and appears white on the screen due to a higher degree of signal reflection. The distance from the measuring point to the respective structure is calculated by the time it takes for each reflected signal to return. Two- and three-dimensional ultrasound transcutaneous
imaging is rapid and non-invasive. Transthoracic and transesophageal scanning is in widespread use. The recent technique of invasive intraluminal vascular imaging is of increasing interest in arterial assessment.

The Doppler principle

is used in ultrasonographic imaging and makes imaging of blood flow possible. If it is reflected upon a moving object, such as the red blood cells, the high frequency of the ultrasonographic signal changes. The signal changes towards higher frequency if the ultrasonic wave is reflected on an object moving towards the receiver and vice versa.

The frequency change is utilized to compute blood flow velocity. Depending on the speed and the angle of the moving object in respect to the receiver, the signal can be visualized in a designated color. Blood flow can thus be visualized on the screen concomitantly with the tissue images.

In the present articles, the Brachial artery (all papers), the Carotids (paper II), and the LV of the heart (paper III) were examined ultrasonographically.

Magnetic Resonance Imaging (MRI)

allows detailed picturing of tissues based on the magnetic properties of protons. Without the drawbacks of methods using ionizing radiation and without the nephrotoxicity of iodinated contrast agents, vascular imaging can be performed with or without contrast. A Gadolinium (Gd) chelate with paramagnetic properties can be given intravenously to neutralize the protons nearby, enabling enhanced luminal visualization. This method is used in paper IV to map the arterial tree.

X-ray angiography

is up to now the most commonly used method, allows luminal visualization of vessel segments by use of intravasal administration of radiopaque contrast. Intervention of stenotic, occlusive, or aneurysmatic lesions by thrombolysis, ballooning, stenting, or coiling can be performed.

Computed Tomography (CT) and Positron Emission Tomography (PET)

can be used with or without contrast enhancement, allowing detailed images of the vascular wall and surrounding tissues.
Physiology of ischemia and hyperemia

Blood flow

is steered by several mechanisms. Autoregulatory and local effects, neural activity, and hormonal signals all primarily lead to luminal alterations. Tissue ischemia is a strong local stimuli for increase in resistance vessel diameter, lowering their resistance and thus facilitating re-perfusion. A doubled radius thereby gives fully a sixteen-fold increase in flow.

Vascular resistance

is calculated by Hagen-Poiseuille’s law where \( L \) = length of the vessel and \( V \) = viscosity of the fluid. The \( r \) = radius of the vessel affects blood flow to the power of four.

\[
R = \frac{L \times V}{r^4} \times \frac{8}{\pi}
\]

and

\[
F = \frac{\Delta P}{R}
\]

The flow \( F \) equals the difference in pressure (\( \Delta P \)) divided by the resistance of the vessel \( R \) (Guyton, textbook of Medical Physiology 8th edition 1991)

Reactive hyperemia

If the Brachial artery is occluded, the tissues distal to the occlusion become ischemic. Hypoxia, hypercapnea (high concentration of CO\(_2\)) and decreasing pH lead to dilation of the vascular bed within the ischemic area. Consequent loss of vascular peripheral resistance allows effective re-perfusion as the occlusion is released. Blood can then flow with high velocity into the tissues fed by the Brachial artery on cuff deflation. A brief high-flow state through the Brachial artery is induced, denoted reactive hyperemia.

The high velocity blood flow (BF) causes friction on the endothelium of the arterial wall. Denoted “shear stress” this friction has been proposed as the trigger for endothelial release of NO [49, 50] [51, 52].
Shear stress

Shear stress is directly related to the velocity and viscosity of blood but inversely related to the vessel diameter. Small-diameter vessels are seen to dilate more in response to reactive hyperemia, possibly due to a higher shear stress stimulus [53]. Basal NO release is likely larger in the microcirculation and contributes more to the vessel tone than in the conduit vessels [45]. On the other hand, a yet unknown endothelium-derived hyperpolarising factor (EDHF) seems to be released mainly by smaller arteries.

Flow patterns in the Brachial artery

In conduit vessels, like the brachial artery, blood flow is mostly laminar, as if fluid tubes of decreasing diameter made out of red blood cells and plasma were placed inside of each other. The widest “tube” or “layer” of blood cells is slowed down by the shear stress. Interaction with cells and particles brought by the blood stream also occurs at this level. Each inner layer, however, is less affected by friction to concurrent layers, and therefore the blood cells in consecutive layers flows at a slightly higher velocity.

The result is a “parabolic blood flow,” where the blood in the center of the artery has a slightly higher velocity than the outer layers. When blood flow velocity increases, as in reactive hyperemia, the relative increase in velocity is highest in the center of the vessel. For the study of peak values of blood flow velocity, the choice of measuring point in the vessel is thus important.

Figure 6. Schematic illustration of the laminar parabolic character of blood flow through a muscular artery. Ill:SJJ

\[ BF \ [\text{ml/min}] = \text{VTI} \times \text{VA} \times \text{HR} \]

BF = blood flow, VTI = velocity time integral (area under curve), VA = vessel area HR = heart rate, which is considered constant when assessing Brachial hyperemia under standardized conditions.
BFI [%] = \frac{(BF_h - BF_r)}{BF_r}

BFI = blood flow increase, BF_h = blood flow during hyperemia, BF_r = blood flow at rest

**shear stress = shear rate \times blood viscosity**

Blood viscosity, maintained strictly at a constant level, is normally three to four times that of water. Plasma levels of cells and molecules contribute less to the viscosity than the blood hematocrit, temperature, and the flow rate (blood volume flow through a vessel per time unit). In our setting, at standardized temperature and time of day, after overnight fasting and all subjects within the normal range in hematocrit, blood viscosity could be considered constant and of negligible value compared to the other factors determining shear stress, namely the vessel diameter and the blood flow velocity.

**shear rate = \frac{4 \times V_{peak}}{vessel \ diameter}**

V_{peak} = peak velocity of blood as measured in the centre of the vessel. The vessel diameter is measured at end diastole, when the diameter is minimal.
Hyperemic blood flow in the Brachial artery

In studies on dogs, the degree of dilation upon reperfusion of a conduit artery in response to a period of ischemia had been shown related to endothelial function. In 1992, Celermajer et al. showed that CV risk factors and endothelial function related to conduit artery dilation after induced hyperemia. The Brachial artery was assessed in individuals with established coronary artery disease as well as symptom-free children and young adults of high risk due to exposure of CV risk factors and compared to the response in healthy controls. Reduced dilation and thus greatly reduced blood flow was found in the former two groups. Thus, a non-invasive way to determine endothelial function and thereby estimate CV risk was presented [3].

However, in several following studies, the relationship between vascular risk factors and FMD was rather poor. The relation between FMD and CV risk was seen mainly in low-risk populations [54]. Moreover, a high dependency on operator skills caused raised questions about the value of FMD.

Microvascular function controls blood flow and oxygen transport but the correlation between measures of conduit vessel dilation and microvascular function is weak [55]. Largely mirroring arterial compliance, and therefore macrovascular function, FMD was of low clinical value assessing aged populations with rigid arteries [56].

There was renewed interest for the hyperemic blood flow in 2004 when Mitchell and co-workers in the Framingham Heart Study first showed that the hyperemic blood flow velocity in both systole and diastole were determinants of FMD. The diastolic velocity, mainly dependent on microvascular dilation, was more closely related to FMD than the systolic velocity [57].

When adding the mean hyperemic diastolic blood flow velocity to regression models relating the major CV risk factors to FMD, the relationship between these risk factors and FMD was markedly attenuated.

This finding suggested that the previously reported association between FMD and CV risk factors were mainly due to the effects of the risk factors on the vasodilation in the resistance arteries governing the hyperemic blood flow, rather than a direct effect on FMD in the Brachial artery.
Technical aspects of evaluating Brachial hyperemia by FMD

Method

To measure FMD, the baseline longitudinal diameter of the Brachial artery at rest is first registered ultrasonographically. Forearm ischemia is then induced by applying a blood pressure cuff inflated to 30-50 mmHg above systolic blood pressure (SBP). After 5 minutes the cuff is deflated. During ischemia, dilation of the resistance vessels is caused by local factors. Rapid re-perfusion of the tissues is necessary, wherefore blood flow through the fending artery is rapidly increased upon cuff release. As mentioned, this phenomenon is called reactive hyperemia.

The Brachial artery diameter and the degree of FMD are determined again during hyperemia. The FMD, although just 5–15% of the resting diameter, commonly increases blood flow by 400–700% to the area recovering from ischemia [3]. Maximal dilation response occurs at around 1 min after cuff release in healthy subjects [58]. About 70% of the dilation at this time is attributable to NO [59]. Short duration of stimuli and strictly standardized conditions are necessary for reproducibility and adequate NO response.

Preconditions and pitfalls of correct FMD evaluation

Corretti and co-authors evaluated technical aspects of FMD [58]. FMD is highly dependent on the nature of the shear stress stimulus [60] and it has been shown that not all FMD is NO dependent [61]. Therefore, strictly specified standard conditions should be followed in order to correctly assess NO dependent response in the Brachial artery [53, 62]:

- Time of day
- Fasting 8—12 hours before the study (including caffeine, vitamin C, tobacco, or high fat foods)
- No vasoactive medications for at least four half-lives
- Phase of menstrual cycle should be taken into account
- Temperature constant
- Quiet surroundings
- Supine position
- Period of rest before assessment
- Occlusion cuff distal to the site of measurement.
- 5 minutes of occlusion
- No ischemic handgrip excersise
Blood flow velocity

Velocity increase is a large, initial feature of reactive hyperemia. Both the systolic and the diastolic velocities are increased in the initial seconds of hyperemia. As the blood flow is increased, the blood flow velocity is rapidly normalized. After roughly 2 minutes of reperfusion, blood flow returns to baseline [58]. However, the dilation of the Brachial artery persists for 10-20 min, i.e. substantially beyond the hyperemic phase.

![Figure 7 The interrelation of blood flow velocity (V_BF), vessel diameter (Diameter) and blood flow (BF) through the Brachial artery upon reactive hyperemia. Ill:SJJ](image)

Blood flow velocity in hyperemia of the Brachial artery

Rest vs. hyperemia

Peak blood flow velocity was measured centrally in the Brachial artery according to Mitchell et al. [57] under standardized conditions.

\[ V_{\text{max}} [\text{m/s}] = \text{peak blood flow velocity} \]

For calculation of the *mean* blood flow velocities in systole and in diastole, the velocity curves of systole and diastole were traced separately, and the duration of each phase was recorded. The *mean* velocity was calculated by dividing the VTI (m) by the time (s) in the systolic and diastolic phase, respectively.
The area under the curve, i.e. the velocity time integral (VTI), was then calculated for systole and diastole, respectively. The calculations were performed by the ultrasonic device.

\[ V_{\text{mean}} [\text{m/s}] = \frac{\text{VTI}}{\text{time}} \]

The area under the curve, i.e. the velocity time integral (VTI), was then calculated for systole and diastole, respectively. The calculations were performed by the ultrasonic device.

VTI

VTI, although presented as an area, equals the distance in meters (m) from the measuring point that the blood has traveled during the course of the time, given in seconds (s). Indicated by yellow and blue colors in Figure 10, the VTI area is reshaped to illustrate the mean value of the blood flow velocity. The blood flow velocity along the endothelial lining is the velocity determining shear stress of the endothelium, the stimulus for NO release.

Figure 8. Doppler curve of the blood flow velocity through the Brachial artery during reactive hyperemia. Ill: The PIVUS study. Doppler by Jan Hall, Lars Lind

Systolic and diastolic blood flow velocity

In hyperemia, the large initial peak velocity of systole is followed by an elevated diastolic blood flow velocity if the function of resistance arteries is well preserved. Reperfusion of the tissues by increased blood flow through the ischemic capillary bed is then rapid. Compared to resting state, blood flow velocity in the Brachial artery is markedly increased throughout the cardiac cycle.

Increase in shear stress or, as hypothesized in the present thesis, the actual velocity increase, could be a stimulus for endothelial NO release and
consecutive dilation of the artery. As the artery dilates, blood flow increases, and the blood flow velocity thereby decreases.

On the other hand, if the resistance arteries are rigid and fail to dilate in response to the local ischemic stimulus, the large systolic peak in velocity is instead followed by a small or absent velocity increase during diastole. The blood is impeded on its way through the arteries and in some cases even pushed in the backward direction due to wave reflection
The present thesis
Aims of the studies

Overall aim

of the study was to explore the value and usefulness of measuring hyperemic blood flow velocity in the Brachial artery in relation to cardiovascular function and cardiovascular risk factors.

Specific aims:

1. To investigate the relative importance of hyperaemic systolic and diastolic blood flow velocity in the forearm regarding both FMD and cardiovascular risk factors.
2. To investigate the relations between SDFV ratio in the Brachial artery and different characteristics of Carotid atherosclerosis.
3. To further investigate the SDFV ratio’s value as a marker of vascular risk in relation to echocardiographically determined left ventricular geometry.
4. To assess the SDFV ratio in relation to established markers of vascular function and global atherosclerosis.
Material and methods

Participants

Data in the present study is based on participants of the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) cohort. Recruited from the register of community living, they were invited within two months of their 70th birthday. Participation rate was 50.1% (1016/2025), of which 50.2% women (table 1). 306 participants (30.1%) were subject to whole body magnetic resonance angiography (WBMRA). The study was approved by the ethics committee of the University of Uppsala, Sweden.

A questionnaire on medical history, regular medication and smoking habits was completed by the participants. A history of coronary heart disease was present in approximately 10%. Four percent reported stroke and 9% diabetes mellitus. Antihypertensive medication was most prevalent 32% of the 45% reporting any cardiovascular medication. Fifteen percent reported use of statins, while oral antiglycemic drugs and insulin were reported in 6% and 2% respectively (see Lind et al 2005 [56] for details). A similar profile was found among the 306 who underwent WBMRA (table 8).

An evaluation of cardiovascular disorders was carried out in 100 consecutive non-participants due to the modest participation rate. Prevalence of diabetes, congestive heart failure and stroke was somewhat higher among the non-participants. The PIVUS participants and non-participants were similar regarding cardiovascular drug intake, history of myocardial infarction, coronary revascularisation, antihypertensive medication, insulin treatment and statin use.
Table 1. Descriptive data and statistics of the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) cohort. www.pivus.uu.se

<table>
<thead>
<tr>
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<th>PIVUS cohort</th>
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<tr>
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<td>Height (m)</td>
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<td>Weight (kg)</td>
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<td>Waist circumference (cm)</td>
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<td>Waist/hip ratio</td>
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<td>Current smoker (%)</td>
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<td></td>
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<tr>
<td>Antihypertensive medication (%)</td>
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<tr>
<td>Statin use (%)</td>
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<tr>
<td>Myocardial infarction (%)</td>
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<tr>
<td>Stroke (%)</td>
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<td>DBP (mmHg)</td>
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<td>IVRT (ms)</td>
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<td>E/A ratio</td>
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<td>EF (%)</td>
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<td>LVMI (g/m²⁷)</td>
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<td>EDV (%)</td>
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<tr>
<td>EIDV (%)</td>
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<td>FMD (%)</td>
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<td>CCAD (%/mmHg)</td>
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<tr>
<td>SV/PP ratio (ml/mmHg)</td>
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<td>Blood flow increase (%)</td>
<td>600 (1.84)</td>
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<td>Systolic mean blood flow velocity (m/s)</td>
<td>1.17 (0.25)</td>
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<tr>
<td>Diastolic mean blood flow velocity (m/s)</td>
<td>0.56 (0.15)</td>
<td></td>
</tr>
<tr>
<td>SDFV ratio</td>
<td>2.16 (0.45)</td>
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</table>

Means (SD) or proportions (%) are given. SBP = Systolic blood pressure, DBP = Diastolic blood pressure, BMI = Body Mass Index, HDL = high density lipoprotein, LDL= low density lipoprotein, EDV= Endothelium dependent vasodilation, EIDV = Endothelium independent vasodilation, FMD = Flow mediated dilatation, CCAD = Common Carotid Arterial Distension, SV/PP ratio =Stroke volume to pulse pressure ratio, TAS= Total atherosclerosis score, SDFV ratio = Systolic to diastolic blood flow velocity ratio during hyperemia of the Brachial artery.
Methods

The participants were examined in the morning, asked not to smoke or take any medication and to be fasting from midnight. Fasting blood glucose and lipid variables were analysed by standard laboratory techniques. Blood pressure was measured by a calibrated mercury sphygmomanometer to nearest mmHg after at least 30 min of rest in the supine position. The average of three recordings was used.

The Framingham risk score (FRS) including age, gender, cholesterol, blood pressure, diabetes and smoking was calculated and used as a comprehensive measure of the major CV risk factors [25].

Brachial artery monitoring

The Brachial artery was assessed by external B-mode ultrasound (Acuson XP128, 10 MHz linear transducer, Acuson Mountain View, California, USA). Imaging was performed 2-3cm above the elbow for measures of the Brachial artery diameter according to the recommendations of the International Brachial Artery Task Force [42]. To document baseline blood flow velocity, a pulsed Doppler measurement of the Brachial artery blood flow velocity was performed. Projection of the sampling volume was in the center of the vessel.

Figure 9. Monitoring of Brachial blood flow velocity by Doppler ultrasonography.

By inflating a blood pressure cuff placed distal to the elbow, the Brachial artery was occluded by applying a pressure of at least 50mm Hg above systolic blood pressure, causing ischemia of the forearm.

After five minutes, blood flow was re-established by rapidly deflating the cuff, causing a reactive hyperemia. Following cuff-release, blood flow velocity was measured for 15 seconds. Thereafter, the Brachial artery diameter was scanned at 30, 60 and 90 seconds following cuff-release,
determining the flow mediated dilatation (FMD). Determination of the diameter was performed in end-diastole (at the R-wave of the electrocardiogram).

The ultrasonographic measurements were recorded on videotapes for later analysis. Analyses were performed on the same device as the recordings. After 1-3 seconds following cuff release the highest values were seen. The diameter as well as the peak systolic and diastolic blood flow velocities in hyperemia were determined by the average of at least three different cardiac cycles.

The velocity time integral (VTI) in systole and diastole was thereafter traced and calculated. Mean blood flow velocity was calculated by dividing the VTI by the time of the respective phase of the cardiac cycle.

The SDFV ratio was calculated as the ratio of the systolic to diastolic mean blood flow velocities during hyperemia.

Figure 10. Schematic presentation of the measurements of blood flow velocities at rest and during hyperemia. At rest (upper panel), maximal blood flow was measured in systole at peak flow as indicated by the arrow. Mean blood flow velocity in systole was calculated as the velocity-time integral in systole divided by the systolic time interval. No measurements of the diastolic velocity were performed at rest due to the limited number of recordings with a definite positive velocity-time integral.

During hyperemia (lower panel), maximal blood flow was measured in both systole and diastole at the corresponding peaks as indicated by the arrows. Mean blood flow velocity in systole was calculated as the velocity-time integral in systole divided by the systolic time interval. Mean blood flow velocity in diastole was calculated in a similar way using the velocity-time integral in diastole and the diastolic time interval. The mean blood flow velocity is schematically illustrated by the yellow and blue bars.

Total blood flow velocity during hyperemia was calculated as the velocity-time integral for both the systolic and diastolic parts divided by the time interval for both systole and diastole. Ill: Lars Lind and SJJ
As the blood flow velocity in diastole was positive only in a part of the subjects at rest, we only evaluated the diastolic blood flow velocity in hyperemia.

Blood volume flow at rest and during hyperemia were calculated from the mean blood flow velocity and the resting diameter.

The blood flow increase induced by hyperemia was given as a percentage of resting blood flow in accordance with previous investigators [3, 58].

FMD was defined as the maximal diameter seen at either 30, 60 or 90 seconds following cuff release in relation to resting diameter. One individual analysed FMD and the indices of blood flow velocity by another.

At our laboratory, the reproducibility and coefficient of variation (C.V) for resting Brachial diameter and FMD were previously reported as 3% and 29% respectively [63]. Measurements on reproducibility regarding the different blood flow velocity variables, in 31 randomly chosen individuals of the PIVUS sample, ranged from 5.7% to 10.3% in C.V. Eighty four (84) participants were excluded from SDFV ratio calculations.

Carotid artery imaging

Ultrasonographic imaging of the Carotid artery was obtained with the same equipment. The common Carotid artery (CCA), the bulb and the internal Carotid artery (ICA) were visualised and the occurrence of plaque was recorded at both sides. The IMT was evaluated in the far wall of the CCA 1-2 cm proximal to the bulb. The given value for carotid artery IMT is the mean value from both sides.

For IMT and echogenicity assessment of the Carotid arteries, the image was then digitised and imported into the AMS (Artery Measurement Software) [36, 64]. A maximal 10 mm segment with good image quality was chosen for analysis. The borders of the IMT of the far wall and the inner diameter of the vessel were automatically identified by the programme. IMT was calculated through measuring the diameter from around 100 discrete points through the 10 mm long segment. If found not appropriate at visual inspection, this automated analysis could be manually corrected.

An ultrasonographic region of interest (ROI) was depicted manually around the intima-media segment evaluated for IMT. IM-GSM was calculated by analysis of the individual pixels within the ROI on a scale from 0 (black) to 256 (white). The blood was used as a reference for black and the adventitia of the Carotid vessel for white. A ROI was also placed manually around plaques for measurement of plaque area and GSM.

Plaque size was graded into four groups according to a previously used classification [34]. If the IMT was locally thickened more than 50%
compared to the surrounding IMT, a plaque was considered small. A plaque was denoted moderate if the plaque area was more than 10 mm². Plaques were regarded as flow-limiting if the velocity was increased distal to the plaque. Occluded carotid arteries were also included in this latter group.

The mean length of the evaluated segment was 6.9 (SD 1.9) mm when subjects with a segment recording less than 3 mm were excluded, leaving 945 subjects with valid recordings. The coefficients of variation were 7.2% for Carotid artery IMT, 7.5% for IM-GSM and 8.3% for GSM in plaque [64].

Echocardiography

Imaging was performed using a 2.5 mHz comprehensive two-dimensional cardiac ultrasound unit equipment, the same as for the Brachial artery recordings.

LV dimensions were measured with M-mode online from the parasternal projections, using a leading edge convention. Measurements included interventricular septal thickness (IVS), posterior wall thickness (PWT), left ventricular diameter in end-systole and end-diastole (LVESD, LVEDD). Left ventricular relative wall thickness (RWT) was calculated as (IVS+PWT)/LVEDD.

Left ventricular mass (LVM) was determined from the Penn-convention and indexed for height to the power of 2.7 to obtain left ventricular mass index (LVMI). The participants were further separated into four different categories of LV geometry, according to Ganau et al. [65, 66]

![Figure 11. Schematic left ventricular categorisation according to Ganau et.al 1992. III: SJJ](image)

A normal LV geometry (n=391) was considered to be present if LVMI was < 51 g/m².7 and RWT < 0.45%. If LVMI was normal, but RWT > 0.45% the
LV geometry was denoted concentric remodeling (n=236). Concentric LVH was defined as LVMI above the threshold for LVH together with RWT > 0.45% (n=140). If RWT was below this cut-off for RWT and LVMI was increased, categorisation into the eccentric group of LVH (n=75) was made. The ejection fraction (EF) was calculated from the M-mode recordings according to Teichholz formula.

The left ventricular diastolic filling pattern of the mitral inflow was obtained by placing transducer in apical 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 (IVRT) was measured between aortic valve closure and the start of mitral flow using the Doppler signal from the area between mitral flow and the left ventricular outflow tract.

Presence of a restrictive filling pattern was evaluated in subjects with an impaired LV systolic function. This pattern was considered to be present if E/A-ratio was >1.5 and IVRT was < 96 ms.

The invasive forearm technique

Forearm blood flow was measured by venous occlusion plethysmography (Elektromedicin, Kullavik, Sweden). After evaluation of forearm blood flow at rest, intra-arterial infusion of drugs were given locally during five minutes for each dose. Dosages were 50ug/minute for Acetylcholine (Clin-Alpha, Switzerland) to evaluate endothelium-dependent vasodilation (EDV) and 10ug/minute of Sodium nitroprusside (SNP), (Nitropress, Abbot, UK) to evaluate endothelium-independent vasodilation (EIDV) in forearm resistance vessels.

Endothelium-dependent and independent vasodilation respectively, were defined as the forearm blood flow during infusion of Ach or SNP respectively minus forearm blood flow while subject at rest, then divided by the forearm blood flow while subject at rest.

Carotid artery compliance

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

By echocardiographic imaging (2.5MHz transducer, Acuson XP 124, California, USA), using Teichholz formula, ejection fraction and stroke volume were calculated. The stroke volume to pulse pressure ratio was calculated as stroke volume divided by Aortic pulse pressure achieved by pulse wave analysis.

Pulse wave analysis

The peripheral Radial pulse wave was continuously recorded by a micromanometer tipped probe (Sphygmocor, Pulse Wave Medical Ltd, Australia) applied to the surface of the skin overlying the Radial artery. The mean values of around ten pulse waves were used for analyses. Based on transfer functions, Aortic systolic and diastolic blood pressure were calculated from the Radial recordings with the Sphygmocor software.

Total peripheral resistance

Estimation of the total peripheral resistance (TPRI) was given as the Cardiac index (CI) divided by the mean arterial pressure (MAP) minus three (an estimation of the central venous pressure in the right atrium of the heart).

Magnetic resonance angiography

Imaging was performed on a 1.5 Tesla MRI system (Gyroscan Intera, Philips Medical Systems, Best, the Netherlands) with a 25 mT/m gradient system, using the standard quadrature body coil.

The whole body was scanned in the supine position using a 3D RF-spoiled T1-weighted gradient echo sequence before and after injection of 40 ml Gd-DTPA-BMA (OmniscanTM, GE Healthcare, Oslo, Norway) at a rate of 0.6 ml/s. The acquired slice thickness was 4 mm with a resolution of 1.76 x 1.76 mm.

The arterial tree was divided into 26 vessel segments:

Internal Carotid arteries, common Carotid arteries including the Brachiocephalic trunk on the right side, thoracic Aorta, abdominal Aorta, Renal arteries, common Iliac arteries, external Iliac arteries, common Femoral arteries, superficial Femoral arteries, Popliteal arteries, Tibio-peroneal trunks, anterior Tibial arteries, Peroneal arteries and the posterior Tibial arteries.

Only the first 3-5 cm of the Renal arteries and the bulb of the internal Carotid arteries were assessed due to low spatial resolution distally. The
right and the left sides were evaluated separately. Imaging did not include the Coronary or Cerebral arteries.

The 26 arterial segments were categorized into five territories:

1. the Carotids including internal Carotid artery (ICA) and common Carotid artery
2. the Aorta including both the thoracic and abdominal part
3. the Renal arteries
4. the Pelvic/upper limbs including common Iliac artery, external Iliac artery (EIA), common Femoral artery (CFA), superficial Femoral artery (SFA) and Popliteal artery (POP)
5. the lower legs including Tibio-peroneal trunk (TPT), anterior Tibial artery (ATA), Peroneal artery (PA) and posterior Tibial artery (PTA).
Evaluated segments were sorted into five groups:
- no stenosis
- 1-49% reduction of lumen diameter
- 50-99% reduction of lumen diameter
- occlusion
- aneurysm.

A normal vessel was defined as absence of vessel wall irregularities. Each segment was graded by its most severe stenosis. Segments not possible to evaluate were allocated into four groups; 1. venous overlap 2. motion
artefacts 3. poor contrast filling and 4. other reasons such as artefacts from prostheses.

In order to obtain a comparable graded number reflecting the atherosclerosis in each territory, an atherosclerotic score was calculated for each territory. A normal vessel segment received null points, less than 50% stenosis was given one point and 50% reduction or more of the vessel diameter including occlusions was given two points.

The points for the vessel segments in a territory were summarized. That sum was then divided with the maximum sum that would be achieved if all included segments had a more than 50% stenosis or occlusion. The quotient was multiplied by 100, giving a maximal atherosclerotic score (AS) of 100 per territory. The total atherosclerotic score, TAS, defined as the sum of the AS in the five territories, maximum 500.

Aneurysms and vessel segments that could not be evaluated were excluded from the calculations.

Statistical analysis
Non normally distributed variables were log-transformed in order to achieve a normal distribution. Differences in blood flow variables between men and women as well as between the assessed groups of respective risk factors were evaluated by analysis of variation (ANOVA). The relationship between continuous variables were evaluated by multiple linear regression analysis adjusting for gender and for traditional risk factors in the FRS. Two-tailed significance levels were set with p< 0.05 regarded as significant. In papers I-III, Statview (SAS inc., NC, USA) was used for calculations.

In paper IV, STATA 10 (College Station, USA) was used for calculations. All variables (used in paper IV) were normally distributed, except TAS, where around one third of the sample had a zero value. Therefore, the relations between blood flow velocity variables and the total atherosclerosis score were evaluated by censored (tobit) multiple regression adjusting for gender. As the Framingham risk score is a comprehensive risk estimate previously found to be related to both TAS [67] and hyperaemic blood flow [68] we used this score to adjust for CV risk factors.
Results and discussion

Paper 1

Results

Blood flow velocity variables in men and women
All blood flow velocity variables, except the hyperaemic peak and mean velocity in diastole, were significantly different between men and women (see table 2 paper 1). We therefore always adjusted for gender.

Relation to the Framingham Risk Score (FRS)
Both the peak and the mean velocities in systole measured at rest were related to the FRS. The inverse relation was found for the diastolic mean blood flow velocity in hyperemia.

The hyperemic systolic to diastolic blood flow velocity ratio was significantly related to the FRS (r=0.18, p=0.0001).

These connections were valid also after adjustment for antihypertensive and lipid-lowering medication in subjects with and without cardiovascular medication.

Relationship to flow mediated dilation (FMD)
Both the hyperaemic peak and mean systolic and diastolic velocity, as well as the hyperaemic total mean velocity and the resting systolic velocities exhibited significant positive relations to FMD.

FMD was negatively related to the hyperaemic peak and mean velocity in systole when divided by its diastolic counterpart. However, no significant relation between FMD and the hyperaemic blood flow increase was found.
Table 2. *Relationship between blood flow velocity variables and flow mediated dilation (FMD) and the Framingham Risk Score (FRS).*

<table>
<thead>
<tr>
<th></th>
<th>FMD</th>
<th>FRS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rest</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak Velocity (s)</td>
<td>0.10 (0.0045)</td>
<td>0.12 (0.002)</td>
</tr>
<tr>
<td>Mean Velocity (s)</td>
<td>0.14 (0.0001)</td>
<td>0.10 (0.0021)</td>
</tr>
<tr>
<td><strong>Hyperemia</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak Velocity (s)</td>
<td>0.11 (0.0009)</td>
<td>0.12 (0.0002)</td>
</tr>
<tr>
<td>Peak Velocity (d)</td>
<td>0.15 (0.0001)</td>
<td>-0.06 (0.073)</td>
</tr>
<tr>
<td>Mean Velocity (s)</td>
<td>0.14 (0.0001)</td>
<td>0.08 (0.013)</td>
</tr>
<tr>
<td>Mean Velocity (d)</td>
<td>0.19 (0.0001)</td>
<td>-0.08 (0.016)</td>
</tr>
<tr>
<td>Total Mean Velocity</td>
<td>0.18 (0.0001)</td>
<td>0.02 (0.52)</td>
</tr>
<tr>
<td>Blood Flow increase (%)</td>
<td>-0.002 (0.95)</td>
<td>-0.15 (0.0001)</td>
</tr>
<tr>
<td><strong>Hyperemic Ratios</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak Velocity (s) / (d)</td>
<td>-0.08 (0.014)</td>
<td>0.21 (0.0001)</td>
</tr>
<tr>
<td>Mean Velocity (s) / (d)</td>
<td>-0.09 (0.004)</td>
<td>0.23 (0.0001)</td>
</tr>
</tbody>
</table>

Values in the columns are given as the correlation coefficient adjusted for gender, with the p-value in parenthesis. Systolic values are indicated by (s) and diastolic values by (d).

**Relation to the traditional risk factors**

Both of the blood flow velocity parameters measured at rest were positively related to systolic blood pressure (SBP). This was also true for the systolic peak, mean and total velocities during hyperaemia, and the systolic to hyperaemic diastolic peak and mean velocity ratios. The blood flow increase during hyperaemia had a negative relation to SBP,
Table 3. Relationship between blood flow velocity variables and the traditional CV risk factors.

<table>
<thead>
<tr>
<th></th>
<th>SBP</th>
<th>DBP</th>
<th>LDL</th>
<th>HDL</th>
<th>Glucose</th>
<th>BMI</th>
<th>Smoking</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rest</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak velocity (s)</td>
<td>0.22 (0.0001)</td>
<td>-0.12 (0.0005)</td>
<td>0.01 (0.77)</td>
<td>-0.03 (0.33)</td>
<td>0.09 (0.009)</td>
<td>-0.036 (0.27)</td>
<td>-0.04 (0.29)</td>
</tr>
<tr>
<td>Mean velocity (s)</td>
<td>0.15 (0.0001)</td>
<td>-0.09 (0.0093)</td>
<td>0.007 (0.82)</td>
<td>-0.061 (0.05)</td>
<td>0.08 (0.015)</td>
<td>0.04 (0.27)</td>
<td>-0.03 (0.43)</td>
</tr>
<tr>
<td><strong>Hyperemia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak velocity (s)</td>
<td>0.18 (0.0001)</td>
<td>-0.23 (0.48)</td>
<td>0.015 (0.65)</td>
<td>-0.09 (0.78)</td>
<td>0.02 (0.58)</td>
<td>0.03 (0.35)</td>
<td>0.01 (0.78)</td>
</tr>
<tr>
<td>Peak velocity (d)</td>
<td>-0.04 (0.24)</td>
<td>0.01 (0.76)</td>
<td>0.015 (0.64)</td>
<td>0.02 (0.47)</td>
<td>-0.11 (0.0013)</td>
<td>0.001 (0.71)</td>
<td>-0.07 (0.057)</td>
</tr>
<tr>
<td>Mean velocity (s)</td>
<td>0.16 (0.0001)</td>
<td>0.001 (0.99)</td>
<td>-0.003 (0.92)</td>
<td>-0.007 (0.81)</td>
<td>0.008 (0.81)</td>
<td>0.04 (0.18)</td>
<td>-0.02 (0.64)</td>
</tr>
<tr>
<td>Mean velocity (d)</td>
<td>-0.06 (0.60)</td>
<td>0.05 (0.15)</td>
<td>0.008 (0.81)</td>
<td>0.03 (0.31)</td>
<td>-0.11 (0.0005)</td>
<td>0.02 (0.52)</td>
<td>-0.031 (0.35)</td>
</tr>
<tr>
<td>Total mean velocity</td>
<td>0.07 (0.029)</td>
<td>0.05 (0.12)</td>
<td>0.002 (0.96)</td>
<td>0.003 (0.91)</td>
<td>-0.05 (0.17)</td>
<td>0.04 (0.18)</td>
<td>-0.02 (0.58)</td>
</tr>
<tr>
<td>Blood flow increase (%)</td>
<td>-0.16 (0.0001)</td>
<td>0.05 (0.12)</td>
<td>0.02 (0.60)</td>
<td>0.08 (0.010)</td>
<td>-0.16 (0.0001)</td>
<td>-0.031 (0.37)</td>
<td>-0.076 (0.03)</td>
</tr>
<tr>
<td><strong>Hyperemic ratios</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak velocity (s)/(d)</td>
<td>0.22 (0.0001)</td>
<td>-0.02 (0.49)</td>
<td>0.005 (0.87)</td>
<td>-0.03 (0.32)</td>
<td>0.17 (0.0001)</td>
<td>0.009 (0.77)</td>
<td>0.12 (0.0003)</td>
</tr>
<tr>
<td>Mean velocity (s)/(d)</td>
<td>0.27 (0.0001)</td>
<td>-0.05 (0.09)</td>
<td>0.003 (0.91)</td>
<td>-0.05 (0.11)</td>
<td>0.20 (0.0001)</td>
<td>-0.005 (0.87)</td>
<td>0.06 (0.070)</td>
</tr>
</tbody>
</table>

Values are given as the correlation coefficient, followed by the p-value in parenthesis, after adjustment for gender. (s) = systole, (d) = diastole. SBP = systolic blood pressure, DBP = diastolic blood pressure, LDL = low density lipoproteins, HDL = high density lipoproteins, BMI = body mass index. Glucose refers to the fasting blood glucose levels and smoking is expressed in pack years.
The diastolic blood pressure (DBP) exhibited an inverse relation to the peak and mean velocities in systole at rest, but was not significantly related to any of the other blood flow velocity variables.

Fasting glucose levels in the blood were positively related to the peak and mean blood flow velocities during systole at rest and to the hyperaemic systolic to diastolic blood flow velocity ratio using both the peak and mean blood flow velocities. A negative relation between the glucose levels and the hyperaemic blood flow velocities in the diastolic phase, and the hyperaemic increase in blood flow were found.

Smoking was associated with a low blood flow increase during hyperaemia and a high systolic to diastolic blood flow velocity ratio when using the peak, but not the mean, velocity for the calculation. When analysed separately, these relations were seen in both genders.

Discussion
The present study first confirmed findings from Framingham [56] that hyperaemic blood flow velocity was related to FMD and to coronary risk factors. In addition, the present study showed that although hyperaemic blood flow in systole and diastole both were positively related to FMD, they were related to coronary risk in divergent ways.

While hyperaemic systolic velocity was related to coronary risk in a positive way, diastolic velocity was related to coronary risk in an inverse way. Therefore, the systolic to diastolic hyperaemic blood flow velocity ratio was more powerfully related to coronary risk than its individual components. In fact, this ratio was more closely related to coronary risk than FMD in multiple regression analysis (see table 5 paper 1).

In the present study blood flow velocity was evaluated both as the peak velocity and as mean velocity. Both of these measures have previously been used as an estimate of shear stress [56, 69]. However, since we did not measure blood viscosity in our total cohort, we were not able to evaluate shear stress in detail.

It was found that peak velocity and mean velocity during hyperaemia were related to FMD and coronary risk in a similar way. Also the systolic to diastolic hyperaemic blood flow velocity ratio was related to coronary risk with similar strength regardless if this ratio used peak or mean velocity for calculations. Since peak velocity is easier to measure than mean velocity and had a similar reproducibility in both systole and diastole as the mean velocity, peak velocity might be a preferred measure in large scale studies evaluating the impact of hyperaemic blood flow velocity.
Blood flow velocity at rest in diastole was not evaluated in the present study, since a uniform positive flow was only seen in a minority of the subjects, in accordance with findings in Framingham [56]. We found it hard to interpret the impact of a negative diastolic blood flow velocity on shear stress, and therefore this variable was not included in the analysis.

The positive relation found between systolic blood flow velocity at rest and FMD might be explained by the well known inverse relationship between baseline arterial diameter and FMD.

SBP was the major risk factor associated with baseline and hyperemic blood flow velocity in systole, but not in diastole. Fasting blood glucose, on the other hand, was related to baseline blood flow velocity in systole in a positive way and to hyperemic blood flow velocity in diastole inversely. This might support the assumption that arterial stiffness mainly effects systolic blood flow velocity, as systolic blood pressure in the elderly generally is a marker of arterial stiffness. Diabetes mellitus, on the other hand, influences both large and smaller arteries and might therefore affect conduit arterial stiffness, in addition to vasodilation in resistance arteries.

Diabetes was the factor of closest relation to diastolic blood flow velocity, in an inverse way. Previous studies on small arteries in diabetic patients have shown the media-to-lumen ratio to be increased [70] [71] indicating vascular hypertrophy. These previous findings are compatible with the reduced hyperemic blood flow velocity in diastole found in subjects with high blood glucose levels in the present study, as resistance vessel hypertrophy would limit hyperemic blood flow velocity in diastole.

The SDFV ratio is positively related to SBP and inversely to blood glucose in a more powerful way than when the individual components were analysed separately, or when the total mean blood flow velocity was calculated over the whole cardiac cycle. The attenuating power of this ratio was also seen when related to current smoking, which was significantly correlated to the peak SDFV ratio but not to the individual components of the ratio.
Paper 2

Results

The SDFV ratio was directly related (p = 0.018) to the number (0, 1 or 2) of Carotid arteries affected by plaque, adjusting for gender and Framingham risk score. None of the other parameters analysed (mean velocity in systole and diastole respectively, or the blood flow increase from rest to hyperemia) was significantly related to the number of Carotid arteries affected with plaque.

Furthermore, a positive relation of SDFV ratio to plaque size was observed (p=0.035) adjusting for gender and FRS (see table 3 paper 2).

Table 4. Mean blood flow velocity parameters during hyperemia of the Brachial artery in relation to number of Carotid arteries with plaque.

<table>
<thead>
<tr>
<th>Number of Carotid arteries with plaque</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>ANOVA p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 296</td>
<td>n = 299</td>
<td>n = 256</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SV (m/s)</td>
<td>1.16 (0.24)</td>
<td>1.18(0.24)</td>
<td>1.17(0.27)</td>
<td>a: 0.48</td>
</tr>
<tr>
<td>DV (m/s)</td>
<td>0.57 (0.14)</td>
<td>0.56 (0.15)</td>
<td>0.55 (0.18)</td>
<td>a: 0.51</td>
</tr>
<tr>
<td>SDFV ratio</td>
<td>2.10 (0.40)</td>
<td>2.18 (0.45)</td>
<td>2.23 (0.48)</td>
<td>a: 0.0005</td>
</tr>
<tr>
<td>BFI (%)</td>
<td>6.11 (1.77)</td>
<td>6.07 (1.84)</td>
<td>5.81 (1.91)</td>
<td>a: 0.03</td>
</tr>
</tbody>
</table>

SV = systolic blood flow velocity, DV = diastolic blood flow velocity, SDFV ratio = the hyperemic systolic to diastolic mean blood flow velocity ratio, BFI = blood flow increase. Means are given in parenthesis. ANOVA p-value: a: adjusted for gender only, b: adjusted for gender and for FRS.

The systolic mean blood velocity during hyperemia was positively related to IMT (p= 0.0009) but not significantly to plaque GSM or IM-GSM.

The diastolic mean velocity during hyperemia was positively related to both plaque GSM (p=0.004) and IM-GSM (p=0.0005), but not to IMT.

The SDFV ratio was positively related to plaque GSM (p=0.0002), IM-GSM (p=0.0001) and to IMT (p=0.0022), adjusting for gender and Framingham risk score.

IMT and the IM-GSM were both significantly related to the SDFV ratio even after adjustment for the traditional cardiovascular risk factors used for calculating the Framingham Risk Score in two separate multiple regression models.
Table 5. Relationship between mean blood flow velocity variables in the Brachial artery during hyperemia and plaque echogenicity, echogenicity in the intima-media complex (IM-GSM) and intima media thickness (IMT).

<table>
<thead>
<tr>
<th>Plaque echogenicity</th>
<th>IM-GSM</th>
<th>IMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>p-value</td>
<td>CC</td>
</tr>
<tr>
<td>SV (m/s)</td>
<td>-0.04</td>
<td>a: 0.23</td>
</tr>
<tr>
<td>DV (m/s)</td>
<td>-0.12</td>
<td>a: 0.005</td>
</tr>
<tr>
<td>SDFV ratio</td>
<td>0.15</td>
<td>a: 0.01</td>
</tr>
<tr>
<td>BFI (%)</td>
<td>0.08</td>
<td>a: 0.05</td>
</tr>
</tbody>
</table>

IM-GSM = Intima-Media- Grey scale median, IMT = Intima Media thickness, CC = correlation coefficient. SV = systolic blood flow velocity, DV = diastolic blood flow velocity.

Discussion

Summarizing our results, one single parameter, the SDFV ratio, was consistently related to several indices of arteriosclerosis of the Carotid artery.

The SDFV ratio was related to the number of Carotid arteries with plaques, as well as to plaque size and echogenicity. Furthermore, this ratio was also related to IMT and IM-GSM, independently of traditional CV risk factors.

CVD is the cause of around 30% of the deaths world-wide and as high as 50% in the United States and Europe (www.who.int, www.americanheart.org). Morbidity after CVD is a further challenge, personally and for the society, at an unestimatable supportive cost. Lean methods for detecting individuals at risk for CVD are therefore wanted. In this study neither the systolic nor the diastolic velocity was consistently related to arteriosclerosis whereas the SDFV ratio was, suggesting this ratio as a possible marker for atherosclerosis.

The systolic hyperemic blood flow velocity possibly reflects, at least in part, the elastic properties of the conduit arteries since we have found the systolic velocity to be related to systolic blood pressure, a marker of arterial stiffness in the elderly. The diastolic hyperemic velocity, on the other hand, possibly reflects the functional properties of the smaller arteries, since we found it to be mainly related to diabetes, known to cause small-vessel disease.
Thus, the SDFV ratio possibly combines information on properties of both large and small vessel status and will thereby serve as an integrated index of vascular function.

It is however not known if a high SDFV ratio will cause atherosclerosis by mechanical influences on the vascular wall or if atherosclerosis would lead to changes in the blood flow velocity pattern. Only a longitudinal approach with repeated measurements of the SDFV ratio and atherosclerosis could possibly answer that question.

Paper 3
Results
When adjusted for gender only, the SDFV ratio was related to LVMI ($p=0.0002$). This relation was not significant following multiple adjustment for FRS. None of the two compounds of the ratio were significantly related to the LVMI.

The SDFV ratio was significantly increased in subjects with concentric left ventricular remodeling when compared to subjects with normal LV geometry following adjustment for multiple risk factors ($p=0.007$). This relation was also valid when only adjusted for gender ($p=0.01$).

However, the systolic and diastolic mean blood velocities individually were not significantly altered in subjects with concentric LV remodeling when compared to the normal LV group. No significant differences in SDFV ratio, the systolic- nor the diastolic- blood flow velocities separately, were seen in the group with eccentric left ventricular remodeling when compared to the subjects with normal LV geometry.

Subjects with concentric left ventricular hypertrophy had an elevated SDFV ratio following adjustment for multiple risk factors ($p=0.001$) in comparison to the group with normal LV geometry. The relation persisted also when only adjusted for gender ($p=0.0027$).

No significant differences between the concentric left ventricular hypertrophy and the normal LV geometry groups were found when assessing the mean blood velocities of systole or diastole separately.
Table 6. Mean blood flow velocity parameters during hyperemia of the Brachial artery in relation to parameters of cardiac ultrasound.

<table>
<thead>
<tr>
<th></th>
<th>SDFV ratio</th>
<th>S V</th>
<th>D V</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R.C</td>
<td>P (a)</td>
<td>R.C</td>
</tr>
<tr>
<td>IVRT</td>
<td>0.055</td>
<td>0.117</td>
<td>-0.021</td>
</tr>
<tr>
<td>E/A ratio</td>
<td>0.023</td>
<td>0.508</td>
<td>0.025</td>
</tr>
<tr>
<td>EF</td>
<td>-0.066</td>
<td>0.066</td>
<td>-0.117</td>
</tr>
<tr>
<td>LVMI</td>
<td>0.128</td>
<td>0.0002</td>
<td>0.014</td>
</tr>
</tbody>
</table>

SDFV ratio = The systolic to diastolic blood flow velocity ratio during hyperemia of the Brachial artery. S V = Systolic blood flow velocity, D V = Diastolic blood flow velocity. Mean values are given. P(a) = p-value after adjustment for gender only. P (b) = p-value after adjustment for gender, systolic- and diastolic- blood pressure, antihypertensive treatment, body mass index and fasting blood glucose level. R.C = regression coefficient. IVRT = Isovolumetric relaxation time, E/A ratio= Ratio of peak velocity of early (E wave) rapid filling wave and peak velocity of atrial filling wave (A wave). EF= Ejection Fraction, LVMI= Left ventricular mass index determined from LVM determined from the Penn convention and indexed for height to the power of 2.7.
<table>
<thead>
<tr>
<th>Normal LV</th>
<th>Concentric remodeling</th>
<th>Eccentric remodeling</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=842</td>
<td>n=236</td>
<td>n=75</td>
</tr>
<tr>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>SV</td>
<td>1.16 0.25</td>
<td>1.18 0.24</td>
</tr>
<tr>
<td>DV</td>
<td>0.57 0.16</td>
<td>0.56 0.15</td>
</tr>
<tr>
<td>SDFV ratio</td>
<td>2.10 0.44</td>
<td>2.20 0.46</td>
</tr>
</tbody>
</table>

Normal LV group (LVMI < 51 g/m² and RWT < 0.45) was used as a reference. SV = Systolic blood flow velocity, DV = Diastolic blood flow velocity, SDFV ratio = Systolic to diastolic blood flow velocity ratio. Mean values are given. SD = Standard deviation. P(a) = Adjustment made for gender. P(b) = Adjustment made for gender, systolic- and diastolic-blood pressure, antihypertensive treatment, body mass index and fasting blood glucose.
Discussion

It is well known that the arterial tree stiffens with age. An increased systolic blood pressure indicates a stiffening vascular tree with mainly a decreased elasticity of the large, central arteries. Their ability to adjust their diameter to permit an even blood flow is impaired. Gradually, the blood flow velocity through these vessels, as well as the pulse wave velocity, increases. This is reflected by an increased blood flow velocity in systole during hyperemia in this study.

Distribution of the blood to adequately supply the tissues is largely dependent upon the resistance arterioles and the capillary network. In healthy persons, the peripheral arterioles and the precapillary sphincters alter the lumen of the vessel to allow blood flow in response to the need of the tissues. Due to their large common cross-sectional area, the peripheral resistance has a high impact on the blood flow. This is reflected by the blood flow velocity in diastole during hyperemia in this study.

The SDFV ratio combines information regarding large artery stiffness with information on peripheral resistance. These two properties are the most important features determining the afterload of the LV. If an increased afterload is sustained over time, it will cause myocytes to increase in size to maintain a proper ejection fraction and stroke volume. An increased afterload will also induce collagen deposition in the LV wall.

Together, these adaptations will lead to thickening of the LV wall, in this study represented as concentric LV remodeling or concentric LVH. As a result of the Frank-Starling effect, the LV lumen and contractile forces increases to enhance cardiac output (CO) [72]. When this cardiac reserve is exceeded, by volume overload or by the remodeling process following myocardial infarction, eccentric LVH is mainly induced. An increased SDFV ratio might thus signal an increased risk of LVH development.

LVH is a well known predictor of cardiovascular events and sudden death [39, 73]. It should however be pointed out that the SDFV ratio was not elevated in subjects with eccentric LVH.

The fact that the SDFV ratio was related to both concentric remodeling and concentric hypertrophy, but not to eccentric LVH, further supports its ability as a valuable measure of afterload.

Importantly, the relationship between the SDFV ratio and concentric remodeling of the LV was independent of other markers of arterial stiffness and peripheral resistance, such as SBP and DBP.
Table 8. Descriptive data of the PIVUS study participants and the subgroup of 306 subjects who underwent whole body magnetic resonance imaging, WBMRA.

<table>
<thead>
<tr>
<th></th>
<th>PIVUS cohort n = 1016</th>
<th>WBMRA subgroup n = 306</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women (%)</td>
<td>50.2</td>
<td>47.4</td>
</tr>
<tr>
<td>Height (m)</td>
<td>169 (9.1)</td>
<td>169 (9.4)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>77 (14)</td>
<td>77 (14)</td>
</tr>
<tr>
<td>Current smoker (%)</td>
<td>11</td>
<td>7.8</td>
</tr>
<tr>
<td>Antihypertensive medication (%)</td>
<td>32</td>
<td>33</td>
</tr>
<tr>
<td>Statin use (%)</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>Myocardial infarction (%)</td>
<td>7.1</td>
<td>6.9</td>
</tr>
<tr>
<td>Stroke (%)</td>
<td>3.7</td>
<td>3.9</td>
</tr>
<tr>
<td>Angina Pectoris (%)</td>
<td>8.1</td>
<td>7.3</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>150 (23)</td>
<td>149 (22)</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>79 (10)</td>
<td>78 (10)</td>
</tr>
<tr>
<td>Heart rate (beats per minute)</td>
<td>62 (8.7)</td>
<td>61 (8.7)</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>27.0 (4.3)</td>
<td>26.9 (4.1)</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol/l)</td>
<td>5.3 (1.6)</td>
<td>5.3 (1.6)</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.5 (0.42)</td>
<td>1.48 (0.38)</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>3.3 (0.88)</td>
<td>3.3 (0.84)</td>
</tr>
<tr>
<td>Serum Triglycerides (mmol/l)</td>
<td>1.3 (0.60)</td>
<td>1.3 (0.63)</td>
</tr>
<tr>
<td>Serum Cholesterol (mmol/l)</td>
<td>5.4 (1.0)</td>
<td>5.4 (1.0)</td>
</tr>
<tr>
<td>Framingham Risk Score</td>
<td>11.2 (3.3)</td>
<td>11.1 (1.3)</td>
</tr>
<tr>
<td>EDV</td>
<td>446.3</td>
<td>460.6</td>
</tr>
<tr>
<td>EIDV</td>
<td>313.2</td>
<td>318.2</td>
</tr>
<tr>
<td>FMD</td>
<td>5.47</td>
<td>5.13</td>
</tr>
<tr>
<td>CCAD</td>
<td>0.083</td>
<td>0.086</td>
</tr>
<tr>
<td>SV/PP ratio</td>
<td>1.28</td>
<td>1.30</td>
</tr>
<tr>
<td>Blood flow increase (%)</td>
<td>6.00 (1.84)</td>
<td>6.19 (1.81)</td>
</tr>
<tr>
<td>TAS</td>
<td>Only in WBMRA subgroup</td>
<td>0.28 (0.31)</td>
</tr>
<tr>
<td>Systolic mean blood flow velocity (m/s)</td>
<td>1.16 (0.25)</td>
<td>1.18 (0.25)</td>
</tr>
<tr>
<td>Diastolic mean blood flow velocity (m/s)</td>
<td>0.56 (0.15)</td>
<td>0.59 (0.16)</td>
</tr>
<tr>
<td>SDFV ratio</td>
<td>2.16 (0.45)</td>
<td>2.06 (0.39)</td>
</tr>
</tbody>
</table>

Means (SD) or proportions (%) are given. SBP = systolic blood pressure, DBP = diastolic blood pressure, BMI = body mass index, HDL = high density lipoprotein, LDL = low density lipoprotein, EDV = endothelium dependent vasodilation, EIDV = endothelium-independent vasodilation, FMD = flow mediated dilation, CCAD = common carotid artery distensibility, SV/PP ratio = stroke volume to pulse pressure ratio, TAS = total atherosclerotic score, SDFV ratio = systolic to diastolic blood flow velocity ratio during reactive hyperemia of the Brachial artery.

Results

EDV was not significantly related to the SDFV ratio, but to the hyperaemic blood flow increase (p=0.002) in a positive way. EIDV had a positive
relation to both the SDFV ratio and to the hyperaemic blood flow increase (p=0.004 for both), while both the systolic and the diastolic blood flow velocities were inversely correlated with EIDV.

FMD inversely correlated to the SDFV ratio (p=0.004), but both the systolic and diastolic components were correlated to FMD in a positive way. Both Carotid artery distensibility (p=0.0003) and the SV/PP ratio (p=0.0001) were inversely correlated to the SDFV ratio. Similarly, for the systolic blood flow velocity vs. the SV/PP ratio (p=0.006) and borderline significance vs. Carotid artery distensibility (p=0.059). The diastolic blood flow velocity was on the other hand positively related to the SV/PP ratio (p=0.009), but not significantly in relation to Carotid artery distensibility.
Table 9. Blood flow parameters during reactive hyperemia of the Brachial artery in relation to established markers of endothelial function, vascular structure and arterial stiffness.

<table>
<thead>
<tr>
<th></th>
<th>SV</th>
<th>DV</th>
<th>SDFV ratio</th>
<th>BFI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RC (P(a))</td>
<td>RC (P(a))</td>
<td>RC (P(a))</td>
<td>RC (P(a))</td>
</tr>
<tr>
<td>EDV</td>
<td>-0.019 (0.20)</td>
<td>-0.017 (0.069)</td>
<td>0.04 (0.13)</td>
<td>0.34 (0.002)</td>
</tr>
<tr>
<td>EIDV</td>
<td>-0.053 (0.0002)</td>
<td>-0.043 (&lt;0.0001)</td>
<td>0.07 (0.004)</td>
<td>0.30 (0.004)</td>
</tr>
<tr>
<td>FMD</td>
<td>0.009 (&lt;0.0001)</td>
<td>0.008 (&lt;0.0001)</td>
<td>-0.01 (0.004)</td>
<td>-0.001 (0.96)</td>
</tr>
<tr>
<td>CCAD</td>
<td>-0.032 (0.06)</td>
<td>0.011 (0.33)</td>
<td>-0.11 (0.0003)</td>
<td>0.24 (0.052)</td>
</tr>
<tr>
<td>SV/PP ratio</td>
<td>-0.09 (0.0006)</td>
<td>0.044 (0.009)</td>
<td>-0.36 (&lt;0.0001)</td>
<td>0.21 (0.28)</td>
</tr>
<tr>
<td>TPRI</td>
<td>1.01x10 -5(0.29)</td>
<td>-1.18x10 -6(0.85)</td>
<td>1.77x10 -5(0.31)</td>
<td>2.38 x10 -4(0.0008)</td>
</tr>
</tbody>
</table>

Linear regression coefficients are given with p-values in parenthesis. SV = Mean blood flow velocity during systole, DV = Blood flow velocity during diastole, SDFV ratio = the systolic to diastolic blood flow velocity ratio. BFI = Blood flow increase. All measured during reactive hyperemia of the Brachial artery. RC = regression coefficient, P(a)= p-value adjusted for gender. EDV = endothelium dependent vasodilation, EIDV = endothelium-independent vasodilation, FMD = flow mediated dilation, CCAD = Common Carotid artery distensibility, SV/PP ratio = stroke volume to pulse pressure ratio, TPRI = total peripheral resistance index. EDV, EIDV, CCA compliance and SV/PP ratio were log transformed due to a non-normal distribution.

Neither the SDFV ratio, nor its components were related to the TPRI. However, the hyperaemic blood flow increase was positively related to TPRI (p=0.0008).

The SDFV ratio (p= 0.015) and the blood flow increase (p=0.02) were both related to TAS in a positive way when adjusted for gender. No interactions between these two blood flow variables and gender were seen regarding TAS (p>0.50 for the interaction terms).

The relationship was fairly linear since addition of squared terms to the models were not significant (p>0.50 for both squared terms).

After adjustment for cardiovascular risk factors, summarized as the Framingham Risk Score, the SDFV ratio (p=0.057) and the BFI (p = 0.078) both lost in significance in relation to TAS.

The systolic- and diastolic- blood flow velocities were not related to TAS when evaluated separately.
Table 10. *Total atherosclerosis score (TAS) in relation to blood flow parameters of the Brachial artery during reactive hyperemia*

<table>
<thead>
<tr>
<th></th>
<th>Relation to TAS after adjustment for</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gender</td>
<td>Framingham Risk Score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>RC (a)</td>
<td>P (b)</td>
<td>RC (a)</td>
<td>P (b)</td>
<td></td>
</tr>
<tr>
<td>SV (m/s)</td>
<td>0.31</td>
<td>0.77</td>
<td>-0.04</td>
<td>0.71</td>
<td></td>
</tr>
<tr>
<td>DV (m/s)</td>
<td>-0.22</td>
<td>0.17</td>
<td>-0.25</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>SDFV ratio</td>
<td>0.16</td>
<td>0.015</td>
<td>0.12</td>
<td>0.057</td>
<td></td>
</tr>
<tr>
<td>BFI (%)</td>
<td>-0.034</td>
<td>0.02</td>
<td>-0.025</td>
<td>0.078</td>
<td></td>
</tr>
</tbody>
</table>

SV = systolic mean blood flow velocity, DV = diastolic mean blood flow velocity, SDFV ratio = systolic to diastolic mean blood flow velocity, BFI = blood flow increase. RC = regression coefficient, P (a) = p-value adjusted for gender, P (b) = p-value adjusted for Framingham Risk Score.

**Discussion**

The Brachial artery mean systolic to diastolic blood flow velocity (SDFV) ratio was related to established markers of vasodilation and vascular stiffness as well as to a global measure of atherosclerosis in the elderly.

We have suggested that the systolic component of the SDFV ratio is mainly a marker of arterial stiffness, while the diastolic component is more of a measure of the cross-sectional area of the resistance vessels and their ability to dilate.

By creating this ratio, we suggested that we would create and index more powerful than its components, since information on both large artery and resistance artery function are incorporated within the index.

When relating the SDFV ratio to established markers of vasodilation and arterial compliance, it appears that our suggestion that the SDFV ratio contains information on both vasodilation and arterial stiffness may be valid, but that the relationships to its components are more complex than we previously hypothesized.

The systolic and diastolic parts of the ratio are both related to indices of vasodilation and arterial compliance. Furthermore, both the systolic and diastolic parts of the ratio are related to FMD in a positive way, but to EIDV in a negative way.
Both the systolic and diastolic parts of the ratio are related to arterial compliance. However, in the case of the SV/PP-ratio, these relationships are different for the systolic and diastolic blood flow velocities.

The expected negative relationship between the SV/PP-ratio (and Carotid artery distensibility) and the systolic blood flow velocity was found. For the diastolic blood flow velocity, the relationship with the SV/PP-ratio was the opposite, suggesting that a good arterial compliance is necessary for a high diastolic blood flow velocity.

We have previously shown that the measures of arterial compliance in the PIVUS study are not related to the indices of endothelium dependent vasodilation, except for a relationship between FMD and arterial compliance [74]. The fact that both the systolic and diastolic parts of the ratio are related to FMD in a positive way, but to EIDV in a negative way could be true, although the negative relation between EIDV and both the systolic and diastolic blood flow velocities were unexpected and we found it hard to explain the physiology behind these findings.

It is well understood that the blood flow increase induced by hyperemia could be related to both EDV and EIDV, since the hyperaemic blood flow is dependent on vasodilation in forearm resistance vessels. It is also related to a more static measure of vascularisation, the total peripheral resistance index.

Our results are limited to caucasians aged 70. Inference to other age groups or ethnicities should be made cautiously. Low spatial resolution of the WBMRA made assessment of the smaller arteries suboptimal. Due to motion artefacts, the coronary arteries could not be visualised.

In the elderly caucasians of the PIVUS study, the SDFV ratio was related to established markers of both vasodilation and arterial compliance and to global atherosclerosis. Future studies are warranted to elucidate its clinical usefulness for identification of subclinical vascular disease and prediction of subsequent cardiovascular events.
General discussion/ Summary

How can we reduce the global burden of CVD?
Thirty percent, or 20 million of annual global deaths are caused by CVD, and CV morbidity is costly to individuals and societies. Alarmingly, the incidence is increasing in spite of the increased knowledge on, and better methods to diagnose and treat the various clinical conditions associated with CVD such as stroke and myocardial infarction.

In the elderly, better methods are needed to assess the vascular function in order to tailor prevention and treatment of their aging vascular system. In this study based on data from the PIVUS-study participants, aged 70, we aimed to explore the value of blood flow velocity in the forearm in assessing cardiovascular function.

Primarily, ultrasonographic Doppler registration of the systolic blood flow velocity in the Brachial artery during reactive hyperemia was found to be positively related to the Framingham Risk Score. The diastolic counterpart showed an opposite relation to the FRS, as previously determined by other investigators.

*The ratio of the systolic to diastolic blood flow velocity (SDFV ratio) during hyperemia of the Brachial artery was found to be closer related to FRS than its separate components.*

Containing information on both arterial stiffening and dysfunction of the small, resistance vessels, the SDFV ratio could be a valuable marker of vascular function, of additional value to the systolic or diastolic velocities when assessed separately.

Secondly, the SDFV ratio was investigated in relation to markers of Carotid atherosclerosis. The number of plaque, their size and echogenicity as well as IMT and IM-GSM, were found to be more closely related to the SDFV ratio than to its components separately.

In our third paper, the SDFV ratio and its nominator and denominator were assessed in relation to remodeling of the left ventricle. Significant correlations were found with concentric remodeling of the LV and the SDFV ratio, but not to its components alone.
Finally, in our fourth paper, we evaluated the SDFV ratio in relation to the Total Atherosclerosis Score by whole body magnetic resonance imaging of 306 PIVUS participants as well as to established markers of endothelial function in forearm resistance vessels, flow mediated vasodilation and arterial stiffness.

The SDFV ratio was positively related to EIDV while inverse relations to FMD, common Carotid artery compliance and the stroke volume to pulse pressure ratio were found. EDV and the total peripheral resistance were not significantly related to TAS.

The SDFV ratio and the blood flow increase from rest to hyperemia were both significantly related to TAS after gender adjustment. When adjusted for the FRS, both the SDFV ratio (p=0.057) and the blood flow increase (p=0.078) lost somewhat in significance.

In affluent societies, changes in lifestyle in response to technical advancement is not only for the better. Increasing numbers of young individuals suffer the consequences of physical inactivity and often unlimited access to provisions. The “metabolic syndrome”, denoting a cluster of risk factors for CVD such as overweight, diabetes and hyperlipidemia, is becoming more frequent.

In aging, arterial stiffness is seen, mainly affecting the systolic blood flow velocity assessed by rapid, noninvasive ultrasonographic Doppler of the Brachial artery. The diastolic blood flow velocity, evaluated likewise is associated to markers of dysfunctional resistance vessels, associated mainly with elevated blood glucose and hyperlipidemia.

The ratio of the systolic to diastolic blood flow velocity, SDFV ratio, contains information on both arterial stiffness and rigidity of the resistance vessels and is more closely associated to markers of cardiovascular risk and function than its components separately.

The SDFV ratio has, in the PIVUS study in 1016 seventy-year olds of caucasian origin, proven of further value than its components alone and therefore could be a promising marker of cardiovascular function, related to CV risk as the FRS. Hopefully, further studies will elucidate whether the SDFV ratio, in this cohort is indicative of cardiovascular end-points and if the ratio is valuable in settings with other ethnicities or age-groups.
Globalt ökar levnadsstandarden. Världens vanligaste dödsorsak är hjärt - kärl - sjukdom, som står för 30 % av världens frånfall. Trots att vi idag har större kännedom om och bättre möjligheter att behandla dessa sjukdomstillstånd ökar frekvensen. Äldrande befolkningar parallellt med ändrad livsstil hos yngre är möjliga förklaringar. Minskad fysisk aktivitet i alla åldrar och överkonsumtion av födoämnen har lett till en epidemisk utveckling av det metabola syndromet, en sammanfattningsbenämning på ett flertal sjukdomstillstånd som tillsammans sångubblar risken att insjukna i någon hjärt - kärl - katastrof.


Resultaten som visas i denna avhandling är baserade på undersökningar som gjorts i PIVUS-studien (The Prospective Investigation of the Vasculature in Uppsala Seniors). Hälften av de kallade från Uppsala län deltog; 1016 sjuttioåringar, varav hälften män.

Målet med denna avhandling var att utforska värdet av att i ”reaktiv flödesökning” med hjälp av ultraljudsledd Doppler utvärdera blodets flödeshastighet genom underarmens pulssåder som en markör för kardiovaskulär funktion. Reaktiv flödesökning (hyperemi) åstadkoms genom fem minuters yttre kompression av underarmen. Distalt om en uppbåst blodtrycksmanschett skapas ett tillstånd av syrebrist i underarmen. När manschetten töms återfylls Brachialisartären. Man kan då se en uttalad ökning av blodflödet (blood flow increase, BFI) till den ischemiska vävnaden.

Tidigare studier har visat att kärlet uppströms om manschetten vidgar sig när det yttre trycket släpps, (flow mediated dilation, FMD) för att öka blodflödet till vävnaden som lidit syrebrist. Detta fenomen kallas flödesmedierad dilatation och beror på att kärlets innersta cellager, endotelet, frigör kväveoxid (NO) som har en avslappnande effekt på kärlväggens glatta muskelceller. Stimulit för denna NO frisättning tros vara graden av friktion blodet utövar på endotelet när kompressionen släpps. Blodets flödeshastighet längs kärlet under reaktiv hyperemi är en av flera faktorer som påverkar friktionsgraden.


I det påföljande arbetet fann vi att SDFV kvoten var i högre grad relaterad till riskmarkörer för sjukdom i halspulsådern (Arteria Carotis) som används kliniskt för att förutse risken att insjukna i slaganfall (stroke) än de i kvoten ingående hastigheterna separat.

Tredje delarbetet visade att SDFV kvoten också var kopplad till förtjockad vänsterkammarvägg på hjärtat, ett tillstånd som uppstår till följd av ökat pumpmotstånd. De i kvoten ingående systoliska respektive diastoliska flödeskastigheterna gav i detta fall inga påvisbara samband.

I det fjärde delarbetet studerade vi SDFV kvotens relation till både ett flertal etablerade markörer för kärlfunktion samt aterosklerosutbredning visualiserad genom helkropps magnetkamera angiografi som genomförts på 306 av de i PIVUS studien ingående individerna. Även här sågs en högre sambandsgrad till SDFV kvoten än till de respektive ingående parametrarna.

I samtliga delarbeten jämfördes också SDFV kvoten med etablerade metoder för kärlfunktion såsom BFI och FMD och funnit att SDFV kvoten har en högre signifikans och bättre reproducierbarhet.

Sammanfattningsvis har vi funnit att kvoten mellan den systoliska och diastoliska flödeskastigheterna i underarmsartären under reaktiv hyperemi, SDFV kvoten, är en lovande markör för den kardiovaskulära funktionen. SDFV kvoten väger samman funktionsmått av kärlstelhet i både större och mindre kärl och verkar därför vara en bättre indikator på den sammanlagda kärlfunktionen och risken att insjukna i kardiovaskulär sjukdom.

Mätningen har flera fördelar; den tar en kvart i anspråk, och är billig i jämförelse med andra undersökningar med liknande syfte, den är icke invasiv och därmed har den låg komplikationsrisk.
Framtida studier får visa huruvida det med hjälp av SDFV kvoten går att förutsäga risken för kardiovaskulär död och om våra fynd kan överföras även på människor av annan ålder och härkomst.
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"..vi är alla löv på samma träd   "

T.DiLeva
Fam. Fabian; Christina, Peter, Katarina, Helena & Richard with families
and Fam. Öhman; Elsa and Harry, Magnus & Sara with families, for the
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Evvi, Birgit and Annika especially, for support in many ways!

“...and for the times when we're apart
well, then close your eyes and know
these words are comin’ from my heart
and then if you can remember...”

Bacharach, Sager, Warwick

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“I see a rainbow blending now! We’ll have a happy ending
now- taking a chance on love!”

Latouche, Fetter, Duke

Thank You!!
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