Assessment of Atherosclerosis by Whole-body Magnetic Resonance Angiography

Tomas Hansen
Dissertation presented at Uppsala University to be publicly examined in Grönwallsalen, Akademiska sjukhuset, Ing. 70, b.v., Uppsala, Friday, April 27, 2007 at 09:15 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in Swedish.

Abstract

Atherosclerosis is a serious threat to public health and a major cause of morbidity and mortality. In this doctoral research, the feasibility of using whole-body magnetic resonance angiography (WBMRA) was studied as a principal aim both in patients and in an epidemiological setting. Secondary aims were to create a score for assessment of the degree of atherosclerosis with the use of WBMRA and to investigate the correlation between this score and various cardiovascular (CV) risk factors.

WBMRA was found feasible both in atherosclerotic patients and in an elderly population from the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS). All subjects except one completed the examination without any adverse events. A large proportion (93-99%) of the vessel segments could be evaluated and the results of a smaller comparison between WBMRA and conventional invasive x-ray angiography were reasonable regarding the assessed degree of maximum stenosis or occlusion. This indicates the safety and robustness of the WBMRA method.

Unsuspected significant vascular abnormalities were found in patients with atherosclerotic symptoms and significant vascular abnormalities were present in elderly subjects without any self-reported vascular disease. The prevalence rates of vascular abnormalities in the carotid, renal, and inflow and runoff arteries of the lower limbs were estimated in an elderly population. A total atherosclerotic score (TAS) reflecting the degree of luminal narrowing was created for the WBMRA method and was significantly related to Framingham risk score (FRS) and to the amount of abdominal visceral adipose tissue, interleukin-6, and leptin and was inversely significantly related to adiponectin.

Studies with outcome data of the PIVUS cohort are needed for further validation of the WBMRA method and to determine whether TAS can be used as an adjunct for CV risk assessment. Meanwhile, the correlation with FRS indicates that TAS could be of value for this purpose.

Keywords: Atherosclerosis, Magnetic Resonance Angiography, Epidemiology

Tomas Hansen, Department of Oncology, Radiology and Clinical Immunology, Akademiska sjukhuset, Uppsala University, SE-75185 Uppsala, Sweden

© Tomas Hansen 2007


urn:nbn:se:uu:diva-7778 (http://urn.kb.se/resolve?urn=nbn:se:uu:diva-7778)
Mät aldrig bergets höjd förrän du nått toppen. 
Då skall du se hur lågt det var.

Dag Hammarskjöld

Endast den hand som kan sudda ut kan skriva det rätta.

Tage Danielsson

Utan tvivel är man inte klok.

Tage Danielsson

To Marianne,

Victor and Cornelia
On the cover:

A maximum intensity projection acquired from a whole-body magnetic resonance angiography projected onto the “Vitruvian man”, which was drawn by Leonardo da Vinci around the year 1492.
List of Original Papers

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

Whole-Body MRA using a Standard Clinical Scanner in Patients.
*Eur Radiol.* Jan 2006;16(1):147-153

II. Hansen T, Wikström J, Johansson L, Lind L, Ahlström H.:

III. Hansen T, Ahlström H, Wikström J, Lind L, Johansson L.:
A Total Atherosclerotic Score for Whole-body MRA and its Relation to Traditional Cardiovascular Risk Factors.
Submitted

Visceral Adipose Tissue, Inflammation and Adiponectin are Related to Atherosclerosis Assessed by Whole-body Magnetic Resonance Angiography in an Elderly Population.
*In manuscript*
Contents

List of Original Papers ............................................................................................................................... 5
Contents ..................................................................................................................................................... 7
Abbreviations ............................................................................................................................................. 9
Introduction ..............................................................................................................................................11
Atherosclerosis ........................................................................................................................................11
The formation of an atherosclerotic plaque and its complications ....................................................11
Traditional cardiovascular risk factors ............................................................................................ 13
Non-traditional cardiovascular risk factors .................................................................................... ..14
Obesity and inflammation ........................................................................................................ ........14
Scoring systems ................................................................................................................................1 5
Imaging atherosclerosis........................................................................................................ ................ 15
Imaging techniques............................................................................................................. ............. 15
Luminography ..................................................................................................................................17
Magnetic resonance ..........................................................................................................................17
Magnetic resonance angiography (MRA)........................................................................................ 18
Whole-body MRA........................................................................................................................... 21
Study aims ........................................................................................................................................... 23
Principal aim of this investigation............................................................................................ ............ 23
Secondary aims................................................................................................................. ............... 23
Specific aims of individual studies ............................................................................................. .......... 23
Study I ............................................................................................................................................. 23
Study II ............................................................................................................................................ 23
Study III........................................................................................................................................... 23
Study IV........................................................................................................................................... 23
Methods ................................................................................................................................................... 24
Patients................................................................................................................................................. 24
Subjects (PIVUS) ................................................................................................................................. 24
Baseline investigation ......................................................................................................... ............. 24
Laboratory....................................................................................................................................... 24
Digital subtraction angiography .......................................................................................................... 26
Whole-body MRA ............................................................................................................................... 27
Image reconstruction ....................................................................................................................... 27
Evaluation........................................................................................................................................ 27
Atherosclerotic score......................................................................................................................... 29
Segmentation of adipose tissue ......................................................................................................... 29
Statistical methods ......................................................................................................................... 29
Abbreviations

2D Two-dimensional
3D Three-dimensional
ABI Ankle-brachial index
AF Acceleration factor
AS Atherosclerotic score
ApoA-1 Apolipoprotein A-1
ApoB Apolipoprotein B
B-FFE Balanced-fast field echo
BMI Body mass index
CACS Coronary artery calcium scoring
CAD Coronary artery disease
CE Contrast-enhanced
CHD Coronary heart disease
CRP C-reactive protein
CT Computed tomography
CTA Computed tomography angiography
CV Cardiovascular
CVD Cardiovascular disease
CVE Cardiovascular event
DBP Diastolic blood pressure
DSA Digital subtraction angiography
FRS Framingham risk score
FOV Field of view
Gd Gadolinium
HbA1c Haemoglobin A1c
HDL High-density lipoprotein
ICAM Intercellular adhesion molecule
IDF International Diabetes Federation
IEL Internal elastic laminae
IFN-γ Interferon-gamma
IL Interleukin
IMT Intima-media thickness
IVUS Intravascular ultrasound
LDL Low-density lipoprotein
M Magnetisation
M-CSF Macrophage colony-stimulating factor
MCP-1 Monocyte chemotactic protein-1
MDCT Multi-detector computed tomography
MI Myocardial infarction
MIP Maximum intensity projection
MMP Matrix metalloproteinase
MPR Multi-planar reformation
MR Magnetic resonance
MRI Magnetic resonance imaging
MRA Magnetic resonance angiography
PAD Peripheral artery disease
PAI-1 Plasminogen activator inhibitor-1
PIVUS Prospective Investigation of the Vasculature in Uppsala Seniors
PSA Prostate Investigation of the Vasculature in Uppsala Seniors
PVD Peripheral vascular disease
rc Regression coefficient
RF Radio frequency
RFOV Rectangular field of view
ROI Region of interest
SAT Subcutaneous adipose tissue
SBP Systolic blood pressure
SCORE Systemic coronary risk evaluation
SHAPE Screening for Heart Attack Prevention and Education
SMC Smooth muscle cell
SNR Signal-to-noise ratio
T Tesla
TAS Total atherosclerotic score
TE Time to echo
TF Tissue factor
TNF-α Tumour necrosis factor-α
tPA Tissue plasminogen activator
TIA Transitory ischaemic attack
TR Time to repeat
US Ultrasonography
VAT Visceral adipose tissue
VCAM Vascular cell adhesion molecule
VR Volume rendering
vWF von Willenbrandt’s factor
w Weighted
WBMRA Whole-body magnetic resonance angiography
WHR Waist-to-hip ratio
Introduction

This thesis concerns the feasibility of performing whole-body magnetic resonance angiography (WBMRAs) and the application of this method in a clinical setting and in an epidemiological study. A scoring method for use in WBMRAs to assess the degree of atherosclerosis was introduced and related to various factors associated with this disease.

Atherosclerosis

-What is atherosclerosis?

Atherosclerosis is a slowly progressive disease of the arteries which may have acute complications in the forms of myocardial infarction (MI), angina pectoris, stroke and intermittent claudication. Atherosclerosis is a major contributor to death and disability worldwide. The sedentary lifestyle prevalent in western societies, with low physical activity, smoking and poor dietary habits is a major cause of large and escalated socio-economic costs.

Atherosclerotic plaques consist of cells, connective tissue elements, lipids, calcification and debris. The plaques contain different relative amounts of these components, as a result of which different plaques along the plaque spectrum vary in their vulnerability to rupture. Atherosclerosis has a complex pathogenesis. Pathogenetic factors such as dyslipidaemia, hypertension, obesity, diabetes mellitus, dyscoagulation, inflammation and numerous other mechanisms are interlinked in their actions on one another and it takes several decades for symptoms of atherosclerosis to become manifest. With these complex relationships, atherosclerosis is a complicated disorder to investigate scientifically.

Healthcare professionals are faced with the challenge of identifying those individuals who are most likely to suffer from cardiovascular events (CVE) and are likely to benefit most from treatment, and also of giving primary protective advice to the population with the aim of reducing the incidence and prevalence of cardiovascular disease (CVD).

The formation of an atherosclerotic plaque and its complications

-What is a plaque and how does it develop?

The blood vessel wall has a trilaminar structure. Viewed from the inside of the vessel, the first layer is the intima, lined with the endothelial cells that face the bloodstream, follow by the tunica media, consisting normally of smooth muscle cells and matrix. On the outside lies the tunica adventitia with loose connective tissue and containing the fibroblasts. Situated between these layers are the external and internal elastic laminae (IEL).

In the past, atherosclerosis was sometimes believed to consist only of lipid and calcified deposits in the vessels. It seemed natural to depict the vessel lumen in order to assess the degree of stenosis and thereby the obstruction to blood flows. Mechanical intervention, sometimes regarded as “plumbing”, of these “rigid pipes” was the logical choice of treatment and little attention was paid to the vessel wall and other inflammatory components. Nowadays, the composition of the plaque situated in the vessel wall seems more interesting for imaging, and stabilisation of plaques is considered today of utmost importance for prevention of CVE.

The development of atherosclerosis is slow and in childhood it starts as a “fatty streak” containing lipid-filled foam cells, macrophages, and smooth muscle cells. These can progress into “raised fatty streaks” with accumulation of extracellular lipids. In a autopsy study the prevalence of raised fatty streaks in the abdominal aorta was 20% in the 15-19-year age group and increased to 40% in the group aged 30 to 34.

The prevalence of these complicated plaques in the coronary arteries is 2% in the 15-19-year age group and 20% in the group aged 30-34 years. Women show a lag of 5 to 10 years compared with men regarding plaque progression. At ages of about 40 to 50, the plaques can either be large enough to cause an impairment of blood flow, due to a haemodynamically significant stenosis, or rupture, an event which can initiate a thrombus. Both occurrences can cause ischaemic symptoms from the organ supplied by the involved vessel.
The atherogenesis depends both on rheological factors and local factors in the vessel wall and its development is a highly dynamic process. The initial step is considered to be endothelial dysfunction, characterized by reduced nitrous oxide released from the endothelial cells and impaired vasodilatation. The dysfunction might be induced by flow disturbances such as oscillating or low shear stress, flow separation, and flow reversal near vessel branches and bending points. One predilection site for eccentric plaque formation is in the far side of the bulb of the internal carotid artery, which is subject to low and oscillating shear stress.

Exogenous lipids are transported from the intestines to the liver as chylomicrons. Endogenous lipids are transported in the bloodstream, attached to various lipoproteins, and a major part of the cholesterol delivered to peripheral tissues from the liver is carried as low-density lipoprotein (LDL) attached to apolipoprotein B (ApoB) -100. Cholesterol is transported in the reverse direction from the peripheral tissues as high-density lipoprotein (HDL) attached to apolipoprotein A (ApoA). There is a continuous influx and efflux of lipoprotein to and from the vessel wall. An inflammatory response in the intima is initiated by ApoB and oxidative modification of LDL, oxidation which can be caused by several factors. Also, smoking, hypertension, and glycation end-products induced by diabetes will recruit lymphocytes and monocytes to sites of inflammation and endothelial dysfunction in the arterial wall. The endothelial cell then becomes activated and endothelial gene expression is induced. This will result in synthesis of adhesion molecules on the surface of the endothelium, which leads to firm adhesion between the bloodborne leucocytes and the endothelium. Examples of these adhesion molecules are selectins, vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1).

Interactions of monocytes, macrophages and T lymphocytes are major determinants in the subsequent progression of atherosclerosis, which begin to resemble a chronic inflammatory state. Monocytes become attracted from the blood stream to the intima of the vessel wall, partly by oxidised LDL and chemotactic factors, such as monocyte chemotactic protein-1 (MCP-1). This continuous recruitment of monocytes is also regulated by growth factors such as macrophage colony-stimulating factor (M-CSF). The growth of the atheroma continues, with transport of LDL cholesterol from the blood into the vessel wall. Monocytes convert into macrophages, which try to protect the vessel wall from proinflammatory, oxidatively modified LDL particles by phagocytosis of the cholesterol through binding of the ApoB to scavenger receptors on the surface of macrophages. The macrophages finally become "foam cells", filled with lipid droplets containing cholesterol. Phagocytosis is primarily regulated by interleukins (IL).

The next step in the progression of an atherosclerotic lesion is the development of a fibroatheroma, also called a fibromuscular plaque. This plaque is characterised by a lipid core and a fibrous cap consisting of smooth muscle cells and collagen. Activated T lymphocytes located in the media layer secrete interferon-γ (IFN-γ), which activates macrophages and recruits smooth muscle cells into the intima. These smooth muscle cells start to produce extracellular matrix proteins, such as collagen, which form into a fibrous plaque. Neovascularisation in the adventitia also has effects on the atherogenesis and plaque stability.

Some plaques become degenerative and accumulate a large extracellular central lipid-filled necrotic core and display extracellular calcium deposition, fewer smooth muscle cells and a thin fibrous cap. Macrophages, T lymphocytes and mast cells become abundant as the inflammatory activity increases. These cells can be activated by autoantigen or microbes and then release cytokines (tumour necrosis factor-alpha (TNF-α) and IFN-γ) and proteolytic enzymes such as the family of matrix metalloproteinases (MMP). MMP degrades the extracellular matrix and INF-γ limits the collagen production from smooth muscle cells, reducing the stability of the plaque. Such complex, degenerative plaques are more prone to rupture or to become superficially eroded than fibromuscular plaques. Apoptosis of smooth muscle cells in the plaque also contributes to plaque vulnerability. A plaque rupture is most likely to occur at the shoulder, where the cap is usually thinnest and has the
most dense content of inflammatory cells. Mechanical forces from the impact of the blood stream are also responsible for some of the plaque ruptures.

In the event of plaque rupture, the thrombogenic lipid-rich core, which includes macrophage-derived tissue factor (TF), apoptotic cell debris and membrane microparticles, will be exposed to the blood stream. This might initiate the coagulation cascade and the formation of a thrombosis. Superficial erosion of the plaque might also be the trigger. The outcome is moderated by the balance of coagulation and fibrinolysis.\textsuperscript{2,15} Coagulation starts with platelet activation, which occurs in three steps: adhesion, secretion, and aggregation. Adhesion starts with exposure of collagen in the vessel wall through endothelial erosion or plaque rupture. Von Willenbrandt’s factor (vWF) is the dominant mediator in this step. Secretion of granules from the platelet initiates aggregation of multiple platelets to one another and to the vessel wall through binding of fibrinogen to receptors on the platelet surface. The coagulation cascade is also initiated by the granules and a fibrin thrombus builds up. In several conditions such as smoking, diabetes and high levels of LDL-cholesterol, a hyperthrombogenic state may be present.\textsuperscript{4} Inflammatory disease (e.g. systemic lupus erythematosus, rheumatoid arthritis), hypercholesterolaemia, diabetes, mental stress and smoking can cause platelet activation.\textsuperscript{16}

Thrombus formation can have two different outcomes. The thrombus may organise, initiating a process of healing, which can increase the degree of luminal narrowing. This is the most common fate of a plaque rupture and is often clinically silent.\textsuperscript{5} The other outcome is that perfusion of the tissues supported by the affected vessel is decreased to such an extent that ischaemic symptoms may occur from the tissues supplied, in the forms of intermittent claudication, a transitory ischemic attack (TIA), angina pectoris, limb gangrene, stroke or MI.

An important marker of the coagulation system is fibrinogen, which is synthesised in the liver. Fibrinogen is both an acute phase protein and a coagulation factor. Another marker is vWF, which is synthesised in the endothelial cells. In the fibrinolytic system, the balance between tissue plasminogen activator (tPA), derived from endothelium, and plasminogen activator inhibitor 1 (PAI-1), which is derived from both endothelium and adipose tissue, is of importance.

In study II (paper II) of the present investigation the distribution and severity of vascular stenoses, occlusions and aneurysms were studied in an elderly population. In study III (paper III) a total atherosclerotic score (TAS) was created, and the relation between this score and cardiovascular (CV) risk factors was examined in study IV (paper IV).

**Traditional cardiovascular risk factors**

- *What contributes to progression of atherosclerosis?*

In a review of 122 000 patients with coronary heart disease (CHD), four risk factors (smoking, diabetes, hyperlipidaemia and hypertension) were found to be present in 80-85% of the cases.\textsuperscript{17} These are considered as the major traditional CV risk factors. In the INTERHEART study with 15 000 cases of MI included from 52 countries, it was concluded that nine risk factors accounted for 90% of the CVE (Table 1).\textsuperscript{18}

<table>
<thead>
<tr>
<th>Traditional CV risk factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking</td>
</tr>
<tr>
<td>Dyslipidaemia</td>
</tr>
<tr>
<td>Hypertension</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>Obesity</td>
</tr>
<tr>
<td>Stress</td>
</tr>
<tr>
<td>Low physical activity</td>
</tr>
<tr>
<td>Low dietary vegetables</td>
</tr>
<tr>
<td>Alcohol</td>
</tr>
</tbody>
</table>
Non-traditional cardiovascular risk factors

*What more is to be found?*

The traditional risk factors for CVD cannot identify all individuals who will suffer from a future CVE. The possible percentage number of such individuals, among those without such risk factors, has been disputed and some scientists have considered it to be as high as 50%, creating a “50% myth”\(^ {15, 19-21}\). Others have concluded that the traditional CV risk factors can explain 75-100% of the CVE\(^ {17}\). However, the unexplained events have been a major driving force for the ongoing pursuit of new CV risk factors. There is a search for additional markers or methods with predictive value over and above that of scoring systems based on traditional CV risk factors. Both imaging and biochemical markers could be included and should be widely accessible, inexpensive, non-invasive, and have a high sensitivity, specificity and reproducibility\(^ {9, 22}\). A variety of biochemical markers for inflammation (C-reactive protein (CRP), IL-6, TNF-\(\alpha\)), coagulation (fibrinogen), fibrinolysis (PAI-1, homocysteine) blood lipids (ApoB / ApoA-1 ratio) and obesity (leptin, adiponectin), as well as such measures as obesity distribution (body mass index (BMI), visceral adipose tissue (VAT), subcutaneous adipose tissue (SAT), waist-to-hip ratio (WHR) and waist circumference) are under evaluation for these purposes in different studies. The ultimate goal is to be able to further predict the risk of each individual for CVE in order to limit treatment to high-risk individuals\(^ {22}\).

**Obesity and inflammation**

The hypothesis was recently proposed that adipose tissue produces proinflammatory cytokines which activate the innate inflammatory system\(^ {16}\). Obesity causes a local invasion of macrophages in the adipose tissue. For example, IL-6 is produced in adipocytes and causes elevation of the circulating levels of acute phase proteins, such as CRP, by increasing their synthesis in the liver. Circulating inflammatory markers may derive from multiple sites such as the liver, adipose tissue and other inflammatory tissue. This makes it difficult to measure each tissue-specific contribution. As in the case of adipocyte-produced IL-6, the amount of CRP produced in the adipose tissue is unknown\(^ {16}\).

The distribution of adipose tissue seems to be more important for the development of CV diseases caused by atherosclerosis than the amount of fat, and the WHR predicts more CV events than does BMI\(^ {18}\). Adipose tissue can also be assessed by magnetic resonance imaging (MRI) and computed tomography (CT) and segmented into abdominal SAT and VAT. VAT has been shown to be an independent risk factor for future MI in elderly women\(^ {21}\) and predictive for onset of coronary artery disease (CAD) in middle-aged men\(^ {24}\), and for all-cause mortality in men\(^ {25}\). The VAT is more inflammatorily active than SAT\(^ {26}\).

Although associated, the exact mediators between increased fat mass in general and VAT in particular, and atherosclerosis are yet to be determined. Increased levels of traditional CV risk factors may partly explain the increased risk for CVD, but an obesity-associated inflammatory status and elevated levels of adipocyte-derived hormones or adipokines may be contributory. Obesity is often clustered with other CV risk factors, e.g. hypertension, dyslipidaemia, insulin resistance and diabetes\(^ {27-29}\), and the recent International Diabetes Federation (IDF) definition of the metabolic syndrome states that abdominal obesity is a prerequisite for the diagnosis (www.idf.org/webdata/docs/Metac Syndrome_def.pdf).\(^ {30}\) A waist circumference cut-off level of over 94 cm in men and over 80 cm in women is considered as central obesity. These levels are valid for white Europeans and different cut-off levels are used for other ethnic groups. Furthermore, increased VAT causes a low-grade inflammation due to infiltration of macrophages in the adipose tissue\(^ {31}\), which may aggravate the atherosclerotic process. Moreover, the adipose tissue is now recognised as an important endocrine organ, and several hormones, adipokines and other vasoactive factors are produced by the adipocyte or by the infiltrating macrophages.

Adiponectin possesses antiatherosclerotic, antidiabetic, and antiinflammatory properties and plasma levels of adiponectin are inversely related to the amount of visceral fat. In previous studies, adiponectin has shown an inverse relationship to carotid artery intima-media thickness (IMT), a marker for subclinical atherosclerosis\(^ {32}\), and also to the presence of CAD\(^ {33}\) or coronary artery cal-
Introduction

cification. It has also been found in some but not all studies that low adiponectin levels predict future CVE.

In contrast, circulating levels of leptin increase with obesity and women have markedly higher levels than men. Leptin has diverse actions related to satiety and metabolism. It has previously been shown that high levels predict development of first-ever MI and stroke, mainly in men. Leptin may promote atherosclerosis through several mechanisms, as recently reviewed by Beltowsky.

In the present research, measures of adipose tissue distribution, adipokines and markers of inflammation obtained in the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) cohort were examined in study IV and related to the total atherosclerotic score assessed by whole-body magnetic resonance angiography.

Scoring systems

How can the CV risk be estimated in an individual?

The traditional CV risk factors form the basis of the Framingham risk score (FRS), which is a well validated scoring system for CV risk assessment. FRS is based on an epidemiological study conducted in a medium-sized city in America with a white middle-class population. The risk score provides an assessment of the likelihood of suffering from cardiovascular morbidity and mortality a ten-year period and comprises age, gender, blood pressure, smoking, diabetes, and HDL- and LDL-cholesterol. The relation between FRS and TAS as assessed by WBMRA was analysed in study III.

Another scoring system is the Systemic Coronary Risk Evaluation (SCORE), which is based on a study comprising 200 000 subjects from 12 European countries. SCORE gives the risk for CV mortality, and not morbidity. Among the European countries, Sweden now has the first national version of SCORE, which gives the risk for this specific country (www.heartscore.org). The traditional CV risk factors included in SCORE are: gender, age, smoking, systolic blood pressure (SBP), and cholesterol. Diabetes and known CVD are not included.

Guidelines from the European task force on prevention of cardiovascular disease have turned towards the systemic nature of atherosclerosis from merely referring to coronary heart disease. An apparently healthy individual with a risk of fatal CVD of more than 5% over a 10-year period is at high risk and should require lifestyle intervention and appropriate medication. These guidelines turn to those with the highest CVD risk, as this task force considers that preventive efforts are most effective in this group. It is stated that subclinical organ damage has clinical relevance by reason of the fact that left ventricular hypertrophy, carotid artery plaques and endothelial dysfunction increase the risk for CV morbidity. The guidelines suggest that MRI plaque characterisation, coronary artery calcium scoring (CACS) and IMT could be included in sophisticated risk assessment models.

In an article from the Screening for Heart Attacks Prevention and Education (SHAPE) task force, IMT and CACS have been proposed as further imaging tools in addition to traditional risk estimation such as the FRS. Other tools mentioned are MRI of the aorta and endothelial dysfunction measurements. In a population testing positive for atherosclerosis, less than 10% will suffer from a near-term event. Further risk stratification could then continue with markers for disease activity in the population based solely on the presence of atherosclerosis. It is also suggested that the perspective of the “vulnerable patient” rather than just the vulnerable plaque may contribute to risk estimation. This term includes the vulnerability of the myocardium to arrhythmias and the tendency of the blood to aggregate or dissolve a thrombus. Examples of markers for this vulnerability are left ventricular hypertrophy, fibrinogen, and PAI-1.

Imaging atherosclerosis

Imaging techniques

Why perform assessment of atherosclerosis?

Different imaging techniques have been considered for detection of subclinical pathology as supplements to or improvement of cardiovascular risk assessment, especially in patients with an intermediate cardiovascular risk, or as surrogate endpoints in studies. Questions have been raised, however, concerning the value of such surrogate markers as
replacements for hard clinical endpoints, such as MI.45

Ultrasonography (US) is an example of these techniques, where differences in acoustic impedance are used to create contrast between tissues. Plaques can be evaluated and heterogeneous and echolucent (dark) plaques are associated with lipids, inflammatory cells and haemorrhage in the plaque, whereas homogeneous and echogenic (bright) plaques are mostly fibrous or calcified.13, 46 The intima-media thickness can also be measured with a resolution of approximately 0.4 mm with 7-8 MHz, and is used in several studies as a measure of global atherosclerosis.47-50 By US the area of a plaque in a stenosis can be measured and the degree of stenosis of a vessel lumen can be estimated, and in addition the direction and velocity of the blood flow can be determined. No ionising radiation is involved. With the use of contrast agent, tissue perfusion and enhancement are possible. The method is user dependent, which is a drawback. Also, in some patients the body configuration and degree of co-operation affect the assessment, for instance in renal artery stenosis. The intima and media thickness cannot be measured separately, and the examination can only cover a few sites, commonly the carotid and femoral arteries. In a recent meta-analysis on IMT, six different ways were found of defining the bifurcation and the internal and common carotid artery, making comparisons between studies difficult.51 In a recent study in which the carotid vessel wall was measured with a non-invasive high frequency transducer (resolution 0.07 mm, penetration depth 1.4 cm, 25 MHz), it was possible to separate the intima from the media. When subjects with and without CVD were compared, an interindividual difference in intima thickness and intima-media thickness ratio was found, suggesting a potential value of assessing the vessel layers separately and calculating the ratio between intima and media. When the intima and media layers were added as a surrogate for IMT, no significant differences were found.52

Intravascular US (IVUS) is an invasive method primarily applied in the coronary or carotid arteries and using a 30 MHz transducer. With this method, the area of an atheroma and of a stenosis can be assessed.53 Coronary artery calcium scoring performed with CT or electron beam CT is a method utilising the differences in attenuation of x-ray beams in various tissues. This method only assesses the amount of calcium in the coronary vessels, which is presented as a calcium score such as the Agatston score or as a “volume” score.54 CACS is an estimate of the chronic plaque burden and indirectly reflects the plaque composition in the coronary arteries. However, plaques with large amounts of calcium are considered to be stable and less prone to rupture, and there is an increased likelihood that with increasing calcium scores there will also be an increase in the frequency of vulnerable plaques. The spatial resolution is 0.5 mm, but the temporal resolution is a limitation. At present, the fastest multislice CT scanner requires approximately 300 ms for performing a rotational scan to create a single image and this cannot entirely eliminate motion artefacts of the coronary arteries. It has been reported that in asymptomatic individuals in an intermediate risk category, as judged by FRS, a high CACS could predict more CVE than FRS itself in both men and women.34, 55 A CACS of zero cannot, however, rule out the possibility of a future CVE.56

The ankle brachial index (ABI) is not an imaging technique but is used for assessment of atherosclerosis. With this technique the ratio is calculated between the blood pressures in the ankle arteries and the brachial arteries. An ABI below 0.90 is considered to be indicative of generalised atherosclerosis in the general population.57 In a large study with 6880 unselected patients visiting general practitioners, the prevalence of ABI< 0.90 was 20% in men and 18% in women. The patients with a low ABI also had a higher odds ratio for other manifestations of atherosclerosis such as cerebro- or cardiovascular events.58 A systematic review has indicated that a low ABI, over and above the conventional risk factor profile, may help to identify asymptomatic individuals at increased risk for CVD.57 A recently introduced technique for assessment of atherosclerosis is arterial wall imaging using high resolution MRI. The most common examined arteries include the aorta and carotid and coronary vessels.59 The area of the plaque and stenosis can be measured on transverse images and the plaque
composition can be evaluated on the basis of signal characteristics of fibrous tissue, lipids, haemorrhage, thrombi, and calcification as well as of contrast enhancement.60

Luminography

Various methods are used to depict the lumen of a blood vessel. All modalities that only visualise the lumen underestimate the extent of atherosclerosis, like merely imaging the hole in the doughnut and not the doughnut itself.61 A major part of this underestimation is caused by arterial remodelling.62 Also, an attempt to visualise a complex angiographic silhouette with a two-dimensional (2D) approach has its limitations. It can also be difficult to determine the most severe degree of stenosis in certain cases. When measuring the grade of a stenosis, a comparison is made with a vessel of “normal” appearance, but the wall of this vessel might also be affected by atherosclerosis, underestimating the severity of the disease.

The gold standard for depicting the lumen is considered to be catheter-based invasive angiography. The reason for this is its superior spatial resolution,61 which lies in the range of 1.0 to 2.2 line pairs/mm, depending on the zoom factor using digital equipment (1.0 line pair/mm is approximately equivalent to a resolution of 0.5 mm). In the days when conventional films were used, the resolution was approximately 4 line pairs/mm. It is also possible to perform pressure measurements and therapeutic intervention in the same procedure and also to obtain dynamic information. The drawbacks are the use of ionising radiation and of iodine contrast agents with nephrotoxicity, potential hazards of arterial puncture, and the limitation of 2D information. The use of biplane equipment and rotational angiography can overcome the 2D limitation and create a sense of 3D.

Computed tomography angiography (CTA) is an easily applied method with a possibility of post-processing reconstructions, including multi-planar reformation (MPR) and volume rendering (VR) techniques. The spatial resolution is higher than in magnetic resonance angiography (MRA) and the scan is faster. Drawbacks are the same as with conventional angiography, except for the use of venous instead of arterial puncture. Large calcium deposits in the vessel wall can cause artefacts and obscure the lumen.

Magnetic resonance angiography does not use ionising radiation and the contrast agent administered is less nephrotoxic than iodinated contrast agents. Three-dimensional (3D) imaging can be achieved with the possibilities of post-processing, and repeated scanning is possible by virtue of the fast sequences employed, enabling dynamic information to be obtained. The features of different k-space sampling techniques are a unique advantage of the magnetic resonance (MR) method. The sensitivity and specificity of dedicated MRA as compared with digital subtraction angiography (DSA) are typically well above 90%.63 Drawbacks are the relatively low spatial resolution. Recently, also, serious adverse events have been reported in patients with severe renal impairment with the use of a commonly used gadolinium (Gd) chelate contrast agent.64 Despite consideration of these limitations, MRA is becoming more accepted as a minimally invasive road-mapping method for assessing the vessel lumen for the degree of stenosis, occlusions and aneurysms as well as for evaluating organ morphology and function. The following sections in this part of the thesis will focus mainly on MRA.

Magnetic resonance

-How does magnetic resonance work?

The following explanation of magnetic resonance is highly simplified and is only intended as a brief overview.

An atomic nucleus that consists of an uneven number of protons or neutrons, such as the hydrogen nucleus, possesses an angular momentum and is called “spin”. An unpaired proton has a charge (net magnetic dipole momentum) and spin, which together create a magnetic momentum vector. Nuclei with a magnetic momentum are often referred to simply as “spins”. This is analogous to a ball that spins around its axis. The net magnetization (M) of multiple spins is zero on account of random orientation that equals out the magnetization.

When nuclei are placed under the influence of a strong static magnetic field, for instance inside an MR scanner, the spins in the body will become
Assessment of Atherosclerosis by Whole-body Magnetic Resonance Angiography

aligned in parallel and antiparallel directions along the external magnetic field $B_0$. The spins move, i.e. precess, around the axis of $B_0$ with the Larmor frequency determined by the strength of the external magnetic field. The axis of the balls circulates around the $B_0$ axis. The greater the strength of the magnetic field (=higher Tesla (T)), the higher the frequency. The spins can have two energy levels, a low (spin up, parallel) and a high (spin down, antiparallel). There is a slight majority of low energy spins aligned parallel with $B_0$. This creates a net magnetization $M_0$ aligned parallel with $B_0$. $M_0$ increases in proportion to $B_0$, so the larger the field strength, the higher is $M_0$. The amplitude of this net magnetization $M_0$ is proportional to the signal later received; this is the reason why a stronger magnetic field $B_0$ gives a higher signal.

A radiofrequency (RF) alternating voltage is then applied across a coil inside the scanner and induces a flow of alternating current. Thereafter a magnetic field $B_1$ will be applied in the body inside the MR scanner with the frequency of the alternating voltage. The duration of this RF pulse is commonly in the range of milliseconds. The energy of the emitted photons (package of radiant energy) from the RF pulse can only be absorbed by the spins in the body if they are in resonance, hence the name magnetic resonance. Some spins then change to a higher energy level as a result of the energy transfer; i.e. they become excited. This results in a smaller magnetic vector pointed in the parallel direction in the longitudinal ($M_z$) plane owing to the fact that more spins are now antiparallel than before, because of their higher energy level. The protons also start to precess in phase with one another, and a magnetic vector in the transverse ($M_{xy}$) plane builds up.

After the RF excitation is terminated, two events occur at the same time. The protons lower their energy level to the equilibrial state and the longitudinal magnetisation $M_z$ returns exponentially with the longitudinal relaxation time $T_1$. $T_1$ is defined as the time when 63% of $M_z$ has returned. Energy from the protons can more easily be transferred to surrounding tissue, for example fat and water, if the precession frequency is the same. This is the case with fat (rapid energy transferral = signal increases rapidly = short $T_1$), but for spins situated in water then energy deposit is more difficult (slow energy transferral = signal increases slowly = longer $T_1$).

The protons also get out of phase, with consequent loss of transverse magnetisation $M_{xy}$ with the transverse relaxation time $T_2$. $T_2$ is defined as the time when 37% of $M_{xy}$ remains. The dephasing is due to local magnetic field and external magnetic field inhomogeneity. There is greater local field inhomogeneity in fat than in water, resulting in a short $T_2$ for fat (faster dephasing = faster signal decay = signal remains shorter) and a long $T_2$ for water (slower dephasing = slower signal decay = signal remains longer). Hydrogen nuclei are very abundant in water and fat in the human body. The relaxation time constants $T_1$ and $T_2$ are different for tissues, but are the same for any specific tissue in the body; it is this difference, with the contribution of proton density, that creates the contrast in the MR images. MR is especially superior for creating contrast in soft tissues.

A moving magnetic field will induce an alternating electric voltage in the receiver coil, which in turn will induce an alternating current. This moving magnetic field can only be used for induction in the receiver coil in the $M_{xy}$ plane, and not in the $M_z$ plane. The alternating current is the MR signal received and is later used for creating the images.

The pulse sequence is the order of the RF pulse. The spatial localisation of the examined volume is accomplished with the use of gradients that are applied in a certain order in the pulse sequence. The time for repetition (TR) and time to echo (TE) determine the contrast between tissues, and the sequence is weighted (w) as T1w, T2w or proton density. In a T1w sequence, TR and TE are chosen so that tissues with short $T_1$ values are bright and those with long $T_1$ are dark. The pulse sequence used for angiography in the present studies is a 3D T1w spoiled gradient echo pulse sequence. The TR used was 2.5 ms and the TE 0.94 ms.

Magnetic resonance angiography (MRA)
Magnetic resonance angiography is an easily performed procedure, which is considered safe in view of its lack of both ionising radiation and the potential nephrotoxicity of iodinated contrast agents used in invasive catheter-based x-ray arterial angiography. MRA is used routinely and with expand-


Introduction

ng indications as a diagnostic tool for the evaluation of vascular diseases.\textsuperscript{63, 65}

MRA can be carried out without the use of contrast agents. Such techniques are called time-of-flight (TOF) or phase contrast (PC) angiography. They depend on the flow effects of non-contrast enhanced blood, and the images are based on the movement of blood. On the other hand, contrast enhanced MRA (CE-MRA) relies on Gd chelates, which are used with the purpose of increasing the signal of the blood on T1w images.\textsuperscript{66, 67} The approach with CE-MRA is not so prone to artefacts, on account of the flow independence and the fact that in-plane saturation effects are avoided. The use of Gd also allows a faster scan.

The desired effect of this decreased T1 in closely situated molecules is that blood appears as the brightest tissue (= highest signal) in the images instead of fat. It is not the Gd itself that appears bright on the images, which is the case with iodinated contrast agents used in x-ray. With this approach, the signal from the vessel is much less disturbed by saturation effects and by turbulent or slow flow, which can disturb MRA without contrast enhancement. The higher signal-to-noise ratio (SNR) can be used to decrease the scan time. The major drawback of CE-MRA is that the time frame for acquiring diagnostic images just of the arterial system is limited, as the method is of the “bolus chase” category.

Factors that influence the scan time are TR, TE and flip angle. The spatial resolution is determined by matrix size, partition thickness and field of view (FOV). The field strength of the main magnetic field, and the maximum amplitude and slew rate of the gradient system, the latter of which is used for spatial encoding, are important factors for a fast scanning procedure such as MRA.

When using a 3D technique, as in this work, it is possible to create thinner slices in the desired plane and achieve higher SNR than with 2D techniques. The reason for this is that the entire imaging volume is excited at the same time in 3D, in contrast to 2D, in which the slices are excited one at a time.

k-space

After the acquisition of spatial frequency (imaging) data from the object, the data are collected in the k-space. The k-space data do not correspond directly to the image data, as they are not acquired pixel by pixel and different portions of the k-space (frequencies) determine certain features in the image. The central portion of the k-space consists of low frequencies and carries information about contrast, the grey-scale, in the image. The peripheral portions consist of higher frequencies and carry information about the resolution, which determines the sharpness of the edges in the image. The way in which the image data are collected in the k-space is important for the contrast and resolution of the images.

There are several different ways of collecting data in the k-space, which have their advantages and drawbacks. The best arterial depiction will be achieved if the very centre of the k-space is collected during the peak arterial enhancement period. If linear sampling is used, this peak period will occur after half the time for the scan has elapsed. This sampling method was used for the cranial station in these studies. If centric elliptic sampling is used, then the central portion of the k-space will be sampled first.\textsuperscript{68} This method was used for the three remaining stations in the present studies. Later when venous enhancement occurs, the peripheral portions are sampled. These high frequencies will not contribute to the contrast of the image, but instead carry information about the edges in the image.

The k-space is symmetrical and the sides are images of each other. A partial Fourier acquisition (synonyms half-scan, partial echo) is therefore a way of significantly decreasing acquisition time but at the cost of decreasing SNR.\textsuperscript{69} The matrix size determines the number of lines in the k-space. With more lines, the spatial resolution increases for a given FOV. The drawback with a smaller voxel is that the amount of tissue enclosed in that voxel will decrease and lower the SNR. The scan time will also increase with more lines.

Zero-filling is a scheme to interpolate more voxels between the acquired voxels in the k-space. In the present studies the matrix was expanded from 256 to 512 by zero-filling. This does not improve the acquired slice thickness but improves the quality of reconstructed images and reduces partial volume averaging errors by creating overlapping slices.
Assessment of Atherosclerosis by Whole-body Magnetic Resonance Angiography

Contrast agent

The mechanism of action of a conventional extracellular Gd-based contrast agent is that it lowers the T1 in the blood to an extent depending on the concentration of Gd contrast agent during the image acquisition. Gd is paramagnetic, which means that it acts like a small magnet and influences small fluctuating local magnetic fields, enabling relaxation. Gd works in an indirect manner, altering the magnetic properties of closely situated hydrogen atoms and slowing the molecular tumbling time, thus enhancing proton relaxation. More rapid energy transfer from the spins to the lattice is achieved and the T1 relaxation time for that tissue in the immediate vicinity of the paramagnetic molecule is shortened.

In combination with a heavily T1 weighted sequence, the use of Gd creates a higher signal from the blood. The purpose is to achieve a higher signal in the blood than in the surrounding tissue. The T1 value of adipose tissue at 1.5T is 270 ms, of muscle 600 ms, and of native blood 1200 ms. A common value of T1 of blood with contrast agent is 50 to 100 ms. If the scan parameters are adjusted to these values, blood appears as the brightest object in the images and the surrounding tissue is suppressed. In order to further reduce the surrounding tissues, subtraction of a pre-contrast scan from the contrast enhanced scan can also be performed.

A conventional extracellular Gd contrast agent is injected intravenously in the arm. The bolus passes through the pulmonary circulation, where its leading and trailing edges often become diluted, after which it enters the systemic arterial circulation. This will usually take 15-25 seconds. Thereafter the Gd enhances various capillary beds in tissues such as the liver, kidneys or musculature and subsequently venous enhancement will occur. The time frame between the start of the arterial filling and the start of venous enhancement is very short.

Vascular enhancement is a transient and dynamic process and timing is the key for creating evaluative images. If the veins are also filled with contrast, it is possible that they may obscure the arteries, depending on the anatomical location of the vessels. This is most pronounced in the renal, lower leg, and neck regions. With a CE-MRA examination it is therefore a challenge to scan the arteries in the arterial phase before venous enhancement occurs. The scan is very fast, necessitating a low matrix size, which decreases the spatial resolution.

Several factors influence the transit time of the contrast bolus through the vascular system. Of these, cardiac output and the injection rate and volume are the most important. A low cardiac output will increase the Gd concentration. The rate of injection influences the maximum arterial Gd concentration and thus the signal from the vessel, and also affects the duration of the plateau phase. A fast injection creates the desired high signal but with a short plateau, and a slow injection creates a signal not so high but with a longer plateau. With a short plateau it is easier to miss the central k-space sampling and artefacts are more abundant. Venous enhancement is also earlier with a fast rate. The volume of contrast agent should be sufficiently large for the duration of injection to allow for some timing errors. A common dose is 0.2 mmol/kg body weight. An approximation which usually works is that the contrast volume should be given at a rate sufficient for half the duration of the scan, and a common injection rate is 2 ml/sec. It is of the greatest importance in CE-MRA to collect the central k-space portions when the arterial concentration of Gd is at its maximum, in order to achieve the highest signal intensity from the arteries and the best background suppression. To estimate the proper timing either a test bolus or fluoroscopic triggering is used. In the present studies 40 ml of contrast agent was injected at a rate of 0.6 ml/sec and a test bolus method was used.

Post-processing

Post-processing of the images is an important step in the achievement of images with the appearance of those in conventional angiography, whereby better understanding of spatial relationships is obtained. Examples of post-processing procedures are maximum intensity projections (MIP) and multi-planar reformations. An MPR displays a planar reconstruction in a different direction than that which was originally acquired. The voxel is not altered in size or intensity. In MIP, an imaginary ray is projected in the 3D data volume and the highest voxel value becomes the highest pixel value in the 2D MIP image. The drawback with MIP images is that asymmetrical luminal narrowing can
Introduction

be missed and that small vessels with low signal can be masked by background noise and be invisible on the MIP. In order to avoid this, the source images always have to be reviewed and are the basis for interpretation.

Additional techniques include surface rendering, which demands a segmentation of voxels with a threshold value. Voxels with intensity above the threshold are visible and those with intensity below are invisible. The purpose of surface rendering is to highlight voxels of tissue boundaries and display them with preservation of the anatomical spatial relationships in 3D. Limitations include difficulties in selecting the appropriate threshold and the fact that with an inappropriate threshold structures may not be displayed. A concept developed from surface rendering is volume rendering. The entire 3D data set is displayed in a translucent manner as either opaque or transparent, depending on the voxel intensity; the brightness and colour, also, are related to the intensity of the voxel. The advantage of VR over surface rendering is that the users do not need to define a threshold for surface boundaries. The VR technique is ideal for presenting an examination to the clinicians. For comparisons of measurements of vessel diameter, MIP and VR measurements with user defined settings, have been found to be comparable and in good agreement with DSA, which is regarded as the gold standard.\textsuperscript{70}

Whole-body MRA

With the introduction of the ultrafast high performance gradient system it became possible to extend the bolus chase method to include more stations into the concept of WBMRA. With this approach, it was suddenly possible for the first time to examine the arterial system from the supra-aortic vessels down to the distal runoff vessels with the same method, in the same subject, and at the same time. The WBMRA concept aims at large anatomical coverage. With the use of the bolus chase method, the scanning of the multiple stations has to be fast, before venous enhancement occurs or the concentration of the contrast agent has diminished in the arteries. This rapid scanning procedure demands a reduced number of lines in the k-space, which in turn lowers the spatial resolution.

The WBMRA method became available to our group in 1999 as representing one of the first sites in the world using a Philips scanner. Simultaneously, a group in Essen in collaboration with Siemens Medical Solutions made their way into the world of WBMRA. The initial reports were made in the early 2000s,\textsuperscript{71} and since then the number of articles has been increasing continuously, reporting studies of feasibility, applications and further development of the WBMRA method.

The WBMRA techniques used by these two groups have both similarities and differences. The major differences are that the method applied by our group only uses the built-in body coil of the scanner and does not require the addition of surface coils. The advantage of surface coils compared with the body coil is an increased SNR. Disadvantages are possible discomfort for the patient due to the closely applied coils over the whole body and the need for manually repositioning the table top for each of the five stations in the initial studies with surface coils. The acquired voxel size in the present studies with the built-in body coil was 1.76 x 1.76 x 4 mm, and this was reconstructed by zero-filling to 0.88 x 0.88 x 2.0 mm. The acquired voxel size with the surface coil method was 1.7 x 1.5 x 3 mm and the reconstructed voxel size was 0.8 x 0.8 x 1.9 mm.\textsuperscript{71} The surface coil method has been further refined with the use of phased-array coils, parallel imaging and multiple receiver channels, enabling faster scanning with a higher spatial resolution, typically 1.6 x 1.0 x 1.5 mm.\textsuperscript{72}

The feasibility of performing WBMRA was investigated in patients and in an elderly population in studies I and II respectively.
Assessment of Atherosclerosis by Whole-body Magnetic Resonance Angiography
Study aims

Principal aim of this investigation

The overall purpose was to assess the feasibility of performing WBMRA in patients and in an epidemiological setting.

Secondary aims

To create a score for assessment of the degree of atherosclerosis.

To explore the correlation of CV risk factors and markers to the degree of atherosclerosis expressed as an atherosclerotic score.

Specific aims of individual studies

Study I

To evaluate the technique of WBMRA with a clinical scanner in patients.

Study II

To investigate the feasibility of WBMRA in a clinical scanner for assessing atherosclerosis in different vascular territories in a cohort of elderly 70-year-old subjects.

Secondary aims were to estimate the prevalence and distribution of atherosclerotic abnormalities in this cohort of 70-year-old subjects, and to determine whether the degrees of atherosclerosis in different vascular territories are related to one another.

Study III

To create a scoring system for WBMRA that allows estimation of atherosclerosis in the arterial tree from the carotid arteries to the lower leg arteries, as one weighted index, the total atherosclerotic score (TAS), and to determine whether the traditional CV risk factors included in the Framingham risk score were related to TAS in an elderly population.

Study IV

To determine whether the amounts of abdominal visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) were differentially related to atherosclerosis as assessed by WBMRA in a mainly asymptomatic elderly population. A further objective was to address the question whether traditional CV risk factors, inflammation, or actions of adipokines could explain the hypothesised relationship between VAT and atherosclerosis.
Methods

Patients
In study I, thirty-three patients (median age 68 years, range 10-90 years, 10 females and 23 males) entered the study non-consecutively during the period September 2001 to September 2002, referred from a vascular surgeon. The MRA indications were: investigations of suspected or known stenoses, occlusion or aneurysm in 29 patients, assessment of patency of vascular grafts in four patients, suspected vasculitis in one patient and suspected vascular aplasia in one patient. Thus in two patients there was more than one indication. The study was carried out with the approval of the ethical committee and with informed consent.

Subjects (PIVUS)
In studies II-IV, 307 subjects (145 women, 162 men) were randomly recruited over a three-year period (November 2002 - November 2005) from a population-based cohort study, namely the Prospective Investigation of the Vasculature in Uppsala Seniors (www.medsci.uu.se/pivus/pivus), comprising 1016 participants. The primary aim of PIVUS was to evaluate the predictive power of three different tests of endothelium-dependent vasodilatation to predict future cardiovascular events. Eligible for that study were all subjects aged 70 living in the municipality of Uppsala, Sweden. The subjects of the PIVUS study were chosen from the Population Register of the municipality and were invited in randomised order within two months from their 70th birthday.

Of the 2025 subjects invited to participate in PIVUS, 1016 subjects were investigated, giving a participation rate of 50.1%. Some characteristics of the total sample (PIVUS study) and of the WBMRA subjects are given in Table 2. The mean length of time between the basic investigation and WBMRA was 16 months (range 3 to 24 months). Subjects with a pacemaker, valvular prosthesis or intracranial clips, and those with claustrophobia were excluded from the WBMRA examination. The study was approved by the Ethics Committee of the University of Uppsala and the participants gave their written informed consent.

Baseline investigation
The participants in the PIVUS study were asked to answer a questionnaire about their medical history, smoking habits and regular medication. All subjects were investigated in the morning after an overnight fast. No medication or smoking was allowed after midnight. After recordings of height, weight, and abdominal and hip circumferences, an arterial cannula was inserted in the brachial artery for blood sampling and later regional infusions of vasodilators. During the investigation, the subjects lay supine in a quiet room maintained at a constant temperature. Blood pressure was measured with a calibrated mercury sphygmomanometer in the non-cannulated arm to the nearest mmHg after at least 30 min of rest and the average of three recordings was used. Hypertension was defined as systolic blood pressure over 140 mmHg and/or diastolic blood pressure over 90 mmHg or on antihypertensive treatment. Diabetes was defined as fasting blood glucose level over 6.2 mmol/l or treatment with antidiabetic medication. Characteristics of the subjects are given in Tables 2, 3 and 4.

As the participation rate in the PIVUS study was only 50%, we carried out an evaluation of the cardiovascular status and medications in 100 consecutive persons who were invited to participate in that study but declined, to see if there was any bias in the selection. A comparison was also made in these respects between the total PIVUS sample and the WBMRA subsample (Table 4).

Laboratory
Lipid variables and fasting blood glucose were measured in the PIVUS cohort by standard laboratory techniques. Leptin and adiponectin were analysed with double-antibody radioimmunoassays (Linco Res., St. Louis, MO, USA). The total coefficient of variation for leptin was 4.7% at both low (2–4 ng/ml) and high (10–15 ng/ml) levels, and for adiponectin it was 15.2% at low (2–4 µg/ml) levels and 8.8% at high (26–54 µg/ml). IL-6 and TNF-α were analysed on the Evidence® array biochip analyzer (Randox Laboratories Ltd., Crumlin, UK). The functional sensitivity of IL-6 was 0.3 pg/ml and of TNF-α 1.8 pg/ml.
Methods

Table 2.
Basic characteristics and major cardiovascular risk factors in the subjects of PIVUS and whole-body MRA studies.

<table>
<thead>
<tr>
<th></th>
<th>PIVUS subjects</th>
<th>WBMRA subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>1016</td>
<td>306</td>
</tr>
<tr>
<td>Women (%)</td>
<td>50.2</td>
<td>47.4</td>
</tr>
<tr>
<td>Height (cm)</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>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/min)</td>
<td>62±8.7</td>
<td>61±8.7</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/l)</td>
<td>5.4±1.0</td>
<td>5.4±1.0</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>3.3±0.88</td>
<td>3.3±0.84</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.5±0.42</td>
<td>1.5±0.38</td>
</tr>
<tr>
<td>Serum triglycerides (mmol/l)</td>
<td>1.3±0.60</td>
<td>1.3±0.63</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>Current smoking (%)</td>
<td>11</td>
<td>7.8</td>
</tr>
</tbody>
</table>

No significant differences were found in any variables between the two sample sets. Values are means ± SD or medians with 10th to 90th percentiles. PIVUS=Prospective Investigation of Vasculature in Uppsala Seniors, WBMRA=whole-body magnetic resonance angiography, SBP=systolic blood pressure, DBP=diastolic blood pressure, HDL=high-density lipoprotein, LDL=low-density lipoprotein.

Table 3.
Additional characteristics and biochemical markers in the subjects of PIVUS.

<table>
<thead>
<tr>
<th></th>
<th>PIVUS subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Framingham risk score</td>
<td>11.1±3.3</td>
</tr>
<tr>
<td>Adiponectin (ng/ml)</td>
<td>6.4 (2.2-18.2)</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>10.5 (3.4-28.4)</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>4.1 (1.2-66.1)</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>3.7 (2.3-7.1)</td>
</tr>
</tbody>
</table>

Values are means ± SD or medians with 10th to 90th percentiles. PIVUS=Prospective Investigation of Vasculature in Uppsala Seniors IL-6=Interleukin-6. TNF-α=Tumour necrosis factor-alpha.
Assessment of Atherosclerosis by Whole-body Magnetic Resonance Angiography

Table 4.
Self-reported history of cardiovascular disorders and regular drug intake in the investigated PIVUS sample, in 100 non-attendees, and in the whole-body MRA sample. The results are given in percent.

<table>
<thead>
<tr>
<th>PIVUS subjects</th>
<th>Not attending</th>
<th>WBMRA subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>1016</td>
<td>100</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>7.1</td>
<td>7.9</td>
</tr>
<tr>
<td>Stroke</td>
<td>3.7</td>
<td>6.7</td>
</tr>
<tr>
<td>Angina pectoris</td>
<td>8.1</td>
<td>13.8</td>
</tr>
<tr>
<td>CABG/PTCA</td>
<td>5.3</td>
<td>5.6</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>3.8</td>
<td>6.9</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>8.7</td>
<td>16.9</td>
</tr>
<tr>
<td>Any regular drug</td>
<td>70</td>
<td>64</td>
</tr>
<tr>
<td>Any CV drug</td>
<td>45</td>
<td>52</td>
</tr>
<tr>
<td>Any antihypertensive medication</td>
<td>32</td>
<td>36</td>
</tr>
</tbody>
</table>

No significant differences in the basic characteristics and major cardiovascular risk factors were found between the total PIVUS sample, the WBMRA sub-sample and those not attending the basic investigation. PIVUS=Prospective Investigation of Vasculature in Uppsala Seniors, WBMRA=whole-body magnetic resonance angiography, CABG/PTCA=coronary revascularization. CV=cardiovascular.

Digital subtraction angiography

In study I, DSA was performed in accordance with the local clinical routine in 14 of the included 33 patients. In the standard procedure, 100 to 150 ml ioxaglat 200 mg/ml (Hexabrix®, Gothia, Billdal, Sweden) was used with a 4 F catheter (Cordis, Johnson & Johnson, New Jersey, USA). A monoplanar x-ray unit (DVI-S, Philips Medical Systems, Best, the Netherlands) was used, except for one biplanar examination of the carotid arteries (Integris, Philips Medical Systems, Best, The Netherlands). Catheterisation was performed via the femoral artery in the groin. Images of the relevant arterial territories were obtained after injection of an iodinated contrast agent. Frontal and oblique projections were acquired as deemed necessary to answer the clinical question. Selective catheterisation was performed to one brachiocephalic trunk, one common internal artery, one internal carotid artery, two renal arteries, and four iliofemoral segments. In the remaining cases the contrast agent was administered into the aorta. The vascular surgeon decided whether DSA was necessary or not, depending on the MRA result, the clinical status and symptoms.

In study I, the numbers of segments as listed in the following were investigated with DSA: 3 internal carotid arteries, 3 common carotid arteries, 2 subclavian arteries, 2 abdominal aortas, 8 renal arteries, 16 common iliac arteries, 14 external iliac arteries, 9 common femoral arteries, 8 superficial femoral arteries, 7 popliteal arteries, 1 tibio-peroneal trunk, 2 anterior tibial arteries, 2 peroneal arteries, 2 posterior tibial arteries. The time interval between the DSA and MRA was 0 to 358 days, with a mean of 88 days and a median of 57 days. In two cases the DSA preceded the MRA and in 12 cases the MRA was performed first. Findings in other vessel segments than those asked about in the referral note from the clinicians were denoted unsuspected findings.
Whole-body MRA

The WBMRA examination was carried out with the standard quadrature body coil in a 1.5T Gyroscan Intera scanner using standard MobiTrak® software (Philips Medical System, Best, the Netherlands). The subject was placed in the supine position feet-first on the table, to which an extension of the table top was attached, allowing for larger coverage. The arms were placed over the patient’s head to avoid foldover.

The WBMRA examination was divided into four stations. The 1st station included the supra-aortic arteries and the thoracic aorta. The 2nd station contained the abdominal aorta, including the renal arteries, and the 3rd station started at the external iliac arteries and continued to the popliteal arteries. The 4th and last station continued for a varying distance below the ankle. An overlap of 3 cm between each station gave a maximum total length of coverage of 171 cm. In studies II-IV breath-holding was performed only for the 2nd station. In study I, it was performed for the 2nd station covering the abdominal aorta, renal arteries, aortic bifurcation and pelvic arteries and also, for the 1st station covering the supra-aortic arteries and the thoracic aorta in the first few patients. In the majority of the patients only one breath-hold during the 2nd station was performed.

A 3D RF-spoiled T1w gradient echo acquisition was carried out at these four stations, beginning with the 4th station, prior to the injection. The scan time for each station was 17 sec. The table top was moved automatically with the table. The scan time for the non-contrast enhanced images was 87 sec, including instructions for breath-holding and table movement, which took 4 sec each for the three movements. Thereafter 40 ml of gadodiamide (Omniscan™, GE Healthcare, Oslo, Norway) was injected intravenously with an automated injector (MR Spectris®, Medrad, Pittsburgh, PA, USA) at a rate of 0.6 ml/sec in 67 sec and flushed with 20 ml of saline solution. The stations were scanned in reversed order, with the 1st station first, during the contrast administration during another 87 sec period.

The measured voxel size was 1.76 x 1.76 x 4.0 mm, and this was reconstructed by zero-filling to 0.88 x 0.88 x 2.0 mm, which gives a 1.54 mm³ volume of reconstructed voxel. Linear k-space sampling was used for the 1st station. For the other stations a method of randomly segmented centric view order (Centra®, Philips Medical System, Best, the Netherlands) was used.

Image reconstruction

WBMRA data were post-processed with subtraction of pre-contrast from post-contrast data. MIP images were obtained from the subtracted series with a 512 x 512 matrix at 45° on each side from the coronal plane in a total of nine images in every station. Seventy-five axial MPR images over the renal arteries were acquired from the unsubtracted contrast-enhanced series, with a slice thickness of 1.5 mm and zero gap in a 512 matrix. Source images, MPR and MIP images were all interpreted on a workstation.

Evaluation

The time required for evaluation of each of the WBMRA examinations in these studies was approximately 5 to 15 minutes. 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 posterior tibial arteries. The right and left sides were evaluated separately. Only the first 3 to 5 cm of the renal arteries and only the bulb of the internal carotid arteries were assessed. In study I the subclavian arteries were also assessed, yielding 28 vessel segments.

If a vessel segment could be evaluated, the finding in that segment was allocated to one of five groups: no stenosis; 1-49% reduction in lumen diameter; 50-99% reduction in lumen diameter; occlusion; or aneurysm. If a segment could not be evaluated, the reason for this was noted and categorised into one of four groups: venous overlap; motion artefacts; poor contrast filling; and other reasons (e.g. susceptibility artefacts from knee prosthesis). Each segment was graded only according to the most se-
vere degree of stenosis. Significant atherosclerotic abnormality was defined as a reduction of the vessel diameter by 50% or more, or occlusion. Stenosis was measured in the narrowest part of the vessel and compared with the normal vessel diameter, using both the source and MIP images. Aneurysm was defined in the abdominal aorta as a lumen diameter of 3 cm or more, and elsewhere as a 50% dilation of the vessel diameter compared with the nearest apparently normal vessel. One radiologist evaluated all WBMRA examinations blinded to other information. In study I, lesions in the internal carotid arteries were graded in accordance with NASCET criteria, with a diameter reduction of 70% or more considered significant.

In study II, a randomly selected consecutive series of 30 subjects from the PIVUS cohort were evaluated by another radiologist blinded to all other information. The radiologist who initially interpreted the images made a new evaluation of the same 30 subjects, blinded to the previous report and the other radiologist’s report.

The 26 vessel segments were categorised into five territories: 1. the carotids, including the internal carotid artery and common carotid artery; 2. the aorta, including both the thoracic and abdominal parts; 3. the renal arteries; 4. the pelvic/upper limbs, including the common iliac artery, external iliac artery, common femoral artery, superficial femoral artery and popliteal artery; 5. the lower legs, including the tibio-peroneal trunk, anterior tibial artery, peroneal artery and posterior tibial artery (Fig. 1).

In study I the concept of territories was not used.

Figure 1.
The 26 vessel segments are divided into 5 territories; 1. the carotids, including the internal carotid artery and common carotid artery, 2. the aorta, including both the thoracic and abdominal part, 3. the renal arteries, 4. the pelvic/upper leg territory, including the common iliac artery, external iliac artery, common femoral artery, superficial femoral artery, and popliteal artery, 5. the lower legs, including the tibio-peroneal trunk, anterior tibial artery, peroneal artery, and posterior tibial artery. The territories are projected on a coronal maximum intensity projection of a whole-body magnetic resonance angiography.
Methods

Atherosclerotic score

In order to obtain a comparable graded number reflecting the atherosclerosis in each territory, an atherosclerotic score (AS) was calculated for each territory in studies II-IV. A normal vessel segment received null point, 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 summed. That sum was then divided by the maximum sum that would be achieved if all included segments had a more than 50% stenosis or occlusion. The ratio was thereafter multiplied by 100. Hence, each territory could attain a maximum AS of 100.

A total atherosclerotic score was then calculated as the sum of the five territories, giving a maximum total of 500 points for the TAS value. Aneurysms and vessel segments that could not be evaluated were excluded from the calculations. In study I the concept of atherosclerotic score was not used.

Segmentation of adipose tissue

In study IV the distribution of abdominal adipose tissue was segmented. The segmentation was based on one MR image in each of 286 subjects; exclusion of the remaining subjects was due to missing information on a technical basis. An axial Balanced Fast Field Echo (B-FFE) sequence, with the parameters 10 mm slice thickness, 450 mm rectangular field of view (RFOV), 256x256 matrix size, and 70% scan percentage, was used as a scout view to plan the acquisitions. The same scan was also used for segmenting the distribution of abdominal visceral and subcutaneous adipose tissue at the L4-L5 intervertebral level. The distribution of each of the two adipose tissues was identified and manually contoured with a region of interest (ROI) on one axial image in each subject, using the software package ImageJ (http://rsb.info.nih.gov/ij/). The numbers of pixels in the ROI were then converted to an area (cm²). In VAT the aorta, inferior vena cava, common iliac vessels, parenchymal organs and bowel were excluded, while smaller vessels in the abdomen were included. Fat dispersed in the muscles was excluded from SAT. The imaging was not done on the same occasion as the PIVUS baseline investigation and no restrictions in terms of fasting or time of day were applied.

Statistical methods

In studies II and III, differences between groups regarding nominal variables were evaluated by means of contingency tables and the chi-square test. Relationships between non-normally distributed pairs of variables were tested by means of Spearman’s correlation analysis. The Mann-Whitney U test was used to compare TAS between men and women. Two-tailed significance values are given, with p<0.05 regarded as significant. Inter- and intra-observer variability was quantified with kappa statistics. A k-value of <0.20 denotes poor agreement, 0.20-0.40 fair agreement, 0.41-0.60 moderate agreement, 0.61-0.80 good agreement, and more than 0.80 denotes excellent agreement. The statistical program package StatView (SAS inc, NC; USA) was used for calculations.

In study IV, censored regression was used for statistical calculations, since 33% of the subjects showed no stenoses. The calculations were based on 282 subjects because of missing values. First, separate regression models were used to correlate TAS to VAT, SAT and BMI respectively, with adjustment for gender. Thereafter, using TAS as dependent variable and VAT and SAT as independent variables together with gender, the traditional risk factors included in the FRS, as well as BMI, were introduced as independent variables in the model. Subsequently, IL-6, TNF-α, adiponectin and leptin were added as independent variables to the model. Skewed independent variables were log transformed. A negative regression coefficient implies an inverse relation. The statistical program package STATA 8.0 (Texas, USA), Tobit procedure, was used for the calculations.
Assessment of Atherosclerosis by Whole-body Magnetic Resonance Angiography

Results

Feasibility of whole-body MRA in patients, Study I

All patients underwent the complete examination. 923 vessel segments were examined and 856 (93%) could be evaluated. Among these evaluable vessel segments, there was a reduction of the vessel diameter of at least 50% in 38 segments (4%), occlusion in 86 segments (10%) and aneurysm in 5 segments (0.6%). These observations were made in 26 of the 33 patients, leaving 7 patients without vascular abnormalities. In 17 of the 33 patients not all vessel segments could be evaluated (range 0-13 non-evaluable segments per patient, median value 1, mean value 2.6). Totally 67 vessel segments were non-evaluable. The reasons were as follows: poor contrast filling (n=31), motion artefacts (n=20), venous overlap (n=12) and other reasons (n=4). Twenty grafted vessel segments were evaluated.

Of the 14 patients who underwent both WBMRA and DSA, the two methods showed agreement in all the imaged segments in 10 patients (72 out of 79 segments). In 3 segments MRA overgraded the stenosis (1 abdominal aorta and 2 common iliac arteries) and in 4 segments it undergraded the stenosis (1 common iliac artery, 1 external iliac artery and 2 renal arteries) compared with DSA.

In nine patients, unsuspected pathology was found on the WBMRA (Table 5). These unsuspected findings had no influence on the treatment and did not require further investigation.

<table>
<thead>
<tr>
<th>Regions</th>
<th>No. of clinically suspected regions</th>
<th>No. of stenoses of more than 50% in the clinically suspected regions</th>
<th>No. of clinically suspected regions partly non-evaluable</th>
<th>No. of patients with additional stenoses of more than 50%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carotid and subclavian arteries</td>
<td>3</td>
<td>2</td>
<td>Lower leg (1), renal (1), renal and femoral-popliteal (1)</td>
<td></td>
</tr>
<tr>
<td>Thoracic and renal arteries</td>
<td>1</td>
<td></td>
<td>Carotid and iliac (1)</td>
<td></td>
</tr>
<tr>
<td>Infrarenal aorta to popliteal arteries</td>
<td>16</td>
<td>3</td>
<td>Carotid and renal (3), carotid (1), renal (1)</td>
<td></td>
</tr>
<tr>
<td>Distal to popliteal arteries</td>
<td>12</td>
<td>8</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Whole body</td>
<td>6</td>
<td>3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5. Number of clinically suspected regions with their pathological findings, and number of patients with additional stenoses of more than 50% in these regions.
Feasibility of whole-body MRA and the prevalence of atherosclerosis in an elderly population, Study II

The examination could be performed in 306 of the 307 subjects. One subject had a vasovagal reaction before administration of the contrast agent and the WBMRA could therefore not be completed. No adverse event occurred. No significant differences in basic characteristics or major cardiovascular risk factors were found between the total PIVUS sample, the WBMRA sub-sample and those not attending the baseline investigation (Table 4). The mean length of time between the baseline investigation and WBMRA was 16 months, with a range from 3 to 24 months.

In total, 7956 vessel segments were evaluated, with a success rate of 99.3%. Of the successfully evaluated 7905 segments, 7186 (90.9%) were normal. The results for the different vessel segments are presented in Table 6. The most frequent significant atherosclerotic abnormalities (≥50% stenosis, occlusion) were found in the lower leg (72%), especially in the anterior and posterior tibial arteries. The most frequently non-evaluable segments were also located in the lower leg territory (67%). Luminal narrowing of 50% or more was present in 1.5% of the renal arteries and in 1.8% in the bulb of the internal carotid arteries. Two percent had an infrarenal abdominal aneurysm.

On an individual basis, 97 subjects (32%) showed no abnormalities and 112 subjects (37%) had only luminal reductions of less than 50%. Ninety-four subjects (31%) had at least one significant atherosclerotic abnormality, 41 (13%) had only one segment with a significant atherosclerotic abnormality, 31 (10%) had two such segments, and 22 (7%) had more than two. In 27 subjects (9%) at least one segment was non-evaluable.

The subjects were divided into two groups according to the presence or absence of self-reported vascular disease. The calculations were based on 299 subjects because of missing values. In the group without self-reported vascular disease (n=230), 85 subjects had normal vessels, 86 had stenosis of <50% and 59 had ≥50% stenosis or occlusion. In the group with self-reported vascular disease (n=69), the corresponding figures were 14, 22 and 33 respectively.

The intra-observer reproducibility was good (kappa value =0.73), with agreement in 94% of the segments. The inter-observer reproducibility was excellent (kappa value =0.83), with agreement in 77% of the segments between two observers.

When a correlation matrix was calculated for the AS in the five different vascular territories they were all found to be significantly interrelated (r=0.44-0.58, p<0.0001 for all comparisons).

Table 6.
Numbers of normal and abnormal segments, and numbers of segments that could not be evaluated.

<table>
<thead>
<tr>
<th></th>
<th>ICA</th>
<th>CCA</th>
<th>AORTA</th>
<th>REN</th>
<th>CIA</th>
<th>EIA</th>
<th>CFA</th>
<th>SFA</th>
<th>POP</th>
<th>TPT</th>
<th>ATA</th>
<th>PA</th>
<th>PTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>504</td>
<td>608</td>
<td>521</td>
<td>568</td>
<td>554</td>
<td>591</td>
<td>600</td>
<td>505</td>
<td>541</td>
<td>600</td>
<td>476</td>
<td>581</td>
<td>537</td>
</tr>
<tr>
<td>Stenosis &lt;50%</td>
<td>92</td>
<td>84</td>
<td>34</td>
<td>46</td>
<td>13</td>
<td>12</td>
<td>92</td>
<td>58</td>
<td>6</td>
<td>41</td>
<td>12</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Stenosis ≥50%</td>
<td>11</td>
<td>9</td>
<td>3</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>2</td>
<td>31</td>
<td>2</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occlusion</td>
<td>1</td>
<td></td>
<td>3</td>
<td>9</td>
<td>3</td>
<td>59</td>
<td>5</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aneurysm</td>
<td>1</td>
<td>6</td>
<td></td>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not evaluable</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-motion</td>
<td>1</td>
<td>1</td>
<td></td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>5</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-venous filling</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-no contrast</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-other</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>7</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ICA=internal carotid artery, CCA=common carotid artery, AORTA=thoracic and abdominal aorta, REN=renal artery, CIA=common iliac artery, EIA=external iliac artery, CFA=common femoral artery, SFA=superficial femoral artery, POP=popliteal artery, TPT=tibio-peroneal trunk, ATA=anterior tibial artery, PA=peroneal artery, PTA=posterior tibial artery.
The introduction of a total atherosclerotic score for whole-body MRA and its relation to the Framingham risk score, Study III

Framingham risk score correlated to total atherosclerotic score (r=0.30, p<0.0001) (Table 7). Of the parameters included in the FRS, male gender (p<0.0001), systolic blood pressure (p=0.0002), cigarette pack-years (p=0.0008) and HDL-cholesterol (p=0.008) contributed to the significance, while blood glucose and LDL-cholesterol did not. The atherosclerotic scores for the five territories were also separately correlated to FRS (p<0.0001) using Spearman rank correlation.

The frequency distribution of TAS is given in Figure 2. In the scattergram (Fig. 3) relating FRS to TAS, there were very few subjects with a low FRS and a high TAS, while a low TAS did not rule out a high FRS.

Table 7.
The correlation between FRS, with its parameters, and TAS.

<table>
<thead>
<tr>
<th></th>
<th>r value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRS</td>
<td>0.30</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Male gender</td>
<td>-</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>-0.15</td>
<td>0.008</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>0.09</td>
<td>0.12</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>0.21</td>
<td>0.0002</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>0.14</td>
<td>0.016</td>
</tr>
<tr>
<td>Blood glucose</td>
<td>0.09</td>
<td>0.11</td>
</tr>
<tr>
<td>Cigarette pack-years</td>
<td>0.23</td>
<td>0.0008</td>
</tr>
</tbody>
</table>

TAS=total atherosclerotic score, FRS=Framingham risk score, HDL=high-density lipoprotein, LDL=low-density lipoprotein.
Results

Figure 2.
The frequency distribution of the total atherosclerotic score (TAS). The y-axis gives the number of observations and the x-axis the TAS value.

Figure 3.
The relationship between the Framingham risk score (FRS) and the total atherosclerotic score (TAS) (r=0.30, p>0.0001). TAS is denoted on the y-axis and FRS on the x-axis.
The relation between the total atherosclerotic score and measures of obesity, inflammation and adipokines, Study IV

In different separate regression models, VAT was related to TAS (regression coefficient (rc)=0.00012, p=0.005) when adjusted for gender, while SAT and BMI were not related to TAS (rc=0.00018, p=0.491 and rc=0.0072, p=0.23 respectively). Both adiponectin and leptin were related to TAS (rc=0.12, p=0.001 and rc=0.089, p=0.027 respectively). A negative regression coefficient implies an inverse relation.

When the traditional cardiovascular risk factors included in FRS (gender, blood pressure, smoking, diabetes, HDL-cholesterol, and LDL-cholesterol) were introduced as independent variables together with VAT, SAT and BMI in a multiple regression model with TAS as dependent variable, VAT was still significantly related to TAS (p=0.049) (Table 8).

When IL-6 and TNF-α were added as independent variables to the model presented in Table 8 (with FRS as a substitute for HDL-cholesterol, LDL-cholesterol, diabetes and smoking), IL-6 (p=0.016), but not TNF-α, together with FRS (p<0.0001) and VAT (p=0.030), was significantly related to TAS. However, when adiponectin and leptin were also added to the regression model, the relationship between VAT and TAS was no longer significant (p=0.18). Now it was found that adiponectin (p=0.021), but not leptin, together with IL-6 (p=0.032) and FRS (p<0.0001), was significantly related to TAS (Table 9).
Results

Table 8.
A multiple censored regression model including measures of adiposity distribution and traditional cardiovascular risk factors as independent variables, with TAS as dependent variable.

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Regression coefficient</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAT</td>
<td>0.0011</td>
<td>0.049</td>
</tr>
<tr>
<td>SAT</td>
<td>-0.00017</td>
<td>0.699</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.17</td>
<td>0.642</td>
</tr>
<tr>
<td>Female gender</td>
<td>-0.51</td>
<td>0.007</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>-0.0052</td>
<td>0.477</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>0.023</td>
<td>0.444</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>0.0054</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0.12</td>
<td>0.098</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.31</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

TAS=total atherosclerotic score, VAT=visceral adipose tissue, SAT=subcutaneous adipose tissue, BMI=body mass index, HDL=high-density lipoprotein, LDL=low-density lipoprotein. A negative regression coefficient implies an inverse relation.

Table 9.
A multiple censored regression model including measures of adiposity distribution, traditional cardiovascular risk factors, markers for inflammation, and adipokines as independent variables, with TAS as dependent variable.

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Regression coefficient</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAT</td>
<td>0.00083</td>
<td>0.18</td>
</tr>
<tr>
<td>SAT</td>
<td>-0.00036</td>
<td>0.43</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.012</td>
<td>0.31</td>
</tr>
<tr>
<td>Female gender</td>
<td>-0.13</td>
<td>0.11</td>
</tr>
<tr>
<td>FRS</td>
<td>0.031</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.039</td>
<td>0.032</td>
</tr>
<tr>
<td>TNF-α</td>
<td>-0.00095</td>
<td>0.99</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>-0.083</td>
<td>0.021</td>
</tr>
<tr>
<td>Leptin</td>
<td>0.088</td>
<td>0.15</td>
</tr>
</tbody>
</table>

TAS=total atherosclerotic score, VAT=visceral adipose tissue, SAT=subcutaneous adipose tissue, BMI=body mass index, FRS=Framingham risk score, IL-6=interleukin-6, TNF-α=tumor necrosis factor-alpha. A negative regression coefficient implies an inverse relation.
Discussion

Summary of findings
In these studies, WBMRA was feasible in both severely ill atherosclerotic patients and in an elderly population. The WBMRA method was used in the present studies for assessing concomitant vascular disease, graft patency, and multiple aneurysms, for example. The prevalence of vascular abnormalities in the carotid, renal, and inflow and runoff arteries of the lower limbs was determined in an elderly population, the PIVUS cohort. A total atherosclerotic score was created for use with the WBMRA method. TAS reflects the degree of luminal narrowing and was found to be significantly correlated with FRS, a well established CV risk assessment score which includes the traditional CV risk factors. The degree of atherosclerosis, as assessed by TAS, was also significantly related to VAT, IL-6, adiponectin and leptin.

The intra- and inter-observer reproducibility was good to excellent, and nearly all subjects underwent the complete examination without any adverse events, indicating the safety and robustness of the WBMRA method. A large proportion (93-99%) of vessel segments were evaluable and a small comparison with conventional angiography yielded reasonable results regarding the degree of maximum stenosis or occlusion.

Significant atherosclerotic abnormalities were present in some subjects without clinically evident vascular disease, and unsuspected significant vascular abnormalities were found in patients with atherosclerotic symptoms. Correlations between atherosclerotic abnormalities in different vascular territories were found for all territories and the atherosclerotic scores for the five vascular territories were also separately significantly related to FRS, emphasizing that atherosclerosis is a systemic disease.

Further studies of outcome data for the PIVUS cohort are required for further validation of the WBMRA method and to address the question whether TAS can be used as an adjunct for CV risk assessment. Meanwhile, the correlation with FRS indicates that TAS might be of value for this purpose.

Role of whole-body MRA in the assessment of atherosclerosis
Atherosclerotic induced luminal narrowing is denoted “atherosclerosis” throughout this thesis. The reason for this is that substantial atherosclerosis can be present in the vessel wall despite a normal appearance of the lumen with use of imaging modalities that only assess the lumen. The main reason for this normal appearance is the occurrence of remodelling of the outer wall of the vessel.

Is there a place for whole-body MRA in clinical practice?
In study I of the present investigation, WBMRA was used in severely ill atherosclerotic patients for assessing concomitant disease, graft patency, and multiple aneurysms. All patients completed the WBMRA examination and 93% of the vessel segments could be evaluated. In that study, 9 out of 33 patients showed abnormalities in vessels in which no abnormalities were suspected. These findings did not, however, lead to further investigation or treatment. Other studies with WBMRA have similarly revealed unsuspected vascular pathology.

In study II, significant vascular abnormalities were observed in 59 out of 230 subjects without CV symptoms. Further, in study III the atherosclerotic scores for the five territories were separately correlated to FRS (p<0.0001), emphasising that atherosclerosis is a systemic disease.

WBMRA is well suited for repeated examinations in patients with systemic diseases. This is due to the lack of ionising radiation and to the large coverage of the vessels. Vasculitis is such a disease affecting large or medium-sized arteries, with examples such as polyarteritis nodosa, which carries a high likelihood of aneurysm in several locations, and Takayasu arteritis, which causes multiple stenoses.

Atherosclerosis is also a systemic disease, and WBMRA can be used to evaluate other vascular regions than those giving rise to symptoms. In patients with clinical symptoms, the likelihood is high that other vascular areas will be affected by significant asymptomatic disease. In a study in which 249 patients with peripheral arterial occlusive disease were assessed by WBMRA, 52 patients (73 segments) displayed significant vascular...
Discussion

pathology, which in 9 patients (11 segments) demanded surgical or interventional therapy.78

Is there a place for whole-body MRA in science?
The WBMRA method was feasible in an elderly population included in an epidemiological study. Only one out of 307 subjects did not complete the WBMRA examination and 99.3% segments were evaluable.

In the present study III, a total atherosclerotic score, reflecting the degree of atherosclerosis, was created for the WBMRA method. This score can be related to other variables for assessing the contribution of that variable to the extent of atherosclerosis. In study III TAS was found to be significantly related to a CV risk assessment tool (FRS) including the traditional CV risk factors. In study IV, TAS was found to be significantly related to the amounts of VAT, adiponectin, leptin and IL-6.

The WBMRA method has not been studied with regard to prediction of future cardiovascular events, as have IMT,51 CACS54, 56 and the ankle-brachial index.57 Thus no outcome data are yet available to validate the WBMRA method as a predictive tool for assessing CV risk. All imaging methods have their advantages and disadvantages in comparison with one another. The advantages of WBMRA are that no ionising radiation is involved and a large anatomical coverage is achieved. In addition, cardiac function and viability (late enhancement) can be studied and segmentation of visceral and subcutaneous abdominal adipose tissues can be performed in the same examination time of approximately 45 minutes and with use of the same contrast injection. However, the WBMRA method needs to be further studied in other cohorts and thoroughly validated regarding prediction of future CVE before the step from science to screening can take place.

The total atherosclerotic score
The present finding (study III) of a close relationship between FRS and TAS supports the idea that the globally assessed atherosclerotic burden (TAS) is related to the coronary risk, as assessed by FRS. Furthermore, all five vascular territories were related to FRS, with only minor differences between the territories.

Whole-body atherosclerosis as measured by MRA has not previously been expressed as a score. Although it is of interest to study individual arteries or territories, it would be of value to be able to compress the overwhelming amount of data obtained at WBMRA into a single score (TAS). The relations between this score and other variables could then be studied, for example in epidemiological investigations.

If every vessel segment included in the TAS were to be weighted as equal to one another, a 50% stenosis in a peripheral artery would be weighted as equal to a 50% stenosis in the internal carotid artery. This does not seem to be a relevant way to create a meaningful score. In the present study, vessels were therefore arranged into five territories and the sum for each territory was then divided by the maximum achievable sum based on the number of included segments. In the final step, the scores for each territory were summed to form a TAS. With this procedure, a stenosis in the lower leg territory will not contribute as much to the TAS as a stenosis in, for example, the carotid territory. This would otherwise be the effect, on account of the different numbers of segments included in a territory. No attempt was made to give the territories different weights, because of the current lack of outcome data.

One limitation of TAS is that it measures the luminal narrowing and not the true plaque burden or the vulnerability of the plaque. At the present state of hard and software for an MR scanner, it is feasible to examine plaques locally in a number of vessel regions for composition and size, but it is not possible to assess the true plaque burden or vulnerability of the whole arterial tree. In the future it will be possible to perform MR sequences for characterisation of plaque morphology at the same examination in order to add information on plaque vulnerability to TAS.80 In the present work, only the bulb of the internal carotid artery was evaluated, because of problems with low spatial resolution. In future studies this could be overcome with use of a similar technique with higher spatial resolution.81, 82 The TAS does not reflect either the lengths or number of stenoses in a certain vessel segment, or atherosclerotic aneurysms. Coronary
arteries are not included in the WBMRA examination, which means that a major contributor to CVE is not assessed. Further studies will therefore be needed to establish the link between TAS and coronary atherosclerosis. Atherosclerosis is, however, a systemic disease, and if stenosis is present in one vascular region, it will be highly likely that there will be concomitant stenoses in other territories.

The final validation of TAS will be carried out by a prospective follow-up study regarding future cardiovascular events (myocardial infarction, stroke, or intermittent claudication) in the PIVUS cohort. Whether individuals with a low TAS also have a lower CV risk remains to be shown in that study. The atherosclerotic scores from the different territories can then be weighted differently in relation to one another when calculating their correlation to outcome data, in order to validate TAS as a prognosis tool for CV disorders.

Is there a place for whole-body MRA in screening?

With WBMRA, an overview over the whole arterial tree is possible without the risks involved in ionising radiation or arterial cannulation, and with the use of a less nephrotoxic contrast agent in conventional doses compared with iodinated contrast agents. On the basis of this overview, a possible further development of the WBMRA concept could be to perform further acquisitions at sites with atherosclerotic plaques with higher resolution scans, in order to determine the degree of stenosis more accurately or to achieve plaque characterisation.

When investigating asymptomatic subjects, the purpose of the examination and what findings are relevant to report must be clear beforehand. The detection of asymptomatic disease is different from the scenario concerning symptomatic patients. In the latter, the examination will be required to provide information for potential preoperative planning. This demands higher resolution in order to determine the degree of stenosis more precisely and to assess the possibilities of either surgery or endovascular intervention.

In recent studies, whole-body MR screening in conjunction with WBMRA has been evaluated for detection of pulmonary lesions, colon polyps, cerebral infarction and intracranial aneurysms, and assessments of myocardial viability, perfusion and contractibility and of valvular function. This concept of assessing abnormalities could be realised in a “one stop shop” examination in order to detect diseases at an early stage before they become symptomatic with a potentially worse prognosis regarding mortality and morbidity.

Screening for asymptomatic pathology is controversial. The major issues are the potential benefits of the detection of such a lesion for the individual versus a number of concerns, namely the potential side effects of the possible treatment, the number of individuals that would need to be treated to prevent the occurrence of an event, the sensitivity and specificity of the test employed in relation to the prevalence in the population, and the cost to the society of the screening procedure itself.

The justification of screening is still debated regarding certain diseases, for example screening for prostate cancer by a blood test that measures the level of prostate specific antigen (PSA). Prostate cancer has a relatively high prevalence in the population but the majority of these tumours do not cause any symptoms during an individual’s life span. An affected person may not be aware of his malignant tumour, which may or may not remain clinically asymptomatic. The awareness of a potentially life-threatening disease, through screening, might just create anxiety for the person concerned. A choice will have to be made between remaining untreated or choosing treatment with its potential side effects. In the case of breast cancer or cancer of the cervix, these are potentially curable diseases when detected early, and screening could save lives. Some people want to know about the presence of asymptomatic diseases and be able to take proper precautions, while others would rather not be told or are not interested. The individual should consider whether he or she wants to be aware of asymptomatic disease before participating in a screening programme.

A common first symptom of previously subclinical, asymptomatic atherosclerosis is an MI or stroke, which could be fatal or cause major morbidity. Nowadays effective risk-lowering therapies for CV diseases are available, and thus individual,
preventive actions could have been sufficient to avoid these events.

In a recent publication from the Swedish Medical Products Agency, it is stated that there is not enough evidence to justify screening for CV risk factors in the population.83 No screening outside the frame of the national health services should be undertaken, as every measurement of CV risk factors should be followed by information to the patient concerning the assessment of the findings and advice regarding possible actions.

Validation of the whole-body MRA method

In study II, the intra- and inter-observer reproducibility was investigated. The kappa value, which reflects the level of agreement, was good to excellent. Similar results have been reported from other studies with WBMRA.84, 85 Repeated examinations of the same subject, yielding reproducibility, have not yet been performed and this is a current limitation of this method. Results of validation against DSA, which is regarded as the gold standard among angiographic methods, were only available for a limited number of vessel segments in study I, though with a reasonable outcome.

A total validation of the whole arterial tree compared to DSA is not possible owing to the hazards of ionising radiation and iodinated contrast agents with DSA. The validations of WBMRA in previous studies have shown a high sensitivity and specificity for the pelvic and lower limb arteries in comparison with DSA. No systematic validation against DSA has been performed for the renal, aortic and carotid arteries. Various methods have been used, however, for confirmation of vascular abnormalities found on WBMRA, such as US, dedicated MRA or DSA, with reasonably high agreement.74, 76-78, 81, 82, 84, 85

Limitations

The WBMRA method is designed for large anatomical coverage. The basis of WBMRA is the chase of the bolus in the arteries, which limits the time span for obtaining evaluable images. With longer scan times, the contrast bolus will have passed from the arteries to the veins, and venous enhancement will be too pronounced. Scanning must therefore be fast, with the result that the resolution is limited. The FOV is also enlarged from the usual maximum 400 mm to 450 mm in order to extend the possible length to be examined in one station. This results in deviation of the margins, including the vessels. This does not seem to be a problem for assessment of vasculature, on account of the 3 cm overlaps of the stations.

In the smaller vessels in the lower leg or neck, with diameters of only 2-3 mm, the vessel consists of only 2-3 voxels. Bearing this in mind, grading of a stenosis was sometimes difficult, and in some cases it was also difficult to differentiate a severe stenosis from an occlusion. Hence, the creation of a total atherosclerotic score was also an attempt to lower the impact of the misinterpretations that would certainly be made from the images concerning the degree of stenosis.

In a study with 2D TOF MRA in which a known diameter of a vessel phantom was compared with the diameter in the images, it was found that ≥3 pixels/diameter yielded an accurate diameter. For low resolution images (<1.3 pixels/diameter), large overestimations of the diameter occurred. For grading stenoses, diameters are usually measured relative to the nearest normally appearing vessel segment. Inadequate resolution in a stenosis (<1 pixel/diameter) and adequate resolution in the adjacent portion of the vessel could lead to an underestimation of the stenosis.86

Another limitation shared with all luminography modalities, such as WBMRA and DSA, is that only the lumen is visualized and not the vessel wall with its plaques. The vessels cannot be considered as rigid pipes and plaque formation is a dynamic process. Owing to the vascular remodelling phenomenon first described by Glagov et al 1987,62 the arteries tend to enlarge their area defined within the IEL in response to early plaque formation. The trigger for outward remodelling is thought to be a response from the vessel in an attempt to preserve normal shear stress and wall tension in the blood vessels when a plaque is present. In the coronary arteries, this phenomenon maintains an apparently normal vessel lumen until a reduction of approximately 40% is reached.62, 87 Overcompensation of the lumen size may also occur in early stages of plaque formation. Finally, the dilation of
Assessment of Atherosclerosis by Whole-body Magnetic Resonance Angiography

the outer walls of the vessel cannot compensate for the increased area of the plaque, and luminal narrowing is visible on luminography. A paradoxical response may also be present, with shrinkage of the IEL area in response to plaque formation. This is more frequent in the femoral arteries. Compensatory enlargement is very frequent in the renal arteries, with only 2% of the plaque mass visible on luminography and the remaining 98% obscured.

Degradation of the extracellular matrix in the vessel wall is part of the remodelling process. This allows the vessel to expand and one contributor to the degradation is MMP. The remodelling can also be inappropriate and cause an increase in inflammatory activity in the plaque, rendering it more vulnerable to rupture. The matrix degradation might also explain why aneurysms develop as a part of overcompensated remodelling. Methods that visualise the vessel wall, such as IVUS, are more able to reveal the true plaque burden.

In epidemiological studies, age and gender are almost invariably adjusted for in the calculations. When only a certain age group is included, adjustments for the effects of age are automatically made. This is also a limitation of studies II-IV, because of the inability to draw conclusions other age groups than the age 70 of the investigated group. The sample in studies II-IV consisted only of Caucasians, precluding conclusions regarding other ethnicities. However, the strengths of this large population-based sample should be emphasised.

A potential selection bias in studies II-IV could be the fact that the studies are based on subjects aged 70. These elderly subjects could imply a survival selection bias with an overrepresentation of survivors less affected by atherosclerosis in the PIVUS study. If the included subjects had constituted a younger group or represented a broader age span, the results would probably be different. In a study of the prevalence and morphology of carotid atherosclerosis assessed as intima-media thickness by US, it was found that in men there was a linear increase in carotid plaques up to the age of 65, after which the increase levelled off. Younger women had fewer plaques than men up to the age of 49. Thereafter, the plaque prevalence accelerated in a curvilinear manner and in the age group 75-84 more women than men had carotid plaques. The prevalence of soft plaques, which considered rupture prone, was higher in men of all age groups. This combined with the finding that the amount of plaques is quite similar in elderly men and women, could be a reason for the observed higher frequency of coronary deaths in elderly men.

The interval between the baseline investigation and WBMRA was quite long (range 3 to 24 months) in studies II-IV, as well as between DSA and WBMRA in study I (mean 88 days, median 57 days, range 0 to 358 days). It is possible that during these times the atherosclerosis may have progressed, which could potentially lower the power of the studies. Atherosclerosis is a slow process, however, and it is unlikely that this would have had a large effect on the results.

A limitation of study IV was that the distribution of adipose tissue in the abdomen was measured in a single slice at the traditional level of the L4-L5 intervertebral space. If the whole abdomen had been included in the measurements of VAT and SAT, the results might have been different. A study aimed at identifying the slice with the highest association with obesity-related health risk indicators showed that a slice 10 cm above L4-L5 in men and 5 cm above in females had an association with risk indicators that was similar to or stronger than that of the total VAT volume. However, when the VAT areas at L4-L5, 5 cm above L4-L5, and at the L3-L4 level were each compared with the total VAT mass, the abilities of these three slices to predict total VAT mass were comparable.

Future directions, MRA

MRA is gaining acceptance as a robust, minimally invasive procedure which alone can serve as a preoperative investigation in the clinical environment. The trend nowadays is towards higher field strength (3T) and a combination of parallel imaging with surface multi-channel coils. In order to further improve MRA, the signal to noise ratio should be increased so that the spatial resolution can be maximised.

An increased field strength, 3T, means that the relaxivity of the contrast agent also increases, resulting in a higher blood signal intensity. At 3T, the T1 of the tissues is also 20-40% longer than at 1.5T, which gives better background suppression.
and an improved contrast-to-noise ratio. Surface multi-channel coils are closer to the investigated area and also contribute to an increase in SNR, in comparison with the use of the body coil.

This increased signal could be used in two ways: either to keep the scan time unchanged and increase the spatial resolution or to shorten the scan time while maintaining the spatial resolution. The scan time can be reduced up to a factor of 2 to 4, when going from 1.5T to 3T.

A problem with parallel imaging is that SNR decreases by at least the square root of the acceleration factor, but if higher field strength is used, there is still enough signal present from the tissue. This approach could also compensate for the problem with high energy deposition in 3T systems.

Recently, a blood-pool agent (MS-325) has been approved for MRA in Europe. The T1 relaxivity is 6-8 times higher than for a conventional extracellular Gd contrast agent. This binds to serum proteins such as albumin and circulates in the blood, enabling scanning for up to 30-60 minutes. With this substance, it is possible to both perform a conventional fast pass angiography and later make acquisitions in steady state, permitting high resolution images (voxel size 0.5 x 0.5 x 0.5 mm) to be obtained over the areas of interest, by virtue of the possibility of a longer scan time. Venous enhancement is present in the steady state images, but with the higher spatial resolution arteries can usually be distinguished from veins, with the possible exception of those in patients with severe atherosclerosis. With a multi-station approach including surface coils and parallel imaging, MRA with MS-325 showed a sensitivity and specificity of over 95% for detection of 75% stenosis in the carotid and lower extremity arteries, as compared with conventional angiography.95

Future directions, plaque characterisation

Angiography describes only the lumen of the vessels, as a "luminogram", and provides no information about the wall of the vessels and the plaques themselves. Owing to compensatory vascular enlargement, “positive remodeling”, large plaques can only be seen as mildly stenotic when examined by angiography. The composition of the plaque seems more interesting as a determinant for subsequent disruption. Increasing interest is being focused on plaque characterisation by various imaging modalities and contrast agents. Assessment of the composition of the plaque, as a potential indirect marker of plaque stability, could be of more value than assessment of the lumen as a predictor of future CVE or for monitoring therapy. As an example, fibrous cap rupture identified by high resolution MRI of carotid plaques exhibited a high association with recent stroke or TIA.98 Different specifically targeted contrast agents designed to localise and assess specific characteristics of unstable plaque, such as MMP, are under development.13, 99

Cardiovascular risk factors

The traditional CV risk factors can explain the majority of future cardiovascular events, but not all. Traditional CV risk factors were found to be related to TAS in the present study III, and novel markers for CV risk were related to TAS in study IV. Most of the traditional CV risk factors were related to the degree of atherosclerosis expressed as TAS, such as male gender, smoking, hypertension, and elevated HDL-cholesterol. This was an expected finding and supports the idea that the proposed TAS is related to the process of atherosclerosis.

Surprisingly, diabetes was not significantly related to TAS. One explanation for this could be that in these elderly subjects an impaired glucose level will have played out its influence on the amount of atherosclerosis or that anti-diabetic treatment will have been effective. Unfortunately haemoglo-
bin A1c (HbA1c), a marker for the long-term glucose level, was not available in the PIVUS study. With that marker, other results could possibly have been obtained.

Nor was LDL-cholesterol significantly related to TAS. In a multi age and ethnic cohort study, oxidised LDL-cholesterol was found to be related to the presence of subclinical atherosclerosis as assessed by carotid US, ABI, and CACS. In a longitudinal population-based study with 30 years of follow-up, LDL-cholesterol lost some power for predicting future MI between the ages of 50 and 70, but LDL-cholesterol was still a significant predictor at the age of 70.

Novel CV risk factors related to adipose tissue were investigated in study IV. The amount of abdominal VAT was related to the degree of atherosclerosis, while BMI and SAT were not. The relationship with VAT was still significant after adjustment for gender, SAT, BMI, IL-6, TNF-α, and the traditional CV risk factors.

The relation between VAT and TAS became non-significant when adiponectin was introduced into the multiple regression models, indicating that this relationship can be mainly explained by the adiponectin levels. The action of adiponectin consists of a potential inhibition of several major atherosclerotic mechanisms, including expression of adhesion molecules on the surface of endothelial cells and subsequent adhesion and migration of inflammatory cells in the vascular wall; uptake of oxidised LDL-cholesterol by scavenger receptors on the surface of foam cells; and migration of smooth muscle cells into the intima layer of the vessel wall, attracted by various growth factors. This significant relation between adiponectin and TAS indicates that adiponectin could be the pathophysiological link between visceral obesity and atherosclerosis.

Study IV is the first study, to our knowledge, that has included adipose tissue segmentation, adipokines and assessment of subclinical atherosclerosis in a cohort comprising both sexes. One previous study, and the only one as far as we know, included measures of adipose tissue distribution as assessed by CT, adipokines and evaluation of subclinical atherosclerosis by IMT in a cohort of young apparently CV healthy women. In that study, hypoadiponectinaemia and subcutaneous, but not visceral, abdominal adipose tissue were related to increased IMT. In our study IV, SAT was not found to be related to TAS, which could indicate that the effect of the adipose tissue distribution on the amount of atherosclerosis is influenced by gender and age.

It has previously been reported that the WHR was better related to myocardial infarction than was BMI. We found in study IV that BMI and VAT were only moderately related to each other, indicating that these two measures carry different information. BMI was not significantly related to TAS in study IV.

Leptin levels have previously been shown to predict CVE and to relate to increased IMT in the carotid artery. In study IV, leptin was significantly related to TAS in the univariate analysis, but did not affect the relationship between VAT and TAS, and the significance of its relationship to TAS was lost after addition of adiponectin to the multiple censored regression models. This finding suggests that leptin did not play an important role in the development of atherosclerosis in this elderly cohort.

No significant interactions were seen in study IV between adiponectin and gender or leptin and gender (data not shown). Adjustments were therefore made for gender in the multiple censored regression models and no stratification by gender was made.

Another plausible explanation for the increased atherosclerosis observed in association with visceral fat accumulation could be actions of other inflammatory components expressed in the adipose tissue, such as TNF-α or IL-6. TNF-α is primarily expressed in macrophages invading the visceral adipose tissue and is a potent inhibitor of adiponectin synthesis. IL-6 is partly synthesised in the visceral adipose tissue and is a major promoter of production of acute phase reactants, such as CRP, which has been linked to CV events but not to subclinical atherosclerosis. It seems that CRP and the degree of subclinical atherosclerosis carry different information and could be used as complementary risk markers. In study IV, IL-6, together with VAT, was found to be an independent predictor of TAS, suggesting that IL-6 is not the mechanism whereby VAT influences TAS. TNF-α was not significantly related to either VAT or TAS.
Conclusions

From the findings in study I it is concluded that WBMRA is feasible with a standard clinical scanner, with a table top extension, in patients. The clinical relevance of the technique and its role in prognostic predictions and monitoring of therapy have to be determined.

In study II it was found that WBMRA could be carried out and successfully interpreted in a random population sample of elderly subjects aged 70. The study confirmed that WBMRA in a 1.5T clinical scanner can be used for quantifying atherosclerosis in different vascular territories in one single examination. Significant atherosclerotic abnormalities were present in a substantial proportion of the 70-year-old subjects without self-reported vascular disease. This method could be useful as a tool for assessing atherosclerosis in an epidemiological setting.

It is concluded from the results of study III that atherosclerosis in the carotid and renal arteries, aorta and arteries in the upper and lower parts of the legs as evaluated by WBMRA, as well as a weighted index, namely the total atherosclerotic score, was related to traditional CV risk factors included in the Framingham risk score.

Study IV led to the conclusion that in an elderly population VAT was related to TAS independently of gender, total obesity (BMI), and the amounts of SAT, IL-6 and TNF-α as well as of the traditional CV risk factors included in FRS. However, adiponectin attenuated the relationship between VAT and TAS, suggesting that adiponectin is an important link between visceral adiposity and atherosclerosis. Leptin was related to TAS in the univariate analysis, but this relationship lost its significance in the multiple censored regression models.

The fact that WBMRA, a minimally invasive, non-radiation technique, could be used successfully both in patients and in a large cohort of elderly citizens in an epidemiological study without adverse events, makes this a safe and robust method.

The full usefulness of WBMRA and the total atherosclerotic score in epidemiological settings still needs to be validated against the outcome data of the PIVUS cohort. The full value of WBMRA as a complementary screening tool also needs to be related to these outcome data.
Assessment of Atherosclerosis by Whole-body Magnetic Resonance Angiography

Summary in Swedish

Bedömning av ateroskleros med magnetisk resonans angiografi av hela kroppen.

Ateroskleros är en dominerande orsak till sjukheter och dödsfallet och kan utvärderas på flera olika sätt, t.ex. med angiografi och ultraljud. Magnetisk resonans angiografi (MRA) är en minimalt invasiv metod som inte använder sig av joniserande strålning.

Det övergripande syftet med denna avhandling var att utvärdera möjligheten att utföra magnetisk resonans angiografi av hela kroppen (WBMRA) med patienter (studie I) samt även i en större epidemiologisk studie med 70-åriga individer (studier II-IV). Specifika delmål var att skapa en mall för bedömningen av graden av ateroskleros i form av en poängsumma, ”total atherosclerotic score” (TAS) för en individ, samt att utvärdera relationerna mellan denna poängsumma och olika kardiovaskulära riskmarkörer.


Samstämmigheten av resultaten mellan två olika bedömare och mellan två bedömningar av samma bedömare var god och alla 33 patienter (studie I) respektive 306 av 307 stycken av de 70-åriga individerna (studie II-IV) kunde genomföra undersökningsen. Endast en individ fick en vasovagal reaktion före kontrastinjektionen. En hög andel av kärelseyen gick att utvärdera (93-99%). I en mindre jämförelse med konventionell angiografi, vilken betraktas som referensstandard, var överensstämmelsen relativt god avseende graden av stenos eller förekomst av ocklusion mellan de två metoderna. Detta indikerar att WBMRA metoden är säker och robust.

Hos 9 av 33 patienter med ateroskleros hittades oväntade signifikanta kärlförändringar som ej misstänktes kliniskt. Även hos 70-åriga individer utan självrappartera aterosklerotiska symptom (n=230), återfanns ≥50% stenoser och ocklusioner (n=59) samt aneurysm. Hos 70-åriga individer med symptom (n=69) sågs signifikanta kärlförändringar hos 33 individer. Bukaortaaneurysm sågs i 2% av individerna och signifikanta kärlförändringar (stenoser ≥50% eller ocklusioner) sågs i 1,5% av njurararterna, 1,8% av karotiskärlen, 1,1% av bäcken- och lårarterna samt 6,2% av underbensarterna. Cirka en tredjedel av dessa 70-åriga individer från Uppsala hade ingen lumenförträngning av kärlen, en tredjedel hade endast icke signifikanta kärlförändringar och den resterande tredjedelen hade stenoser ≥50% och eller ocklusioner.
Statistiskt signifikanta korrelationer hittades mellan graden av kärlförändringar i de fem olika kärlterritorierna. Den aterosklerotiska poängsumman för de fem olika kärlterritorierna var även enskilt signifikant relaterad mot ett väl validerat kardiovaskulärt riskbedömningsprotokoll, nämligen ”Framingham risk score” (FRS). Den totala aterosklerotiska poängsumman (TAS) var även signifikant relaterad till FRS, vilket understryker att ateroskleros är en systemisk sjukdom.

TAS var också signifikant relaterad till mängden abdominell visceral fettvävnad (VAT), interleukin-6, leptin samt inverst relaterad till adiponectin i en enkel regressionsanalys, men inte till mängden subkutan fettvävnad eller body mass index (BMI). I en multipel regressionsanalys var VAT relaterat till TAS trots ett flertal andra kardiovaskulära markörer. Adiponectin uttrycks specifikt av fettvävnad och dess blodhalt är minskad vid fetma. När denna variabel adderades till regressionsanalysen föll den signifikanta relationen mellan VAT och TAS och adiponectin blev istället signifikant relaterad till TAS. Detta kan tala för att adiponectin kan vara den patofysiologiska länken mellan bukfetma och kärlförändringar.

I denna avhandling visades att WBMRA är möjlig att använda för bedömning av samtidig kärlsjuka i flera olika kärlområden både hos patienter och för epidemiologisk forskning. Prevalensen av kärlförändringar i karotisartärer, njurartärer samt aorta, bäcken, lår och underbensartärer i form av stenoser, ocklusioner och aneurysm fastställdes i en grupp av 70-åriga individer från Uppsala. En mall för bedömning av graden av ateroskleros skapades för WBMRA. Ytterligare studier behövs för att bedöma den eventuella ytterligare nyttan av TAS relativt konventionella metoder, såsom exempelvis FRS, för att bedöma den framtidiga risken för kardiovaskulära händelser hos en individ. I en sådan studie kommer de framtidiga aterosklerotiska sjukdomshändelserna i form av hjärtinfarkt, kärlkramp, slaganfall eller hjärtód att relateras mot den aterosklerotiska poängsumman (TAS). I väntan på denna studie indikerar relationen till FRS att TAS kan vara av potentiellt värde för detta syfte.
Acknowledgements

This thesis is not the work of one person alone. To be able to produce a thesis, one needs a team of interested persons who are willing to contribute with work, time, a will to teach, and competence. I have been fortunate enough to have these prerequisites and I will remember this journey with gratitude and joy.

I wish to express my sincere appreciation to everyone who has contributed to the necessary efforts and supported me in all forms.

“No one named, no one forgotten”
References


Assessment of Atherosclerosis by Whole-body Magnetic Resonance Angiography


Assessment of Atherosclerosis by Whole-body Magnetic Resonance Angiography


References


Appendix

Vitruvian man

On the cover is a maximum intensity projection acquired from a whole-body magnetic resonance angiography projected onto the “Vitruvian man”, which was drawn by Leonardo da Vinci among the year 1492. The drawing was made with pen and brown ink with wash over metalpoint on paper. The accompanying notes were written in mirror writing. The drawing depicts a nude male in two superimposed positions and simultaneously inscribed in a circle and square. The drawing is also called the “Canon of Proportions” and is believed to symbolise the material existence by the square and the spiritual existence by the circle. Thus Leonardo attempted to depict the correlation between these two aspects of human existence and his belief that the well balanced proportions of the human body represented the perfect relationship between material and spiritual existence.

An ancient Roman architect, Vitruvius, wrote: “Similarly, in the members of a temple there ought to be the greatest harmony in the symmetrical relations of the different parts to the general magnitude of the whole. Then again, in the human body the central point is naturally the navel. For if a man can be placed flat on his back, with his hands and feet extended, and a pair of compasses centered at his navel, the fingers and toes of his two hands and feet will touch the circumference of a circle described therefrom. And just as the human body yields a circular outline, so too a square figure may be found from it. For if we measure the distance from the soles of the feet to the top of the head, and then apply that measure to the outstretched arms, the breadth will be found to be the same as the height, as in the case of plane surfaces which are completely square.”

(Marcus Vitruvius, De Architectura, Book III, Chapter 1, p 3).

Vitruvius also wrote that:
- a palm is the width of four fingers
- a foot is the width of four palms
- a cubit is the width of six palms
- a man’s height is four cubits (and thus 24 palms)
- a pace is four cubits
- the length of a man’s outspread arms is equal to his height
- the distance from the hairline to the bottom of the chin is one-tenth of a man’s height
- the distance from the top of the head to the bottom of the chin is one-eighth of a man’s height
- the maximum width of the shoulders is a quarter of a man’s height
- the distance from the elbow to the tip of the hand is one-fifth of a man’s height
- the distance from the elbow to the armpit is one-eighth of a man’s height
- the length of the hand is one-tenth of a man’s height
- the distance from the bottom of the chin to the nose is one-third of the length of the head
- the distance from the hairline to the eyebrows is one-third of the length of the face
- the length of the ear is one-third of the length of the face

The innovation in this drawing by Leonardo as compared to previous drawings is his observation that the square can not have the same centre as the circle, which has the navel as centre.

http://en.wikipedia.org